

Temporal Accumulation Patterns of Paeonol, Paeoniflorin, and Tannic Acid in Leaves of Five Tree Peony (*Paeonia suffruticosa*) Cultivars

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Abstract

As a significant by-product of the tree peony industry, tree peony leaves are a valuable source of bioactive compounds, making the analysis of their composition and accumulation patterns vital for exploitation. This study systematically investigated the temporal accumulation patterns of three bioactive compounds—paeonol, paeoniflorin, and tannic acid—in the leaves of five major tree peony cultivars ('Luoyanghong', 'Fengdan', 'Jingyu', 'Erqiao', and 'Huhong') across different growth stages in Henan, China. Using HPLC and UV-Vis methods, we quantified the contents of these components at five key harvest timepoints from March to September. The results revealed distinct temporal dynamics. Paeoniflorin content decreased sharply after flowering, with reductions of 70%-80% by September compared to March levels across all cultivars. Paeonol accumulation was cultivar-dependent, generally peaking in May-July; for instance, 'Jingyu' reached 761.48 µg/g in July, while 'Fengdan' showed a declining trend from 554.05 µg/g in March. Tannic acid content was highest in spring (e.g., 'Luoyanghong' at 643.98 mg/g in April), then declined significantly by autumn, with reductions of 50%-60% across cultivars. These findings demonstrate significant temporal and varietal variations in bioactive compound accumulation in tree peony leaves. The study provides a scientific basis for optimizing harvest timing to maximize the yield of specific compounds, supporting the valorization of tree peony leaf resources in pharmaceutical and functional product applications.

Introduction

Tree peony (*Paeonia suffruticosa Andrews*) is a unique woody and precious plant species native to China, boasting ornamental, medicinal, and oil-using values (Guo et al., 2022). In recent years, with the strong support of government industrial policies, the tree peony industry, particularly the oil-using tree peony sector, has achieved large-scale development. Oil-using tree peony is now cultivated across 22 provinces and more than 200 counties and cities in China, with core production areas concentrated in Henan, Shandong, Anhui, Shaanxi, and other regions. As of now, the cultivation area of oil-using tree peony in China has reached about 130,000 hectares, with an annual output of 53,000 t of tree peony seed oil. The industry scale and economic benefits continue to rise steadily (National Forestry and Grassland Administration., 2023).

With the rapid advancement of the oil-using tree peony industry, the resource utilization of its by-product, tree peony leaves, has gradually emerged as a key direction for industrial upgrading. Studies have shown that tree peony leaves contain a variety of high-value active ingredients, including paeonol, paeoniflorin and tannins (Zheng et al., 2023; Yang et al., 2017). These ingredients show significant potential in both drug development and functional health products. Through the optimization and comprehensive utilization of the extraction process of the active components of tree peony leaves, the resource utilization rate of the oil-using tree peony industry can be effectively improved, and the added value of the industrial chain can be significantly increased. However, there are still notable gaps in current academic research on these key active ingredients in tree peony leaves: there is a lack of systematic investigation into the accumulation patterns of active ingredients across different tree peony varieties,

and particularly, a clear understanding of the dynamic changes in these components during different growth stages has yet to be established.

Therefore, this study selected five representative tree peony (*Paeonia suffruticosa* Andr.) cultivars, namely Luoyanghong, Fengdan, Jingyu, Erqiao, and Huhong, as research objects. High-performance liquid chromatography (HPLC) and ultraviolet-visible spectrophotometry (UV-Vis) were employed to systematically determine and analyze the spatiotemporal accumulation characteristics of paeonol, paeoniflorin, and tannins in their leaves. The objectives were to clarify the differential accumulation patterns of active ingredients among different tree peony cultivars and identify the optimal harvest period when the content of each ingredient meets the medicinal standards. This research provides a theoretical basis for the high-value utilization of tree peony leaf resources and promotes the transformation of the oil-using tree peony industry from "single oil production" to "multi-component collaborative development."

Materials and Methods

Plant material

Leaf samples of five tree peony varieties were collected at the tree peony garden of Henan University of Science and Technology during three seasons in 2024—Figure 1: spring (March 22nd, pre-flowering; April 15th, flowering; May 31st, post-flowering), summer (July 31st), and autumn (September 31st). The full names and abbreviations of the cultivars are listed in Table 1. The tree peony leaf variety was identified by Professor Wang Erqiang, a researcher at the Peony Research Institute of the Luoyang Academy of Agricultural and Forestry Sciences. Healthy tree peony plants free from pests and diseases were selected. Subsequently, uncontaminated leaves from the mid - upper canopy of the selected plants were collected. Place the collected tree peony leaves in an electric heating constant-temperature drying oven at 40°C until constant weight is achieved. Then grind the dried leaves into powder, seal powder in a container, and store it at room temperature away from light for subsequent analysis. Dried plant specimens are stored at Henan University of Science and Technology.

