Analysis of Differences in Medicinal Component Contents of *Magnolia officinalis* at Different Altitudes and Their Causes

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Abstract [**Objectives**] To analyze the differences in medicinal component contents of *Magnolia officinalis* across different altitude gradients and explore their causes. [**Methods**] In this experiment, *M. officinalis* trees aged 15 - 20 years growing at four altitudes (1 301, 1 444, 1 573, and 1 643 m) were selected as experimental materials. Leaf traits, soil physicochemical properties, and medicinal component contents were investigated, and the relationships among leaf traits, soil physicochemical properties, and medicinal components were analyzed. [**Results**] With increasing altitude, the specific leaf area (SLA) of *M. officinalis* significantly increased, while stomatal density, vein density, leaf thickness, and mesophyll tissue thickness decreased. Soil total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), and organic matter contents (OM) decreased significantly with altitude, whereas available potassium (AK) showed the opposite trend. The contents of medicinal components magnolol and honokiol in *M. officinalis* also significantly decreased with altitude. Correlation analysis revealed that, in addition to altitude, soil physicochemical properties (pH, TP, OM) and leaf traits (leaf thickness, palisade tissue thickness, SLA) were significantly correlated with magnolol and honokiol contents. [**Conclusions**] *M. officinalis* at lower altitudes exhibited better growth and higher magnolol and honokiol contents, which may be attributed to higher soil nutrient availability in low-altitude regions. This study provides guidance for selecting cultivation sites and optimizing planting patterns for *M. officinalis*.

Key words *Magnolia officinalis*, Altitude, Medicinal components, Leaf traits, Soil physicochemical properties

1 Introduction

Magnolia officinalis, a deciduous tree in the Magnoliaceae family, reaches 5 - 15 m in height, with brown bark and stout branchlets that are pale yellow or grayish-yellow. As a renowned traditional Chinese medicine, *M. officinalis* is listed in the *Pharmacopoeia of the People's Republic of China*. Its bark and flower buds are used medicinally: the former eliminates dampness, resolves phlegm, and relieves abdominal distension, while the latter exhibits aromatic damp-resolving and qi-regulating properties[1]. Recent studies have expanded its medicinal use to root bark, seeds, and buds, which share similar therapeutic effects to the bark[2-4]. The efficacy is closely linked to the content and quality of bioactive components, primarily magnolol and honokiol in *M. officinalis*. These components directly determine its medicinal and economic value. For instance, the 2015 edition of the *Chinese Pharmacopoeia* stipulates that the total content of magnolol and honokiol in medicinal *M. officinalis* must not be less than 2.0%.

The quality of medicinal plants depends on the types and concentrations of bioactive components, which are influenced by genetic factors, cultivation practices, and environmental conditions. China boasts abundant medicinal plant resources, yet secondary metabolites vary due to genetic differences among species, resulting in species-dependent characteristics. For example, studies on *Leontopodium nanum* and *Polygonum viviparum* have demonstrated that biomass correlates with altitude[5-6]. Variations in altitude lead to differences in soil properties, light, water, temperature, and oxygen levels, which collectively affect the morphology, physiology, and growth of medicinal plants. *M. officinalis* grows in mountainous regions at 300 - 1 800 m altitude. Existing studies have analyzed its yield and magnolol/honokiol contents across elevations, revealing significant variations in both yield and component concentrations. Notably, magnolol and honokiol contents show a positive correlation with increasing altitude[2,7-8]. Additionally, stand age, silvicultural practices, and fertilization significantly influence its growth and bioactive component accumulation[9-12]. Although studies have documented altitudinal differences in magnolol and honokiol contents—the key bioactive components of *M. officinalis*—the underlying causes remain poorly understood.

This study aims to establish relationships among leaf traits, soil physicochemical properties, and medicinal components of *M. officinalis*; analyze how soil properties affect its bioactive components; and explore the causes of altitudinal differences in medicinal efficacy. The findings will identify optimal growth environments for enhancing quality and productivity, offering critical insights for future cultivation and development.

