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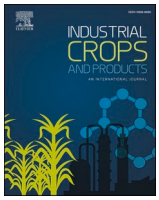

Short communication

Prioritization of germplasm resources for *Epimedium* breeding: Chemotype oriented discovery using cross latitude scale resource evaluation

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ABSTRACT

Epimedium is a traditional medicinal plant used healthcare consumption for thousands of years, and also serves as a highly sought-after raw material in the pharmaceutical industry. In recent years, wild resources of this kind have been gradually dwindling, therefore, how to select high-quality *Epimedium* resources for breeding and cultivation is crucial. In the present work, a total of 34 batches of *Epimedium* germplasm resources were collected over a distance of more than 1000 kilometers and across 5 ° of latitude. After a year of cultivation of the homogeneous gardens, the characters of a collection of 34 *Epimedium* plants were investigated based on the quantification of physical and chemical parameters. The results of the correlation analysis showed that the length of the first node appears to be longer when *Epimedium* plants are enriched with epimedin C and magnoflorine in the sample E02. By classifying the contents of six major bioactive components detected and analyzed, it was found that E02 and E26 were the populations of *Epimedium* with high quality chemotypes. Thus, E02 and E26 could be further studied and bred to provide excellent core resources for the protection of *Epimedium* germplasm, and based on correlation analysis, the length of the first node could perhaps serve as a selection indicator. In summary, this study proposes an effective discovery method that can be used for the chemotype oriented breeding of *Epimedium* plants, and also provides a solid foundation for the utilization of other medicinal plants in the future.

1. Introduction

Epimedium is a genus containing more than 50 species of herbal plants, distributed mainly in China, Herba Epimedii is the aerial parts of *Epimedium* species (Ma et al., 2011; Wu et al., 2003). *Epimedium* plants are widely used as a traditional Chinese medicine for the prevention of various diseases such as cardiovascular diseases (Kim and Shim, 2019), menstrual irregularity (Zhang et al., 2007), chronic nephritis (Zhao et al., 2022) and immunological dysfunction (Lu et al., 2023). Due to the above effects, *Epimedium* was in huge market demand, with 20,000 tons per year required in the Chinese market alone (Zhao et al., 2024), and its market price remains high, reaching about 200 China Yuan (CNY) per kilogram or 30 United States dollar (USD) per kilogram (Yang et al., 2022). It can be seen that *Epimedium* has high planting value, while the quality of artificially cultivated products is unstable, and this is often reflected in the content of active ingredients. Therefore, it is essential to investigate the variation in the composition of active metabolites among different *Epimedium* species, which will help in selecting breeding resources of *Epimedium* with high content of active compounds.

Previous phytochemical studies have shown that a variety of components can be extracted from the dried leaves of *Epimedium* plants, including flavonoids (Li et al., 2012), flavonoid glycosides (Zhang et al., 2008), lignans (Ma et al., 2011; Wu et al., 2003), xanthones (Ma et al., 2011; Wu et al., 2003), alkaloids (Zhao et al., 2025), and acids (Zhao

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et al., 2024). In particular, flavonoid glycosides such as epimedin A, epimedin B, epimedin C (Zhang et al., 2009), icariin (Miyase et al., 1989), baohuoside I, and icaritin are considered to be the major bioactive components, which are frequently used as the marker compounds for evaluating the quality of *Epimedium* plants (Gani et al., 2023; Naseer et al., 2015). Multi-omics analysis provides a new approach for the exploration and utilization of medicinal plants (Zhu et al., 2024; Qin et al., 2022). In order to learn more information about the accumulation of active components in *Epimedium*, researchers have started to unravel the biosynthetic pathway of flavonoid-derived bioactive components by integrated transcriptional and phytochemical analyses (Huang et al., 2015; Zeng et al., 2013). However, most studies focus only on a single species of the *Epimedium* genus (Wang et al., 2010; Xue et al., 2023; Su et al., 2018), or just a few species (Xu et al., 2021; Bae et al., 2020), the comparative analysis of active components in different *Epimedium* species is not comprehensive, and these studies have not explored how to classify *Epimedium* populations into different chemotypes based on a single component. This is important for the quality development and commercialization of *Epimedium*. The chemotype of different germplasm resources should be accurately assessed to effectively broaden the genetic base, thereby facilitating the direct breeding of *Epimedium*.

Therefore, in the present study, different species of *Epimedium* from various regions of China were collected and the main phenotypic characteristics of 34 *Epimedium* populations grown under similar environmental conditions and cultivation practices were evaluated in detail. Then, the high performance liquid chromatography (HPLC) method has been established for the quantitative analysis of bioactive constituents. To further determine the association between phenotypic characteristics and phytochemical composition, a correlation analysis was performed according to the quantitative results. Finally, according to the quantitative results of the active compounds, the 34 *Epimedium* populations were divided into three chemotypes, including extremely high, intermediate, and extremely low chemotypes. The aim of this study was to provide a basis for the targeted breeding of the chemical components of *Epimedium* plants based on the chemotype classification of *Epimedium* populations. The results of this study will provide a reference for the screening of potential germplasms of *Epimedium* with excellent physicochemical traits.

