Variation of leaf morphological and effective components of Eucommia ulmoides natural female population and its response to climatic factors

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Variation of leaf morphological and effective components of *Eucommia ulmoides* natural female population and its response to climatic factors

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Abstract: *Eucommia ulmoides* Oliv. is a significant medicinal and economically valuable tree species endemic to China. Female E. *ulmoides* plants are particularly prized for their fruits and seeds, which serve as important industrial, medicinal, and oil resources, offering a longer industrial chain and higher economic value compared to male plants. However, the morphological variation in leaves among natural female populations, the dynamics of pharmacologically active compounds, and their interactions with climatic and geographical factors remain poorly characterized. In this study, we examined five leaf morphological traits (e.g., leaf length, leaf width) and quantified twelve bioactive compounds in 126 mature E. *ulmoides* individuals from 10 populations across four provinces. Among the analyzed compounds, six major constituents chlorogenic acid (CGA), asperuloside (ASP), aucubin (AU), geniposidic acid (GPA), rutin (RT), and isoquercitrin (IQ) accounted for 96.25% of the total active ingredient content, while the remaining six minor components collectively represented only 3.75% (each < 0.1%). The six major compounds exhibited substantial variability, with coefficients of variation ranging from 36.67% (CGA) to 92.56% (AU) and Shannon diversity indices spanning from 1.61 (RT) to 2.01

(CGA). Significant inter-population differences were detected, with explanatory power values ($\hat{P}^2$) as follows: CGA (0.24), AU (0.34), GPA (0.84), ASP (0.52), RT (0.15), and IQ (0.40). Across populations and leaf phenotypic traits, chemical composition showed strong associations with perimeter, length, and shape index. Notably, GPA exhibited a positive correlation with CGA and AU but negative correlations with the other nine compounds, suggesting that its biosynthesis may regulate the accumulation of CGA, IQ, ASP, and RT. While individual climatic and geographical factors showed weak associations with leaf traits and bioactive compounds, their combined effects played a more substantial role in shaping phenotypic and biochemical variation. These findings provide mechanistic insights into how female E. *ulmoides* plants optimize morphological and biochemical traits in response to environmental heterogeneity. Moreover, this study provides a scientific foundation for optimizing male-to-female planting ratios, advancing precision cultivation, and promoting the high-value utilization of E. *ulmoides* resources.

Key words: Female *Eucommia ulmoides*; Natural population; Active constituent; Phenotypic variation

1. INTRODUCTION

Eucommia ulmoides Oliv. (*Eucommiaceae*), a dioecious deciduous tree endemic to China, is the sole living representative of its family. As a rare and endangered Category II protected species with over 2000 years of documented cultivation history, it represents both significant ecological and economic value [1,2]. The species has gained particular attention due to the abundance of bioactive compounds present in its leaves, bark, and other tissues, establishing it as an important industrial and medicinal tree species currently cultivated across 29 Chinese provinces [1,2]. While the bark has been traditionally used in Chinese medicine, its utilization faces significant challenges. The extended growth cycle required for bark maturation (typically 10-15 years) combined with the destructive nature of harvesting (frequently leading to tree mortality) has resulted in resource scarcity and elevated costs, thereby hindering sustainable development [3]. The main active components and pharmacological effects of *Eucommia* leaves are similar to those of *Eucommia* bark in traditional Chinese medicine [4], indicating high medicinal value. Among them, iridoid compounds such as aucubin, geniposidic acid and asperuloside have the effects of scavenging free radicals, reducing blood glucose and protecting the liver [5]. Chlorogenic acid and other phenylpropanoids, which are phenolic compounds, have antibacterial, antiviral, anticancer, and immune-boosting properties. Isoquercitrin and rutin, which are flavonoids, have multiple physiological activities, including antioxidant, anti-depressant, anti-inflammatory, anti-tumor, and vasodilatory effects [6,7]. It can replace *Eucommia* bark to a certain extent [8,9] and be applied in food, health products, functional feed and other fields [10]. HFD induced hyperlipidemia mouse model showed that there was no significant difference between *Eucommia ulmoides* leaves and *Eucommia* bark in the anti-hyperlipidemia effect [11].

Sexual specialization in dioecious trees, due to different reproductive costs, leads to distinct morphological, growth, and spatial distribution characteristics. Sexual segregation may lead to more efficient resource utilization by both female and male plants [12]. In a homogeneous garden, the analysis of six major active components (chlorogenic acid, aucubin, geniposidic acid, asperuloside, isoquercitrin, and total flavonoids) in the leaves of female and male *Eucommia ulmoides* from different sources shows that the content of these six active components in the leaves of male *Eucommia ulmoides* is slightly higher than that of female plants, but there is no significant difference [13]. The content of gutta percha in the leaves of female *Eucommia ulmoides* is significantly higher than that of male plants at all times, making female plants more suitable as leaf gum sources ($p<0.01$) [14]. Additionally, the fruits of female plants are rich in gutta percha, and the seeds are rich in fatty acids, with over 60% of the total fatty acids being α-linolenic acid, which can be used to produce high-grade plant oil. Therefore, female plants have a longer industrial chain and broader development potential, making them more valuable.

China holds 99% of the world's *Eucommia ulmoides* resources, which are widely cultivated in China (24°50' – 41°50 'N, 76°00' – 126°00' E), mainly in provinces south of the Qinling Mountains, including Shanxi, Sichuan, Guizhou, Hubei, Hunan, and others [15,16]. Due to genetic mutations, ecological isolation, and natural selection, *Eucommia ulmoides* exhibits intraspecific differentiation, characterized by differences in morphology, anatomy, growth and development, and adaptability, with leaves being the most sensitive to environmental changes [17-19]. The accumulation of pharmacologically active components in *Eucommia ulmoides* is influenced by both genetic and environmental factors. The content of glycosides such as pinoresinol diglucoside, aucubin, and geniposidic acid varies by origin [20]. The polyphenols and flavonoids in *Eucommia ulmoides* leaves from different sources are diverse, with male leaves containing slightly higher levels than female leaves [21]. However, the variation patterns of leaf traits, particularly the content of active ingredients, among different female plants of different species have not been reported. This study employs modern analytical techniques, including ultra-high-performance liquid chromatography-tandem mass spectrometry and mixed linear models, to systematically investigate the phenotypic and active ingredient content variations in the leaves of 10 natural populations of *Eucommia ulmoides* at both the sex and population levels. By studying the correlation between traits and climatic and geographical factors, the study aims to uncover the environmental response mechanisms behind changes in leaf morphology and the accumulation of important active ingredients in *Eucommia ulmoides*, providing a basis for optimizing the planting structure of female and male plants, promoting the targeted cultivation and high-quality utilization of *Eucommia ulmoides* resources.

2. METHODS

2.1 Plant material and Climatic factors of Populations

2.1.1 Instrument

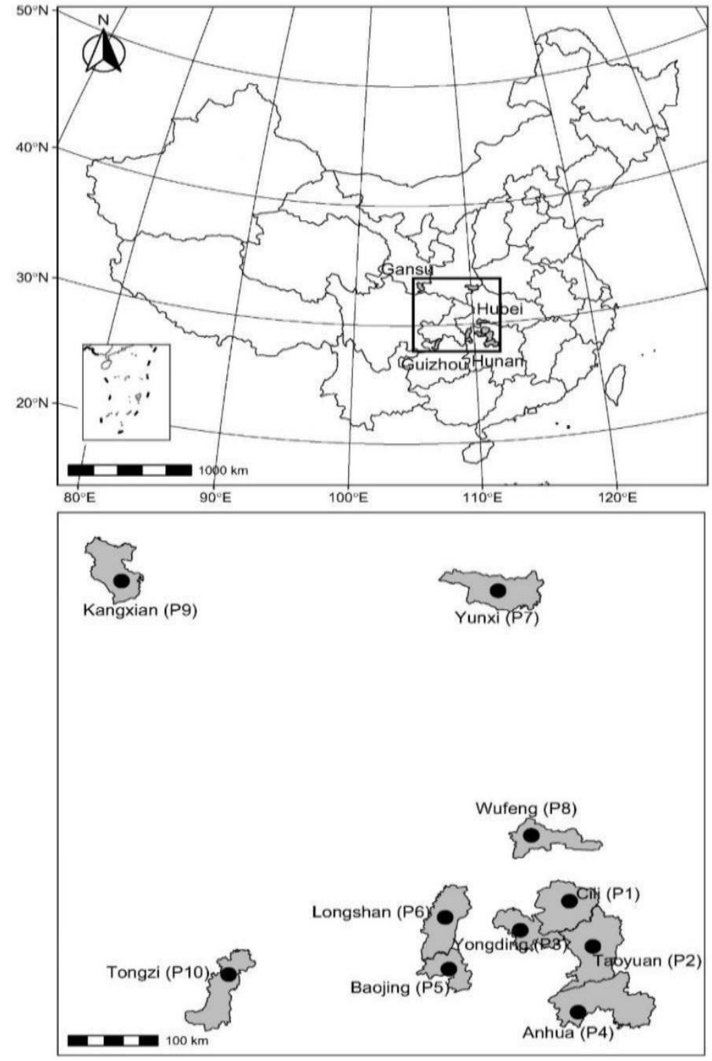
Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS): Waters Xevo TQD triple quadrupole mass spectrometer (American Waters Corporation, equipped with automatic sampler, quaternary pump, column temperature box, vacuum deaerator, and Masslynx 4.1 mass spectrometry workstation), AL-204 electronic balance (Mettler Toledo , American), XO-5200DT ultrasonic cleaning machine (Nanjing Xianou Instrument Manufacturing Co., Ltd., China), ultra-pure water system (Haisi Instrument Technology (Shanghai) Co., Ltd., China). Chromatographic column: Waters BEH C18 chromatography column (100mm×2.1mm, 1.7 μm).