2 Materials and methods

2.1 Experimental materials The 12 batches of *M. officinalis* samples tested in this experiment were collected in late May 2023 from Bazicun Village, Suojiang Qiang Township, Pingwu

Received: January 13, 2025 Accepted: March 15, 2025

Supported by National Key Research and Development Program of China in 2018 (2018YFC1708005); the Southwest Minzu University Research Startup Funds (RQD2023020).

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County, Mianyang City (Table 1). These samples were identified as *M. officinalis* of the Magnoliaceae family by Assistant Researcher Feng Jingqiu from Southwest Minzu University. Within the same site, three trees with similar DBH (diameter at breast height) were selected at 5-meter intervals. Bark sections approximately 50 cm in length were stripped from 1 m above ground level. The bark from each tree constituted one batch sample, which was air-dried in shade in the laboratory, ground into powder, passed through a No. 3 sieve, and stored for later use. Two mature leaves per tree were collected and transported to the laboratory for leaf trait observation and anatomical experiments. After bark collection, soil samples were collected from three points (0 – 20 cm depth) 1 m away from the main trunk of each selected tree. Surface litter was removed, and the subsamples were homogenized into one composite sample per tree. These soil samples were air-dried in shade for physicochemical property analysis.

Table 1 Sample information of *Magnolia officinalis*

Altitude of collection site//m	Location	Cultivation pattern	Slope aspect	Cultivation duration//year
1 301	104°21′53″ E; 32°9′59″ N	*M. officinalis*-tea forest (managed)	Sunny slope	16 – 17
1 444	104°22′29″ E; 32°9′39″ N	*M. officinalis* pure forest (managed)	Sunny slope	15 – 16
1 573	104°22′29″ E; 32°9′39″ N	*M. officinalis* pure forest (managed)	Shady slope	15 – 16
1 643	104°22′03″ E; 32°9′36″ N	*M. officinalis*-broadleaf forest (mixed forest)	Shady slope	20

2.2 Instruments and reagents DGU-20A5R high-performance liquid chromatography (HPLC) system (Shimadzu Corporation, Japan); KQ-250DE digital ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd., China); ME204E/02 electronic balance (Mettler Toledo Instrument Shanghai Co., Ltd., China); Wonda-Sil C_{18}-WR column (4.6 mm × 150 mm, 5 μm). Magnolol reference substance (RFS-H00411801016) and honokiol reference substance (RFS-H00502204013) were purchased from Chengdu Refines Biological Technology Co., Ltd., China; other reagents included deionized water, FAA fixative solution, and chromatographic-grade methanol.

2.3 Determination of leaf morphology and anatomical structure Specific leaf area (SLA): Six mature leaves per altitude gradient were collected from different plants. After measuring leaf area, leaves were labeled and oven-dried at 65° C to constant weight. SLA was calculated as the ratio of leaf area to leaf dry weight.

Vein density: A small section from the midrib region of *M. officinalis* leaves was prepared as temporary slides. Seven fields of view were imaged under an optical microscope. Vein length and field area were measured using ImageJ software, and vein density was expressed as vein length per unit area.

Stomatal density and stomatal area: Clear nail polish impressions were made on both abaxial and adaxial surfaces of midrib-adjacent leaf regions. After air-drying, the polish films were peeled off, mounted on slides, and observed under an optical microscope. Stomatal density was calculated as stomatal count per unit area. Stomatal length and width were measured using ImageJ software, and stomatal area was derived accordingly. Six replicates were performed for each treatment.

Leaf anatomical thickness, palisade tissue thickness, and cuticle thickness: Leaf sections from the midrib region were fixed in FAA solution, embedded in paraffin, and stained with safranin-fast green for transverse sections. Leaf thickness and palisade tissue dimensions were observed under an optical microscope and quantified using ImageJ software.

2.4 Determination of soil nutrients Soil pH, total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), available potassium (AK), and organic matter (OM) were analyzed by Nanjing Ruiyuan Biotechnology Co., Ltd. using the following methods: potentiometry for pH, Kjeldahl method for TN, NaOH fusion-molybdenum antimony anti-spectrophotometry for TP, NaOH fusion for TK, alkaline hydrolysis diffusion for AN, spectrophotometry for AP, ammonium acetate extraction for AK, and potassium dichromate volumetric method for OM.

