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Multi-Indicator Comprehensive Quality Evaluation of *Turpinia arguta* (Lindl.) Seem Herbs at Different Harvesting Periods

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Abstract: This study aimed to investigate differences in the leaf biomasses, chemical compositions, and pharmacological activities of *Turpinia arguta* histocultured seedlings and live seedlings at different harvesting periods (S1, S2, S3, and S4) in order to determine the optimal harvesting period. Eleven indexes, including biomass, key active components, secondary metabolites, nutrient content, and antioxidant activity, were evaluated by high-performance liquid chromatography and colorimetric methods during different harvesting periods. The weights of the 11 indexes were calculated by principal component analysis, and then a comprehensive quality evaluation was performed. The results showed significant differences in leaf biomasses, key active components, secondary metabolites, nutrient contents, and antioxidant activities between the different harvesting periods. The highest quality score was obtained for the S4 period, indicating that the quality of *T. arguta* was the best at this time and that the S4 period was the most suitable harvesting period. At this period (S4), the medicinal component content, antioxidant activity, and comprehensive quality score of the histocultured seedlings of *T. arguta* were higher than those of the live seedlings, indicating the importance of tissue culture technology in enhancing the quality of *T. arguta*. This study provides more novel and abundant information and reference for determining the appropriate harvesting period of *T. arguta*, with the aim of providing newer scientific guidance for the management of herbs.

Keywords: herb quality evaluation; harvesting period; *Turpinia arguta*

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1. Introduction

Turpinia arguta, belonging to the family Staphyleaceae [1], is an evergreen shrub native to the Jiangxi, Hunan, and Fujian Provinces. The dried leaves, known as *Turpiniae folium*, are utilized for medicinal purposes [2]. This herb, originally used in traditional remedies in Anyuan County, Jiangxi Province, lacks historical documentation, but is locally referred to as "moth medicine" based on its application [3]. Characterized by a bitter taste and cold nature, *T. arguta* offers benefits such as heat clearance, detoxification, antibacterial and anti-inflammatory effects, blood circulation enhancement, bruise dissipation, pharyngeal swelling reduction, cough suppression, and phlegm expectoration [4], and it is traditionally used to treat pharyngolaryngitis, tonsillitis, upper respiratory tract infections, and tumors [5]. Modern proprietary Chinese medicines, such as Shanxiangyuan tablets [6], granules, and lozenges derived from *T. arguta*, have been introduced. Previous studies have identified various chemical constituents, including volatile compounds, flavonoids, megaterpenoids, polyphenols, and alkaloids, in *Turpiniae folium* [4,7], which exhibits pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, analgesic, and immune-enhancing effects [8,9].

Active ingredients are substances with pharmacological activities that are the material basis for traditional Chinese medicine to exert disease prevention and treatment effects.

Flavonoid components are the most widely studied and pharmacologically active components of *T. arguta* [10,11], especially ligustroflavone and rhoifolin, which were referenced as the key active components in the 2020 edition of the Chinese Pharmacopoeia for quality control of this herb [2]. However, Chunrong et al. emphasized that it is difficult to fully reflect the quality of *T. arguta* herbs by using only its contents of chasteberry glycosides and wild laccinosides as indicators [4]. The secondary metabolites of medicinal plants such as polyphenols, flavonoids, and alkaloids, have a variety of biological activities, which are important material bases for herbs to exert medicinal effects and are key indexes for evaluating the quality of herbs [12]. Soluble proteins (SP), soluble sugars (SS), and soluble starches (SST) are pivotal for plant growth, yield optimization, and quality enhancement. These key nutrients not only influence the plant's own development but also directly impact the quality and the nutritional value of agricultural products [13], which provides a scientific basis for using the nutritional value to evaluate the health benefits of herbs. In terms of pharmacological activity, Xin et al. [14] showed that *T. arguta* has powerful antioxidant activity, which provides reliable experimental support for using *T. arguta* as a natural antioxidant. The antioxidant activity of *T. arguta* was examined by DPPH radical scavenging and hydroxyl radical scavenging assays, which can better reflect the quality of medicinal plants, ensure their safety and effectiveness in clinical applications, and provide scientific basis for their applications in the field of medicine.