Table 1. Name and abbreviation of studied tree peony.

Cultivar abbreviation	Cultivar name
LYH	Luoyanghong
EQ	Erqiao
FD	Fengdan
HH	Huhong
JY	Jingyu

Chemicals and reagents

Paeonol, paeoniflorin, and tannic acid standards (purity $\geq 98\%$) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Chemical structures are shown in Figure 2. Acetonitrile (HPLC grade) was purchased from Hubei Changtai Xingye Technology Co., Ltd., and 85% phosphoric acid (HPLC grade) was acquired from Tianjin Kermel Chemical Reagent Co., Ltd. All experiments utilized C'estbon purified water. The instruments employed in this study were an E2695-2489 ultra-high performance liquid chromatograph provided by Waters Corporation and an Infinite 200 PRO microplate reader obtained from Henan Dequan Xingye Trading Co., Ltd.

Leaf size measurement

From March 22 to April 21, the maximum length and width of 10 leaves from each tree peony variety were measured every 3 days using a vernier caliper. Calculate the average leaf length and width for each cultivar, and construct growth curves with observation dates on the x-axis and average leaf length and width on the y-axis to quantitatively analyse the natural growth patterns of tree peony leaves.

Preparation of sample solution

The preparation protocol for paeonol sample solutions was developed based on the Chinese Pharmacopoeia (2020 Edition) and the method reported by Wang Xuejie et al.(Jiexue W et al., 2025), with minor modifications. Precisely weighed 0.25 g of dried tree peony leaf powder was mixed with 6 mL of petroleum ether, soaked for 30 minutes, shaken for 10 seconds, and then the petroleum ether was discarded to remove chlorophyll. 25 mL of methanol solution was added and shaken well. All samples underwent ultrasound-assisted extraction at room temperature ($25 \pm 5\text{ }^{\circ}\text{C}$) for 30 minutes (100 W, 45 kHz), were cooled, reweighed, and made up to the original weight with methanol. Then, they were centrifuged at 3000 r/min for 10 minutes, and the supernatants were filtered through a 0.22- μm microporous membrane. The subsequent filtrates were taken as the sample solutions.

The preparation protocol for Paeoniflorin sample solutions was based on the method reported by Pan Xu et al.(Xu et al., 2023), with minor modifications. 0.5 g of dried tree peony leaf powder was precisely weighed and mixed with 25 mL of 50% ethanol solution, shaken well. Ultrasound-assisted extraction was carried out at room temperature ($25 \pm 5\text{ }^{\circ}\text{C}$) for 30 minutes (100 W, 45 kHz). After cooling, it was reweighed and made up to the original weight with 50% ethanol. The mixture was then centrifuged at 3000 r/min for 10 minutes, and the supernatant was filtered through a 0.22- μm microporous membrane. The subsequent filtrate was diluted tenfold with 50% ethanol to obtain the sample solution.

The preparation protocol for tannin sample solutions was based on the method reported by Makkar (Makkar, 2003) , with minor modifications. 0.2 g of dried tree peony leaf powder was precisely weighed and mixed with 10 mL of 70% acetone solution. Ultrasound-assisted extraction was conducted at room temperature ($25 \pm 5\text{ }^{\circ}\text{C}$) for 20 minutes (100 W, 45 kHz). After cooling, it was reweighed and made up to the original weight with 70% acetone. Then, it was centrifuged at 4000 g for 10 minutes at $4\text{ }^{\circ}\text{C}$, and the supernatant was collected as the sample solution, which was kept on ice.

Preparation of standard solutions

Paeonol standard and paeoniflorin standard were precisely weighed. They were then dissolved in methanol to prepare individual standard stock solutions with concentrations of 1 mg/mL and 500 µg/mL, respectively. Subsequently, gradient dilutions were carried out using methanol for each of these solutions. The series of working standard solutions were filtered through a 0.22-micron (0.22-µm) microporous filter. Then, 1.5 milliliters (1.5 mL) of the filtered solution was transferred into sample vials and analyzed using a High-Performance Liquid Chromatograph (HPLC). Obtained the calibration curves for paeonol ($y = 20514x - 27315$, $R^2 = 0.9989$) and paeoniflorin ($y = 25856x - 46527$, $R^2 = 0.9995$), respectively.

Tannic acid standard was precisely weighed and dissolved in distilled water to prepare a tannic acid standard stock solution with a concentration of 0.4 mg/mL. Then, gradient dilutions were performed using distilled water for this solution. Subsequently, the analysis was performed using a microplate reader. The standard curve for tannic acid ($y = 4712.9x + 7.3$, $R^2 = 0.9945$) has been obtained.