2.5 Determination of bioactive components in *M. officinalis*

2.5.1 Chromatographic conditions. The column was packed with octadecylsilane-bonded silica gel. The mobile phase consisted of methanol-water (78 ∶ 22, *v/v*) with a detection wavelength of 294 nm. The theoretical plate number for the magnolol peak was not less than 3 800. A 4 μL aliquot of each reference solution and the test solution was injected into the HPLC system for analysis.

2.5.2 Preparation of reference solutions. Accurately weighed magnolol and honokiol reference substances were dissolved in methanol to prepare solutions containing 40 μg/mL magnolol and 24 μg/mL honokiol, respectively.

2.5.3 Preparation of test solutions. Approximately 0.2 g of powder (passed through a No. 3 sieve) was accurately weighed into a stoppered conical flask, mixed with 25 mL methanol, and soaked for 24 h. The mixture was filtered, and 5 mL of the filtrate was transferred to a 25 mL volumetric flask, diluted with methanol to the mark, and homogenized.

2.6 Data processing One-way ANOVA in SPSS 20.0 (SPSS Inc, Chicago, IL, USA) was used to assess differences in leaf anatomical traits, soil properties, and bioactive component contents across altitudes at $P = 0.05$. Correlation analysis among variables was also performed. Figures were generated using Sigma Plot 10.0 (Systat Software Inc., CA, USA) and Adobe Photoshop 14.0 (Adobe Systems Inc., CA, USA).

3 Results and analysis

3.1 Methodological investigation

3.1.1 Investigation of linear relationship. Magnolol and honokiol were diluted with methanol to prepare a series of mixed reference solutions with gradient concentrations: magnolol (85.40, 21.35,

10.68, 5.34, 2.67, 0.67 μg/mL) and honokiol (80.20, 20.05, 10.03, 5.34, 2.51, 0.63 μg/mL). A 4 μL aliquot of each concentration was injected into the HPLC system under chromatographic conditions. Peak areas were recorded, and calibration curves were plotted with concentration (X) as the abscissa and peak area (Y) as the ordinate. Regression equations were established (Table 2). The results showed that magnolol had good linearity in the range of 0.67 – 85.40 μg/mL, and honokiol had good linearity in the range of 0.63 – 80.20 μg/mL.

Table 2 Regression equation and linear range

Compound	Regression equation	r	Linear range // μg/mL
Magnolol	$Y = 7.266 \times 10^3 X - 8.339 \times 10^3$	0.999 3	0.67 – 85.40
Honokiol	$Y = 8.000 \times 10^7 X - 8.946 \times 10^3$	0.999 3	0.63 – 80.20

3.1.2 Precision test. The test solution was injected six times (4 μL each) under the chromatographic conditions specified in Section **2.5.1** to measure peak area of magnolol and honokiol. The relative standard deviations (*RSD*s) of peak area were 0.15% for magnolol and 0.10% for honokiol ($n = 6$), indicating excellent instrument precision.

3.1.3 Stability test. The test solution (Batch: 13-1) was analyzed at 0, 2, 4, 6, 8, 12, and 24 h according to the chromatographic conditions in Section **2.5.1** to record peak area. The results showed that the *RSD* of peak areas was 0.12% for magnolol and 0.17% for honokiol ($n = 7$), confirming solution stability within 24 h.

3.1.4 Repeatability test. Six portions of the same *M. officinalis* sample (Batch: 16-2) were precisely pipetted and prepared into test solutions according to the method described in Section **2.5.3**. The samples were then injected and determined under the chromatographic conditions described in Section **2.5.1**, and the peak area was recorded to calculate the contents of magnolol and honokiol. The results showed that the *RSD*s of content determination were 1.63% for magnolol and 1.58% for honokiol ($n = 6$), demonstrating good method repeatability.