2.1.2 Reagents and Materials

Reference products include Chlorogenic acid (CGA, batch number RP210916, content 99.78%), Asperuloside(ASP, batch number RP220211, content 98.27%), Caffeic acid (CA, batch number RP220610, content 99.95%), Protocatechuic (PC, batch number RP220605, content 99.99%), Aucubin (AU, batch number RP201206, content 99.47%), Geniposidic acid (GPA, batch number RP200624, content 99.85%), Geniposide (GP, batch number RP190629, content 99.57%), Catechin (CC, batch number RP211109, content 99.89%), Pinoresinol diglucoside (GPD, batch number RP220122, content 99.42%), Rutin (RT, batch number RP210601, content 98.65%), Isoquercitrin (IQ, batch number RP220404, content 99.18%) and Astragalin (AG, batch number RP220517, content 99.77%) were both purchased from Chengdu Medsun Technology Co., Ltd. in China; Acetonitrile (batch number P2800855, mass spectrometry purity) and Methanol (batch number P2798320, chromatographic purity) were both purchased from Tedia Corporation in the United States; Formic acid (batch number P2670894, mass spectrometry purity) was purchased from Shanghai TixiAi Chemical Industry Development Co., Ltd. in

China; The water is pure Watsons water.

In September to October 2022, 126 female *Eucommia* leaves were collected from 10 natural populations in Hunan, Hubei, Guizhou, and Gansu provinces (Fig. 1). To minimize the influence of genetic relationships, a distance of 30 meters was maintained between samples within each population. Fresh, mature leaves were collected from the upper branches of the tree canopy in the east, south, west, and north directions, free from obvious pests or diseases, and brought back to the laboratory. Ten leaves were selected for the determination of LL, LW, PL, and LP, and the LI (LL/LW) was calculated. The leaves, weighing approximately 1 kg, were dried at low temperatures according to the 2020 edition of the Chinese Pharmacopoeia, ground, sieved through a 60-mesh screen, and bagged for analysis of active ingredients. Climatic variables were obtained from Climate AP v3.10 over the period 1991–2020 for each



sampling population using latitude, longitude and elevation as inputs [22].

Fig. 1 Locations of the ten populations sampled of *E. ulmoides*

Table 1 information of population and geographical and climatic factors

Population	Sample size	LONG	LAT	ELEV(m)	MAT	MCMT	MAP	AHM	DD5	RH
P1(Cili, Hunan)	57	110.17-111.30	29.09-29.60	71-808	16.76	5.08	1477.63	18.18	4323.39	74.09
P2(Taoyuan, Hunan)	9	111.25	29.37	250-280	17.19	5.49	1505.00	18.09	4463.22	74.89
P3(Yongding, Hunan)	8	110.49-110.79	29.10-29.24	179-672	17.28	5.65	1396.00	19.55	4493.75	74.88
P4(Anhua, Hunan)	10	110.6-111.60	28.05-28.36	110-387	17.46	5.76	1667.00	16.57	4556.80	75.50
P5(Baojing, Hunan)	3	109.68-109.70	28.80	470-570	17.07	5.57	1370.00	19.77	4425.33	75.00
P6(Longshan, Hunan)	5	109.48	28.88	700-720	16.40	5.00	1382.00	19.10	4198.60	76.00
P7(Yunxi, Hubei)	12	110.51-110.62	32.89-32.95	420-780	15.07	2.72	839.50	29.90	3830.67	67.92
P8(Wufeng, Hubei)	3	110.54-110.63	30.08-30.09	960-1390	12.03	0.60	1642.00	13.43	2910.67	73.33
P9(Kangxian, Gansu)	9	105.88-105.89	33.16-33.17	1076-1098	11.82	0.22	906.22	24.13	2863.89	69.00
P10(Tongzi, Guizhou)	10	106.88	27.72-27.73	924-978	15.97	5.20	1096.30	23.68	4026.40	77.00

LONG, longitude; LAT, latitude; ELEV, elevation; MAT, mean annual temperature; MCMT, mean coldest monthly temperature; MAP, mean annual precipitation; AHM, annual heat-to-moisture index; DD5, degree-days above 5 °C; RH, relative humidity.

2.2 Measurement of leaf morphological and Active ingredient

2.2.1 Determination of leaf morphological

The LA-S plant leaf image analyzer of Hangzhou wanshen Detection Technology Co., Ltd. was used to measure the leaf length (LL), leaf width (LW), and leaf perimeter (LP). The petiole length (PL) was measured with a vernier caliper. The leaf shape index is the ratio of leaf length to width.

2.2.2 Preparation of reference solution

Weigh an appropriate amount of CGA, ASP, CA, PC, Au, GPA, GP, CC, PDG, RT, IQ, and Ag reference substances, add 50% methanol to dissolve, and prepare a reference solution with a concentration of 1 mg/ml. Take an appropriate amount of the reference solution and mix it to obtain the reference solution. Store the mixed solution at 4℃ in the refrigerator and keep it away from light.

2.2.3 Preparation of test sample solution

Weigh 0.5000 g of *Eucommia ulmoides* leaves passing 60 mesh sieve into a 50 ml centrifuge tube, add 20 ml of 50% methanol, weigh the total weight of the centrifuge tube and the sample, sonicate (power 300 W, frequency 40 kHz) for 40 min, cool to room temperature, re-weigh, and use 50% methanol to make up for the weight loss. After mixing, centrifuge at 9000 r/min for 8 min, take the supernatant and pass it through 0.22 μm organic membrane to obtain the test sample solution. Due to the different response of mass spectrometry to compounds, the samples were diluted 10 times.

2.2.4 The active ingredients of the samples were determined by UPLC-MS/MS

2.2.4.1 Chromatographic condition

The C18 column was used, the mobile phase was 0.1% formic acid aqueous solution (phase A) and acetonitrile (phase B). The gradient elution conditions were: 0-1 min, 5% - 25% B; 1-4 min，25%-32% B； 4-7 min，32%-35% B； 7-8 min，35%-50% B； 8-9 min，50-90% B； 9-9.5 min，90%-

5% B； 9.5-14 min，5%B； The flow rate was 0.25 ml/min, the column temperature was 30℃ , and the injection volume was 3 μ L.

2.2.4.2 Mass spectrometry conditions

The electrospray ionization source (ESI), positive and negative ion multiple reaction monitoring mode (MRM), capillary voltage of 3.2 kV, ion source temperature of 110 ℃, desolvation temperature of 500 ℃, desolvation gas flow of 800 L/ h, and cone hole gas flow of 50 L/h were used. The taper hole voltage and collision energy were optimized by manual tuning.

2.2.4.3 Active ingredient determination

Refer to and improve the method as literature [23], process the samples according to the method of 2.2.3, and determine the content of each active ingredient under the conditions of 2.2.4.1 and 2.2.4.2.