The quality of Chinese herbal medicines is closely related to the harvesting process, and timely harvesting is an important prerequisite for quality standardization in the Chinese medicine industry [15]. Timely harvesting is crucial to the production of herbs, and a reasonable harvesting period not only ensures the yield and time cost of the herbs but also stabilizes the quality of the herbs. The Chinese Pharmacopoeia indicates that *T. arguta* should be harvested in the summer and fall when the leaves are in full bloom, but the summer and fall seasons span half a year, which does not provide effective guidance for the harvesting period. Tao Xiuhua and other researchers [15] have solved the problem of the artificial cultivation of *T. arguta* and established a rapid propagation system of histoculture by using stem segments of a good single plant (one-year live seedling) as the explant. Because the "cultured herbs" obtained from artificial culture (not wild or cultivated) may show variations during the tissue culture process, it is necessary to evaluate their quality in comparison with that of traditional field-grown herbs [16] to ensure the effectiveness of their market application. In this context, the aim of this study was to systematically analyze cultured herbs and annual traditional herbs at different harvesting periods from various aspects (biomass, nutrients, secondary metabolites, key active ingredients, antioxidant activity) and to conduct a comprehensive evaluation using principal component analysis (PCA) to compare the quality of *T. arguta* herbs at different harvesting periods and to clarify the optimal harvesting period of *T. arguta* herbs, which would be helpful for a comprehensive scientific assessment of the quality of *T. arguta* herbs. This will contribute to a comprehensive scientific understanding of the quality of *T. arguta*, providing a reference for the quality control of this plant.

2. Materials and Methods

2.1. Plant Material, Reagents and Equipment

The experimental materials were obtained from the greenhouse and herbal medicine nursery of the Jiangxi Academy of Forestry (Figure 1). The *T. arguta* tissue-culture seedlings (cultured herbs) for testing were planted in the greenhouse on 1 September 2023, with one harvesting period every three months and a total of four harvesting periods, namely, S1 (1 September 2023), S2 (1 December 2023), S3 (1 February 2024), and S4 (1 May 2024). At each harvesting period, 20 plants with good growth conditions were randomly selected for sampling in three replications, and the above steps were repeated three times within each harvesting period. On 1 June 2024, 20 well-grown, one-year live *T. arguta* plants (traditional herb) were randomly selected from the herb nursery for the CK experiment (replicated three times). After the samples were collected, they were quickly placed in a liquid nitrogen holding

tank for temporary storage and subsequently transferred to a −80 °C refrigerator for long-term freezing and preservation. To ensure the reliability of the experimental data and the consistency of the results, we implemented strict horticultural management measures for all the plants involved in the experiment. These measures were designed to minimize the effects of environmental factors such as soil, water, and temperature on plant growth. The identification of all samples was carried out by Senior Engineer Leung Yue Lung, an expert in plant taxonomy, and all of them were confirmed to be derived from this species.

Figure 1. Different harvesting periods of *Turpinia arguta* tissue-culture seedlings and one-year-old *T. arguta* live seedlings.

The main drugs and reagents were as follows: Standard ligustroflavone, rhoifolin, gallic acid, quercetin (HPLC grade, purity ≥ 98%) were purchased from the Yuan Ye Company (Shanghai, China). Matographically pure MeOH (purity ≥ 99.9%) was purchased from the Merck Company (Rahway, NJ, USA). The DPPH free radical and hydroxyl radical kit and the soluble protein kit were purchased from Keming Company (Suzhou, China). Folin–Ciocalteu reagent was purchased from the McLean Company (Shanghai, China), and the rest of the reagents were analytically pure.

The main equipment was as follows: HPLC (SIL-20A, Shimadzu, Japan), UV spectrophotometer (759S, Shanghai Prism Technology Company, Shanghai, China), and thermostat (HWS-080, Shanghai Jinghong Company, Shanghai, China).

2.2. Measurement of Leaf Biomass

The leaves from the above different harvesting periods were taken in paper bags and dried at 85 °C until achieving a constant weight; the weight was recorded, and the dry weight/g of the leaves at this point was taken as the biomass of the leaves.

2.3. Nutrient Determination

2.3.1. Determination of Soluble Proteins Content

To determine the SP content, we employed a method adapted from Bradford et al. [17]. A sample weighing 0.3 g was ground using a 60-mesh sieve and placed into a 20 mL centrifuge tube. We then added 15 mL of distilled water and subjected the mixture to 30 min of ultrasonication using an ultrasonic cleaner. After ultrasonication, the homogenate was centrifuged at 4000 rpm for 10 min. The supernatant was collected into a 50 mL volumetric flask, while the residue underwent two additional extractions using the same procedure. Finally, the supernatants were combined and the volume adjusted to 50 mL in the flask. A standard curve was plotted for the different gradients using bovine serum albumin standards with the Coomassie Blue G-250 staining method, and the SP content of the samples was calculated.

2.3.2. Determination of Soluble Sugar and Soluble Starch Contents

The contents of SS and SST were quantified following the procedure outlined by Li Zhongguang [18]. In brief, 0.1 g of leaf samples, dried at various growth stages, was finely ground using a mortar and pestle and mixed with 8 mL of deionized water to create a homogenate. This mixture was then transferred to a test tube and heated in a water bath at 100 °C for 30 min. After allowing it to cool to room temperature, the supernatant was separated by centrifuging at 24 °C at 4000 r/min (10 min). The process was repeated twice, and all collected supernatants were combined in a 50 mL volumetric flask, diluted

with deionized water, and labeled as the soluble sugar sample solution. The residual pellet from this extraction was further processed by adding 8 mL of deionized water and heating at 80 °C for 15 min, followed by adding 2 mL of 9.2 mol/L $HClO_4$ and heating for an additional 15 min. The subsequent centrifugation (24 °C, 4000 r/min, 10 min) and steps mirrored the SS extraction process. The absorbances of various glucose and starch concentrations were measured through anthrone colorimetry [19], allowing for the construction of a standard curve. The sample absorbances were similarly assessed, and the contents of the SS and SST were derived from the standard curve.