HPLC analysis

With reference to the Chinese Pharmacopoeia (2020 Edition), high-performance liquid chromatography (HPLC) was employed to analyze the standard and sample solutions. Chromatographic conditions employed for paeonol determination were as follows. A Waters Hypersil BDS-C18 chromatographic column (5 µm, 4.6 mm × 250 mm) was utilized. The mobile phase consisted of methanol-water (45:55, V/V) with isocratic elution. The flow rate was set at 1.0 mL/min, the column temperature was maintained at 30 °C, and the injection volume was 20 µL. The samples were detected at a wavelength of 274 nm.

Chromatographic conditions employed for paeoniflorin determination were as follows. A Waters Hypersil BDS-C18 chromatographic column (5 µm, 4.6 mm × 250 mm) was used. The mobile phase was acetonitrile-0.1% phosphoric acid solution (15:85, V/V) with isocratic elution. The flow rate was set at 1.0 mL/min, the column temperature was maintained at 30 °C, and the injection volume was 20 µL. The samples were detected at a wavelength of 230 nm.

Under this condition, the retention time of paeonol is approximately 19.5 minutes, while that of paeoniflorin is around 11.5 minutes. Qualitative analysis of the components in the sample solutions was conducted based on the corresponding retention times of the standard substances eluted under the same conditions. The contents of paeonol and paeoniflorin in each sample were calculated from the peak areas using the standard curves. The quantitative results for each compound are expressed per gram of plant dry weight.

Colorimetric reaction and spectrophotometric analysis

The total tannin content of tree peony leaf extract was determined by the Folin-Ciocalteu colorimetric method, as described by Makkar (Makkar, 2003) and Unban et al (Unban et al., 2020). A pipette was used to transfer 0.01 mL of the tannin sample solution into a test tube. It was then mixed with 0.99 mL of

distilled water. Subsequently, 0.5 mL of Folin-Ciocalteu reagent (FCR) was added and 2.5 mL of a 20% sodium carbonate (Na_2CO_3) solution. The mixture was vortexed thoroughly to ensure uniformity and then incubated in the dark at room temperature ($25 \pm 5^\circ\text{C}$) for 40 minutes. After incubation, 200 μL of the mixture was transferred into a 96-well plate, and the optical density was measured at 725 nm using a microplate reader. Each sample was analyzed in triplicate. The total polyphenol content was calculated using a standard curve.

Polyvinylpolypyrrolidone (PVPP) was added to bind with tannins in the sample solution, forming precipitates that separated tannins from other phenolic compounds. The specific steps were as follows: 0.6 g of PVPP was weighed out and added to a mixture containing 0.25 mL of sample solution and 4.75 mL of distilled water. The mixture was vortexed thoroughly and then allowed to stand undisturbed at 4°C for 15 minutes. An appropriate amount of the mixture was transferred into a centrifuge tube and centrifuged at 4000 r/min for 10 minutes, and the supernatant was collected. The optical density of the obtained supernatant was determined using the Folin-Ciocalteu reagent (FCR), following the same method as described above. Using the tannic acid standard curve, the content of unabsorbed simple phenols remaining in the solution after precipitation was calculated. The tannin content was obtained by subtracting the content of unabsorbed polyphenols from the total phenolic content. The experimental results were expressed as tannic acid equivalents per gram of plant dry weight (mg TAE/g DW).

Quantitative determination of constituents

The calculation formulas for the contents of paeonol, paeoniflorin, and tannins were as follows:

$$c = \frac{x \times v \times f}{m} \text{ (mg/mL)} \quad (1)$$

In Equation (1): c is the content of active components in tree peony leaves; x is the concentration calculated by substituting into the standard curve, in mg/mL; v is the volume used to make up the sample solution to the final volume, in mL; f is the dilution factor; m is the sample mass, in g.

Data analysis

Data acquisition and processing were performed using the Empower software integrated with the E2695-2489 High-Performance Liquid Chromatograph (HPLC). Experimental results were presented as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range test were employed to analyze the significance of differences at the 0.05 level. Data analysis was conducted using Microsoft Excel 2016 and SPSS 26.0 software. Graphical representations were created using GraphPad Prism 8.0 and ChemDraw 23.1.1 64-bit software.

Results and Discussion

Growth Patterns of Tree Peony Leaves

The growth trends of five types of tree peony leaves in the Luoyang region were generally consistent. As shown in Fig. 3, leaf growth is most rapid from March to early April, stabilizing by mid-to-late April. The width of LYH, EQ, HH, and JY leaves increased from 5–7 cm to 8–9 cm, while their length increased from 5–6 cm to 7–8 cm, representing an increase of approximately 2–3 cm in both dimensions. In contrast, FD leaves exhibit a distinctive narrow, elongated shape. Their width increases from approximately 2 cm to 6 cm (an increase of about 4 cm), while their length extends from 5 cm to 11.5 cm (an increase of about 6.5 cm). Consequently, FD exhibited a significantly higher growth rate in April compared to the other four varieties.