2.3 Statistical analysis

2.3.1 Principal component analysis

Principal component analysis (PCA) was used to reduce the variability and to explore the data structure and relationships between populations. Prior to PCA, Leaf phenotypes and active ingredient content of *Eucommia ulmoides Oliv* was standardised using the Z-score method. The PCA was performed using the R function ‘prcompt’. Biplot of the PCA was drawn using the ‘fviz_pca_biplot’ function in in the R package factoextra (version 1.0.7) [24]. The first three principal components (PCs) were used as indirect variables in the subsequent analysis.

2.3.2 Linear mixed model

A linear mixed model was fitted to examine the influence of population on Leaf phenotypes and active ingredient content of *Eucommia ulmoides Oliv* and PCs. The model was as follows:

$$y = \mu + Z_{pop} + e \quad (1)$$

where y is the vector of individual tree phenotypic values for a response variable, μ is the vector of the overall mean, pop refers to the vectors of the random population effect, and e is the vector of the residual error effects. Z is an incidence matrix linking phenotypic records (y) to estimates of effects. The random effects are assumed to be normally distributed $pop \sim N\left(0, \sigma_{pop}^2 I\right)$, $e \sim N(0, \sigma_e^2 I)$, where σ_{pop}^2 and σ_e^2 are the population and residual error variances, respectively, and I is an identity matrix.

Variance components of the random effects were estimated by the restricted maximum likelihood (REML) approach, using the average information REML algorithm [25] implemented in the statistical software ASReml-R (ver. 3.0, Queensland Department of Primary Industries and Fisheries, Brisbane, Australia [26], package in R software (ver. 3.1.2) [27]. Approximate standard errors of the estimated parameters were estimated using a standard Taylor series expansion method with ASReml-R [28]. The statistical significance of the population variance components for each leaf phenotypes and phytochemotype trait was determined by using a two-tailed likelihood ratio test (LRT) [29], whereby the test is conducted against the null hypothesis that the estimated parameter is equal to zero.

Multiple comparisons and statistical significance at $p < 0.05$ were used to compare differences between populations using the 'TukeyHSD' function in R, which incorporates a sample size adjustment that produces meaningful intervals for slightly unbalanced designs.

Coefficients of variation (CV) and Shannon–Wiener index (H) was used to evaluate the magnitude of variation and the population diversity for each leaf phenotypes and phytochemotype trait. The CVs at population and phenotypic level were calculated, respectively, as follows:

$$CV_{pop} = \hat{\sigma}_{pop}/\bar{x} \times 100\% \quad (2)$$

$$CV_{phen} = \hat{\sigma}_{phen}/\bar{x} \times 100\% \quad (3)$$

where $\hat{\sigma}_{pop}$ and $\hat{\sigma}_{phen}$ are standard deviation of $\hat{\sigma}_{pop}^2$ and $\hat{\sigma}_{phen}^2$, respectively. $\hat{\sigma}_{phen}^2 =$

$\hat{\sigma}^2_{pop} + \hat{\sigma}^2_e$. $\bar{x}$ is the arithmetic mean of the phenotypic data of the leaf phenotypes and phytochemotype trait.

The leaf phenotypes and phytochemotypetrait was converted to a ten-grade variable: the values $< \bar{x} - 2\sigma$ were assigned to the first grade; the remaining grades were set in steps of 0.5σ [30]. Then the H was calculated as:

$$H = -\sum p_i (ln p_i) \quad (4)$$

where p_i indicates the percentage of each grade.

The amount of population variation (also known as "population differentiation coefficient") was estimated as:

$$\hat{P}^2 = \hat{\sigma}^2_{pop} / \hat{\sigma}^2_{phen} \quad (5)$$

Bivariate analysis was performed to determine the correlations between two different leaf phenotypes and phytochemotype traits at population and phenotypic levels. The bivariate analysis was based on the following linear mixed model:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} + \begin{bmatrix} Z_{11} & 0 \\ 0 & Z_{12} \end{bmatrix} \begin{bmatrix} pop_1 \\ pop_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \quad (6)$$

where y is the vector of individual tree observations; the subscripts 1 and 2 refer to Leaf phenotypes and phytochemotypetrait 1 and 2; μ is the vector of fixed overall mean; *pop* and *e* are the vectors of random population effect and residual errors.

The correlations at the population ($\hat{r}_{pop}$) and phenotypic ($\hat{r}_{phen}$) levels were calculated upon the variance and covariance parameters estimated from the bivariate analyses for all possible combinations of leaf phenotypes and phytochemotype traits using the following formulas:

$$\hat{r}_{pop} = \frac{\hat{\sigma}_{pop(12)}}{\sqrt{\hat{\sigma}^2_{pop(1)} \times \hat{\sigma}^2_{pop(2)}}} \quad (7)$$

$$\hat{r}_{phen} = \frac{\hat{\sigma}_{phen(12)}}{\sqrt{\hat{\sigma}^2_{phen(1)} \times \hat{\sigma}^2_{phen(2)}}} \quad (8)$$

where $\hat{\sigma}_{pop(12)}$ and $\hat{\sigma}_{phen(12)}$ are the estimated covariance between leaf phenotypes and phytochemotype trait 1 and 2 at population and phenotypic level; $\hat{\sigma}^2_{pop(1)}$ and $\hat{\sigma}^2_{pop(2)}$, $\hat{\sigma}^2_{phen(1)}$ and $\hat{\sigma}^2_{phen(2)}$ denote the estimated population and phenotypic variance components for leaf phenotypes and phytochemotype trait 1 and 2, respectively.

2.3.3 Relationships between fatty acid content and climatic factors

Pearson's correlation analysis was used to examine the relationships between the population effects obtained from equation (3) and climatic factors at the population level. The coefficients and statistical significance were obtained using the R function 'cor.test'.

3. RESULTS

3.1 Variation diversity of leaf morphology, composition and Content of *Eucommia ulmoides* at the species level

Table 2 shows that among the five leaf traits, the mean values of leaf length, leaf width, leaf shape index, petiole length and leaf perimeter are 127.33mm, 61.68mm, 2.07, 15.76mm and 330.71mm respectively. The coefficient of variation of the five leaf traits ranges from 11.22 (LW) to 18.22 (PL), and the diversity index ranges from 1.94 (LP) to 2.01 (LL, LW).

Table 2 Variation of morphology, composition and content of *Eucommia* leaves at species level

	Traits and codes	n	mean	sd	min	max	range	CV_{phen}	H_{phen}
LL	Leaf length (mm)	126	127.23	15.23	89.97	175.99	86.02	11.97	2.01
LW	Leaf width (mm)	126	61.68	6.92	43.36	84.56	41.20	11.22	2.01
LI	Leaf shape index	126	2.07	0.24	1.66	2.77	1.11	11.38	2.00
PL	Petiole length (mm)	126	15.76	2.87	8.41	29.85	21.44	18.22	1.98
LP	Leaf perimeter (mm)	126	330.71	49.34	224.16	477.58	253.42	14.92	1.94
CGA	Chlorogenic acid (%)	126	3.57	1.31	0.18	6.18	6.00	36.67	2.01
ASP	Asperuloside (%)	126	0.14	0.10	0.00	0.58	0.58	74.01	1.69
CA	Caffeic acid (%)	126	0.03	0.07	0.00	0.32	0.32	232.24	0.60
PC	Protocatechuic (%)	126	0.04	0.03	0.01	0.36	0.35	89.89	1.41
AU	Aucubin (%)	126	1.34	1.24	0.03	6.57	6.54	92.56	1.74
GPA	Geniposidic acid (%)	126	0.74	0.58	0.06	2.54	2.48	77.59	1.82
GP	Geniposide (%)	126	0.05	0.05	0.00	0.18	0.18	109.59	1.53
CC	Catechin (%)	126	0.02	0.02	0.00	0.12	0.12	99.25	1.30
PDG	Pinoresinol diglucoside (%)	126	0.05	0.05	0.00	0.21	0.21	85.86	1.65
RT	Rutin (%)	126	0.13	0.12	0.01	0.86	0.85	89.11	1.61
IQ	Isoquercitrin (%)	126	0.24	0.21	0.01	0.91	0.90	88.13	1.79
AG	Astragalin (%)	126	0.05	0.03	0.01	0.22	0.21	57.85	1.86

Among the 12 active ingredients, the relative contents of chlorogenic acid, asperuloside, aucubin, geniposidic acid, rutin and isoquercitrin were relatively high, accounting for 96.25% of the total active ingredient content. Among them, the relative content of chlorogenic acid was the highest (3.57%, accounting for 55.78%), aucubin was the second (1.34%, accounting for 20.94%), and rutin was the lowest (0.13%, accounting for 2.03%). The relative contents of the other six active ingredients were low, all below 0.1%, accounting for 3.75%. The coefficients of variation of 12 active ingredients ranged from 36.67 (CGA) to 232.24 (CA), and the diversity index ranged from 1.94 (LP) to 2.01 (LL, LW). For the six main active ingredients with relative content greater than 0.1g/kg, the coefficient of variation ranged from 36.67 (CGA) to 92.56 (AU), and the diversity index ranged from 1.61 (RT) to 2.01 (CGA).