2. Materials and methods

2.1. Plant materials and planting sites

Epimedium plants from different area were transplanted to the homogeneous nursery of Lushan Botanical Garden, Lushan City, China (29°32'N, 115°58'E). The homogeneous garden experiment for *Epimedium* plants was established at 1021 m elevation within Lushan Mountain under deciduous broad-leaved canopy. Cultivation utilized raised beds (height: 25 cm; width: 1.2 m) with understory light manipulation maintaining 50–70 % summer canopy closure; in the homogeneous garden, the number of effective cultivated plants from the same wild population was more than 30. Key site parameters include: precipitation: 1833.5 mm/yr (multiyear mean); thermal regime: −18℃ (min) to 32℃ (max); RH: 80 % (annual mean of hyper-humid); edaphic conditions: acidic soil (pH 5.9–6.3), 40–50 % volumetric water content at 10 cm depth (sunny days); litterfall: 0.8 kg/m^2/yr; management: semi-ecological planting, spring-summer weeding (2–3 cycles/yr), mountain spring irrigation, and guaranteed drainage ditch functionality during monsoon period. After a complete growth and metabolism cycle (one year) of cultivation, a total of 34 *Epimedium* varieties were selected for our study. Plants specific information was recorded in Table S1.

2.2. Measurement of phenotypic traits

Three to six robust plants of each *Epimedium* variety with normal growth, no obvious defects, no pests, and no diseases were randomly selected as test sample plants. Seven morphological characteristics of each Herba Epimedii as follows were recorded using a vernier caliper or ruler (Fig. 1): length of the first node (FL), the length from the ground to the first branch in centimeters (cm); width of the first node (FW), the thickest part of the first node in millimeters (mm); petiole length (PL), the length from the beginning of the first or second node branch to the bottom of the leaf blade in cm; petiole width (PW), the thickest part of the petiole in mm; leaf length (LL), the longest section of the leaf blade parallel to the main vein in cm; leaf width (LW): the widest part of the leaf blade in cm; leaf area (LA), took a picture with a camera and measured the leaf area with Image J software in square centimeters (cm^2). There were more than one petiole and leaf blade of the same plant, which were measured separately, and the data were unified after obtaining multiple sets of data for collation and analysis.

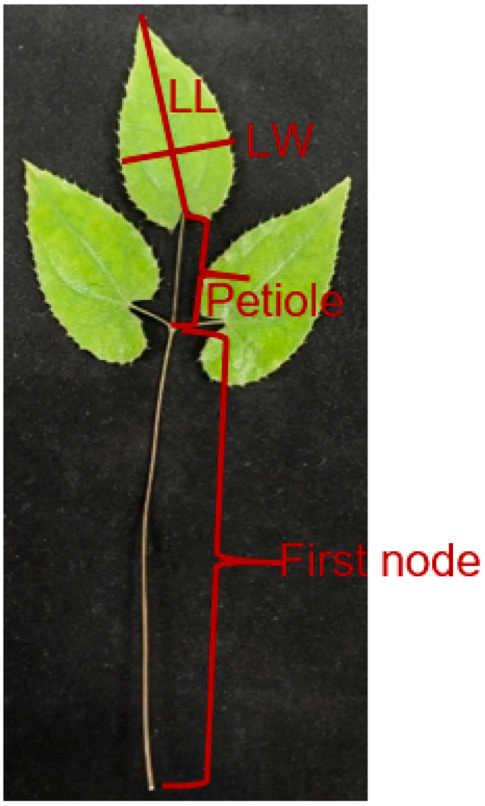

Fig. 1. The main phenotypic traits of *Epimedium* plant.

2.3. Chemical analysis

Methanol, acetonitrile, and formic acid were HPLC grade, and supplied by Merck (Darmstadt, Germany). The standards magnoflorine, epimedin A, epimedin B, epimedin C, icariin, baohuoside I, icariside I, icaritin, hyperoside, quercitrin, chlorogenic acid, cryptochlorogenic acid, and caffeic acid, were purchased from Chengdu Must Biotechnology Co., Ltd. (Chengdu, China). All *Epimedium* samples were collected from Lushan Botanical Garden. 100 mg of each sample was mixed with 10 mL of methanol and then sonicated for 30 min. The solutions were filtered (polyvinylidene fluoride membrane, 0.22 μm) and then used directly for HPLC analysis.

HPLC-DAD analysis was performed on an Agilent 1260 system consisting of degasser, pump, autosampler, temperature-controlled column compartment, and DAD, applying an XBridge C18 (4.6 ×250 mm; 3.5 μm) column at a temperature of 30 °C. Aqueous formic acid (0.1 %) was used as eluent A and acetonitrile as eluent B at a flow rate of 0.8 mL/min with the following gradient: 0–10 min 13–27 % B; 10–22 min 27–32 % B; 22–35 min 32–100 % B; 35–36 min 100–13 % B; 36–44 min

Table 1
Quantitative phenotypic traits of 34 *Epimedium* accessions.