3.1.5 Recovery rate. 0.1 g of *M. officinalis* sample from the same batch (Batch No.: 16-2) was accurately weighed, and a specific amount of reference standard was added in an approximate 1 : 1 ratio (sample content to reference standard). The test solution was prepared according to the method outlined in Section **2.5.3**, and six parallel preparations were made. The samples were injected and analyzed under the chromatographic conditions specified in Section **2.5.1**. The recovery rate of the test samples was calculated. The average recovery of magnolol was 106.71% (*RSD* = 3.12%), and the average recovery of honokiol was 106.96% (*RSD* = 1.10%). The results indicated that the method demonstrated satisfactory accuracy (Table 3).

Table 3 Recovery rates of magnolol and honokiol

Compound	Sample weight // g	Background content // mg	Addition // mg	Measured content // mg	Recovery rate // %	Average recovery rate // %	*RSD* // %
Magnolol	0.100 1	0.870 9	0.870 4	1.794 9	106.15	106.71	3.12
	0.100 0	0.870 0	0.870 4	1.782 5	104.84		
	0.100 8	0.877 0	0.870 4	1.784 0	104.20		
	0.100 6	0.875 2	0.870 4	1.847 3	111.68		
	0.100 1	0.870 9	0.870 4	1.827 5	109.90		
	0.100 5	0.874 4	0.870 4	1.775 1	103.48		
Honokiol	0.100 1	1.011 0	1.010 0	2.092 5	107.08	106.96	1.10
	0.100 0	1.010 0	1.010 0	2.089 2	106.85		
	0.100 8	1.018 1	1.010 0	2.085 5	105.68		
	0.100 6	1.016 1	1.010 0	2.161 6	108.61		
	0.100 1	1.011 0	1.010 0	2.172 3	107.87		
	0.100 5	1.015 1	1.010 0	2.082 1	105.65		

3.2 Leaf structural traits across altitudes Leaf structural traits varied significantly across altitudes (Fig. 1 and Table 4). For *M. officinalis* at 1 301 m altitude, the stomatal density was recorded as (595.51 ± 51.55) counts/mm^2, vein density as (1.23 ± 0.04) mm/mm^2, leaf thickness as (192.98 ± 21.64) μm, palisade tissue thickness as (95.66 ± 8.88) μm, and specific leaf area (SLA) as (179.30 ± 14.75) μm^2/g. For *M. officinalis* at 1 444 m altitude, the stomatal density was measured at (546.06 ± 47.27) counts/mm^2, vein density at (1.27 ± 0.10) mm/mm^2, leaf thickness at (168.33 ± 11.49) μm, palisade tissue thickness at (81.34 ± 5.45) μm, and SLA at (239.29 ± 16.47) μm^2/g. At 1 573 m altitude, the stomatal density of *M. officinalis* was found to be (352.94 ± 34.15) counts/mm^2, vein density (1.13 ± 0.04) mm/mm^2, leaf thickness (46.50 ± 13.42) μm, palisade tissue thickness (76.34 ± 4.89) μm, and SLA (302.49 ± 14.24) μm^2/g. For specimens at 1 643 m altitude, the stomatal density

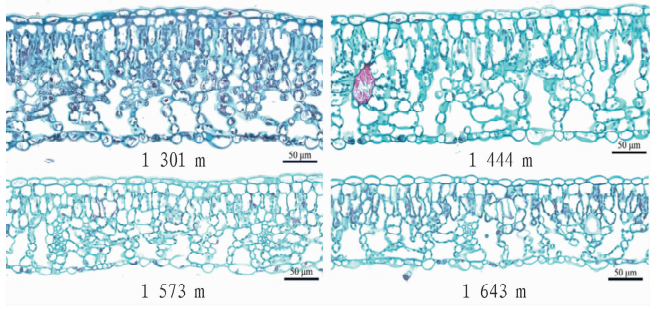


Fig. 1 Anatomical characteristics of *Magnolia officinalis* leaves across altitudes