3.2 Variation characteristics of morphology, composition and Content of *Eucommia* leaves at the population level

Variations in leaf morphology and active constituents across *Eucommia ulmoides* populations are presented in table 3. Multiple comparisons at the population level showed that the P6 population had the largest leaf circumference (399.91 mm^2) and the second largest leaf length (146.55 mm) and leaf width (65.49 mm) among the five leaf phenotypes indexes. This population had the characteristics of large leaves. The P8 population had the largest leaf length (148.14 mm) and the smallest leaf width (57.44 mm). Meanwhile, the leaf shape index (2.57) and petiole length (21.93 mm) were the largest. This population had the characteristics of narrow and long leaves. The petiole length (13.52 mm), leaf circumference (295.96) and leaf shape index (1.97) of the P3 population were the smallest, and this population had the characteristics of small round leaves.

Among the six active ingredient indexes with relatively high content, the ones with relatively high content of CGA and GPA were P5 (4.23%, 1.66%), P6 (4.36%, 2.03%), P7 (4.58%, 1.59%), P8 (4.46%, 1.29%) and P9 (4.67%, 1.17%); The groups with higher relative contents of IQ and ASP were: P1 (0.34%, 0.17%), P3 (0.46%, 0.29%) and P10 (0.33%, 0.22%); The relative content of RT was higher in P1 (0.19%) and P10 (0.16%); The relative content of AU was higher in P2 (2.78%), P3 (2.04%) and P6 (2.81%).

The relative contents of aucubin (2.81%) and geniposidic acid (2.03%) in P6 population were the highest; The relative content of chlorogenic acid (4.67%) in P9 population was the highest; the P1 group had the highest relative content of rutin (0.19%); The relative contents of asperuloside (0.29%) and isoquercetin (0.46%) were the highest in P3 population; the P4 group had relatively low levels of the main active components in the leaves. PDG, the key active ingredient in *Eucommia* bark, was detected in the leaves of P1, P2, P3 and P10 populations.

Table 3 Multiple comparisons of leaf, component and content of 10 populations

Traits	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
LL	121.95 ± 13.22 c	133.52 ± 10.63 abc	123.56 ± 11.47 bc	139.32 ± 17.55 ab	132.03 ± 6.67 abc	146.55 ± 5.67 a	125.78 ± 11.55 bc	148.14 ± 24.37 a	132.18 ± 13.31 abc	122.45 ± 18.49 bc
LW	61.66 ± 6.96 ab	66.75 ± 6.75 a	63.67 ± 9.17 ab	61.98 ± 5.86 ab	64.09 ± 0.74 ab	65.49 ± 11.04 ab	59.81 ± 4.97 ab	57.44 ± 5.28 b	59.38 ± 6.46 ab	58.37 ± 5.38 ab
LI	1.99 ± 0.19 de	2.01 ± 0.15 cde	1.97 ± 0.25 e	2.26 ± 0.3 bc	2.06 ± 0.1 bcde	2.27 ± 0.3 b	2.11 ± 0.19 bcde	2.57 ± 0.20 a	2.23 ± 0.13 bcd	2.10 ± 0.23 bcde
PL	15.71 ± 2.37 bc	16.4 ± 2.07 bc	13.52 ± 2.8 c	16.73 ± 2.04 bc	14.65 ± 2.13 bc	16.96 ± 2.86 b	16.19 ± 2.6 bc	21.93 ± 7.06 a	14.18 ± 2.47 bc	15.06 ± 3.59 bc
LP	305.9 ± 34.1 de	353.5 ± 40.13 bc	295.96 ± 20.69 e	383.36 ± 43.12 abc	373.25 ± 14.78 abc	399.91 ± 45.18 a	341.44 ± 29.16 cd	389.97 ± 58.16 ab	386 ± 42.29 ab	298.98 ± 35.71 e
CGA	3.40 ± 1.35 abcd	2.51 ± 1.49 d	3.1 ± 1.61 bcd	2.84 ± 1.45 cd	4.23 ± 0.14 abc	4.36 ± 0.23 ab	4.58 ± 0.38 ab	4.46 ± 0.27 ab	4.67 ± 0.48 a	3.6 ± 0.72 abcd
ASP	0.17 ± 0.1 bc	0.09 ± 0.05 cd	0.29 ± 0.07 a	0.06 ± 0.01 d	0.06 ± 0 d	0.06 ± 0.01 d	0.06 ± 0.01 d	0.05 ± 0.01 d	0.05 ± 0.02 d	0.22 ± 0.09 ab
CA	0.04 ± 0.08 b	0.12 ± 0.14 a	0.02 ± 0.02 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0.01 ± 0 b
PC	0.05 ± 0.04 a	0.03 ± 0.04 a	0.02 ± 0 a	0.02 ± 0.01 a	0.02 ± 0 a	0.01 ± 0 a	0.02 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.01 a	0.05 ± 0.01 a
AU	1.03 ± 1.08 bcd	2.78 ± 2.24 a	2.04 ± 0.73 ab	0.47 ± 0.29 d	1.80 ± 0.29 abcd	2.81 ± 0.81 a	1.99 ± 1.26 abc	0.52 ± 0.32 d	1.3 ± 0.17 bcd	0.71 ± 0.48 cd
GPA	0.41 ± 0.23 e	0.72 ± 0.47 d	0.36 ± 0.28 e	1.03 ± 0.27 c	1.66 ± 0.07 b	2.03 ± 0.42 a	1.59 ± 0.33 b	1.29 ± 0.1 c	1.17 ± 0.18 c	0.18 ± 0.08 e
GP	0.07 ± 0.05 ab	0.05 ± 0.07 b	0.1 ± 0.04 a	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0.08 ± 0.03 ab
CC	0.01 ± 0.01 b	0.01 ± 0.01 b	0.06 ± 0.04 a	0.01 ± 0 b	0.02 ± 0.01 b	0.01 ± 0 b	0.01 ± 0 b	0.01 ± 0 b	0.02 ± 0.02 b	0.01 ± 0 b
PDG	0.08 ± 0.03 a	0.03 ± 0.04 b	0.08 ± 0.04 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b	0.09 ± 0.02 a
RT	0.19 ± 0.15 a	0.04 ± 0.05 b	0.10 ± 0.05 ab	0.05 ± 0.05 ab	0.05 ± 0.01 ab	0.08 ± 0.02 ab	0.09 ± 0.02 ab	0.09 ± 0.03 ab	0.09 ± 0.01 ab	0.16 ± 0.1 ab
IQ	0.34 ± 0.22 ab	0.15 ± 0.15 bc	0.46 ± 0.23 a	0.05 ± 0.03 c	0.06 ± 0.01 c	0.08 ± 0.01 c	0.07 ± 0.01 c	0.07 ± 0.01 c	0.07 ± 0.01 c	0.33 ± 0.11 ab
AG	0.06 ± 0.04 a	0.04 ± 0.03 a	0.06 ± 0.02 a	0.03 ± 0.02 a	0.05 ± 0 a	0.05 ± 0.01 a	0.05 ± 0.01 a	0.03 ± 0.01 a	0.04 ± 0.01 a	0.06 ± 0.02 a

Different letters between different groups indicate significant differences ($p < 0.05$)

3.3 Principal component analysis of morphology, composition and content of *Eucommia* leaves

The cumulative contribution rate of the principal components of the five eigenvalues ($\lambda > 1$) was 74.65% (Table 4). PC1 explained 34.56% of the total variation of the data, and the load on LP, GP, PDG and IQ was large (absolute value of load ≥ 0.3) (Fig. 2). PC2 explained 14.07% of the total variation, with a larger load on LL, CGA, AU, GPA, and AG. PC3 explained 11.42% of the total variation, with a significant load on LL, LW, PL and CGA. PC4 explained 7.82% of the total variation, with a larger load on LI, ASP, CA, AU, and RT. PC5 explained 6.78% of the total variation, and the load on PL, ASP, CA and PC was larger.

