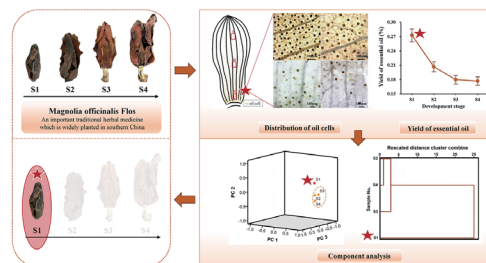


Assessment of Quality in Essential Oil from *Magnolia officinalis* flos at Different Developmental Stages by Anatomy, GC-MS, and Chemometric Methods

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Abstract: *Magnolia officinalis* Flos (Houpohua), a valued traditional Chinese medicine, suffers from inconsistent quality due to vague pharmacopeial harvesting criteria. To resolve this, we integrated multi-scale analytical approaches—including microscopic histochemistry, gas chromatography-mass spectrometry (GC-MS), and chemometrics (n=120 samples)—yielding three critical discoveries: (1) A newly established 4-stage developmental classification (S1-S4) pinpointed S1 (bud length: 4.1–6.0 cm) as the biosynthetic optimum, exhibiting peak essential oil content (0.27% w/w) and maximal oil cell density (236.35 ± 7.09 cells/mm²); (2) Spatial mapping revealed a 40.03-fold oil cell gradient between basal (115.29 ± 3.45 cells/mm²) and apical tepal regions, indicating tissue-specific accumulation patterns; (3) Multivariate analysis uncovered a developmental metabolic shift dominated by caryophyllene-to-caryophyllene oxide conversion (explaining 94.06% of PC1 variance), synchronized with oil cell maturation. Mechanistically, post-S1 yield reduction stems from cell dilution (tepals expansion) and oxidative transformation. This work not only establishes evidence-based harvesting standards but also proposes a novel quality assessment paradigm integrating structural and metabolic markers for medicinal plants.



Key words: *Magnolia officinalis* flos, essential oil, oil cell, GC-MS, chemometrics

1 Introduction

The Magnoliaceae family represents a phylogenetically significant basal angiosperm lineage, comprising 17 genera and approximately 347 extant species worldwide¹. This family exhibits its highest species diversity in Southeast Asia, with China serving as its primary biodiversity hotspot. Magnoliaceae species have been extensively utilized in traditional medicine, particularly in East Asia. The dried flower buds of *Magnolia biondii*, *Magnolia denudata*, and *Magnolia sprengeri*, officially documented as “Xinyi” in the *Pharmacopoeia of the People’s Republic of China*² are rich in bioactive compounds, including terpenoids and alkaloids. These constituents have demonstrated significant pharmacological properties, particularly anti-inflammatory and antihypertensive effects^{3, 4}. Among these species, *M. officinalis* Rehd. et Wils. and its variant *M. of-*

ficialis var. *biloba* Rehd. et Wils. are considered geo-authentic medicinal materials, predominantly cultivated in the Yangtze River basin provinces (e.g. Hubei and Sichuan). Cultivated varieties currently account for over 90% of commercial medicinal products⁵. According to pharmacopeial standards, the flower buds must be harvested before the final flowering stage in spring². Modern pharmacological investigations have revealed that *M. officinalis* lower buds exhibit broad-spectrum antimicrobial activity and modulate gastrointestinal dysfunction through the 5-hydroxytryptamine (5-HT) signaling pathway⁶. Furthermore, they demonstrate multi-target therapeutic potential, including anxiolytic and anti-allergic effects.

The essential oil of *M. officinalis* flos exhibits broad pharmacological activities, including significant *antioxidant and antibacterial effects*⁷, *anti-inflammatory*

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properties via NF- κ B suppression⁸⁾, neuroprotective and analgesic actions in neuropathic pain models⁹⁾, and *gastro-intestinal regulation* through gut microbiota modulation¹⁰⁾. These diverse bioactivities highlight *M. officinalis* flos's therapeutic potential, though further research is needed to optimize its bioavailability and stability for clinical applications. Essential oils, the key bioactive components determining the medicinal value of *M. officinalis* flower buds^{11, 12)}, exhibit significant variations in content during pre- and post-flowering stages¹³⁾. Oil cells, characteristic structures of Magnoliaceae plants, serve as the primary sites for essential oil synthesis and accumulation¹⁴⁾. During bud development, the morphology, quantity, and distribution patterns of oil cells undergo dynamic changes, which play a critical regulatory role in the biosynthesis and accumulation of essential oils^{15–18)}.

As a vital medicinal plant of Magnoliaceae plants, the essential oils of *M. officinalis* buds have garnered significant attention due to their dual pharmacological activity and aromatic properties. However, research on the dynamic changes of essential oils during bud development and their relationship with oil cell development remains limited^{13, 19)}. To resolve these questions, we implemented an integrative approach combining advanced analytical techniques with cellular morphology studies. GC-MS with its exceptional sensitivity (detection limit 0.01 ng for major terpenoids), allowed comprehensive profiling of essential oil compounds across different developmental stages²⁰⁾. We coupled this with microscopic analysis to track oil cell differentiation and multivariate statistical methods to uncover hidden patterns in the complex metabolic data²¹⁾.

Our investigation reveals for the first time how the physical development of oil-producing structures directly influences the accumulation of valuable medicinal compounds in *M. officinalis* buds. The findings provide scientific validation for traditional harvesting practices while offering new insights for quality control in medicinal plant production. More broadly, this work establishes a framework for studying secondary metabolite development in other important medicinal species, bridging the gap between cellular biology and phytochemistry research.

2 Materials and Methods

2.1 Plant material

M. officinalis specimens were collected during March–April 2023 from a natural population in the Tuheba Formation (Taipingcao; 29° 52' 15" N, 109° 12' 43" E; mean annual temperature 16°C, rainfall 1500 mm), Damucun Village, Banqiao Town, Enshi City, Hubei Province, China. Flower organs were morphologically authenticated by Wenguang Wang (Engineer, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences) and confirmed as *M. officinalis*. A voucher specimen (HP20230316) was deposited following standard herbarium protocols in the Chinese Medicine Resource Specimen Museum (HUST-CMRS), College of Pharmacy, Hubei University of Science and Technology. Based on bud length gradients, samples were classified into four developmental stages (Table 1): S1: 4.1–6.0 cm; S2: 6.1–8.0 cm; S3: 8.1–9.0 cm; S4: 9.1–10.0 cm.