2.4. Determination of Secondary Metabolites

2.4.1. Extraction of Plant Samples

The dried samples were ground and passed through a 40-mesh screen. Approximately 0.02 g of each sample was extracted in 2 mL of 80% ethanol solution at 60 °C for 2 h with agitation. The mixture was then centrifuged at 10,000× *g* for 10 min at 25 °C. The resulting supernatant was collected and refrigerated at 4 °C for subsequent analysis of the total polyphenols, flavonoids, alkaloids, and antioxidant activity.

2.4.2. Determination of Total Polyphenol Content

The total polyphenol content was determined spectrophotometrically using the Folin–Ciocalteu reagent according to Wojdyło et al. [20]. A standard curve was prepared using gallic acid, and the results were expressed as gallic acid equivalents (mg/g, dry weight). For the assay, a mixture consisting of 0.1 mL of each sample extract, 0.2 mL of the Folin–Ciocalteu reagent, and 2 mL of deionized water was prepared. Subsequently, 1 mL of 20% sodium carbonate was added to the mixture. After incubation in the dark at 20 °C for 1 h, the absorbance was measured at a wavelength of 760 nm.

2.4.3. Determination of Total Flavonoid Content of Leaf Blade

The total flavonoid content was quantified using the aluminum chloride colorimetric assay, with quercetin serving as the reference standard [21]. The results were reported as quercetin equivalents (mg/g, dry weight). The assay procedure involved combining 0.25 mL of the sample extract with 1.25 mL of distilled water. $NaNO_3$ was then introduced, and the mixture was incubated in darkness for 6 min. Subsequently, 0.15 mL of 10% $AlCl_3$ solution was added, followed by another 5-min dark incubation. The reaction was completed by adding 0.5 mL of NaOH and 0.275 mL of distilled water. Absorbance measurements of both the control and sample solutions were performed at 510 nm, using a reagent blank as reference.

2.4.4. Determination of Total Alkaloid Content of Leaf Blade

The determination of the total alkaloid content in the leaf samples was conducted using a modified version of the method outlined by Lahare et al. [22]. This analytical approach is grounded in the chemical reaction between the alkaloids and bromocresol green (BCG). The process involved several key steps: First, a DMSO-based denaturation of the plant extract (1 mg/mL) was performed, followed by acidification with 2N HCl and subsequent filtration. The resulting solution was then transferred to a separatory funnel, where it was combined with equal volumes of bromocresol green solution (0.05%, 5 mL, 75% alcohol solution as solvent) and phosphate buffer (0.013 mol/dm^3, pH 7.4). After vigorous agitation, chloroform extractions were carried out in incremental volumes (1–4 mL). These extracts were collected in a 10 mL volumetric flask and brought to volume with additional chloroform. The final step involved spectrophotometric analysis, measuring the absorbance of the prepared solution at 470 nm.

2.5. Determination of Active Ingredient Content

Leaf samples from various growth stages, initially stored at −80 °C, were oven-dried to a constant weight at 60 °C and pulverized in liquid nitrogen. The extraction followed the

Chinese Pharmacopoeia protocol [2]. The ligustroflavone and rhoifolin concentrations were analyzed using HPLC, with detection at 336 nm. The mobile phase consisted of methanol and 0.5% aqueous phosphoric acid (43:57), as per Hu et al. [23].

2.6. Determination of In Vitro Antioxidant Activity of Different T. arguta Samples

2.6.1. DPPH Free Radical-Scavenging Rate (SR)

The DPPH radical-scavenging activity of the *T. arguta* leaf extracts was evaluated using a modified protocol based on Wu et al. [24]. In brief, a 0.1 mL aliquot of each sample extract was mixed with an equal volume of 0.1 mmol/L DPPH solution, ensuring thorough homogenization. The reaction mixture was then incubated for 30 min at room temperature, enabling sufficient interaction between the sample and the DPPH. Spectrophotometric measurements were performed at 517 nm to determine the absorbance. Each sample was analyzed in triplicate to ensure reproducibility. The SR was quantified using the following equation: SR (%) = (Ac − As)/Ac × 100, where Ac represents the absorbance of the control (blank) and As denotes the absorbance of the sample.