Table 4 Principal component analysis of phenotype and ingredients of *Eucommia* leaves

	PC1	PC2	PC3	PC4	PC5
LL	0.25	-0.31	0.33	-0.24	0.06
LW	0.10	-0.05	0.63	0.16	-0.17
LI	0.16	-0.28	-0.28	-0.42	0.22
PL	0.12	-0.21	0.33	-0.27	0.35
LP	0.32	-0.28	0.19	-0.06	0.01
CGA	-0.10	-0.45	-0.30	0.02	0
ASP	-0.28	-0.05	0.17	-0.33	-0.42
CA	-0.19	-0.23	0.12	0.46	0.41
PC	-0.21	-0.01	0.11	0.08	0.42
AU	-0.07	-0.42	0.08	0.31	-0.33
GPA	0.27	-0.33	-0.20	0.13	-0.19
GP	-0.33	-0.07	0.16	-0.05	-0.13
CC	-0.20	-0.18	-0.03	0.06	-0.24
PDG	-0.36	0.06	0.16	-0.13	0.18
RT	-0.23	-0.09	-0.02	-0.45	-0.04
IQ	-0.37	-0.15	0.04	-0.07	0
AG	-0.27	-0.30	-0.15	0.07	0.12
Eigen value	5.88	2.39	1.94	1.33	1.15
Proportion of Variance	34.56	14.07	11.42	7.82	6.78
Cumulative Proportion	34.56	48.63	60.05	67.87	74.65

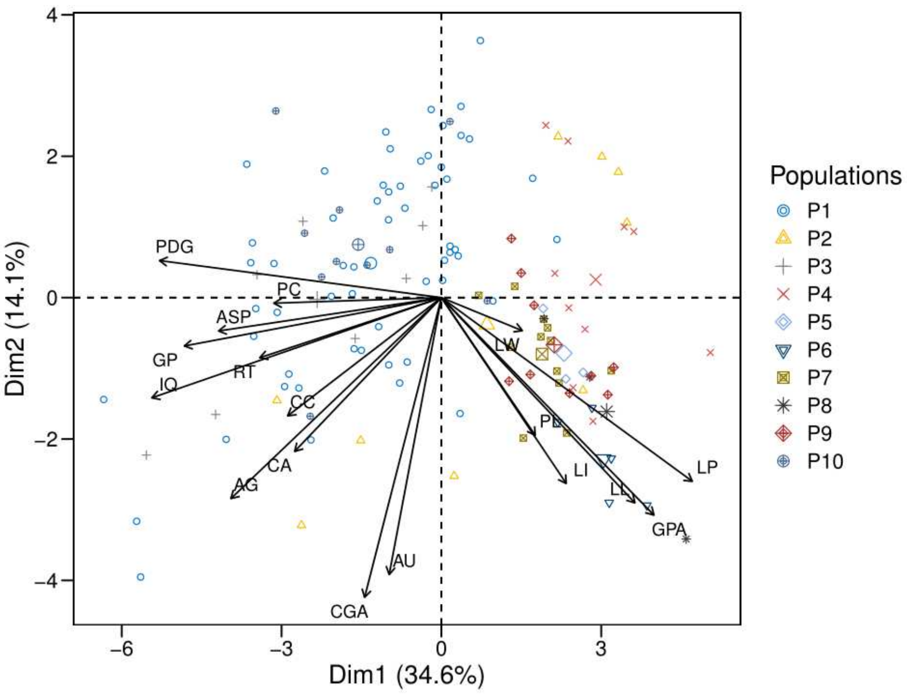


Fig. 2 Biplot of the principal component analysis based on 17 characteristics of *Eucommia* leaves

3.4 Variance analysis of morphology, composition and content of *Eucommia* leaves

According to table 5, except for leaf width (LW) and Astragalin (AG), other traits were significant among populations (PL, $p<0.05$). The population effect variance of five leaf traits ranged from 0.03 (LW) to 0.53 (LP). The group effect variance of 12 active ingredients ranged from 0.06 (IQ) to 0.84 (GPA), and that of 6 main active ingredients ranged from 0.15 (RT) to 0.84 (GPA). Among the 17 leaf traits and active ingredient traits, the population variance of LP, GPA, ASP, CC and PDG was higher ($\hat{P}^2 > 0.50$), and the population variance of 6 active ingredients with higher content was CGA (0.24), Au (0.34), GPA (0.84), ASP (0.52), RT (0.15) and IQ (0.40). The group effect variance of PC1 was higher (0.61), while PC2 (0.22) and PC3 (0.21) were lower.

In general, the population selection of LP, GPA, PDG, CC, ASP and other traits is relatively effective. The group effect predicted by BLUP was similar to the results of multiple comparisons (Table 4). The P6, P7, P8 and P9 populations can select individuals with large leaves (LP) and high CGA content, and the P6 population can select individuals with high GPA content at the same time; the P1, P3 and P10 populations can select individuals with high ASP and PDG (the main effective component of *Eucommia* bark), and the P3 population can select individuals with high CC content at the same time.

Table 5 Variance analysis of morphology, composition and content of *Eucommia* leaves

Traits	$\hat{\sigma}_{pop}^2$	$\hat{\sigma}_e^2$	$\hat{P}^2$	CVpop/%	CVphen/%	CVres/%
LL	58.135 (39.343) ***	187.934 (24.672)	0.24 (0.13)	5.99	12.33	10.77
LW	1.645 (3.366) ns	46.863 (6.165)	0.03 (0.07)	2.08	11.29	11.1
LI	0.021 (0.013) ***	0.042 (0.006)	0.33 (0.14)	6.97	12.14	9.94
PL	2.509 (1.811) *	7.22 (0.955)	0.26 (0.14)	10.05	19.79	17.05
LP	1459.291 (770.059) ***	1276.782 (167.559)	0.53 (0.14)	11.55	15.82	10.8
CGA	0.452 (0.299) ***	1.426 (0.187)	0.24 (0.12)	18.82	38.35	33.41
ASP	0.007 (0.003) ***	0.006 (0.001)	0.52 (0.14)	58.47	81.41	56.65
CA	0.001 (0.001) **	0.004 (0.001)	0.19 (0.11)	103	239.25	215.94
PC	0 (0) ***	0.001 (0)	0.14 (0.09)	33.47	88.84	82.3
AU	0.587 (0.351) ***	1.148 (0.151)	0.34 (0.14)	57.3	98.54	80.17
GPA	0.373 (0.181) ***	0.07 (0.009)	0.84 (0.07)	82.25	89.62	35.59
GP	0.002 (0.001) ***	0.002 (0)	0.49 (0.14)	82.55	117.83	84.09
CC	0 (0) ***	0 (0)	0.57 (0.13)	85.42	113.43	74.63
PDG	0.001 (0.001) ***	0.001 (0)	0.67 (0.11)	70.29	85.81	49.21
RT	0.002 (0.001) ***	0.011 (0.001)	0.15 (0.09)	33.58	87.3	80.58
IQ	0.019 (0.011) ***	0.029 (0.004)	0.40 (0.14)	57.34	90.62	70.18
AG	0 (0) ns	0.001 (0)	0.06 (0.07)	14.39	58.08	56.27
PC1	4.011 (2.06) ***	2.602 (0.341)	0.61 (0.13)			
PC2	0.552 (0.386) ***	1.99 (0.261)	0.22 (0.12)			
PC3	0.441 (0.298) ***	1.618 (0.212)	0.21 (0.12)			

*, ** and *** denote significance for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

3.5 Correlation analysis of morphology, composition and content of *Eucommia* leaves

From Fig. 3, it can be seen that chlorogenic acid is positively correlated with leaf shape index (r=0.26, P < 0.05), negatively correlated with leaf width (r=-0.20, P < 0.05), and highly significantly positively correlated with geniposidic acid (r=0.73, P < 0.001), and negatively correlated with other 10 active ingredients, among which, it has the highest negative correlation with caffeic acid (r=-0.98, P < 0.001). Aucubin has a low correlation with five leaf traits, positively correlated with caffeic acid (r=0.26, p<0.05) and chlorogenic acid (r=0.43, p<0.001), and negatively correlated with rutin (r=-0.66, p<0.001).