2.2 External morphology observation

Five replicates of floral buds per developmental stage were randomly selected, and their total lengths were measured using a digital vernier caliper (accuracy: 0.01 mm) with data recorded in centimeters (cm).

2.3 Tissue clearing treatment

Well-developed and intact flower buds of *M. officinalis* were selected as samples. The specimens were thoroughly rinsed with sterile deionized water to remove surface impurities, ensuring cleanliness. After washing, under aseptic conditions, the flower bud samples were dissected into uniform tissue blocks of 1 cm × 1 cm using a sterilized scalpel. The excised tissue blocks were then transferred into 10 mL sterile centrifuge tubes, with 7 mL of 5% NaOH solution added to each tube for initial clearing at a constant temperature of 25°C. After 4 days, the NaOH solution was replaced with a 2% concentration, and the treatment continued until complete tissue transparency was achieved^{17, 18)}.

Following the clearing process, the tissue blocks were transferred into 3% H₂O₂ solution for bleaching at room temperature for 5 minutes. Subsequently, the samples were rinsed three times with sterile distilled water (5 minutes each) to thoroughly eliminate residual bleaching agents. After treatment, the tissue samples were placed

Table 1 Developmental stages of *M. officinalis* flower buds.

	S1	S2	S3	S4
Length (cm)	4.46 ± 0.32a	7.48 ± 0.41b	10.23 ± 0.58c	12.75 ± 0.67d
Growth rate (cm/day)	—	0.11 ± 0.02a	0.09 ± 0.01b	0.07 ± 0.01c
n	20	20	20	20

Data were analyzed by one-way ANOVA followed by Tukey's test. Different superscript letters within a row indicate significant differences ($p < 0.05$). "—" indicates no data/not applicable.

onto clean glass slides, covered with a drop of deionized water, and sealed with coverslips. Finally, the distribution characteristics of oil cells were observed and recorded using an optical microscope (Olympus BX53) equipped with a digital imaging system under $40\times$ to $100\times$ magnification. To ensure data representativeness, at least five different fields of view were examined for each sample, followed by detailed documentation and analysis.

2.4 Essential oil extraction

The collected *M. officinalis* flower samples were dried in an electric blast drying oven (Model DHG-9070A) at a constant temperature of 60°C for 12 h until a constant weight was achieved. The dried samples were pulverized using a high-speed grinder (Model FW-100) and sieved through a 60-mesh sieve (aperture: $250\text{ }\mu\text{m}$). The resulting powder was stored in a desiccator for further use.

The extraction process was performed following Method A for essential oil determination as specified in the *Chinese Pharmacopoeia*²¹, with optimized experimental parameters. Precisely 5.0 g (accurate to 0.0001 g) of the powdered sample was weighed and transferred into a 1000 mL round-bottom flask. Then, 300 mL of deionized water and several boiling stones ($3\text{--}5\text{ mm}$ in diameter) were added. After thorough shaking and homogenization, the mixture was soaked at room temperature for 6 h. The flask was connected to a Clevenger-type essential oil extractor, and a spherical condenser was installed. Deionized water was added from the top of the condenser until the scale reached level 3. Heating was controlled using an electric heating mantle (Model DF-101S). The solution was initially heated to 200°C until boiling and then adjusted to 100°C to maintain a gentle boil for 3.5 h ^{22, 23}. After stopping the heating, the apparatus was allowed to cool naturally for 30 min. The stopcock of the essential oil extractor was opened to collect the essential oil into a pre-weighed 100 mL beaker. The inner walls of the condenser and the extractor were rinsed three times with dichloromethane (chromatographic grade, 5 mL each time), and the rinsate was combined into the beaker. The mixed solution was transferred to a 125 mL separatory funnel, and 20 mL of dichloromethane was added for extraction. After phase separation, the upper aqueous layer was discarded. The lower organic phase was collected and dehydrated with an appropriate amount of anhydrous sodium sulfate (analytical grade). After standing for 30 min, the solution was filtered through a $0.45\text{ }\mu\text{m}$ organic membrane. The filtrate was transferred to a 40 mL beaker and concentrated to approximately 1 mL via evaporation in a 55°C water bath (Model HH-4). The concentrated solution was quantitatively transferred to a 1.5 mL brown centrifuge tube. The dichloromethane was completely removed by continued water bath heating to obtain purified essential oil.

The essential oil yield (%) was calculated using the fol-

lowing formula:

$$\text{Essential oil yield (\%)} = (\text{Mass of extracted essential oil} / \text{Mass of sample}) \times 100\%$$

Each sample was independently extracted in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was performed using OriginPro 2021 software, with a significance level set at $p < 0.05$. The obtained essential oil samples were sealed with sealing film and stored at 4°C in the dark for subsequent use.

2.5 GC-MS analysis

GC-MS analysis was conducted using an Agilent 7890A gas chromatograph coupled with a 5975C mass spectrometer (Agilent Technologies, USA) equipped with an HP-5ms capillary column ($30\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness; 5% phenyl-95% dimethylpolysiloxane). The HP-5ms column was specifically selected for its superior resolution of *M. officinalis* flower bud essential oil components, and demonstrated excellent separation efficiency for both low-boiling monoterpenes (e.g. α -pinene) and high-boiling sesquiterpenoids (e.g. β -caryophyllene), with particularly improved peak shapes for oxygenated terpenes (e.g. 1,8-cineole)²⁴.

The oven temperature was programmed as follows: initial hold at 50°C for 5 min, ramped to 130°C at $5^{\circ}\text{C}/\text{min}$, then to 170°C at $2^{\circ}\text{C}/\text{min}$, and finally to 280°C at $10^{\circ}\text{C}/\text{min}$ (5 min hold). This optimized gradient achieved baseline separation of both essential oil-associated monoterpenes (boiling point $150\text{--}180^{\circ}\text{C}$) and higher-boiling sesquiterpene alcohols (boiling point $>220^{\circ}\text{C}$). The injector temperature was maintained at 280°C with a split ratio of 5:1. High-purity helium (99.999%) was used as the carrier gas at a constant flow rate of $1.0\text{ mL}/\text{min}$ (column head pressure: 12.051 psi).