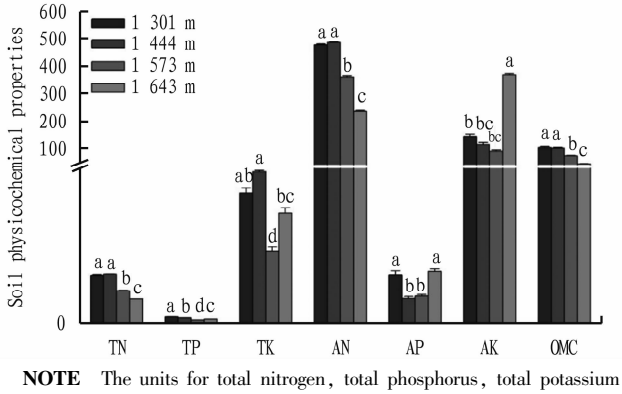
was determined as (466.12 ± 40.35) counts/mm^2, vein density as (1.13 ± 0.05) mm/mm^2, leaf thickness as (143.88 ± 4.88) μm, palisade tissue thickness as (72.54 ± 2.97) μm, and SLA as (270.47 ± 7.82) μm^2/g. Overall, a decreasing trend with increasing altitude was observed in stomatal density, vein density, leaf thickness, and palisade tissue thickness, while an increasing trend was noted in specific leaf area.

Table 4 Leaf trait characteristics of *Magnolia officinalis* across altitudes

Altitude//m	Stomatal density mm^2	Vein density mm/mm^2	Leaf thickness μm	Palisade tissue thickness//μm	Specific leaf area μm^2/g
1 301	595.51 ± 51.55^a	1.23 ± 0.04^a	192.98 ± 21.64^a	95.66 ± 8.88^a	179.30 ± 14.75^c
1 444	546.06 ± 47.27^{ab}	1.27 ± 0.10^a	168.33 ± 11.49^a	81.34 ± 5.45^a	239.29 ± 16.47^b
1 573	352.94 ± 34.15^b	1.13 ± 0.04^a	146.50 ± 13.42^a	76.34 ± 4.89^a	302.49 ± 14.24^a
1 643	466.12 ± 40.35^{ab}	1.13 ± 0.05^a	143.88 ± 4.84^a	72.54 ± 2.97^a	270.47 ± 7.82^{ab}
F	4.493	0.990	2.597	2.902	13.603
P	0.040	0.424	0.088	0.067	0.000

NOTE Different lowercase letters within the same column indicate significant differences ($P < 0.05$).

3.3 Soil physicochemical properties across altitudes Soil pH across four altitudes ranged from 5.37 to 6.33, with no significant differences. Other soil properties showed marked variations among altitudes (Fig. 2). Total nitrogen (TN) content was highest at 1 444 m and 1 301 m (significantly higher than at 1 573 m), and lowest at 1 643 m. Total phosphorus (TP) content decreased with increasing altitude. Total potassium (TK) content peaked at 1 444 m, was lowest at 1 573 m, and was higher at 1 301 m than at 1 643 m. Available nitrogen (AN) generally decreased with increasing altitude. Available phosphorus (AP) was highest at 1 643 m, lowest at 1 444 m, and higher at 1 301 m than at 1 573 m. Available potassium (AK) followed the order: 1 643 m > 1 301 m > 1 444 m > 1 573 m. The content of organic matter decreased with the increase of altitude.



NOTE The units for total nitrogen, total phosphorus, total potassium, and organic matter are g/kg; the units for available nitrogen, available phosphorus, and available potassium are mg/kg.

Fig. 2 Variations in soil physicochemical properties across altitudes

3.4 Bioactive component contents across altitudes A total of 24 batches of medicinal material were analyzed in this study. The content of magnolol was measured to range from 0.77% to 4.76%, with a mean value of 2.03%, while the content of honokiol ranged from 0.18% to 2.83%, with a mean of 0.99%. Among the pharmacologically active components of *M. officinalis* across different altitude gradients, the magnolol content was consistently higher than that of honokiol. As shown in Fig. 3, the contents of magnolol and honokiol were the highest at an altitude of 1 301 m, and the contents of magnolol and honokiol were the lowest at an altitude of 1 643 m. The magnolol and honokiol contents at 1 301 m altitude were both higher than those at 1 444 m altitude. However, the magnolol content at 1 444 m was lower than that at 1 573 m altitude, while the honokiol content at 1 444 m exceeded that at 1 573 m altitude. The magnolol content at 1 573 m altitude was higher than those at both 1 444 and 1 643 m altitudes, whereas the honokiol content was lower compared to specimens at 1 444 and 1 643 m altitudes. Overall, bioactive compound contents in *M. officinalis* decreased with increasing altitude.