Geniposidic acid was positively correlated with leaf circumference, leaf length and leaf shape index, and was negatively correlated with other nine components except chlorogenic acid ($r=0.40$, $P < 0.01$) and aucubin. Isoquercitrin was inversely correlated with geniposidic acid in leaf morphology, i.e., negatively correlated with leaf perimeter, leaf length, and leaf shape index; On the index of effective components, it is opposite to chlorogenic acid, that is, except geniposidic acid which is extremely significantly negatively correlated ($r=-0.54$, $P < 0.001$), it is positively correlated with the other 10 effective components.

The results showed that the correlation between asperuloside and isoquercitrin was highly consistent in leaf morphological indexes and effective component indexes, that is, it was negatively correlated with leaf perimeter, leaf length and leaf shape index; On the index of effective components, except for the extremely significant negative correlation with geniposidic acid ($r=-0.87$, $P < 0.001$), it was positively correlated with the other 10 effective components. The correlation of rutin with asperuloside and isoquercitrin in leaf morphological indicators and effective component indicators was also relatively consistent.

Overall, the correlation between leaf composition and leaf circumference, leaf length and leaf shape index is high, while the correlation with leaf width and node spacing is low. Among the six main components, isoquercitrin, asperuloside and rutin had similar correlations with other indicators. Geniposidic acid is positively correlated with chlorogenic acid and aucubin, but negatively correlated with other nine active ingredients. Therefore, the metabolic synthesis of geniposidic acid in *Eucommia ulmoides* leaves may be the key to the content regulation of effective ingredients such as chlorogenic acid, isoquercitrin, asperuloside and rutin.

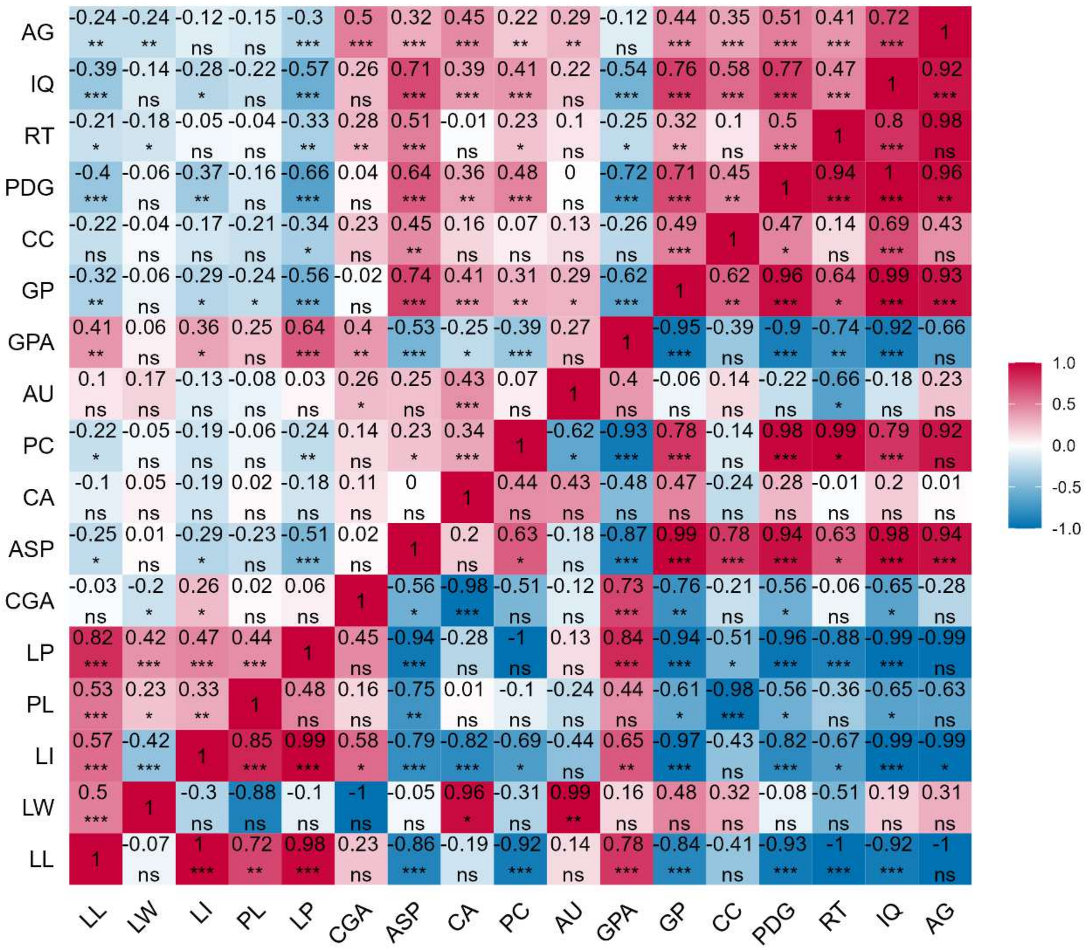


Fig. 3 Correlations among leaf traits and phytophytochemotype. *, ** and *** denote significance for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; See Table 2 for full description of traits.

3.6 Correlation analysis between morphology, composition and content of *Eucommia* leaves and geographical and climatic factors

According to Fig. 4, the correlation between latitude and altitude and 17 traits is consistent; The correlation between longitude and annual rainfall and 17 traits was consistent; Among the temperature related climatic factors, DD5, DD18, EXT, Eref, MAT had the same correlation with 17 traits, while DD0 was opposite to the above climatic factors. DD0 was significantly positively correlated with LL, LW and PL (r = 0.2 ~ 0.39), which may indicate that the earlier the growth period starts, the larger the leaves and the longer the petioles.

Among the 12 active ingredients, CGA, ASP, GPA, GP, PDG and IQ have a certain correlation with most climatic and geographical factors, indicating that these ingredients are affected by the environment to some extent. On the contrary, CA, PC, AU, CC, RT and AG have low or no correlation with most geographical and climatic factors, indicating that the content of these components may be more affected by genetic factors. DD0 has a very significant positive correlation with CGA and GPA, and a very significant negative correlation with ASP, GP, PDG and IQ, while DD5, DD18, EXT, Eref and MAT have the opposite, which may indicate that low temperature is conducive to the generation of CGA and GPA, and with the increase of temperature, it is conducive to the generation of ASP, GP, PDG and IQ. MAP and Eref were significantly negatively correlated with CGA (r=-0.37, **), indicating that drought stress may be conducive to the increase of chlorogenic acid content. AHM was significantly negatively correlated with GPA (r=-0.25, **), indicating that high humidity may not be conducive to its accumulation.

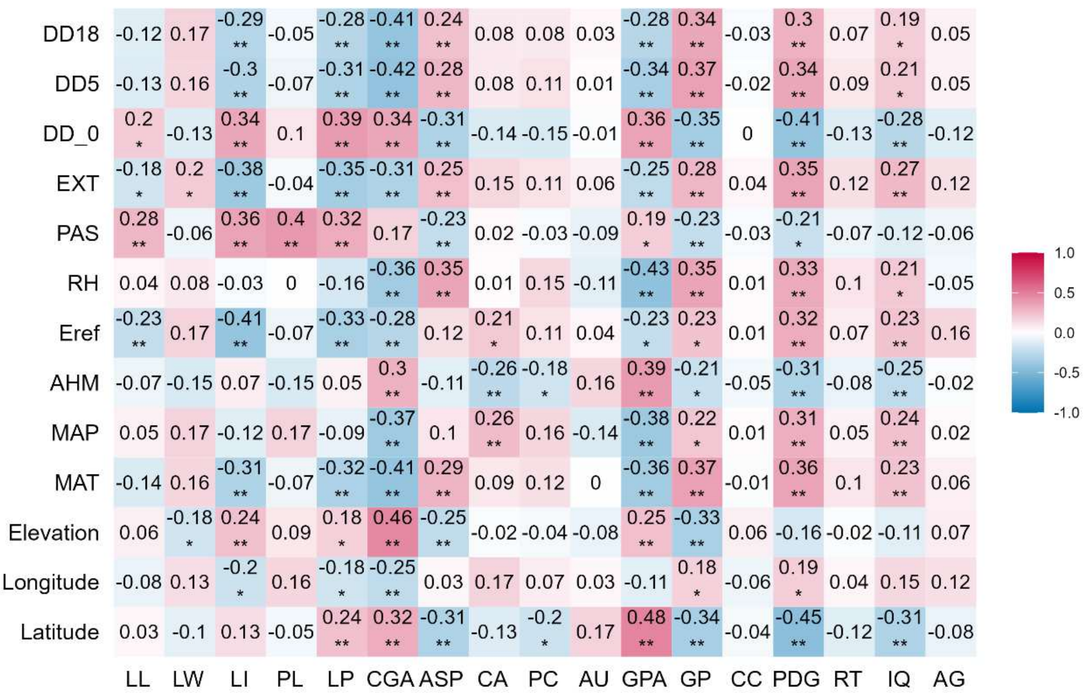


Fig 4 Correlations among leaf traits and phytophytochemotype and climatic factors. *, ** and *** denote significance for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; See Table 1 and Table 2 for full description of traits.