Number	FL[a] (cm)	FW[b] (mm)	PL[c] (cm)	PW[d] (mm)	LL[e] (cm)	LW[f] (cm)	LA[g] (cm^2)
E01	11.60 ± 0.53	0.71 ± 0.13	2.21 ± 0.59	0.32 ± 0.04	2.90 ± 0.38	2.03 ± 0.18	4.95 ± 1.64
E02	21.13 ± 3.37	1.58 ± 0.33	2.26 ± 0.35	0.82 ± 0.19	10.77 ± 2.34	3.59 ± 0.63	32.75 ± 10.93
E03	14.93 ± 3.31	1.13 ± 0.20	2.04 ± 0.48	0.42 ± 0.07	5.32 ± 0.85	3.16 ± 0.70	13.44 ± 6.50
E04	14.80 ± 0.52	1.07 ± 0.06	2.68 ± 0.18	0.48 ± 0.12	7.00 ± 0.61	3.34 ± 0.16	19.34 ± 8.83
E05	15.30 ± 0.30	1.41 ± 0.10	3.54 ± 0.67	0.70 ± 0.07	8.24 ± 0.90	5.02 ± 0.57	27.15 ± 9.60
E06	20.67 ± 1.53	1.73 ± 0.07	3.52 ± 0.16	0.79 ± 0.12	8.60 ± 0.09	4.93 ± 0.27	31.44 ± 6.06
E07	8.13 ± 3.35	0.77 ± 0.18	1.40 ± 1.07	0.37 ± 0.13	3.82 ± 1.58	1.98 ± 0.89	4.70 ± 4.21
E08	9.73 ± 0.46	0.69 ± 0.07	1.64 ± 0.19	0.39 ± 0.10	4.17 ± 0.28	2.98 ± 0.02	9.33 ± 2.39
E09	14.63 ± 1.00	1.11 ± 0.08	2.11 ± 0.37	0.54 ± 0.02	8.52 ± 1.13	3.10 ± 0.30	13.09 ± 2.10
E10	13.73 ± 3.65	0.89 ± 0.10	2.60 ± 1.20	0.52 ± 0.05	6.43 ± 0.67	3.06 ± 0.57	15.46 ± 8.00
E11	13.90 ± 3.44	1.13 ± 0.06	2.34 ± 0.40	0.56 ± 0.02	7.96 ± 0.20	3.17 ± 0.12	15.80 ± 1.10
E12	13.87 ± 3.01	1.67 ± 0.32	3.69 ± 0.40	0.90 ± 0.03	8.80 ± 0.64	5.32 ± 0.33	44.61 ± 6.95
E13	16.63 ± 3.18	1.13 ± 0.13	2.74 ± 0.16	0.54 ± 0.05	6.60 ± 0.91	3.18 ± 0.15	16.31 ± 2.05
E14	9.17 ± 1.72	0.84 ± 0.15	1.94 ± 0.39	0.43 ± 0.06	4.30 ± 0.76	3.16 ± 0.49	11.20 ± 2.51
E15	16.40 ± 0.56	1.30 ± 0.21	2.82 ± 0.12	0.50 ± 0.05	5.60 ± 0.82	3.47 ± 0.47	15.83 ± 1.43
E16	9.27 ± 1.12	0.73 ± 0.08	1.62 ± 0.04	0.37 ± 0.08	3.30 ± 0.07	2.36 ± 0.21	5.05 ± 1.02
E17	7.90 ± 1.31	0.71 ± 0.09	1.61 ± 0.59	0.43 ± 0.06	3.96 ± 0.40	2.31 ± 0.34	3.69 ± 1.52
E18	15.67 ± 4.04	1.44 ± 0.29	1.53 ± 0.35	0.55 ± 0.11	9.03 ± 1.81	3.16 ± 0.49	18.42 ± 9.80
E19	10.40 ± 0.53	0.88 ± 0.10	3.76 ± 0.92	0.39 ± 0.09	3.78 ± 0.33	2.27 ± 0.12	6.83 ± 1.00
E20	13.13 ± 3.41	1.05 ± 0.18	2.17 ± 0.23	0.48 ± 0.02	6.88 ± 0.24	3.58 ± 0.43	16.13 ± 5.53
E21	11.67 ± 0.38	0.99 ± 0.09	2.48 ± 0.25	0.54 ± 0.05	6.49 ± 1.71	3.36 ± 0.28	15.48 ± 4.09
E22	13.37 ± 3.65	0.95 ± 0.21	3.60 ± 0.38	0.46 ± 0.05	4.53 ± 0.30	3.17 ± 0.15	13.72 ± 3.02
E23	11.50 ± 8.26	0.74 ± 0.13	2.14 ± 0.57	0.33 ± 0.03	2.30 ± 0.23	2.03 ± 0.18	3.56 ± 0.23
E24	8.83 ± 2.08	1.07 ± 0.23	1.99 ± 0.61	0.37 ± 0.02	4.06 ± 0.13	2.29 ± 0.36	6.68 ± 2.58
E25	6.87 ± 1.60	0.90 ± 0.19	2.28 ± 0.24	0.41 ± 0.06	4.53 ± 0.61	2.81 ± 0.08	8.31 ± 1.13
E26	16.5 ± 1.80	1.23 ± 0.10	2.06 ± 0.10	0.53 ± 0.08	4.41 ± 0.27	3.17 ± 0.12	10.66 ± 1.58
E27	11.67 ± 2.31	0.97 ± 0.22	2.08 ± 0.18	0.49 ± 0.06	5.12 ± 1.01	2.90 ± 0.20	10.40 ± 1.34
E28	14.80 ± 1.21	1.25 ± 0.09	2.60 ± 0.23	0.69 ± 0.05	6.13 ± 0.56	3.59 ± 0.20	17.75 ± 5.95
E29	8.13 ± 0.23	0.88 ± 0.14	1.57 ± 0.12	0.53 ± 0.05	5.46 ± 0.46	2.48 ± 0.22	11.16 ± 1.61
E30	7.90 ± 1.47	0.83 ± 0.11	2.48 ± 0.34	0.50 ± 0.02	3.46 ± 0.18	2.78 ± 0.19	9.02 ± 0.41
E31	9.67 ± 2.08	0.79 ± 0.09	2.70 ± 0.33	0.35 ± 0.02	3.19 ± 0.10	2.94 ± 0.23	6.06 ± 1.64
E32	8.57 ± 0.59	0.83 ± 0.14	1.91 ± 0.29	0.38 ± 0.03	3.49 ± 0.23	2.24 ± 0.14	4.44 ± 1.47
E33	11.97 ± 5.32	0.92 ± 0.11	2.99 ± 0.65	0.43 ± 0.05	3.94 ± 0.34	3.19 ± 0.20	10.26 ± 3.61
E34	6.17 ± 1.26	0.75 ± 0.05	2.43 ± 0.12	0.38 ± 0.02	2.67 ± 0.29	3.07 ± 0.15	7.80 ± 1.02

Note: Results are shown as the mean ± SD (n = 3–6)
[a] : Length of the first node
[b] : Width of the first node
[c] : Petiole length
[d] : Petiole width
[e] : Leaf length
[f] : Leaf width
[g] : Leaf area

13 % B. The eluate was monitored at a wavelength of 270 nm and 360 nm. The sample injection volume was 5 μL. For absolute quantification, calibration curves were prepared in a concentration range (3.125–100 μg/mL) using external standards in methanol. The standard solutions were stored at 4°C for further analysis.