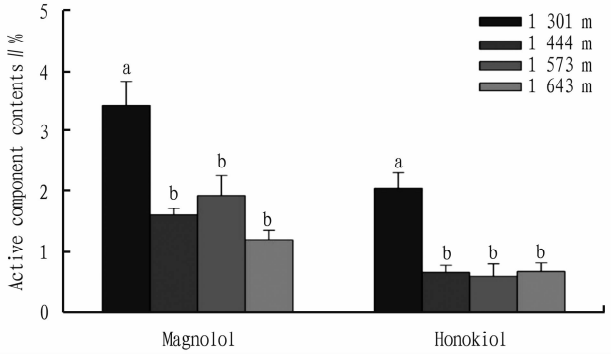


Fig. 3 Variations in pharmacologically active compound content of *Magnolia officinalis* across altitudes

3.5 Correlation analysis Significant correlations were observed among leaf morphology/anatomy, soil properties, and bioactive components (Table 5). Altitude showed significant negative correlations with TN, TP, AN, OM, and SLA ($P < 0.01$), and with magnolol, honokiol, leaf thickness, and palisade tissue thickness ($P < 0.05$). pH was positively correlated with magnolol, honokiol, and leaf thickness ($P < 0.05$). TN was positively correlated with TP, AN, and OM ($P < 0.01$); positively with TK ($P < 0.05$); negatively with AK ($P < 0.05$); and negatively with SLA ($P < 0.05$). TP was positively correlated with TK and stomatal density ($P < 0.01$); with AN, honokiol, OM, leaf thickness, and palisade tissue ($P < 0.05$); and negatively with SLA ($P < 0.01$). TK was positively correlated with stomatal density ($P < 0.01$) and negatively with SLA ($P < 0.05$). AN was negatively correlated with AK ($P < 0.01$) and positively with OM ($P < 0.01$). AP was

positively correlated with AK ($P<0.05$). AK was negatively correlated with OM ($P<0.01$). OM was positively correlated with magnolol, leaf thickness, and palisade tissue ($P<0.05$). Magnolol was positively correlated with honokiol and palisade tissue ($P<0.01$), and with leaf thickness ($P<0.05$). Honokiol was positively correlated with leaf thickness and palisade tissue ($P<0.05$), and negatively with SLA ($P<0.05$). Stomatal density was negatively correlated with SLA ($P<0.05$). Leaf thickness was positively correlated with palisade tissue thickness ($P<0.01$).

Table 5 Correlations among morphological traits, soil nutrients, and pharmacologically active compound contents of *Magnolia officinalis*