Dynamic changes in paeoniflorin content

The paeoniflorin contents of the leaves from the studied tree peony cultivars at different periods are presented in Table 2. The paeoniflorin content of all five tree peony varieties peaks in March. HH leaf paeoniflorin content exhibited a relatively low peak, reaching only approximately 27.56 ± 4.14 mg/g, while the other four tree peony leaf varieties registered around 60 mg/g. The highest peak value was recorded for LYH, reaching approximately 68.45 ± 4.89 mg/g, followed by EQ at about 61.94 ± 7.70 mg/g. From March to April, the paeoniflorin content in tree peony leaves decreased significantly. During this period, paeoniflorin levels in JY and FD varieties dropped by approximately half, while EQ and LYH varieties fell to about one-third of their March levels. By May, paeoniflorin content in all five varieties of tree peony leaves had decreased to below 5 mg/g. By autumn, paeoniflorin levels remained at a low and stable level. This observation indicates that the paeoniflorin content in tree peony leaves exhibits a consistent and marked declining trend during the critical phenological phase from pre-flowering to post-flowering. The coloration of tree peony flowers is primarily regulated by the synergistic interaction of two types of pigments in the petals: flavonoids (such as anthocyanins) and carotenoids. Following tree peony flowering, paeoniflorin content in leaves declines sharply, likely resulting from the combined effects of “reproductive resource allocation” and “competitive inhibition of metabolic pathways.” Both mechanisms are closely linked to the mevalonate (MVA) pathway, which underlies the synthesis of carotenoids and paeoniflorin. Paeoniflorin belongs to pinane-type tricyclic monoterpene glycosides, and its synthesis depends on the MVA (mevalonate) pathway to generate yak 2 phosphate (GPP)(Lee et al., 2024). Carotenoid synthesis requires further polymerization of GPP to GGPP (geranylgeranyl pyrophosphate), a key precursor in carotenoid biosynthesis(Lao et al., 2018; Vranová et al., 2019). Since both paeoniflorin and carotenoid synthesis share GPP as a common substrate, and this substrate sharing relationship may lead to the competitive consumption of GPP. Plants optimize the structure and function of different organs through allometric resource allocation at various life history stages, thereby driving the differentiation of nutritional and reproductive strategies(Duan YN, 2025). During the flowering stage, tree peony plants activate a resource allocation strategy prioritizing reproduction. On one hand, the development of floral organs requires large quantities of pigment substances such as carotenoids and anthocyanins. The synthesis of these pigments necessitates upregulation of MEP/MVA pathway-related

genes (e.g., IPP isomerase)(Li et al., 2021), accompanied by substantial consumption of precursors like GPP and IPP. This leads to GPP concentrations in leaves falling below the threshold for paeoniflorin synthesis, resulting in precursor depletion for paeoniflorin production(Botella-Pavía et al., 2004; Yao et al., 2023; Guo et al., 2022). On the other hand, during the flowering stage, tree peony leaves undergo a functional transition from "secondary metabolite accumulation" to "nutrient export." The expression of the key paeoniflorin-synthesizing enzyme PoDPBT in leaves significantly declines during this period, reaching only 1/5 of its level in the budding stage, thereby weakening the leaves' capacity for paeoniflorin synthesis(Zhang et al., 2023). Under the combined influence of these factors, resource allocation-induced precursor shortages exacerbate competitive inhibition, while competitive inhibition further enhances the flow of precursors toward floral organs, creating a dual blockade on paeoniflorin synthesis. Consequently, paeoniflorin content in leaves declines by 70%-80%, a process consistent with the "metabolic sink-source" functional transition during tree peony flowering(Zhong PX et al., 2012).

Table 2
Paeoniflorin content in tree peony leaves at different harvest times.