2.6.2. Hydroxyl Radical-Scavenging Rate

The hydroxyl radical-scavenging activity of the *T. arguta* folium was evaluated using a modified version of Samak et al.'s method [25]. The procedure involved combining 1 mL of the sample extract with 1 mL each of $FeSO_4$ solution (10 mmol/L), salicylic acid-ethanol solution (10 mmol/L), and H_2O_2 solution (8.8 mol/L) in sequence. After thorough mixing, the preparation was incubated at 37 °C for 30 min. The absorbance was then measured at 510 nm, with the process repeated three times per sample. Distilled water served as the blank control. The hydroxyl radical-scavenging rate (Q, %) was determined using the following equation: Q = (A2 − A1)/A2 × 100%, where A1 and A2 represent the sample and blank absorbances, respectively.

2.7. Statistical Analysis

MS Excel 2010 was used for data analysis, and IBM SPSS Statistics, Version 25, was used for the analysis of variance, the analysis of the significance of differences, and PCA.

3. Results

3.1. Leaf Biomass

As can be seen in Table 1, there was a significant difference in the dry weights of the leaves at different harvesting periods. The leaf dry weights showed an increasing trend, reaching a maximum value of 1.097 g in the S4 period, but was not significantly different from and was slightly lower than that in the CK period.

Table 1. Changes in the dry weight of the leaves of the *Turpinia arguta* at different harvesting periods.

Harvesting Period	Leaf Biomass (mg/g, dw)
CK	1.198 ± 0.077 a
S1	0.215 ± 0.037 d
S2	0.598 ± 0.025 c
S3	0.854 ± 0.075 b
S4	1.097 ± 0.055 a

Note: Statistically significant differences (ANOVA + Duncan's test, $p \leq 0.05$) among the groups are denoted by different lowercase letters.

3.2. Nutrient Determination

As shown in Figure 2, the changes in the soluble protein, soluble sugar, and soluble starch contents of the leaves at different harvesting periods were more complex. The soluble protein content first decreased, then increased, and finally decreased, reaching a maximum value (42.93 mg/g, dw) in the S3 period, which was lower than that of the CK group (45.64 mg/g, dw), and there was a significant difference. The content of soluble sugar first

increased, then decreased, and finally increased, reaching a maximum value (16.37 mg/g, dw) in the S2 period, which was higher than that of the CK period (14.31 mg/g, dw), and there was a significant difference. The content of soluble starch first increased and then decreased, reaching a maximum value (9.53 mg/g, dw) in the S2 period, which was higher than that of the CK period (8.23 mg/g, dw), and there was a significant difference.

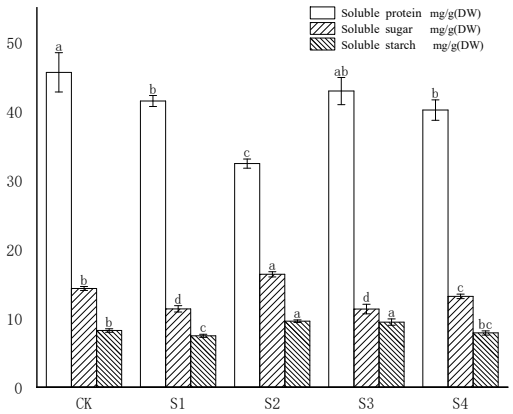

Figure 2. Changes in the nutrient contents of the leaf blades of the *Turpinia arguta* tissue-culture seedlings at different harvesting periods. Note: Statistically significant differences between groups are denoted by lowercase letters. Note: Statistically significant differences (ANOVA + Duncan's test, $p \leq 0.05$) among the groups are denoted by different lowercase letters.

3.3. Secondary Metabolites

3.3.1. Determination of Total Polyphenols, Total Flavonoids, and Alkaloids in Plants

As shown in Figure 3, the total polyphenols and total flavonoids initially increased and then decreased; the total polyphenols and the total flavonoid contents of the leaves at the S3 period reached the maximum value of 22.86 mg/g and 5.80 mg/g (DW), respectively, which were significantly different from those of the CK group and were higher than those of the control group (20.45 mg/g (DW), 5.67 mg/g (DW)). The changes in the alkaloids showed the opposite trend, with the highest alkaloid content being 14.50 mg/g (DW) at the S1 period, which then decreased until reaching the lowest alkaloid content of 8.40 mg/g (DW) at the S4 period, which was significantly different than that of the control group and higher than that of the CK group (5.31 mg/g (DW)).

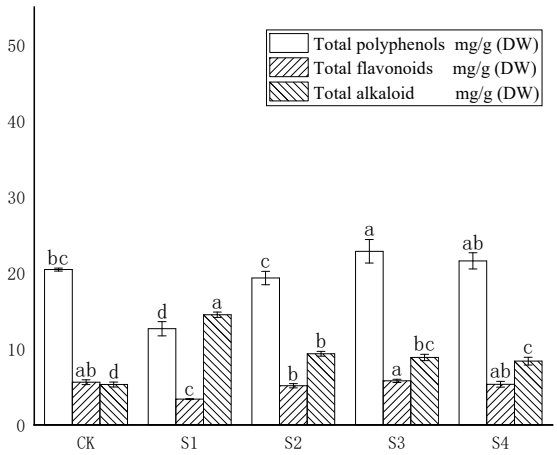

Figure 3. Changes in the total polyphenol, total flavonoid, and alkaloid contents of the leaves of the *T. arguta* tissue-culture seedlings at different harvesting periods. Note: Statistically significant differences (ANOVA + Duncan's test, $p \leq 0.05$) among the groups are denoted by different lowercase letters.