2.4. Statistical analysis

GraphPad PRISM version 8.0.2 (GraphPad Software, USA) was used for statistical analysis. Results are expressed as means ± SD of three to six repetitions (n = 3–6). The quantitative data were subjected to simple descriptive statistics. A one-way analysis of variance (ANOVA) was used to statistically compare the data of different groups and p value < 0.05 was considered as statistically significant. Correlation analysis was applied to determine the relationship among all quantitative index using R Studio. The classification of populations into different chemotype employs a modified Z-score approach. For each tested indicator: A threshold (T) was primarily established based on fundamental data characteristics, then performed a logarithmic transformation on the data and computed the average (A) and median absolute deviation (MAD). For individual measurement (D): if it met Eq. (1), classify into 'extremely high' group; if it met Eq. (2), classify into 'extremely low' group; otherwise, it was assigned to 'intermediate' group.

$$\frac{D - A}{MAD} > T \quad (1)$$

$$\frac{D - A}{MAD} < -T \quad (2)$$

3. Results and discussion

3.1. Analysis of difference in phenotypic traits

The *Epimedium* landraces are characterized by different characters (FL, FW, PL, PW, LL, LW, and LA). Certain variations were observed among the *Epimedium* varieties in terms of the measured quantitative phenotypic traits. The number of FL was in the range of 6.17 (E34) to 21.13 cm (E02) and FW ranged from 0.71 mm (E01 and E17) to 1.73 mm (E06). PL was between 1.40 cm (E07) and 3.76 cm (E19). PW ranged from 0.32 mm (E01) to 0.90 mm (E12). LL was also in the range of 2.30 cm (E23) to 10.77 cm (E02). LW was between 1.98 cm (E07) and 5.32 cm (E12). LA was as small as 3.56 cm^2 (E23) and as large as 44.61 cm^2 (E12) in the *Epimedium* cultivars (Table 1). The observed high variation in the range of the quantitative phenotypic traits in all *Epimedium* landraces may be caused by genetic basis or could be related to environmental adaptations. *Epimedium* landraces should adopt to the changes of rainfall, temperature, soil, and altitude that differ with the collection sites. It should be noted that different cultivation site can also lead to changes in phenotypic traits. The favored medicinal varieties usually cultivated in geo-authentic growing area (Li et al., 2023). However, it is now widely believed that cultivated varieties have

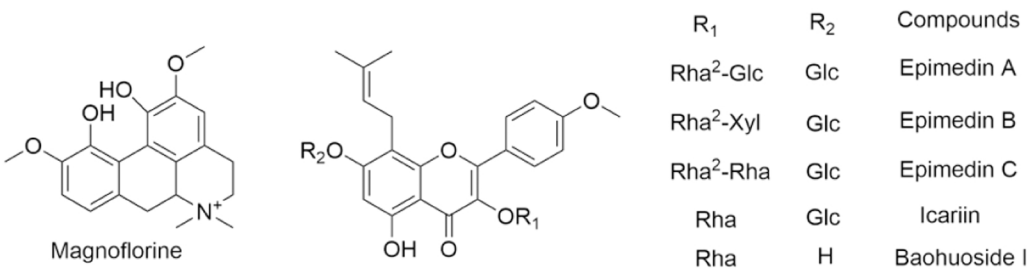

Fig. 2. Chemical structures of magnoflorine, epimedin A, epimedin B, epimedin C, icariin, and baohuoside I (glc: glucose; rha: rhamnose; xyl: xylose).