Mass spectrometric detection was performed in electron ionization (EI) mode at 70 eV , with the ion source and quadrupole temperatures set at 230°C and 150°C , respectively. Full-scan mass spectra were recorded in the range of m/z $35\text{--}550$ with a solvent delay of 3 min. Samples were prepared in dichloromethane ($800\text{ }\mu\text{g}/\text{mL}$) and $1\text{ }\mu\text{L}$ aliquots were injected in triplicate. Compound identification was achieved by: (1) matching mass spectra against the NIST14 library (similarity $>85\%$), and (2) comparing calculated retention indices (using C7-C40 n-alkanes as references) with literature values (deviation $<3\%$). Relative abundances were determined by peak area normalization, while essential oil yield (%) was calculated as (essential oil mass/sample mass) $\times 100$. Statistical analysis (one-way ANOVA, $p < 0.05$) and data visualization were performed using OriginPro 2022 (OriginLab Corporation, USA).

2.6 Multivariate statistical analysis

Hierarchical cluster analysis (HCA) was performed to evaluate the similarity patterns among samples based on their chromatographic profiles. Using SPSS software, we employed an agglomerative clustering algorithm with inter-group linkage and Pearson correlation as the similarity measure^{17, 25}. The distance matrix was constructed using all compounds identified by GC-MS analysis, and the hierarchical clustering results were visualized as a phylogenetic dendrogram illustrating sample relationships. This approach groups samples with similar chemical compositions while maintaining maximal separation between distinct clusters.

Principal component analysis (PCA) was conducted to reduce data dimensionality and identify patterns in the multivariate dataset^{17, 26}. The analysis involved singular value decomposition of the standardized peak area matrix containing all GC-MS-detected components, performed using SPSS software. The resulting principal components were interpreted through both score plots (showing sample clustering patterns) and loading plots (revealing the contribution of individual compounds to the observed variance). The first two principal components (PC1 and PC2) were selected for visualization as they typically capture the majority of the systematic variance in the data.

3 Results

3.1 External morphology of *M. officinalis* flower buds

The flower bud differentiation of *M. officinalis* commenced in March and was completed by early May, forming fully developed flower buds, which then entered a rapid growth phase until blooming. The mature flower buds exhibited a miniature “fingered citron”-like structure (Buddha’s hand-shaped). During early development, the buds appeared dark brown, gradually deepening to a reddish-brown or dark brown hue as they matured, with densely distributed grayish-yellow verrucous protrusions on the epidermis (Fig. 1).

To quantify the developmental patterns of the flower buds, morphological measurements were conducted on dried buds at different developmental stages (S1–S4). As shown in Table 1, the elongation growth of the flower buds displayed distinct stage-specific characteristics: S1–S2 (Rapid Growth Phase): The bud length increased from 4.46 cm to 7.48 cm, with an average growth rate of 0.11 cm/day, indicating that this phase accounted for the majority of elongation growth. S2–S3 (Moderate Growth Phase): The buds exhibited only a 0.48 cm increase (7.48→7.96 cm), and the average growth rate declined to 0.03 cm/day, suggesting a gradual deceleration in elongation. S3–S4 (Growth Plateau Phase): The bud elongation further slowed, increasing by merely 0.34 cm (7.96→8.30 cm), with an average growth rate dropping to 0.02 cm/day, indicating near stagnation in growth during this stage. These findings demonstrate that the developmental cycle of *M. officinalis* flower buds follows a “fast early, slow late”

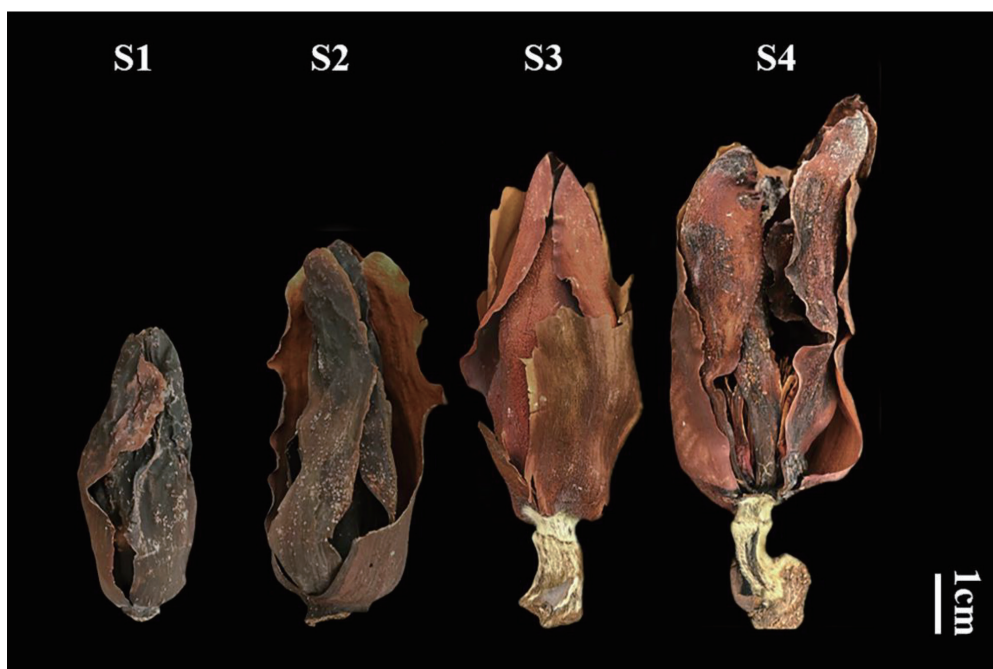


Fig. 1 External morphological characteristics of *M. officinalis* flower buds at different developmental stages. S1–S4: Developmental stages 1–4. Scale bar = 1 cm.