3.3.2. Determination of Ligustroflavone and Rhoifolin Contents

The samples were assessed according to the method of Hu et al. [23]. The contents of ligustroflavone and rhoifolin were calculated from the standard curve of ligustroflavone and rhoifolin during the growth process, and the specific results are shown in the following figures (Figures 4 and 5); the chemical structures of ligustroflavone and rhoifolin are shown in Figures 6 and 7. With the continuous growth of the plantlets, the contents of the two active ingredients were significantly different among the different harvesting periods. The contents of ligustroflavone and rhoifolin increased, and the highest content of ligustroflavone was 0.53% at the S4 period, which was not different from that of the control group but was higher than that of the control group (0.49%); the content of rhoifolin was 0.21%, which was significantly different from that of the CK group and higher than that of the CK group (0.148%).

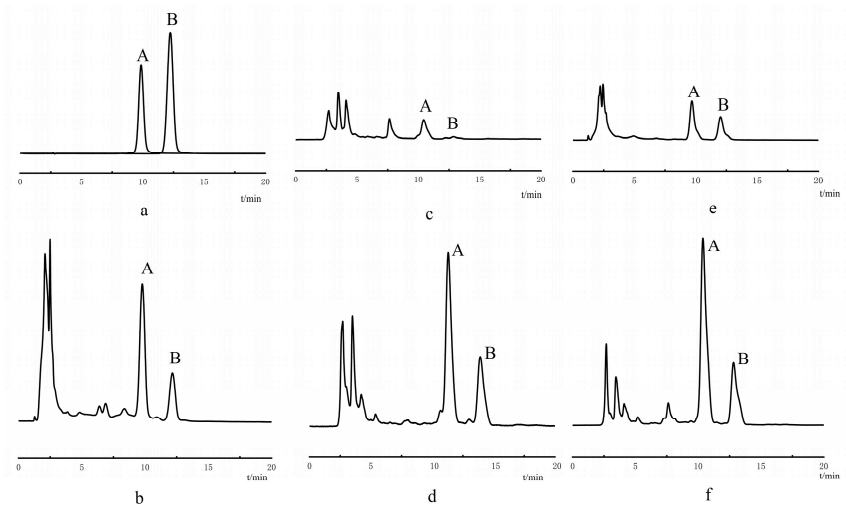

Figure 4. Chromatograms of the leaves of the *T. arguta* seedlings at different harvesting periods, in which peaks A and B are ligustroflavone and rhoifolin, respectively; (**a**) is the chromatogram of the ligustroflavone and rhoifolin standards, and (**b**–**f**) are the chromatograms of CK, S1, S2, S3, and S4, respectively.

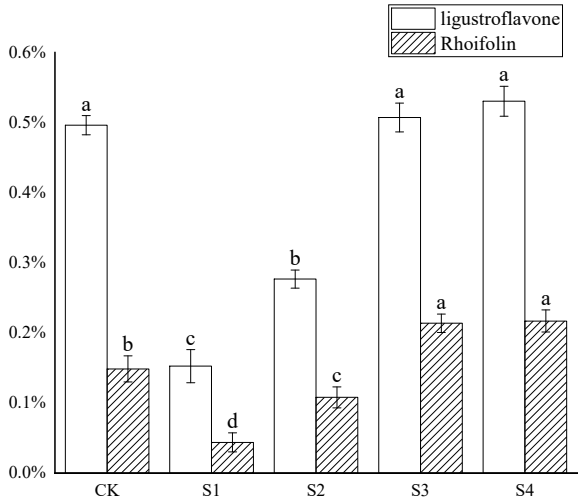

Figure 5. Contents of ligustroflavone and rhoifolin in the leaves of the *T. arguta* tissue-culture seedlings at different growth periods. Note: Statistically significant differences (ANOVA + Duncan's test, $p \leq 0.05$) among the groups are denoted by different lowercase letters.