Variety	Paeoniflorin content (mg/g)					Average value
	March 22nd	April 15th	May 31st	July 31st	September 30th	
LYH	68.45 ± 4.89 ^a	22.93 ± 2.20 ^b	5.72 ± 0.33 ^c	3.00 ± 0.11 ^c	2.33 ± 0.77 ^c	20.49
EQ	61.94 ± 7.70 ^a	19.95 ± 1.81 ^b	4.52 ± 0.72 ^c	4.03 ± 0.29 ^c	3.60 ± 1.53 ^c	16.03
FD	56.75 ± 4.65 ^a	32.99 ± 3.24 ^b	4.28 ± 1.04 ^c	2.42 ± 0.33 ^c	2.07 ± 0.79 ^c	19.70
HH	27.56 ± 4.14 ^a	20.17 ± 0.86 ^b	5.44 ± 1.27 ^c	4.63 ± 0.31 ^c	3.06 ± 0.39 ^c	12.17
JY	60.29 ± 5.54 ^a	30.50 ± 4.76 ^b	4.22 ± 0.54 ^c	3.49 ± 0.12 ^c	2.15 ± 0.37 ^c	20.13
Note. Data are expressed as the mean ± standard deviation from four independent experiments. Different lowercase letters within the same row indicate significant differences by Duncan's multiple range test at the 0.05 level. LYH, Luoyang Hong; EQ, Er Qiao; FD, Feng Dan; HH, Hu Hong; JY, Jing Yu.						

Dynamic changes in paeonol content

Paeonol, as a phenolic compound, is biosynthesized via the phenylpropanoid pathway in plant secondary metabolism. Analysis of paeonol content in leaves from most tree peony cultivars across different time periods reveal a non-monotonic pattern: initial fluctuating increase, mid-stage peak, and subsequent significant decline(Table 3). Specifically, the content of JY leaf paeonol remained at a low level from March to April. Paeonol accumulated rapidly in JY leaves from April and reached a peak value in July (761.48 ± 25.34 μg/g), which was much higher than that of other four varieties. In contrast, paeonol

content in LYH, EQ, and HH leaves peaked in May. Furthermore, the paeonol content in EQ leaves was relatively low, and showed minimal fluctuation throughout the growth period. This trend results from the synergistic effects of genetic characteristics, physiological demands, and environmental factors. During the bud initiation to vegetative growth phase (March-April), leaves undergo cell division and expansion. Despite gradually enhanced photosynthetic capacity, energy and carbon flow primarily support primary metabolism and structural growth(Zong M et al. 2011). As a secondary metabolite, paeonol synthesis may be regulated by early leaf physiological activities, manifesting as a fluctuating upward trend. In the vegetative growth to flowering/fruiting stage (May-July), photosynthesis intensifies, and biomass accumulation peaks, providing ample carbon skeletons and energy for secondary metabolite synthesis. Studies indicate that when leaves fully expand and photosynthetic efficiency reaches its maximum, key enzymes in the phenylpropanoid pathway (e.g., PAL, CHS) are upregulated, significantly promoting paeonol production(Wu et al. 2025). Mature leaves also exhibit higher levels of soluble sugars and proteins than young leaves, offering abundant substrates for secondary metabolism. Consequently, paeonol content in tree peony leaves peaks during May-July. By September, senescent leaves turn yellow, and nutrient translocation reduces paeonol content to levels comparable to March. Environmental factors such as optimal temperature and moderate light intensity activate the phenylpropanoid pathway, enhancing paeonol synthesis(Wu et al. 2025). Additionally, as an antioxidant and defense compound, paeonol's dynamic changes are closely linked to functions like reactive oxygen species scavenging, pathogen resistance, and growth regulation (e.g., in vitro rooting of *Paeonia suffruticosa*) (Rao et al., 2025). Notably, the FD cultivar exhibits a unique trend: high paeonol content in spring, characterized by a March peak of $554.05 \pm 20.4 \mu\text{g/g}$, was followed by a progressive monthly decline to $194.79 \pm 3.48 \mu\text{g/g}$ in September. This pattern may be attributed to the preferential allocation of paeonol to root bark, reflecting metabolic regulatory mechanisms specific to authentic medicinal materials(Zheng et al., 2023).

Table 3
Paeonol content in tree peony leaves at different harvest times.

Variety	Paeonol content (µg/g)					Average value
	March 22nd.	April 15th	May 31st	July 31st	September 30th	
LYH	216.23 ± 9.76 ^d	376.22 ± 5.84 ^b	428.70 ± 11.47 ^a	325.75 ± 10.35 ^c	213.58 ± 15.55 ^d	312.09
EQ	249.14 ± 15.81 ^b	247.57 ± 18.44 ^b	308.44 ± 34.38 ^a	268.67 ± 25.55 ^b	184.78 ± 17.98 ^c	251.72
FD	554.05 ± 20.4 ^a	528.06 ± 13.71 ^b	300.80 ± 7.86 ^c	243.31 ± 23.56 ^d	194.79 ± 3.48 ^e	364.20
HH	246.79 ± 6.05 ^c	318.71 ± 25.91 ^b	434.52 ± 12.36 ^a	258.36 ± 8.41 ^c	192.56 ± 14.40 ^d	290.19
JY	201.58 ± 5.68 ^c	199.69 ± 5.53 ^c	546.66 ± 28.04 ^b	761.48 ± 25.34 ^a	222.52 ± 3.35 ^c	386.39

Note. Data are expressed as the mean ± standard deviation from four independent experiments. Different lowercase letters within the same row indicate significant differences by Duncan's multiple range test at the 0.05 level. LYH, Luoyang Hong; EQ, Er Qiao; FD, Feng Dan; HH, Hu Hong; JY, Jing Yu.