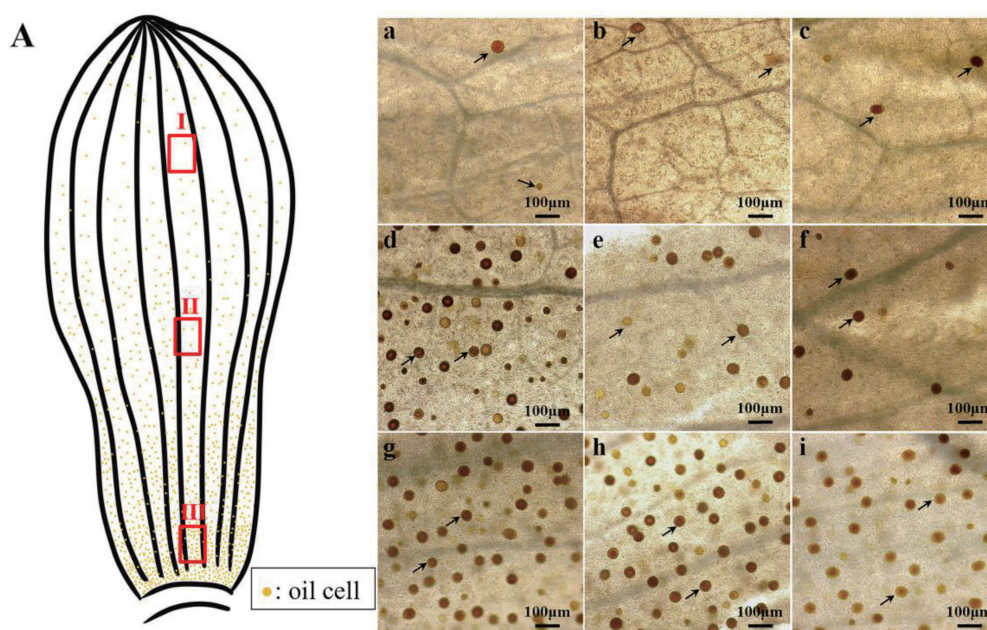


Fig. 2 Distribution of oil cells at different locations in *M. officinalis* flowers.
A: Petal apex (I), mid-region (II), base (III); Petal base. a-c: Petal apex; d-f: Petal mid-region; g-i: Petal base. Bar = 100 μm . Black arrows indicate oil cells.

growth pattern, with 78.43% of total elongation growth concentrated in the S1–S2 phase.

3.2 Distribution characteristics and dynamic changes of oil cells in the perianth of *M. officinalis*

To elucidate the spatial distribution characteristics of oil cells during bud development, this study focused on the perianth tissue, which constitutes the largest proportion of the flower bud. Microscopic observations revealed significant spatial heterogeneity in the distribution density of oil cells across different regions of the same perianth (Fig. 2A): The basal region exhibited the highest density (Fig. 2A-III), reaching $115.29 \pm 3.45 \text{ n/mm}^2$ (Figs. 2g–2i). The middle region showed a moderate density (Fig. 2A-I), with $87.91 \pm 2.64 \text{ n/mm}^2$ (Figs. 2d–2f). The apical region displayed a sharp decline in density (Figs. 2A-II), dropping to $2.88 \pm 0.09 \text{ n/mm}^2$ (Figs. 2a–2c). This gradient distribution suggests a concentration-dependent pattern of oil cell localization.

Through a comprehensive temporal analysis of oil cell density in the perianth base across developmental stages S1–S4 (Figs. 3A–3D), we identified a broad dynamic range spanning from 20.18 to 236.35 n/mm^2 , with a consistent downward trend in overall oil cell density (Fig. 4a). During the initial S1 stage (Fig. 3A), the perianth base exhibited a pronounced enrichment of oil cells, reaching a peak density of $236.35 \pm 7.09 \text{ n/mm}^2$ (Fig. 4a). As the bud development progressed into the S2–S3 stages (Figs. 3B, 3C), a significant expansion in the perianth surface area (Fig. 1) led to a reduction in oil cell density per unit area, declining to

$92.23 \pm 2.77 \text{ n/mm}^2$ and $42.23 \pm 1.27 \text{ n/mm}^2$, respectively (Fig. 4a). By the mature S4 stage (Fig. 3D), the oil cell density reached its nadir at $20.18 \pm 0.61 \text{ n/mm}^2$ (Fig. 4a). The declining oil cell density inversely correlates with perianth expansion.

Using refined tissue clearing techniques for oil cell tracking, we identified five distinct developmental phases (Fig. 3), based on previous frameworks^{13, 14} and essential oil dynamics: Oil-less stage - No observable oil droplets (Fig. 3a); Oil formation stage - Emergence of pale yellow micro-droplets (Figs. 3b, 3c); Oil accumulation stage - Coalescence into golden-yellow droplets (Fig. 3d); Oil saturation stage - Complete lumen filling (Figs. 3e, 3f); Oil degradation stage - Browning and volume reduction (Figs. 3g, 3h).

3.3 Variations in essential oil content of *M. officinalis* flower buds at different developmental stages

In this study, hydrodistillation was employed to extract essential oils from *M. officinalis* flower buds at different developmental stages to investigate their quantitative variation patterns. As illustrated in Fig. 4b, the essential oil content exhibited significant dynamic changes during development, displaying an overall declining trend with an initially rapid and subsequently slower rate of decrease.

At the early developmental S1 stage, the essential oil content reached its peak value of 0.27% (Fig. 4b). As development progressed, the oil content dropped sharply to 0.21% in the S2 stage (Fig. 4b), representing a 22.22% reduction. In the subsequent S3 and S4 stages, the decline in essential oil content continued but at a notably slower rate,