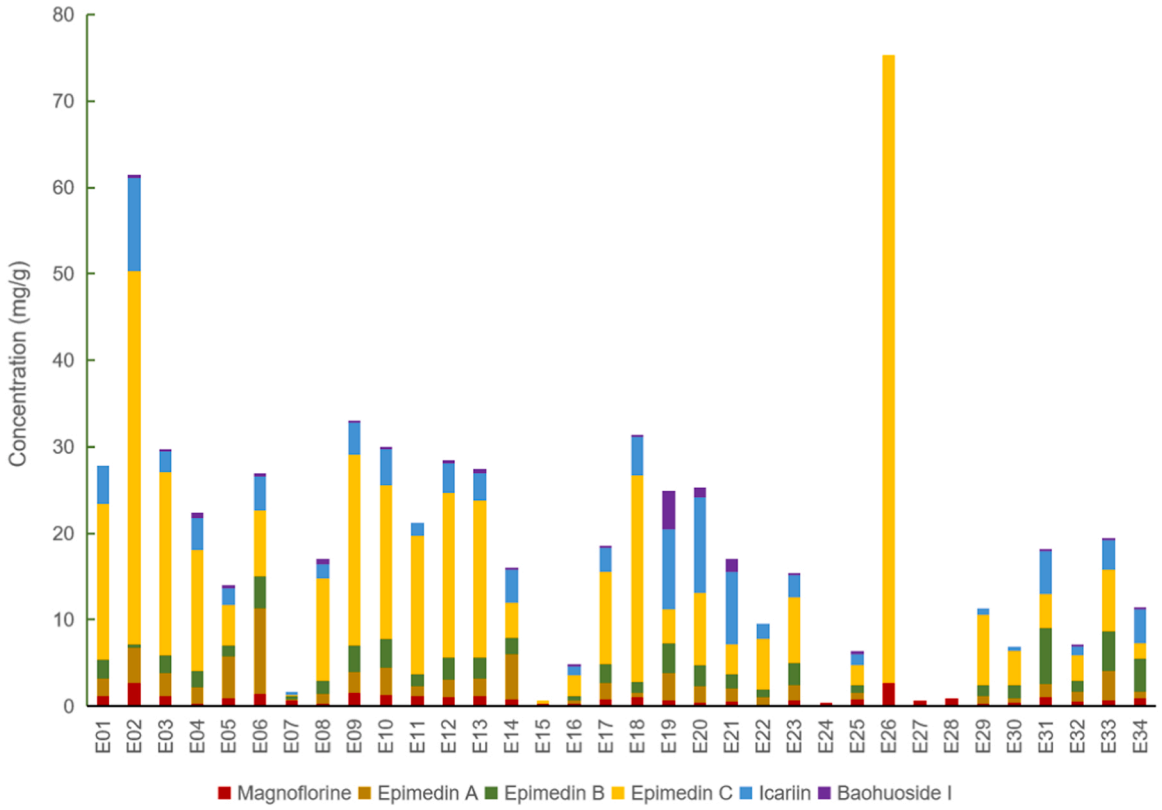

Fig. 3. Mean content of the active components in the studied *Epimedium* accessions (n = 3–6; red, magnoflorine; dark grey, epimedin A; green, epimedin B; yellow, epimedin C; blue, icariin; purple, baohuoside I).

superior physicochemical properties and quality, which is mainly attributed to the domestication process (Xu et al., 2025).

3.2. The content of active chemical compositions

The amounts of active components is an essential index for the evaluation of the quality of *Epimedium* plant, therefore, 13 active components in Herba Epimedii were quantified using HPLC-DAD method. The developed quantitative method was validated by evaluating the limit of detection (LOD), limit of quantification (LOQ), linearity, precision, repeatability, stability, and recovery. LOD and LOQ were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively, under optimized chromatographic conditions. As summarized in Table S2, LODs ranged from 0.01 μg/mL to 0.45 μg/mL, while LOQs spanned 0.17 μg/mL to 1.27 μg/mL across all 13 target compounds. All calibration curves showed excellent coefficients of determination ($r \geq 0.9998$) within the detected concentration range for the corresponding analytes in the samples. Six replicate analyses of mixed standards within 24 h showed RSDs $\leq$ 3.86 %; inter-day precision: duplicate analyses over three consecutive days yielded RSDs $\leq$ 6.18 %; triplicate extractions of samples E20 or E11 demonstrated robust repeatability, with process RSDs ranging from 0.08 % to 5.19 % for all analytes; sample stability was confirmed in E20/E11 powders stored at ambient temperature (23 ± 2°C). RSDs for analyte concentrations remained between 0.45 % and 2.88 % throughout the 48-hour test period (n = 6 time-points); mean recovery rates of 13 compounds spiked into 0.10 g E20/E11 samples were determined via 6 replicates. Results (Table S2) showed recoveries of 97.75~104.52 % with associated RSDs of 0.94–5.52 %, confirming extraction efficiency.

Although we attempted to detect the concentration of 13 components in *Epimedium* accessions, most of the samples contained only 6 compounds including magnoflorine, epimedin A, epimedin B, epimedin C, icariin, and baohuoside I (Fig. 2). It may be because the physicochemical properties of the compound and the detection limits of the analytical instruments, chlorogenic acid, cryptochlorogenic acid, and caffeic acid were not detected. Icariside I, as a secondary glycoside, is not an original storage component in the plant, it may be converted from other major flavonoid glycosides during extraction, processing, or storage, research showed that the content of certain flavonoid glycoside compounds in *Epimedium* changes during heat treatment (Zhao et al.,