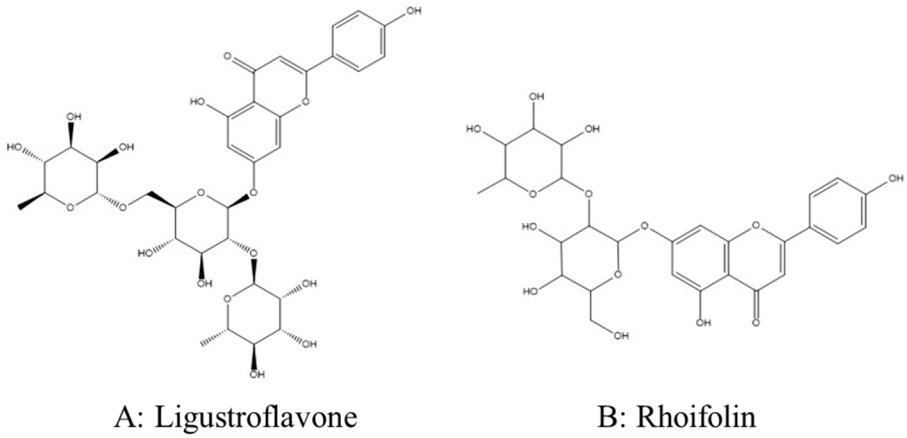

Figure 6. Planar chemical structures of ligustroflavone and rhoifolin, from left to right: (**A**) ligustroflavone and (**B**) rhoifolin.

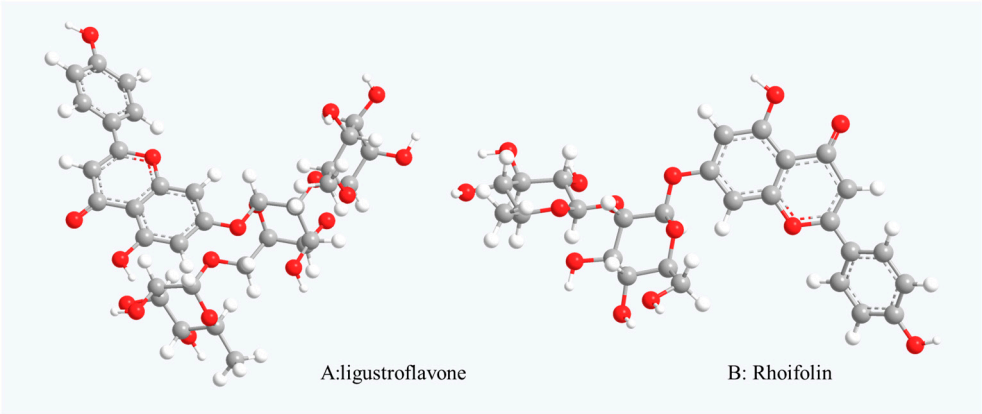

Figure 7. Stereochemical structures of ligustroflavone and Rhoifolin, from left to right, (**A**) ligustroflavone, (**B**) Rhoifolin.

3.4. Determination and Comprehensive Evaluation of Antioxidant Activity

The results of the antioxidant activity of *T. arguta* are shown in Figure 8. The DPPH radical-scavenging and hydroxyl radical-scavenging activities of the samples from different harvesting periods were significantly different, showing the ability to inhibit the oxidative process in both assays. The highest SR was 67.93% in the S3 period, which was lower than that of the control (69.00%) and not significantly different. The hydroxyl radical-scavenging rate was 62.63% in the S4 period, which was significantly different and higher than that of the control (50.90%). When the different antioxidant evaluation methods were selected to determine the antioxidant capacities of the *T. arguta* leaves at different harvesting periods, samples from the same period showed different antioxidant capacities in different antioxidant evaluation systems due to the different response mechanisms of different evaluation methods. For example, in the DPPH method, the S3 leaves had the best antioxidant capacity, while in the hydroxyl radical method, the S4 leaves had the best antioxidant capacity. To comprehensively and systematically evaluate the antioxidant activities of the leaves, the antioxidant potency composite (APC) index was used to comprehensively evaluate the two methods [26]. The APC index (%) of a certain harvesting period was defined as the average value (ratio) of the SR and hydroxyl radical-scavenging rate of that growth period to the maximum value of the SR and hydroxyl radical-scavenging rate of all periods, respectively. The comprehensive evaluation of the antioxidant activities at different harvesting periods showed that the antioxidant capacities of the CK period and the S3 and S4 periods were higher (APC index $\geq$ 85.00%) (Table 2), and the antioxidant capacity of the leaves of the S4 period was the best and was higher than that of the CK group.

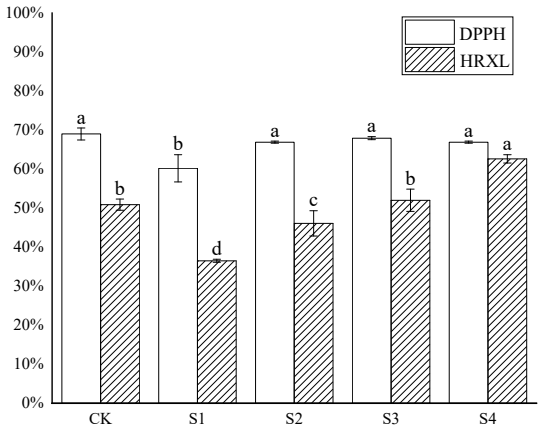

Figure 8. DPPH radical-scavenging and hydroxyl (HDXL) radical-scavenging rates of the *T. arguta* tissue-culture seedlings at different harvesting periods. Note: Statistically significant differences (ANOVA + Duncan's test, $p \leq 0.05$) among the groups are denoted by different lowercase letters.