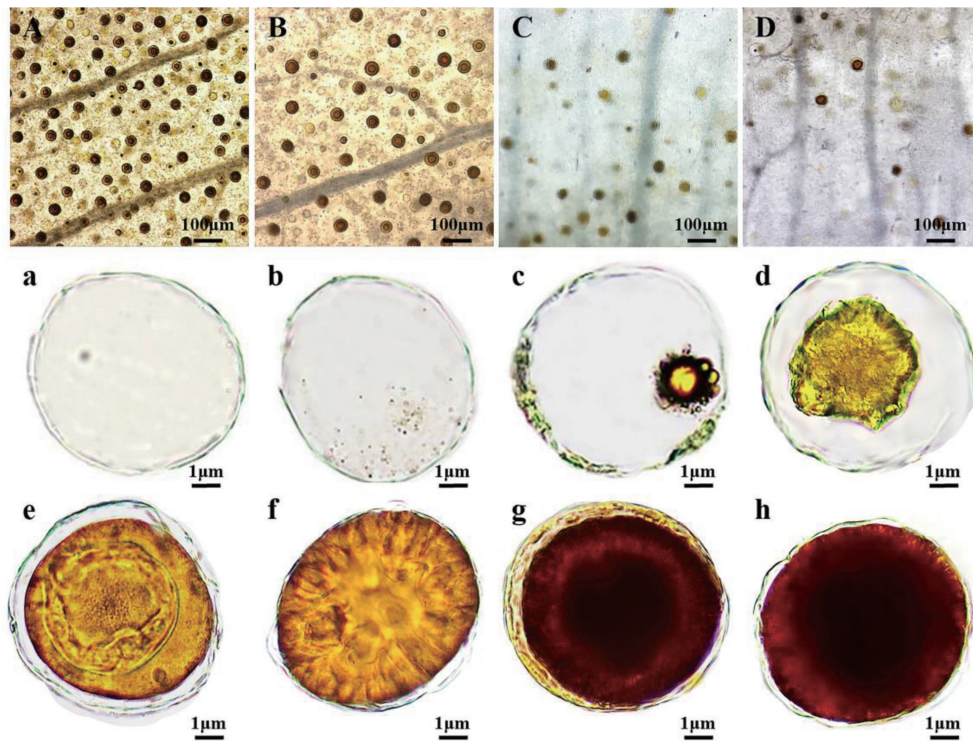


Fig. 3 Developmental morphology of oil cells at the base of the floral perianth. A: Distribution of oil cells at S1 stage; B: Distribution of oil cells at S2 stage; C: Distribution of oil cells at S3 stage; D: Distribution of oil cells at S4 stage. Bar = 100 μm . a: Oil-less stage; b-c: Oil formation stage; d: Oil accumulation stage; e-f: Oil saturation stage; g-h: Oil degradation stage.

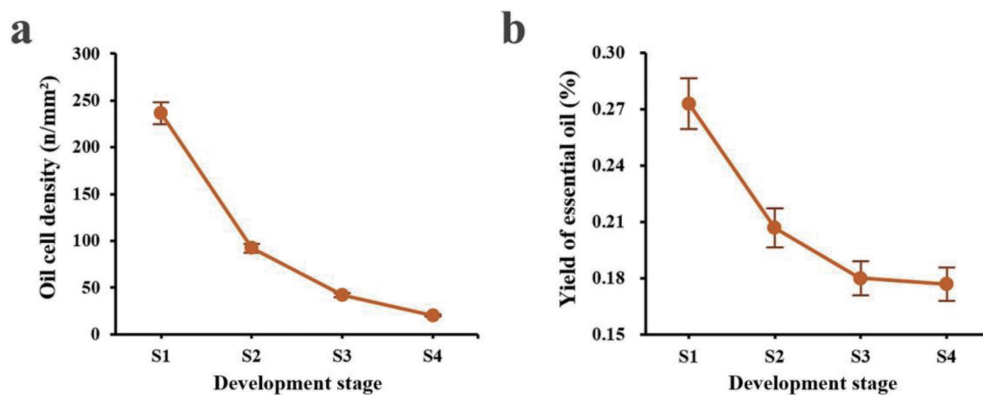


Fig. 4 (a) Changes in oil cell density across different developmental S1-S4; (b) Changes in essential oil content across different developmental S1-S4.

decreasing to 0.18% and 0.17%, respectively (Fig. 4b). Compared to the S2 stage, these reductions corresponded to 14.29% and 5.56%, respectively.

3.4 Proportional analysis of oil cells with differential essential oil accumulation levels

To elucidate the correlation between oil cell developmental stages and essential oil content variations, this study systematically analyzed the proportional changes of oil cells with different essential oil accumulation levels

across developmental phases. The results revealed distinct stage-specific patterns in essential oil dynamics during oil cell development. At the S1 stage, oil cells in the essential oil-saturated phase peaked at 35.37% (Table 2), indicating an active accumulation period. Upon transitioning to the S2 stage, the proportion of saturated-phase oil cells further increased to 42.19%, while those in the formation and accumulation phases significantly decreased to 9.37% and 17.19%, respectively (Table 2), suggesting a decline in biosynthesis and accumulation rates. By the S3 stage, oil cells

Table 2 Proportion of oil cells with varying levels of essential oil accumulation at different developmental stages of *M. officinalis* flower buds.

	S1	S2	S3	S4
Oil-less stage (%)	12.20 ± 0.61a	7.81 ± 0.39b	—	—
Oil formation stage (%)	19.51 ± 0.98a	9.37 ± 0.47b	3.33 ± 0.17c	—
Oil accumulation stage (%)	32.92 ± 1.65a	17.19 ± 0.86b	10.00 ± 0.50c	7.14 ± 0.36d
Oil saturation stage (%)	35.37 ± 1.77a	42.19 ± 2.11b	40.00 ± 2.00c	28.57 ± 1.43d
Oil degradation stage (%)	—	23.44 ± 1.17b	46.67 ± 2.33c	64.29 ± 3.21d

— : not observed; Different superscript letters within the same row indicate significant differences ($p < 0.05$, LSD test). Values represent mean ± SD (n = 5).

entering the essential oil-degradation phase rose markedly to 46.67% (Table 2), signifying the onset of essential oil catabolism and a notable reduction in accumulation activity. At the final S4 stage, degradation-phase oil cells dominated at 64.29% (Table 2), whereas saturated-phase cells dropped to 28.57%. No cells in the formation or accumulation phases were detected, confirming the near-complete cessation of essential oil production. These trends exhibited strong consistency with the overall decline in essential oil content (Fig. 4b), reinforcing the developmental regulation of essential oil metabolism in *M. officinalis* flower buds.

3.5 GC–MS fingerprint analysis of essential oils in *M. officinalis* flower buds at different developmental stages

To elucidate the dynamic changes in chemical composition of essential oils during different developmental stages of *M. officinalis* flowers, we systematically analyzed the essential oil components across four developmental stages (S1–S4) using GC–MS. As shown in Table 3, a total of 26, 25, 26, and 23 compounds were identified in stages S1 to S4, respectively, accounting for 84.70%–88.81% of the total chromatographic peak area (Table 3). Further classification revealed that the essential oils primarily consisted of three major chemical classes: monoterpenes (3.78%–11.95%), sesquiterpenes (68.20%–78.88%), and fatty acid methyl esters (0.34%–3.82%). Among these, sesquiterpenes were the predominant components at all developmental stages, highlighting their crucial role in the essential oil composition of *M. officinalis* flowers. Although the types of essential oil constituents remained similar across developmental stages, their relative abundances varied significantly (Table 3). Five key compounds—caryophyllene (17.31%–30.07%), caryophyllene oxide (18.72%–33.81%), *cis*-calamenene (2.38%–4.60%), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl- (1*Z*,4*Z*,7*Z*)- (2.19%–3.79%), and β -selinene (1.78%–4.19%)—were consistently present at high concentrations. Notably, caryophyllene peaked at 30.07% in stage S1, then gradually declined to its lowest level (17.31%) by S4. Conversely, caryophyl-

lene oxide exhibited an opposite trend, increasing continuously from 18.72% (S1) to a maximum of 33.81% (S4).