Dynamic changes in tannin content

The tannin contents of the leaves from the studied tree peony cultivars at different periods are presented in Table 4. The dynamic changes in leaf tannin content of LYH, EQ, and FD varieties across different growth stages generally followed an upward trend initially, followed by a decline. All varieties reached peak tannin levels in May and exhibited the lowest content in September. Among them, LYH exhibited the highest tannin content. Starting from April, its tannin content increased significantly, maintaining a high level above 600 mg/g from April to July. By September, LYH tannin content dropped sharply to approximately one-third of the July level. Both EQ and FD leaves exhibited peak tannin content exceeding 500 mg/g, but FD leaves showed a greater decline in tannin levels in July compared to EQ. The leaf tannin content of HH and JY varieties exhibited a trend of initial decline followed by recovery and subsequent decrease, with both reaching peak values in March at 483.84 ± 6.74 mg/g and 438.69 ± 12.16 mg/g, respectively. Tannin levels decreased significantly in April, rebounded somewhat in May, but remained below March levels. From July to September, they continued to decline, falling below 200 mg/g. Tannins belong to the class of polyphenolic compounds. In plants, they are primarily synthesized from shikimic acid as a precursor, which is catalyzed by phenylalanine deaminase to produce phenylpropanoid compounds, ultimately polymerizing to form tannins. From a seasonal perspective, all tree peony cultivars exhibit rapid tannin accumulation in their leaves during spring (March-April), which aligns closely with the chemical defense demands of young leaf development(Iqbal et al., 2024). In the early stages of leaf development, when physical defense structures (e.g., cuticle, wax layer) are not fully formed, tree peony leaves compensate by rapidly accumulating chemical defense substances like

tannins, thereby maximizing survival opportunities(Mithöfer et al., 2012; Lamy et al., 2011). The temporal complementarity between chemical and physical defense mechanisms exemplifies the plant's optimized resource allocation strategy. During summer and autumn (June-September), tannin content in tree peony leaves significantly declines, likely due to the metabolic shift away from defense after physical barriers mature in fully developed leaves(Han et al., 2018). Concurrently, environmental factors such as elevated temperatures, potential water stress, and fluctuating light conditions collectively contribute to reduced tannin accumulation by influencing enzyme activity and metabolic pathways(Zhang LH et al., 2010). Notably, in April, some cultivars exhibit unique patterns: HH and JY show decreased tannin content compared to March. This phenomenon may be attributed to carbon allocation competition during the tree peony flowering period (April-May), where phenylpropanoid metabolites are prioritized for reproductive organs, thereby suppressing tannin synthesis. These finding parallels research showing that “reproductive development influences phenolic accumulation” in plants such as olive leaves(Kabbash et al., 2021), reflecting a trade-off mechanism in plant resource allocation.

Table 4
Tannin content in tree peony leaves at different harvest times.

Variety	Tannin content (mg/g)					Average value
	March 22nd	April 15th	May 31st	July 31st	September 30th	
LYH	447.96 ± 3.38 ^b	643.98 ± 11.17 ^a	643.19 ± 11.14 ^a	628.00 ± 13.22 ^a	225.21 ± 1.72 ^c	517.67
EQ	368.32 ± 5.49 ^d	445.10 ± 18.56 ^c	515.27 ± 10.35 ^a	488.57 ± 9.12 ^b	273.48 ± 2.85 ^e	418.15
FD	273.79 ± 3.61 ^d	481.82 ± 3.84 ^b	526.80 ± 7.14 ^a	317.52 ± 1.45 ^c	200.58 ± 2.58 ^e	360.1
HH	483.84 ± 6.74 ^a	400.55 ± 6.49 ^b	476.82 ± 2.74 ^a	291.02 ± 1.55 ^c	199.86 ± 6.63 ^d	370.42
JY	438.69 ± 12.16 ^a	271.28 ± 18.53 ^c	362.19 ± 3.36 ^b	189.38 ± 6.26 ^d	116.01 ± 0.46 ^e	275.51
Note. Data are expressed as the mean ± standard deviation from three independent experiments. Different lowercase letters within the same row indicate significant differences by Duncan's multiple range test at the 0.05 level. LYH, Luoyang Hong; EQ, Er Qiao; FD, Feng Dan; HH, Hu Hong; JY, Jing Yu.						