Table 2. APC index of the antioxidant capacities of the leaves of *T. arguta* at different harvesting periods.

Period	APC Index/%	Arranged in Order
CK	88.87	3
S1	71.35	5
S2	83.63	4
S3	88.98	2
S4	96.56	1

3.5. PCA of Indicators of T. arguta at Different Harvesting Periods

The data for evaluating the best harvesting period of *T. arguta* were standardized and subjected to PCA, and the data with eigenvalues greater than 1 were extracted, and the results are shown in Table 3. According to the results of the principal component analysis, the ligustroflavone, total polyphenols, and total flavonoids (>0.90) were significant factors influencing the quality of *T. arguta*. There were two principal components with eigenvalues greater than 1, and the cumulative variance contribution rate reached 81.596%, indicating that these two principal components can reflect most of the information about the quality of *T. arguta* and can be used as a comprehensive index for its quality evaluation. According to the eigenvalues of the principal components, the principal component scores of the leaves at different harvesting periods were calculated (Table 3), the linear combination expressions of the principal components and the comprehensive evaluation function of the principal components were obtained, and the comprehensive score was calculated as F = F1 × 63.323% + F2 × 20.706%. The higher the composite score, the better the performance of its comprehensive quality. The results are shown in Table 4; the highest score was observed in the S4 period and was greater than that of the CK period, indicating that the best quality of *T. arguta* herbs was obtained at the S4 period and that this period is the most suitable harvesting period for *T. arguta* herbs.

Table 3. Eigenvalues, contributions, and cumulative contributions of the two principal components.

Indicator Components	Principal Component	
	F1	F2
Leaf biomass	0.928	−0.179
DPPH free radical-scavenging rate	0.888	0.197
Hydroxyl radical-scavenging rate	0.851	−0.142
Antioxidant potency composite	0.214	−0.83
Soluble proteins	0.247	0.822
Soluble sugars	0.389	0.693
Soluble starches	0.948	0.079
Total polyphenol	−0.904	−0.089
Total flavonoids	0.943	0.145
Total alkaloids	0.949	−0.293
Ligustroflavone	0.888	−0.221
Rhoifolin	6.927	2.106
Eigenvalue (math.)	60.89	20.706
Contribution rate/%	63.323	82.21
Cumulative variance contribution/%	0.928	−0.179

Table 4. Scores of the first two principal components and comprehensive evaluation at different harvesting periods.

Harvesting Period	Principal Component Score (of a Function)			Arranged in Order
	F1	F2	F	
CK	4.714	−1.168	2.745	2
S1	−12.533	−1.390	−8.159	5
S2	−1.298	3.993	−0.053	4
S3	4.382	−0.721	2.622	3
S4	4.735	−0.713	2.845	1

4. Discussion

4.1. Determination of the Optimal Harvesting Period

The harvesting period is an important factor affecting the quality and yield of medicinal herbs, which has practical guidance for the rational utilization of resources and industrial development. Most researchers have shown that the optimal harvesting period of medicinal herbs is the point in time when the herbs reach the highest concentrations of their active ingredients during the growth process [27]. Medicinal plants are usually characterized by their biologically active chemical constituents, such as polyphenols, flavonoids, and alkaloids, which are the core criteria for quality control [28]. However, due to the complexity and difficulty of separating the constituents of herbs, for many herbs, only a single class of constituents is used as an indicator of whether or not they have met the existing criteria [29,30]. Taking *T. arguta* herbs as an example, early research showed that *T. arguta* was mainly composed of flavonoids [10], of which ligustroflavone and rhoifolin contents were particularly prominent, and it then established a quality evaluation method with ligustroflavone and rhoifolin contents as the core indicators. From the viewpoint of ligustroflavone and rhoifolin content, only *T. arguta* in the S3, S4, and CK periods met the quality standards of the Chinese Pharmacopoeia (ligustroflavoside > 0.3%, rhoifolin > 0.1%), suggesting that these periods were the appropriate harvesting periods for *T. arguta*. However, the determination of the optimal harvesting period of herbs is a complex issue, and the components and biomass accumulation are in a dynamic process [31]. In this study, the combination of the changing patterns of leaf biomasses, active ingredient contents, secondary metabolites, nutrients, antioxidant activities at five levels during the different harvesting periods, and the results of PCA showed that the S4 period was the optimal harvesting period. Compared with a single indicator, PCA provides

a more comprehensive evaluation perspective [32]. This comprehensive evaluation not only reduces the interference of complex factors but also, to a certain extent, avoids the influence of human bias, improves the stability and accuracy of the evaluation results, and provides a solid foundation for the development of scientific quality-control strategies for *T. arguta* herbs. Meanwhile, the leaves of *T. arguta* grew abundantly during the optimal harvesting period (S4), which was also in line with the judgment of the Chinese Pharmacopoeia on the suitable harvesting period of *T. arguta* herbs.