3.6 HCA and PCA analyses

To systematically elucidate the inter-group similarities and differences in essential compositions across developmental stages of *M. officinalis* flower buds, hierarchical cluster analysis (HCA) and principal component analysis (PCA) were employed for multivariate analysis of essential components from S1 to S4 stages.

As illustrated in Fig. 5a, HCA revealed a clear segregation between S1 stage and other developmental stages, forming an independent cluster branch, while S2–S4 stages exhibited high chemical similarity and clustered into another distinct group. This clustering pattern demonstrated significant spatiotemporal correlation with both the decreasing gradient of oil cell density (Fig. 4a) and the dynamic changes in essential oil content (Fig. 4b).

PCA results (Fig. 5b) showed that the first three principal components (PC1–PC3) accounted for 94.06%, 5.06%, and 0.76% of variance, respectively, with a cumulative contribution rate reaching 99.88% (information loss rate merely 0.12%), indicating that the three-dimensional principal component space effectively represented the chemical characteristics of the original data. In the spatial distribution plot, S1 stage samples distinctly deviated toward the negative PC1 direction, while S2–S4 stage samples showed overlapping distribution in the positive PC1 direction. Combined with loading factor analysis, the primary characteristic compounds contributing to PC1 axis were identified as caryophyllene (negative loading) and caryophyllene oxide (positive loading). This distribution pattern exhibited strong consistency with the GC–MS quantitative results, where caryophyllene content progressively decreased while its oxide gradually accumulated during development (Table 3).

Table 3 Chemical composition of essential oils in *M. officinalis* flower buds at different developmental stages by GC-MS analysis.

NO.	Compound	Constitutional formula	RI	RT ^A	Relative content ^B (%)				Identification
					S1	S2	S3	S4	
1	Linalool	C ₁₀ H ₁₈ O	1217	11.52	1.67	0.75	1.39	0.39	GC-MS; RT; RI
2	Borneol	C ₁₀ H ₁₈ O	1321	14.21	1.42	0.66	0.64	tr	GC-MS; RT; RI
3	L- α -Terpineol	C ₁₀ H ₁₈ O	1356	15.29	0.43	0.24	0.39	tr	GC-MS; RT; RI
4	(2 <i>E</i>)-1-Methoxy-3,7-dimethylocta-2,6-diene	C ₁₁ H ₂₀ O	1405	16.87	tr	0.33	7.47	3.56	GC-MS; RT; RI
5	Geraniol	C ₁₀ H ₁₈ O	1436	18.11	1.45	tr	tr	tr	GC-MS; RT; RI
6	L-Bornyl acetate	C ₁₂ H ₂₀ O ₂	1468	19.38	2.52	0.82	1.24	0.62	GC-MS; RT; RI
7	α -Cubebene	C ₁₅ H ₂₄	1532	22.84	0.22	tr	0.19	tr	GC-MS; RT; RI
8	Geranyl butyrate	C ₁₄ H ₂₄ O	1552	23.19	1.48	0.98	0.83	0.23	GC-MS; RT; RI
9	Caryophyllene	C ₁₅ H ₂₄	1577	24.41	30.07	22.94	21.46	17.31	GC-MS; RT; RI
10	<i>trans</i> - α -Bergamotene	C ₁₅ H ₂₄	1591	25.09	1.28	0.98	1.06	0.85	GC-MS; RT; RI
11	1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl- (1 <i>Z</i> ,4 <i>Z</i> ,7 <i>Z</i>)-	C ₁₅ H ₂₄	1605	25.80	3.79	2.65	2.39	2.19	GC-MS; RT; RI
12	(<i>E</i>)- β -Farnesene	C ₁₅ H ₂₄	1610	26.06	1.06	0.54	0.42	0.39	GC-MS; RT; RI
13	β -Selinene	C ₁₅ H ₂₄	1633	27.34	4.19	3.00	2.34	1.78	GC-MS; RT; RI
14	α -Selinene	C ₁₅ H ₂₄	1642	27.81	4.10	2.62	1.71	1.39	GC-MS; RT; RI
15	(<i>Z</i> , <i>E</i>)- α -Farnesene	C ₁₅ H ₂₄	1645	27.98	2.24	tr	tr	tr	GC-MS; RT; RI
16	α -Muurolene	C ₁₅ H ₂₄	1647	28.13	0.39	tr	tr	tr	GC-MS; RT; RI
17	Germacrene D	C ₁₅ H ₂₄	1661	28.87	tr	0.55	0.55	tr	GC-MS; RT; RI
18	<i>cis</i> -Calamenene	C ₁₅ H ₂₂	1671	29.42	4.60	2.93	2.64	2.38	GC-MS; RT; RI
19	Caryophyllene oxide	C ₁₅ H ₂₄ O	1739	33.28	18.72	30.36	25.95	33.81	GC-MS; RT; RI
20	Humulene-1,2-epoxide	C ₁₅ H ₂₄	1770	35.06	2.09	3.27	3.01	3.62	GC-MS; RT; RI
21	Cadinol T	C ₁₅ H ₂₆ O	1805	37.14	2.19	2.40	1.87	2.43	GC-MS; RT; RI
22	Copaene	C ₁₅ H ₂₄	1811	37.50	0.40	0.42	tr	tr	GC-MS; RT; RI
23	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol	C ₁₅ H ₂₆ O	tr	37.56	0.24	0.33	0.67	0.54	GC-MS; RT; RI
24	Neointermedeol	C ₁₅ H ₂₆ O	1817	37.84	1.82	1.17	tr	tr	GC-MS; RT; RI
25	α -Cadinol	C ₁₅ H ₂₆ O	1818	37.92	0.93	1.16	1.29	2.12	GC-MS; RT; RI
26	Cadalene	C ₁₅ H ₁₈	1835	39.00	0.43	3.57	2.66	2.21	GC-MS; RT; RI
27	Methyl palmitate	C ₁₇ H ₃₄ O ₂	2027	50.30	tr	tr	0.23	0.95	GC-MS; RT; RI
28	9,12-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	2121	55.89	tr	tr	0.24	1.25	GC-MS; RT; RI
29	Methyl linolenate	C ₁₉ H ₃₂ O ₂	2125	56.11	0.34	1.09	1.23	2.87	GC-MS; RT; RI
30	9-Tricosene, (<i>Z</i>)-	C ₂₃ H ₄₆	2187	59.81	tr	1.86	1.62	1.68	GC-MS; RT; RI
31	Tetracosane	C ₂₄ H ₅₀	2192	60.11	0.74	2.02	2.53	2.15	GC-MS; RT; RI
	Monoterpenes				8.97	3.78	11.95	4.79	
	Sesquiterpenes				78.77	78.88	68.20	71.01	
	Fatty acid methyl esters				0.34	1.09	1.46	3.82	
	Others				0.74	3.88	4.39	5.08	
	Total				88.81	87.65	85.99	84.70	