Declarations

All authors declare that they have no conflicts of interest related to this study.

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Author Contribution

G.Y. and W.F. wrote the main manuscript text. W.F., Q.S. and L.L. led the experimental execution. G.Y., H.W., and X.H. administered the project, supervised the study and carried out the investigation. L.M., Y.Q., and W.T. led the conceptualization and methodology design. X.H., X.W. and M.Z. performed the validation experiments to verify the results. W.F., S.W., Q.S. led the data curation, formal analysis and visualization. G.Y. acquired the funding. All authors reviewed the manuscript.

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Figures

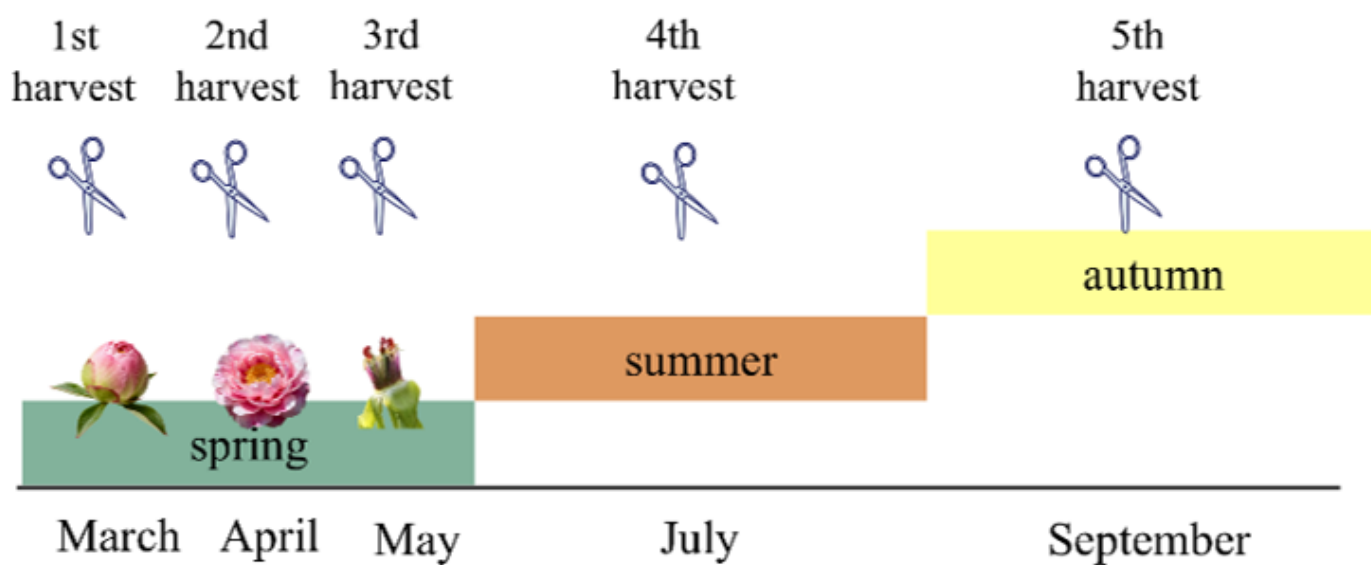


Figure 1

Harvesting time of peony leaves.