4.2. Differences in Changes at Different Harvesting Periods

We observed that the biomass of the plant showed a continuous growth trend with the advancement of the harvesting period, which was due to continuous accumulation of organic matter and growth energy during the developmental cycle, providing a material basis for the exertion of its medicinal value [33,34]. At the same time, this study revealed the complex pattern of change in the nutrient composition of *T. arguta* during different harvesting periods, which was related to the plant's growth and development stages, its physiological and metabolic activities, and its nutrient storage strategies, reflecting the plant's abiotic stress tolerance and related adaptation mechanisms [35,36]. Understanding these changes is important for studying plant growth, development, and regulatory mechanisms during different harvesting periods [37] and helps to optimize both the plant's growth environment and management measures to improve plant yield and quality.

Secondary metabolites of plants, such as polyphenols, flavonoids, and alkaloids, show different trends during different harvesting periods. Initially, the total polyphenol and total flavonoid concentrations showed a steady increase as the harvesting period progressed, which was due to the activation of secondary metabolic activities in response to environmental stresses and an increase in the rate of synthesis [38]. However, this increase is not endless and usually peaks at plant maturity and then begins to decrease [39]. The current quality indicators, i.e., the two flavonoids, ligustroflavone and rhoifolin, showed a gradual decline in the increase at different harvesting periods, although the content generally increased, which further confirms the biological law that the pre-increase is not infinite. The alkaloid content, however, reached its maximum at the early S1 period of harvesting, which is due to the fact that alkaloids are usually produced and stored the most during the early growth stages [40] and may decline over time. This phenomenon has also been observed in other studies [41,42], although the exact trajectory of change may vary depending on the plant species and environmental conditions.

Polyphenols and flavonoids are important secondary products in plants, with good free-radical scavenging and metal-chelating abilities [43] and are the main natural antioxidant substances in plants. In various studies, polyphenols and flavonoids have previously been found to be strongly and positively correlated with antioxidant activity in plants [44,45]. The types, structural characteristics, and contents of polyphenols and flavonoids determine their antioxidant capacities to a certain extent [46–48]; in general, the higher the content, the stronger the antioxidant activity of the plant. The results of this study showed that the total polyphenol and total flavonoid contents of the plants in the S4 period were slightly decreased, while the antioxidant capacities (APC index) of the plants in the S4 period were higher than those in the S3 period. This is because polyphenols and flavonoids can not only radically reduce the rate of reactive oxygen species (ROS) formation [49] but also indirectly enhance the antioxidant capacity of plants by inducing the activities of some antioxidant enzymes [50], such as peroxidase and superoxide dismutase; synergize with other antioxidant substances, such as vitamin C and carotenoids [51]; and jointly improve the antioxidant activity.

4.3. Quality Research on Cultivated and Traditional Medicinal Herbs

With the modernization of Chinese medicine, the use of medicinal plant resources will continue to increase. Apart from wild collection, the field cultivation of medicinal plants is currently the main method of providing herbal medicines. The traditional practice of

such cultivation is often over-farming and domestication, which leads to the gradual loss of the original genetic diversity of medicinal plants during the growth process, as well as the susceptibility to environmental stresses that cause significant degradation of the medicinal constituents and reduction of the overall quality of the plant [52,53]. Under complex biological and environmental conditions, medicinal plant tissue culture ("tissue culture") has become an effective solution to these problems due to its ability to precisely regulate the culture conditions and improve production efficiency [54]. The results of this study showed that the key active ingredients (ligustroflavone and rhoifolin), antioxidant activity, and overall quality of the tissue culture seedlings at the optimal harvesting period (S4) were better than those of the live seedlings; these results not only have preliminary practical value for clinical research and industrialization but also point out the direction for the further optimization of culture strategy and improvement of the quality of medicinal herbs.

5. Conclusions

In this study, a more comprehensive quality evaluation method was established to detect the chemical components and antioxidant activities of *T. arguta* herbs at different harvesting periods. PCA was employed for this comprehensive quality evaluation. The quality of the *T. arguta* tissue-culture seedlings at the optimal harvesting period (S4) was better than that of the live seedlings, which demonstrates the superiority of tissue culture technology to other methods for the propagation of Chinese herbal medicines and provides scientific support for the improvement of the quality of Chinese herbal medicines and propagation technology. Therefore, we recommend further research to explore the potential of *T. arguta* in the development of new herbal medicines and the optimization of propagation techniques. However, there are limitations to this study. Plant growth and development are influenced by a variety of factors, such as geographic location, climate, and soil type. For the cultivation of herbs, we only set a harvesting period. It is therefore difficult to directly generalize the results of the study to other geographical environments and conditions.

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