RI: Retention Index; RT^A: Retention time; ^B: relative abundance of chemical constituents in essential oils across developmental stages (%); GC-MS: Gas chromatography-mass spectrometry; tr: Trace amounts (< 0.20% of total peak area)

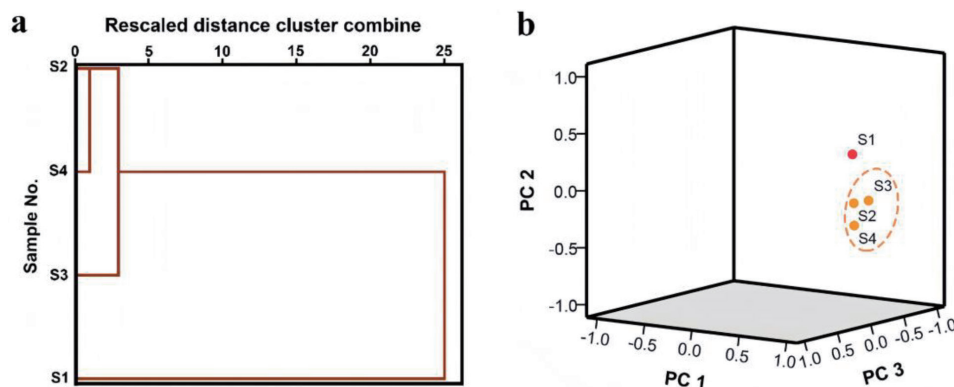


Fig. 5 (a) Dendrogram constructed from GC-MS data of four *M. officinalis* flower samples. Dendrogram based on Pearson correlation ($n=20$); (b) Scatter plot of component scores based on the chemical composition of the samples. PCA scores plot (PC1:94.06%, PC2:5.06%, PC3:0.76%).

4 Discussion

4.1 Regulatory mechanisms of oil cell distribution density and developmental stage on essential oil content

As specialized idioblastic structures, oil cells are the predominant secretory tissues in Magnoliaceae plants and play a crucial role in the biosynthesis and storage of essential oils^{16,17}. Tucker²⁷ systematically investigated 153 Magnoliaceae species across 10 genera and found oil cell distribution in the mesophyll of 88% of the species, indicating their ubiquity in this family. Studies have demonstrated that oil cell density (n/mm^2) exhibits dynamic changes under multi-dimensional regulation, including plant age, seasonal variations, and environmental factors¹⁵. Notably, even within the same species, oil cell distribution density varies significantly across developmental stages. For instance, Li et al.¹⁷ reported that essential oil content in *Cinnamomum cassia* leaves not only positively correlated with oil cell density but also showed a significant coupling relationship with intracellular oil accumulation levels. Similarly, Hu et al.¹⁸ observed that the tepals of *Magnolia zenii* flower buds exhibited higher oil cell density compared to stamens and pistils, accounting for 65%–72% of the total bud weight.

This study focused on analyzing the oil cell distribution density and essential oil accumulation in *M. officinalis* tepals across S1–S4 developmental stages. Our results revealed a significant correlation between oil cell density, developmental progression, and essential oil content. At the initial flowering stage (S1), oil cell density peaked at $236.35 \pm 7.09 \text{ n/mm}^2$ (Fig. 4a), with 35.37% of oil cells in the saturated phase (Table 2), coinciding with the highest essential oil content (0.27%; Fig. 4b). As development advanced to S2, tepal expansion increased their surface area to 1.8 times the initial volume (Fig. 1), leading to a sharp decline in oil cell density ($92.23 \pm 2.77 \text{ n/mm}^2$; Fig. 4a) and a parallel reduction in essential oil content (0.21%; Fig. 4b). By the late blooming phase (S4), tepal area further expanded

to 3.2 times that of S1 (Fig. 1), oil cell density dropped to its lowest level ($42.23 \pm 1.27 \text{ n/mm}^2$; Fig. 4a), and the proportion of degradation-phase oil cells rose to 64.29% (Table 2). Consequently, essential oil content decreased to 0.17% (Fig. 4b). These findings demonstrate a coupling mechanism between dynamic changes in oil cell density and essential oil accumulation patterns. The combined effects of spatial expansion (dilution effect) and metabolic shifts (degradation phase dominance) ultimately drive the decline in essential oil content. This study provides novel experimental evidence for understanding the developmental regulation network of essential oil biosynthesis in Magnoliaceae plants.

4.2 Heterochronic tepal development generates metabolic gradients for essential oil cell patterning

The accumulation and spatial distribution of floral essential oils are regulated by interactions between exogenous environmental factors and endogenous developmental programs. Environmental factors critically modulate this process: climatic conditions such as temperature enhance meristem activity while inhibiting oil cell differentiation at supraoptimal levels²⁸; photoperiod synchronizes developmental timing through phytohormone pathways²⁹; and humidity maintains cellular membrane integrity²⁸. Regarding endogenous developmental programs, Vasconcelos et al.³⁰ reported that in *Eugenia* species, tepals of short-growth-cycle plants displayed significant growth rate gradients within organs, with apical meristem regions growing 2.3 times faster than basal regions. Such heterochronic development likely modulates resource allocation, subsequently affecting the spatial distribution of oil cells³¹.

Our findings reveal that heterochronic growth in tepals occurs not only at the organ level but also forms distinct gradient distribution patterns at the sub-organ scale. Smyth et al.³² demonstrated through live microscopy that growth rates in the basal region of a single tepal were sig-

nificantly lower than in the apical region, a difference negatively correlated with vascular bundle differentiation. Supporting this, Lu et al.³³⁾ provided anatomical evidence that the basal region contains 67% sclerenchyma cells, primarily serving mechanical support and nutrient transport, whereas the apical region is dominated by 82% parenchyma cells, facilitating rapid cell elongation³⁴⁾. This spatial heterogeneity in cell types leads to metabolic compartmentalization: ATPase activity is markedly higher in the apical region³⁵⁾, suggesting a negative correlation between energy metabolism and oil cell distribution.