4. DISCUSSION

Before the implementation of genetic improvement, it is essential to evaluate the difference and diversity of natural populations. Phytochemotype of plants, i.e. plant morphologicals composed of the content, composition or structure of endogenous chemical components in plants, reflect the variation and diversity within species [31,32]. The analysis of plant phytochemotype classification and its formation mechanism has theoretical and practical value in guiding plant development, utilization, quality evaluation and directional cultivation of germplasm. In addition, environmental factors can significantly affect the composition and content of active ingredients in *Eucommia ulmoides* leaves by interfering with the synthesis of plant secondary metabolites, which is an important factor determining the quality of traditional Chinese medicine [33]. The study of the interrelationship between traits and geographical and climatic factors can promote the understanding of these changes. Based on the leaf morphological and phytochemotype of female *Eucommia ulmoides Oliv.* For the first time, this study quantified the leaf morphological, composition and content of 126 female individuals from 10 natural provenances, and combined with geographical and climatic factors, analyzed the relationship between the three, so as to provide the basis for optimizing the planting structure of female and male *Eucommia ulmoides Oliv.* In the later stage, and promoting the directional cultivation and high-quality utilization of *Eucommia ulmoides Oliv*.

4.1 Diversity of phenotypic and phytophytochemotypes of *Eucommia* leaves

The experimental materials of this study were all female plants, and the variation coefficients of leaf length, leaf width, leaf shape index, petiole length and leaf perimeter were 11.97%, 11.22%, 11.38%, 18.22% and 14.92%, respectively; The corresponding coefficient of variation of male and female mixed samples in natural population were 15.34%, 14.15%, 14.92%, 19.62% and 14.87% [11], and the difference was significant (t= 0.03, p < 0.05). The coefficient of variation of mixed samples was slightly higher than that of whole female samples, indicating that gender has a certain impact on the coefficient of variation. In the homogeneous garden, the corresponding coefficient of variation of male and female mixed samples were 10.77%, 11.32%, 8.90%, 18.27% and 11.27%, respectively [34]. The above shows that the phenotypic variation of *Eucommia ulmoides* leaves in both natural forests and peer industrial forests of different provenances belongs to weak variation, which is not prone to differentiation, and the influence of climate and environmental factors on leaf morphological variation is greater than that of gender.

The relative contents of chlorogenic acid, asperuloside, asperuloside, Aucubin, rutin and isoquercitrin were higher, accounting for 96.25% of the total effective components. Among them, the relative content of chlorogenic acid was the highest (3.57%, accounting for 55.78%), aucubin was the second (1.34%, accounting for 20.94%), and rutin was the lowest (0.13%, accounting for 2.03%). The relative contents of the other six active ingredients were low, all below 0.1%, accounting for 3.75%. In the homogeneous garden, the six active ingredients with relatively high content in the mixed male and female leaves of *Eucommia ulmoides Oliv* were basically the same as those in this study, but the content differences were large, and the content from high to low was: AU(3.17%)、CGA(2.21%)、TF(Total flavonoids, 1.59%)、GPA(0.96%)、ASP(0.46%)、IQ(0.34%)[13], the reason for the large difference in content may be that on the one hand, the individuals in the homogeneous garden are the excellent plants after evaluation, on the other hand, it may be related to the fact that most of the effective component content of *Eucommia ulmoides* leaves is susceptible to environmental impact, and it may also be caused by the relatively low content of effective components in leaves, different instruments, methods or experimental errors.

The coefficients of variation of 12 active ingredients ranged from 36.67 (CGA) to 232.24 (CA), and the diversity index ranged from 1.94 (LP) to 2.01 (LL, LW). The coefficient of variation of the six main active ingredients with relative content > 0.1g/kg ranged from 36.67 (CGA) to 92.56 (AU), which was much higher than that of the mixed male and female samples in the homogeneous garden: 19.35 (TF) to 34.42 (IQ), which was consistent with the actual situation of environmental impact on plant morphological and quality traits. The diversity index of the six active ingredients ranged from 1.61 (RT) to 2.01 (CGA), and the diversity index of the active ingredients of the mixed male and female samples in the homogenous garden ranged from 2.12 (CGA/TF) to 2.16 (AU). The higher diversity index in the homogenous garden may be caused by gender, and may also be related to its wider sampling range.

4.2 Influence of geographical and climatic factors on the content of leaves and active ingredients of *Eucommia ulmoides*

The partial Mantel test of leaf, fruit and seed kernel morphology of *Eucommia ulmoides Oliv.* Female plants with geographical and climatic factors showed that the relationship between morphological and geographical distance was not significant (r=0.16, p=0.214), indicating that after excluding the influence of climate differences, simple geographical isolation contributed less to the phenotypic differences, which may indicate that gene flow or historical migration offset the effect of geographical isolation; There was a significant positive correlation between morphological and climate difference (r=0.31, *p*=0.034), indicating that under the control of geographical distance, climate difference can still significantly explain the morphological differences between populations, indicating that environmental adaptation may be the main driver of morphological differentiation [18]. The Mantel test of the leaf morphological of *Eucommia ulmoides Oliv.* Male and female samples showed that the morphological was significantly related to geographical distance (r=0.33, *p*=0.048), climate (r=0.51, *p*=0.004) and altitude (r=0.32, *p*=0.021), but not to longitude (r=0.02, *p*=0.435) and latitude (r=0.28, *p*=0.074) [11]. This study analyzed the correlation between leaf morphological, phytochemotype and geographical and climatic factors of *Eucommia ulmoides* based on BLUP value. The results showed that the correlation between leaf size and effective component content and climatic and geographical factors was low ($r < 0.5$). DD0 was significantly positively correlated with LL, LW and PL ($r = 0.20$-0.39), which may indicate that the earlier the growth period starts, the larger the leaves and the longer the petioles. Among the 12 effective components, CGA, ASP, GPA, GP, PDG and IQ have certain correlation with most climatic and geographical factors, indicating that these components are affected by the environment to some extent; On the contrary, CA, PC, AU, CC, RT and AG have low or no correlation with most geographical and climatic factors, indicating that the content of these components may be more affected by genetic factors. In general, the correlation between leaf morphological and phytochemotype of *Eucommia ulmoides* and single geographical and climatic factors is low, which is more reflected in the comprehensive effect of climatic and environmental factors.

4.3 Limitations of the study

The research mainly focused on the collection of superior single plant seeds of *Eucommia ulmoides*, only collected and detected female samples, ignored the collection and detection of the corresponding male plant leaf samples in the population, and failed to achieve the comparative analysis of *Eucommia ulmoides* leaves in the scale of population and gender at the same time. In addition, the ages of samples in natural populations are not consistent. Whether the differences in sample ages will lead to the differences in leaf composition and content needs further study. Of course, the probability of finding groups with relatively consistent ages in natural forests is generally small. In addition, the sampling scope of this study is relatively small compared with the whole distribution area of *Eucommia ulmoides*, and no obvious correlation between leaf morphological and phytochemotype and a single geographical and climatic factor has been found. Sampling in a larger area, such as the edge area of *Eucommia ulmoides* natural distribution or the area with large terrain and climate differences, may have more obvious rules.

5. CONCLUSIONS

On the five leaf morphology indicators, the P6 population exhibits characteristics of large leaves. The P8 population has the characteristic of narrow leaves. The P3 group has the characteristic of small circular leaves. Among the 12 active ingredients, chlorogenic acid, asperuloside, aucubin, geniposidic acid, rutin, and isoquercetin have relatively high relative contents, accounting for 96.25% of the total active ingredient content.

The correlation between leaf composition and leaf circumference, leaf length, and leaf shape index is high, while the correlation with leaf width and internode spacing is low. The metabolic synthesis of geniposidic acid (GPA) in *Eucommia ulmoides* leaves may be the key to regulating the content of active ingredients such as chlorogenic acid, Isoquercitrin, Asperuloside, and rutin.