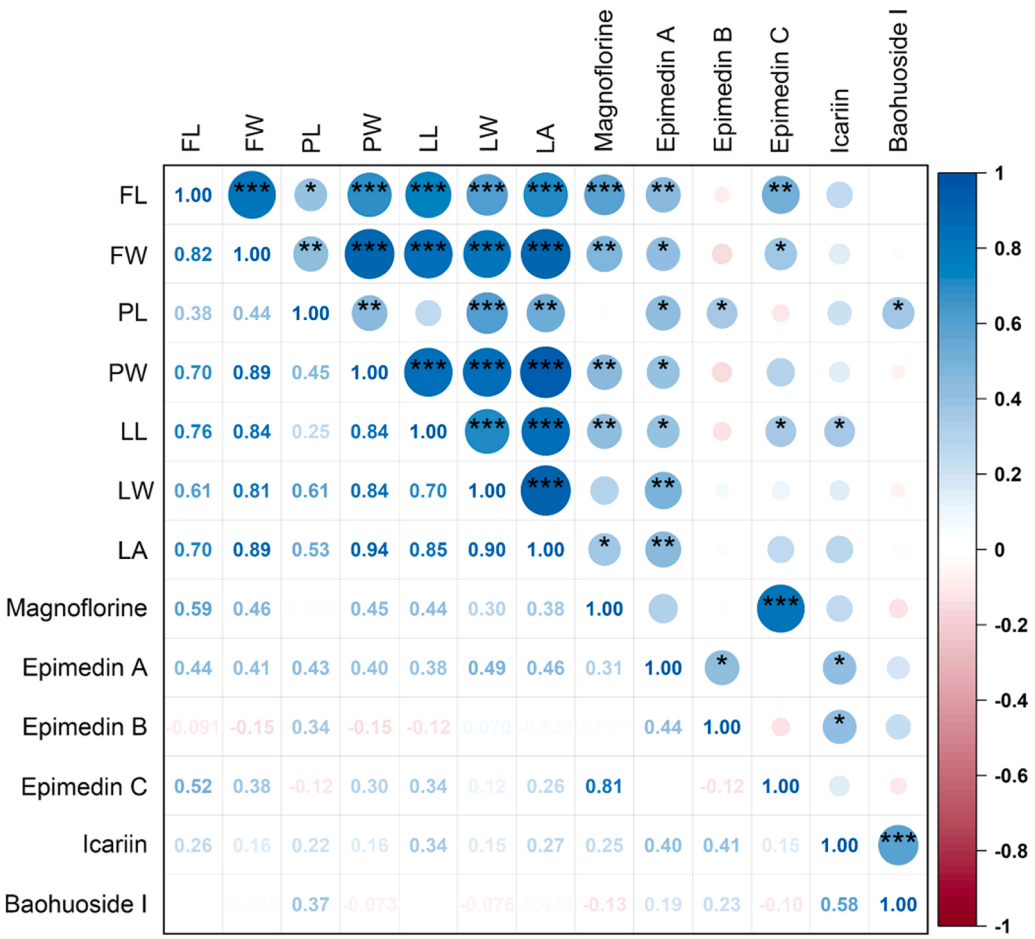

Fig. 4. Correlations between the phenotypic traits and chemical composition of 34 *Epimedium* accessions studied (***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$).

2024). Therefore, Icariside I was not detected in this study either. Icaritin was only present in three samples, E04 (0.06 mg/g), E07 (0.01 mg/g), and E13 (0.08 mg/g). Trace amounts of hyperoside and quercitrin appeared only in a very few samples, which may be due to the fact that the analyzed *Epimedium* samples were composed of different species, leading to large variations in the content of some compounds. These trace compounds are not shown in Fig. 3, only magnoflorine, epimedin A, epimedin B, epimedin C, icariin, and baohuoside I were shown. In our study, the dominant flavonoid glycoside in Herba Epimedii was epimedin C, found in the highest content in sample E26 (72.63 mg/g), followed by E02 (43.18 mg/g). Epimedin A was also abundant in Herba Epimedii, the maximum level and the second highest level were E06 (9.94 mg/g) and E14 (5.19 mg/g), respectively. The highest content of epimedin B was E31 (6.48 mg/g). Icariin was detected with highest content in E20 (10.98 mg/g), closely followed by E02 (10.80 mg/g). Baohuoside I was detected at low levels in some cultivars, whereas E19 (4.48 mg/g) exhibited the highest concentration of baohuoside I. On the other hand, magnoflorine, an alkaloid, naturally occurring in *Epimedium* is responsible for health-related benefits including anti-diabetic, antioxidant and anti-inflammatory effects (Xu et al., 2020). Accession E26 contained the highest content of magnoflorine with 2.66 mg/g.

3.3. Correlation between chemical constituents and phenotypic traits

It is well known that the phenotype of plants refers to the externally observable traits expressed from the plant's genetic information under specific environmental conditions, while secondary metabolites are one of the key intrinsic products of this expression at the molecular level. Owing to the correlation between accumulation patterns of metabolites and phenotypic features, we hypothesized that the phenotypic features could be used to characterize the accumulation of bioactive compounds in *Epimedium* plants. Correlation analysis was conducted to understand the connection between bioactive metabolites and phenotypic traits. As shown in Fig. 4, magnoflorine showed a significantly positively correlated with epimedin C, with a correlation coefficient (r) of 0.81, $p < 0.001$, which may explain the higher levels of magnoflorine and epimedin C in accession E02. The content of magnoflorine and epimedin C was significantly positively correlated with the phenotypic trait FL ($r = 0.59$ or 0.52, $p < 0.001$ or $p < 0.01$), implying that the accumulation of these two bioactive chemicals in *Epimedium* plants may be roughly determined based on the length of FL in the same individual plant.

It is generally accepted that secondary metabolites, such as alkaloids and flavonoids, are essential for mediating the interactions between plants and environment, including defense against insects, herbivores, and even pathogenic microorganisms, as well as coping with various abiotic stresses (Hao et al., 2025). Therefore, the accumulation of compounds such as flavonoid glycosides in *Epimedium* plants serves as a chemical barrier for healthy growth and reducing damage. Previous studies have found a certain correlation between leaf morphology and flavonoid glycoside content: *Epimedium* with high levels of active ingredients tends to have smaller leaves and a lower degree of leaf leathery texture (Gao et al., 2009). In short, combined the plant phenotypic traits parameters with quantitative results, we can find that changes in the external phenotype of plants perhaps affect the content and yield of metabolites in *Epimedium*. However, this is just our preliminary exploration, and the molecular mechanisms behind this result still need to be