To elucidate these mechanisms, we employed tissue-clearing techniques to examine *M. officinalis* tepals (Fig. 2). Quantitative analysis revealed that oil cell density was highest in the basal region (115.29 ± 3.45 n/mm²; Figs. 2g-2i), followed by the middle region (87.91 ± 2.64 n/mm²; Figs. 2d-2f), and lowest in the apical region (2.88 ± 0.09 n/mm²; Figs. 2a-2c). This gradient pattern not only corroborates the observations reported by Wang et al.³⁶⁾ in *Rosa* species but also demonstrates its broader applicability.

These results suggest that growth cycles regulate oil cell differentiation by establishing metabolic gradients through heterochronic organ development. Under rapid growth conditions, the apical region's metabolic dominance diverts oil cell precursors toward energy metabolism, whereas the stable microenvironment in the basal region favors lipid body accumulation and maturation. This mechanism provides a novel theoretical framework for understanding the spatial heterogeneity of plant essential oil composition.

4.3 Coupling mechanism between essential oil composition dynamics and ecological functions

Plant essential oils undergo dynamic changes in accumulation and chemical composition during plant development¹⁶⁾. In *M. officinalis* flowers, we identified a coupling mechanism between essential oil metabolism and energy metabolism across four developmental stages (S1-S4). During the rapid growth phase (S1-S2), essential oil content decreased by 22.22%, showing a strong inverse correlation with flower bud expansion (Fig. 4b). This aligns with Criddle et al.'s³⁷⁾ thermodynamic model, where growth-induced respiration increases thermal energy, accelerating both volatilization and oxidative degradation of oil components.

GC-MS analysis revealed stage-dependent metabolic shifts between caryophyllene and caryophyllene oxide (Table 3). While caryophyllene decreased from 30.07% (S1) to 17.31% (S4), its oxide showed the opposite trend, increasing from 18.72% to 33.81%. This transformation mirrors known essential signaling patterns³⁸⁾ and supports the "pollination attraction metabolic reprogramming" hypothesis³⁹⁾, suggesting an active biochemical regulation process.

Our findings reveal that *M. officinalis* employs a devel-

opmental stage-specific metabolic strategy: storage-type terpenes (e.g. caryophyllene) dominate early growth, while maturation favors production of essential oxygenated compounds (e.g. caryophyllene oxide) that enhance pollinator attraction. This provides new evidence for how plants ecologically optimize secondary metabolite production throughout their life cycle. The identified metabolic balancing mechanism offers insights into the evolutionary adaptation of floral essential chemistry.

4.4 Scientific determination and optimization strategy for optimal harvesting period of *M. officinalis* flower buds

Precise determination of harvesting time is crucial for quality control of traditional Chinese medicines, directly affecting their active component content and clinical efficacy²⁾. This study systematically evaluated the optimal harvesting strategy for *M. officinalis* flower buds by integrating chemometric analysis with oil cell developmental dynamics. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) results (Figs. 5a, 5b) demonstrated that S1 stage samples showed significant separation in PC1 (94.06% contribution) and PC2 (5.06% contribution) spaces, exhibiting fundamentally different chemical profiles compared to S2-S4 stages.

GC-MS fingerprint analysis revealed that the S1 stage (bud length 4.1-6.0 cm) reached peak essential oil content (0.27%; Fig. 4b) with 26 characteristic components identified (Table 3). Sesquiterpenes constituted 78.77% of the total components, with major constituents being β -caryophyllene (30.07%), caryophyllene oxide (18.72%), and cis-calamenene (4.60%). These compounds have demonstrated significant pharmacological activities, including antioxidant⁴⁰⁾ and wound-healing properties⁴¹⁾. Notably, S1 stage exhibited the highest oil cell density (236.35 ± 7.09 n/mm²; Fig. 4a) and maximum essential oil content (Fig. 4b), indicating optimal physiological conditions for secondary metabolite accumulation. Comparative analysis showed continuous decline in total essential oils during S2-S4 stages (Fig. 4b), closely associated with reduced oil cell density due to rapid tepal expansion and activation of oxidative pathways.

Field validation studies confirmed substantial increases in material qualification rates alongside improved processing characteristics. The smaller bud size at this developmental stage translates to greater harvesting efficiency and reduced energy requirements for drying. Economic analysis indicates these operational advantages, combined with market premiums for quality, result in improved profitability despite moderately reduced yield per unit area⁴²⁾.

Based on the research findings, we recommend stage S1 (flower bud length 4.1-6.0 cm) as the optimal harvesting period for *M. officinalis* flowers, which demonstrates four key advantages: (1) Superior chemical composition, with both total essential oil and terpenoid content simultane-

ously peaking; (2) Enhanced pharmacological activity, showing significantly improved anti-inflammatory effects of key components compared to other stages; (3) Stable cytological basis, with oil cells at their peak biosynthesis phase; and (4) Improved economic viability due to premium market valuation of quality-certified medicinal material and optimized harvesting/processing efficiency.

5 Conclusion

The flower buds of *M. officinalis* contain abundant essential oils, but their yield shows a continuous decreasing trend during growth and development. Our study demonstrates that both the distribution density of oil cells and the degree of essential oil accumulation in flower buds are key determinants of essential oil content and chemical composition quality (Figs. 4a, 4b). At the S1 developmental stage (bud length: 4.1–6.0 cm), the oil cell density in the tepal base reaches 236.35 ± 7.09 n/mm² (Fig. 4a), while the essential oil content also peaks (Fig. 4b). Based on these findings, we recommend the S1 stage as the optimal harvesting window for *M. officinalis* flower buds, as it ensures maximum essential oil yield and provides a scientific basis for guaranteeing medicinal material quality.

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Authors' Contributions

ZYL and XYW performed observation of oil cells, essential oil extraction, and wrote the first draft of the manuscript. YW, TYG, and WJM performed GC-MS data processing. QQC and SW performed data validation and manuscript revision and provided funding support. MLH proposed the research concept, supervised the manuscript quality, and provided funding support. All authors have read and approved the final manuscript.

Data Availability

Data are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest

All the authors have no conflicts of interest to disclose.

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