The P6, P7, P8, and P9 populations can choose individuals with large leaves (LP) and high CGA content, and the P6 population can also choose individuals with high GPA content; P1, P3, and P10 populations can choose individuals with high ASP and PDG (the main active ingredients of *Eucommia ulmoides* bark) content, and P3 population can also choose individuals with high CC content. The correlation between the morphological and phytochemotype of leaves and a single geographical and climatic factor is relatively low, and is more reflected in the comprehensive effect of climatic and environmental factors.

Compliance with Ethical Standards

Author contributions

H.L. and C.Q. designed the study and wrote the manuscript. H.G. performed the statistical analysis. L.T., C.T. and C.X. collected the phenotypic measurements and detected the active ingredients. All authors contributed to the article and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration

Ethics approval and consent to participate

The *Eucommia ulmoides* plant material used in this study was collected with the permission of the local forestry regulatory authorities. Hongguo Li carried out the formal identification of the samples. Voucher specimens (voucher numbers: EU2022-F001 to EU2022-F126) have been deposited in the Herbarium of Jishou University, China.

Consent for publication

Not applicable.

Clinical trial number

Not applicable.

Competing interests

The authors declare no competing interests.

Funding:

Conflicts of Interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Zhang M, Liang FN, Sun YW, Han F, Zhou Y, Rong YF, Qiu F, Ding L (2023) Research progress on chemical constituents, pharmacological effects and clinical application of *Eucommia ulmoides*. Chin Tradit Herb Drugs 54(14)
2. Li Y, Kang X (2024) Advances in research on the bioactive compounds and genetic improvement of *Eucommia ulmoides*. MPB 3(1)
3. Zhang L, Li N, Tu W, Li X, Wang C, Shao Z (2019) Simultaneous determination of contents of 8 components in *Eucommia ulmoides* leaves by HPLC. China Pharmacy 3383-3387
4. Xiang LL, Wen YJ, Miao MS (2017) Analysis of chemical pharmacological and clinical application of *Eucommia ulmoides* leaf. Acta Chin. Med 32:99-102
5. Peng M, Zhang M, Zhou C, Zhu J (2006) Analysis of iridoids in *Eucommia* samples with high performance liquid chromatography-electrospray ionization mass spectrometry. J Instrum Anal 25(1):52
6. Li J (2019) Research Progress in the Extraction Methods and Bioactivity of Flavonoids from *Eucommia ulmoides* leaves. Sci Technol Food Ind 40(7):346-350
7. Li X,Wei M, Wu W X, Luo X Q, Yao B,Wu S Y (2025) Inhibitory effect of rutin on the growth and metastasis of osteosarcoma in vitro and in vivo. J Shanghai Jiaotong Univ (Med Sci) 45(1):20-28
8. Wang Z, Sun Y, Han Y, Chen X, Gong P, Zhai P, Yao W, Ba Q, Wang H (2023) *Eucommia* bark/leaf extract improves HFD-induced lipid metabolism disorders via targeting gut microbiota to activate the Fiaf-LPL gut-liver axis and SCFAs-GPR43 gut-fat axis. Phytomedicine 110:154652
9. Wang Z, Yao W, Sun Y, Han Y, Chen X, Gong P, Zhai P, Pei S, Xie J, Ba Q, Wang H (2023) *Eucommia* bark/leaf extract improves lipid metabolism disorders by affecting intestinal microbiota and microbiome–host interaction in HFD mice. J Agric Food Chem 71(7):3297-3314
10. Wang L, Du L Y, Du H Y(2014) Research progress of *Eucommia ulmoides*. Feed Research 404(19):29-31+73
11. Gong HM, Yang M, Wang CC, *et al.* Leaf phenotypic variation and its response to environmental factors in natural populations of *Eucommia ulmoides*. *BMC Plant Biol* **23**, 562 (2023)
12. Mayer SS, Charlesworth D (1991) Cryptic dioecy in flowering plants. Trends Ecol Evol 6(10):320-325
13. Qing J, Wei YX, Wang DH, Wang L, Liu PF, Du HY, Du QX (2018) Study on genetic variation of main active components in leaves of *Eucommia ulmoides*. Acta Bot. Boreal.-Occid. Sin. 38(02):316-323
14. Wang BW, Wang YQ, Mo H, Li XL, Luo MX, Cui KM (1999) Comparison of cytology, apical buds and gutta content between staminate and pistillate of *Eucommia ulmoides* trees. J Integr Plant Biol 41(1)
15. Du HY (2014) China *Eucommia* Pictorial.China Forestry Publishing House: Beijing, China
16. Du HY, Hu W Z, Yu R (2015) The report on development of China's *Eucommia* rubber resources and industry (2014–2015). Social Sciences Academic Press: Beijing, China

17. Deng P, Wang Y, Hu F, Yu H, Liang Y, Zhang H, Wang T, Zhou Y, Li Z (2022) Phenotypic trait subdivision provides new sight into the directional improvement of *Eucommia ulmoides Oliver*. Front Plant Sci 13:832821
18. Wang CC, Gong HM, Feng M, Tian CL (2023) Phenotypic variation in leaf, fruit and seed traits in natural populations of *Eucommia ulmoides*, a relict Chinese endemic tree. Forests 14(3):462
19. Gong HM, Yang M, Wang CC, Tian CL (2023) Leaf phenotypic variation and its response to environmental factors in natural populations of *Eucommia ulmoides*. BMC Plant Biol 23(1):562
20. Dong C, Zhang Z, Shao Q, Yao T, Hu H, Huang J, Huang J, Liang Z, Han, Y (2022) Deciphering the effects of genetic characteristics and environmental factors on pharmacological active ingredients of *Eucommia ulmoides*. Ind Crops Prod 175:114293
21. Wei Y X, Liu P F, Du Q X, Du H Y (2016) Comparison in Contents of Polyphenol and Flavonoid in Leaves of *Eucommia ulmoides* Germplasm. Forest Research 29(4):529
22. Wang T, Wang G, Innes JL, Seely B, Chen B (2017) ClimateAP: An application for dynamic local downscaling of historical and future climate data in Asia Pacific. Front Agric Sci Eng 4(4):448-458
23. Liu X (2022) Comparison of Chemical Composition and Contents inDifferent Parts of *Eucommia ulmoides* and Its Applicationin Chinese Patent Vedicines.(Master's thesis, Nanjing University of Traditional Chinese Medicine). Master's degree
24. Kassambara A, Mundt F (2020) Extract and visualize the results of multivariate data analyses [R package factoextra version 1.0. 7]. Comprehensive R Archive Network (CRAN)
25. Gilmour AR, Thompson R, Cullis BR (1995) Average information REML: an efficient algorithm for variance parameter estimation in linear mixed models. Biometrics 1440-1450
26. Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) ASReml-R reference manual. The State of Queensland, Department of primary industries and fisheries, Brisbane
27. R Core Team. R (2013) R: A language and environment for statistical computing.Vienna, Austria: R Foundation for Statistical Computing
28. Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2009) ASReml user guide release 3.0. Hemel Hempstead, UK: VSN International Ltd
29. Self SG, Liang KY (1987) Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. J Am Stat Assoc 82(398):605-610
30. Li HG, Chen DZ, Xu JS, Liu GJ, Pang XD, Ye JH, Mo XW, Chen HH (2019) Phenotypic diversity and variation in natural populations of *Erythrophleum fordii*, an endangered plant species. Scientia Silvae Sincae 55(04):69-83
31. Rovesti P (1957) Essential oils of some phytochemotypes of aromatic eritrean labiates. Pharm Weekbl 92(23):843-845
32. Eilers EJ, Kleine S, Eckert S, Waldherr S, Müller C (2021) Flower production, headspace volatiles, pollen nutrients, and florivory in Tanacetum vulgare phytochemotypes. Front Plant Sci 11:611877
33. Soro LC, Munier S, Pelissier Y, Grosmaire L, Yada R, Kitts D, Atchibri ALO, Guzman C,Boudard F, Menut C, Robinson J C, Poucheret P (2016) Influence of geography, seasons and pedology on chemical composition and anti-inflammatory activities of essential oils from Lippia multiflora Mold leaves. J Ethnopharmacol 194:587-594
34. Meng YD, Du HY, Wang L, Lv GX, Qing J, He F, Huang HY, Du QX (2022) Diversity analysis of leaf phenotypic traits of *Eucommia ulmoides* germplasm resources. J For Res 35(5):103-112.