Traits	Altitude m	pH	Total nitrogen (TN) g/kg	Total phosphorus (TP) g/kg	Total potassium (TK) g/kg	Available nitrogen (AN) mg/kg	Available phosphorus (AP) mg/kg	Available potassium (AK) mg/kg	Organic matter (OM) mg/kg	Magnolol	Honokiol	Stomatal density counts/mm^2	Vein density mm/mm^2	Leaf thickness μm	Palisade tissue thickness μm	Specific leaf area (SLA) cm^2/g
Altitude//m	1.00	−0.52	−0.90 **	−0.87 **	−0.57	−0.88 **	−0.01	0.54	−0.90 **	−0.70 *	−0.69 *	−0.57	−0.34	−0.66 *	−0.67 *	0.78 **
pH	0.08	1.00	0.36	0.42	0.28	0.37	0.23	−0.20	0.46	0.58 *	0.68 *	0.35	−0.37	0.64 *	0.56	−0.52
Total nitrogen (TN)//g/kg	0	0.24	1.00	0.79 **	0.68 *	0.98 **	−0.34	−0.68 *	0.97 **	0.47	0.41	0.49	0.50	0.54	0.52	−0.60 *
Total phosphorus (TP)//g/kg	0	0.18	0.00	1.00	0.80 **	0.69 *	0.25	−0.13	0.70 *	0.52	0.59 *	0.75 **	0.42	0.63 *	0.60 *	−0.84 **
Total potassium (TK)//g/kg	0.05	0.38	0.02	0.00	1.00	0.55	0.00	0.00	0.53	0.01	0.24	0.76 **	0.44	0.34	0.22	−0.67 *
Available nitrogen (AN)//mg/kg	0	0.24	0	0.01	0.06	1.00	−0.43	−0.79 **	0.99 **	0.49	0.38	0.38	0.45	0.51	0.51	−0.50
Available phosphorus (AP)//mg/kg	0.96	0.48	0.28	0.44	0.99	0.17	1.00	0.72 **	−0.37	0.24	0.39	0.18	−0.17	0.17	0.14	−0.33
Available potassium (AK)//mg/kg	0.07	0.54	0.02	0.68	0.99	0.00	0.01	1.00	−0.78 **	−0.38	−0.16	0.10	−0.19	−0.26	−0.32	0.038
Organic matter (OM)//mg/kg	0	0.13	0	0.01	0.08	0	0.24	0.00	1.00	0.58 *	0.48	0.39	0.38	0.60 *	0.60 *	−0.55
Magnolol	0.01	0.05	0.12	0.08	0.97	0.10	0.45	0.22	0.05	1.00	0.78 **	0.27	0.01	0.66 *	0.74 **	−0.55
Honokiol	0.01	0.02	0.19	0.04	0.46	0.22	0.21	0.62	0.12	0.00	1.00	0.52	−0.20	0.71 **	0.74 **	−0.74 **
Stomatal density//counts/mm^2	0.05	0.26	0.10	0.00	0.00	0.23	0.57	0.77	0.21	0.40	0.08	1.00	0.05	0.41	0.41	−0.90 **
Vein density//mm/mm^2	0.29	0.24	0.10	0.17	0.15	0.14	0.60	0.55	0.23	0.97	0.52	0.88	1.00	−0.12	−0.18	−0.09
Leaf thickness//μm	0.02	0.02	0.07	0.03	0.27	0.09	0.60	0.41	0.04	0.02	0.01	0.18	0.71	1.00	0.95 **	−0.52
Palisade tissue thickness//μm	0.02	0.06	0.08	0.04	0.50	0.09	0.67	0.31	0.04	0.00	0.01	0.18	0.56	0	1.00	−0.54
Specific leaf area (SLA)//cm^2/g	0.00	0.08	0.04	0.00	0.02	0.09	0.30	0.91	0.06	0.06	0.01	0	0.79	0.09	0.07	1.00

NOTE *, $P<0.05$; **, $P<0.01$.

4 Discussion

Altitude directly determines climatic factors. With increasing altitude, parameters such as mean temperature, precipitation, oxygen partial pressure, and UV intensity exhibit predictable patterns[13]. Climatically, temperature decreases with rising altitude[14], while relative humidity increases. As far as plant growth is concerned, with the change of altitude, the light intensity and sunshine time received by plants at different altitudes are different. Thus, altitudinal gradients serve as natural laboratories to study plant environmental adaptation[15]. This study analyzed leaf morphology/anatomy, bioactive components, and soil nutrients of *M. officinalis* across altitudes.

4.1 Effect of altitude on the growth of *M. officinalis* Stomatal density, vein density, leaf thickness, and palisade tissue thickness decreased with altitude (Table 4), contrasting findings from Li *et al.* on 66 grassland species in the Tibetan Plateau[16]. It is generally recognized that plant functional traits, such as leaf thickness and vein density, tend to increase with rising altitude. This is attributed to the fact that thicker leaves and palisade tissues facilitate heat retention in plant leaves, thereby preventing frost damage[17]. Thicker leaves, palisade tissues, and dense veins have been demonstrated to effectively mitigate UV radiation damage, enhance water-use efficiency, promote photosynthetic efficiency, and strengthen stress resistance[13,18]. These findings suggest that the growth of *M. officinalis* in the study area is minimally affected by local ambient temperature, possibly due to insignificant temperature variations across the altitude gradient. Additionally, a significant increasing trend in SLA was observed with rising altitude, which contradicts previous research conclusions[13]. SLA is closely associated with plant photosynthesis; under low-light conditions, higher SLA allows plants to expand leaf area for capturing more light energy, thereby adapting to low-light environments[19]. *M. officinalis* is a tree species, and its growth in native habitats across different altitudes is not hindered by shading from other tree species, with sufficient sunlight availability. In summary, the results indicate that *M. officinalis* in low-altitude regions exhibits higher stomatal density and vein density, coupled with lower SLA, which promotes the accumulation of dry matter and consequently influences its growth.