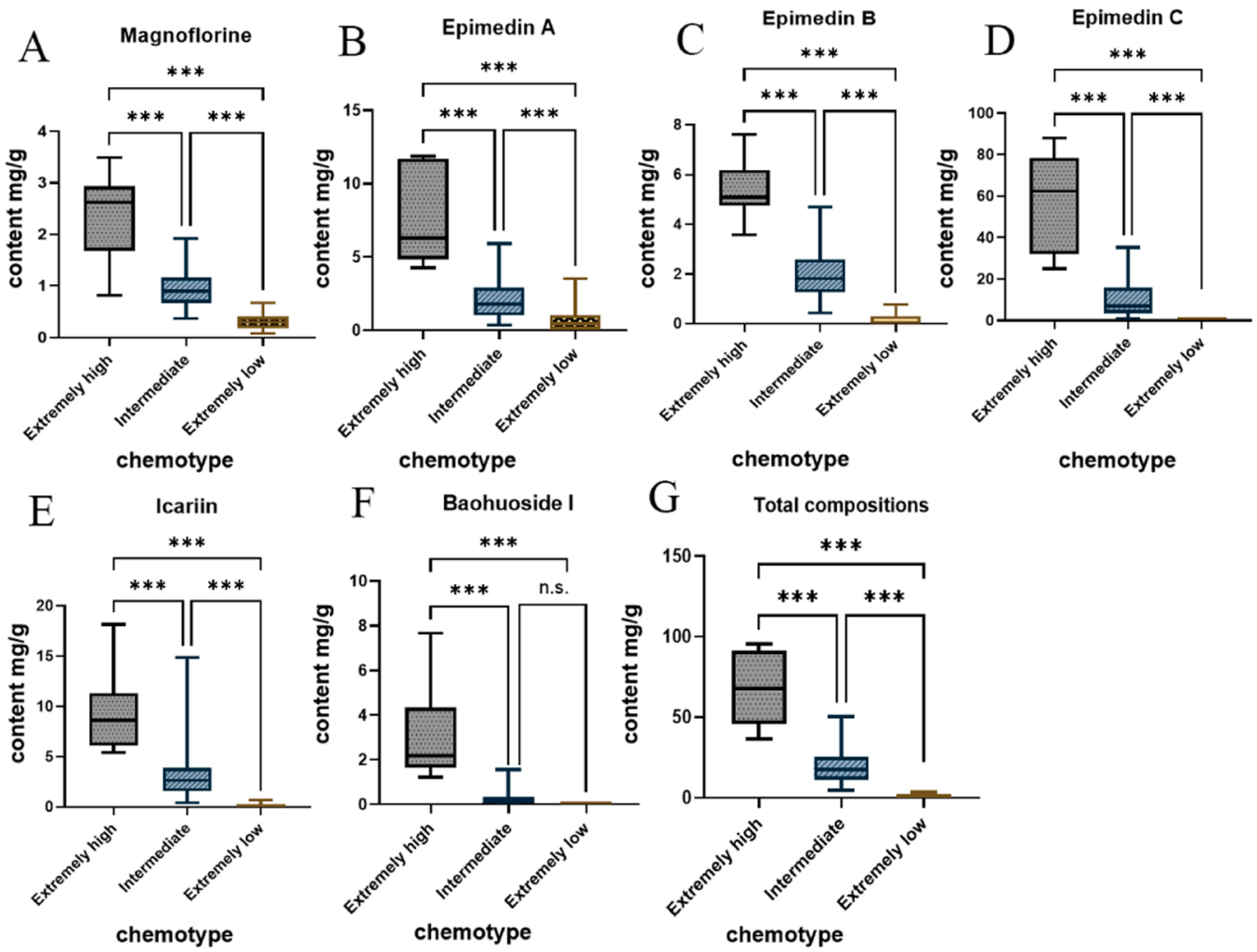

Fig. 5. Chemotype classification of *Epimedium* populations based on different chemical compositions, including extremely high, intermediate, and extremely low types. A to G respectively showed the contents and corresponding populations of magnoflorine, epimedin A, epimedin B, epimedin C, icariin, baohuoside I and total compositions in different chemotypes. Statistical differences were analyzed by one-way analysis of variance (ANOVA), ***$p < 0.001$; n.s.: no significant difference.

studied in depth.

3.4. Determination of different chemotypes

As the material basis of medicinal plant efficacy, the analysis of the structure, composition and content of secondary metabolites have always been the focus of medicinal plant research, so the analysis of phytochemical types is essential. By classifying the contents of six major bioactive components detected and analyzed in this study, the 34 *Epimedium* populations were divided into three chemotypes, including extremely high, intermediate, and extremely low chemotypes, and there were significant differences among these three chemotypes, as shown in Fig. 5 and Table S3. The extremely high chemotypes of magnoflorine and epimedin C were both composed of plants E26 and E02, and because the content of epimedin C was much higher than that of the other five components, the extremely high chemotype of the total chemical composition was also E02 and E26. The extremely high chemotypes of icariin and baohuoside I included E19 and E21, the extremely high chemotypes of icariin also contained an additional E20 and E02 population. Meanwhile, the extremely high chemotypes of epimedin A and epimedin B were composed of E06, E14 and E31, E33, respectively. Combined with the results of correlation analysis, epimedin C and magnoflorine may be important chemical constituents to screen *Epimedium* population with high-quality chemotype.

In order to explore high quality Herba Epimedii, the composition content of extremely high chemotype in *Epimedium* population was further analyzed, as shown in Fig. 6. The results showed that E26 had the highest content of magnoflorine, epimedin C and total compositions, while E02 was only lower than E26, and E02 also ranked second in icariin content. In addition, E20 had the highest icariin content, and E19 had the highest baohuoside I content. E06 and E31 owned the highest contents of epimedin A and epimedin B respectively, however, because the concentration of these two components was not high in these 34 *Epimedium* populations, they did not become the extremely high chemotype of the total components. Through comprehensive and systematic screening, we found that E02 and E26 were the populations of *Epimedium* with high quality chemotypes, which could be further studied to provide excellent resources for subsequent component-targeted breeding of *Epimedium*.