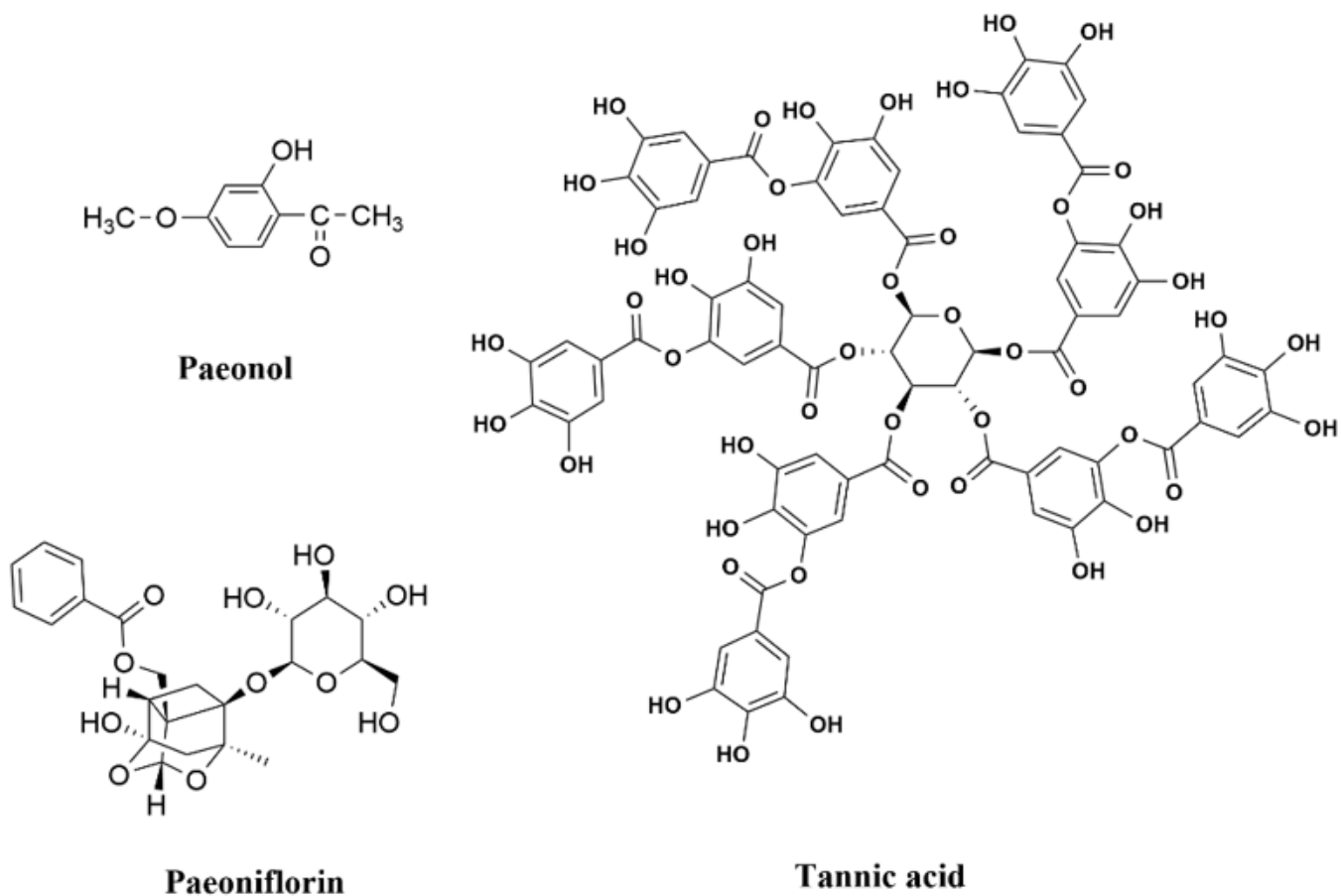


Figure 2

Chemical structure of paeonol, paeoniflorin and tannin acid.

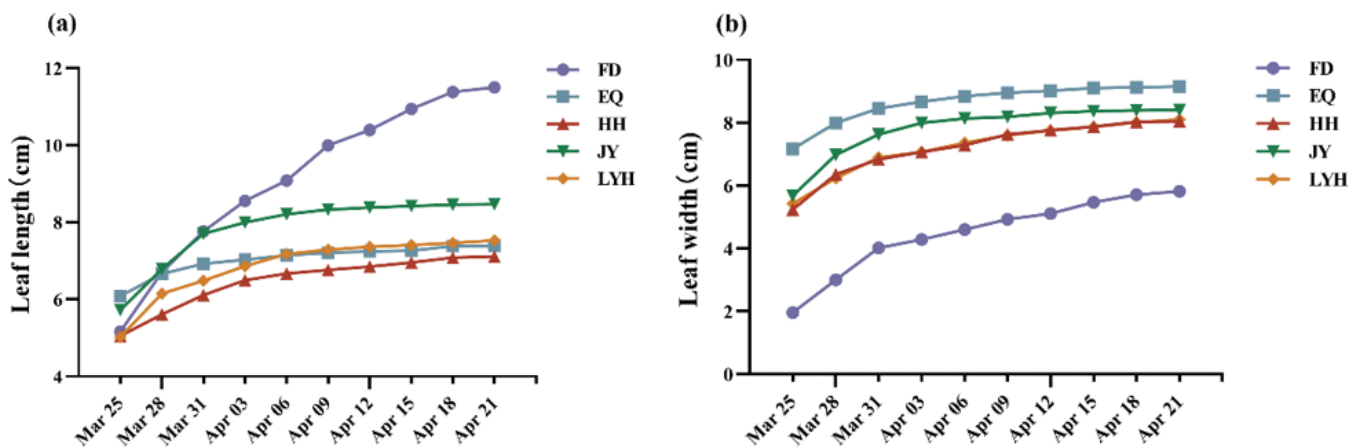


Figure 3

The temporal changes in leaf growth of five different treepeony varieties. (a) Variations in leaf length. (b) Variations in leaf width. Abbreviations: LYH,Luoyang Hong; EQ, Er Qiao; FD, Feng Dan; HH, Hu Hong; JY, Jing Yu.