4.2 Effect of altitude on soil physicochemical properties Studies have revealed that soil nutrient content and chemical properties vary across different vegetation types with increasing altitude, and these variations exhibit correlations with altitude[20]. In this study, significant variations in soil physicochemical properties were identified across altitudes, with soil pH ranging from 5.37 to 6.33, indicating acidic soil conditions in the study area. Total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), and organic matter (OM) content gener-

ally displayed declining trends with increasing altitude. This aligns with findings from Chen *et al.* [21] on soil physicochemical changes in *Forsythia suspensa* root zones across altitudes. The content of available phosphorus (AP) exhibited a U-shaped pattern with altitude, which may be linked to management practices and planting patterns of *M. officinalis* forests at different altitudes. At lower altitudes, artificial fertilization in managed tea plantations likely enhanced soil phosphorus content, while abundant litter diversity at higher altitudes contributed to elevated phosphorus levels. The higher available potassium (AK) content observed in high-altitude regions may be attributed to the diverse litter composition in these areas. As altitude increases, ambient temperature typically decreases, leading to colder climatic conditions[22], which alters soil temperature and microbial activity, thereby impacting soil biological processes and nutrient cycling[23-24]. The results suggest that intercropping tea plants under *M. officinalis* forests provides sufficient rhizospheric soil nutrients, creating favorable conditions for the growth and accumulation of pharmacologically active compounds in *M. officinalis*.

4.3 Effect of altitude on bioactive components of *M. officinalis* In this study, a significant decrease in the contents of both magnolol and honokiol was observed with increasing altitude. This finding contrasts with previous reports suggesting a positive correlation between altitude and the content of pharmacologically active compounds in *M. officinalis*. For instance, earlier studies indicated that the contents of magnolol and honokiol increased with rising altitude[2]. Other studies reported a positive correlation between altitude and magnolol content, whereas honokiol content showed a negative correlation with altitude[25]. Variations in the content of bioactive compounds among medicinal plants across altitudes are attributed to species-specific environmental adaptations, which also serve as key drivers in the formation of region-specific authentic medicinal materials[26]. At higher altitudes, plant growth rates are reduced due to environmental constraints, leading to slower growth of *M. officinalis*. This slower growth may enhance the accumulation of magnolol. *M. officinalis* is vertically distributed between 300 and 1 800 m altitude, where its growth is influenced by temperature, air quality, light availability, and other factors. The most vigorous growth was reported at 1 200 m altitude[7], though exceptional growth in some regions at 2 000 m altitude has been documented due to unique geographical conditions. These results suggest that the declining trend in magnolol and honokiol contents with altitude may be linked to regional thermal gradients. Furthermore, factors affecting the bioactive compounds in *M. officinalis* exhibit significant regional variability, such as soil properties and climatic conditions. In this study, low-altitude areas were characterized by agroforestry systems combining *M. officinalis* and tea plants, where tea plants do not directly shade *M. officinalis*, but human interventions in tea plantation management (*e. g.*, fertilization) significantly altered the rhizospheric soil nutrient profile of *M. officinalis*. For example, fertilization practices enhanced nutrient availability in the root zone of *M. officinalis*. In conclusion, altitude plays a critical role in determining the content of bioactive compounds in *M. officinalis*. These findings provide insights for identifying optimal habitats that promote both growth and medicinal compound accumulation, which holds significant implications for improving medicinal quality (*e. g.*, understory agroforestry systems) and sustainable resource utilization.

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