This classification is based on the determination results of the active ingredient content in *Epimedium* planted in the first year. Since the accumulation of secondary metabolites is closely related to cultivation conditions, this research must first conduct testing on data after one year of common-garden cultivation and obtain first-hand information. This information directly reflects the chemotypic performance of wild medicinal materials following a complete cycle of ex-situ cultivation. The high-content populations of specific compounds we identified through testing should be regarded as crucial breeding resources, because plants

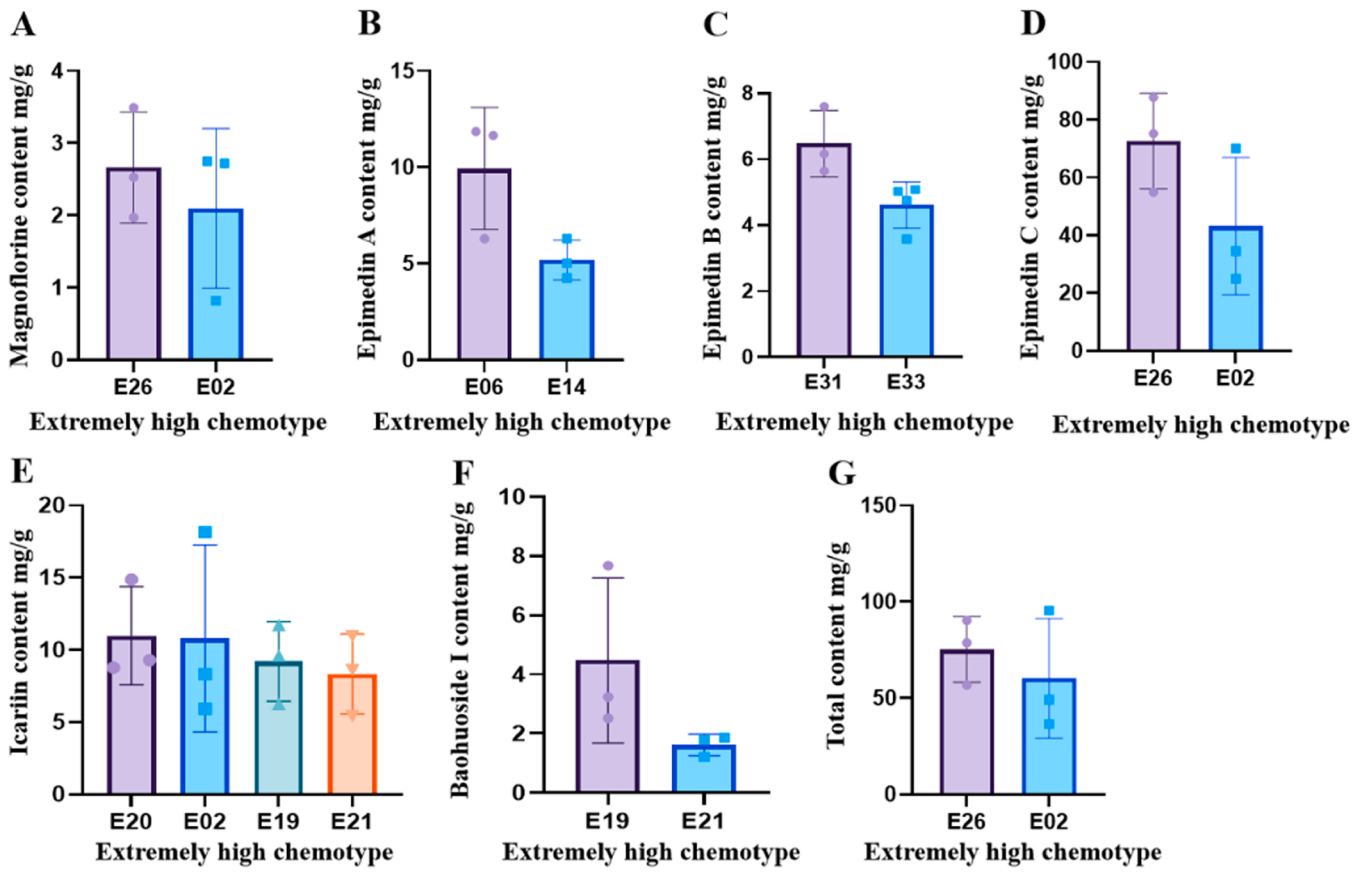

Fig. 6. The content and population of extremely high chemotype in different chemical composition. A to G showed the corresponding populations and contents of the extremely high chemotypes of magnoflorine, epimedin A, epimedin B, epimedin C, icariin, baohuoside I and total compositions, respectively.

at unfamiliar ex-situ cultivation sites, under less-than-ideal horticultural conditions may likely suffer from natural disasters causing failure to survive into the second or third year. Significantly, for the germplasm resources selected for this study, we consecutively tested their chemical compositions through the second and third years, results demonstrated that the target populations obtained exhibit remarkably stable performance in high-yield individual compound content.

4. Conclusion

In this study, we collected 34 batches of *Epimedium* from different sources and established a multi-dimensional evaluation method that integrates phenotypic characteristics and secondary metabolite content, this is the first time such a comprehensive large-scale collection of Epimedium species has been analyzed. The correlation analysis was used to conduct a preliminary exploration of the relationship between chemical composition and phenotypic characteristics. The results showed that the phenotypic data of FL may be further used for predict epimedin C and magnoflorine content in *Epimedium* plants. Furthermore, 34 *Epimedium* populations were divided into three chemotypes, and it was found that sample E02 and E26 were the populations of *Epimedium* with high quality chemotypes, which contain rich content of epimedin C, magnoflorine, and icariin, making them the candidate for breeding. However, it should be noted that this result is preliminary and needs to be further validated at the molecular level. In addition, homogeneous garden cultivation began in 2021, the 34 batches of *Epimedium* measured in this study were samples transplanted to the homogeneous garden for one year, currently, data from the second and third years have also been measured, and further research is ongoing. Overall, this study provides significant guidance for the targeted breeding of *Epimedium* in the future.

CRediT authorship contribution statement

Yan Li: Writing – review & editing, Supervision, Software. **Jiaxin Yang:** Writing – review & editing, Supervision, Investigation. **Chunsong Cheng:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Shufang Yang:** Writing – original draft, Methodology, Data curation. **Qiqing Cheng:** Writing – original draft, Methodology, Investigation, Formal analysis. **Mengting Xiao:** Software, Data curation. **Shimeng Li:** Methodology, Investigation, Formal analysis.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.122365.

Data availability

Data will be made available on request.

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