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Tetrastigma hemsleyanum Diels et Gilg Roots from Different Origins: Pharmacognostical Identification, Content Determination of Functional Composition and *in vitro* Antioxidant Activity

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Abstract: *Tetrastigma hemsleyanum* Diels et Gilg (*T. hemsleyanum*) is a unique and rare Chinese medicinal herb mainly distributed in the south of the Yangtze River in China. In the present study, *T. hemsleyanum* roots from seven different sites in the south of the Yangtze River (Guangxi [Baise, Guilin, and Hechi], Hunan [Fenghuang], and Zhejiang [Hangzhou, Taizhou, and Wenzhou] provinces) were collected, and differences in the macroscopic and microscopic appearance of the roots, content of their active ingredients, and antioxidant activity of the root extracts were analyzed. The Pearson correlation coefficient was used to analyze the correlation between the antioxidant activity and the total flavonoids and total polyphenols contents of *T. hemsleyanum* roots. The results of macroscopic and microscopic root appearance indicated that *T. hemsleyanum* roots from different sites could be clearly differentiated based on size and epidermal characteristics, the color of dry root powder, and the shape and arrangement of the root canal. The results of content determination showed no significant differences ($P>0.05$) between the contents of flavonoids, polyphenols, rutin, and catechin in *T. hemsleyanum* roots from Guilin, Guangxi, and those from Zhejiang and Hunan. However, the contents of quercetin and kaempferol showed a significant difference ($P<0.01$) between *T. hemsleyanum* roots from Guilin, Guangxi, and those from Zhejiang, Hunan, and Guangxi (Baise and Hechi). Furthermore, the root extracts of *T. hemsleyanum* showed a high antioxidant activity ranging from 2.254±0.240 to 14.655±4.528 mg ascorbic acid equivalent/g sample. Pearson correlation analysis showed that the antioxidant activity of *T. hemsleyanum* roots had a higher correlation with the total flavonoids content than with the total polyphenols content. The results of this study provided a reference for the quality evaluation of *T. hemsleyanum* from different origins and provided technical support for subsequent resource development.

Keywords: *Tetrastigma hemsleyanum* Diels et Gilg, Medicinal Plants, Pharmacognostical Study, Flavonoids, Polyphenolics, Antioxidant Activity

Introduction

Tetrastigma hemsleyanum Diels et Gilg, which is also known as the three-leaf climbing vine, stone monkey, snake aconite, and golden wire hanging gourd, is a dicotyledonous vine belonging to the angiosperm phylogeny group. *T. hemsleyanum* is a rare Chinese medicinal herb unique to China and it is mainly distributed in Zhejiang Province, Guangxi Province, Hunan Province, Sichuan Province, Chongqing City, Guizhou Province, Yunnan Province, and other Chinese mainland subtropical areas. The entire *T. hemsleyanum* herb can be used for formulating medications and among all the plant parts of *T. hemsleyanum*, the root has the best medicinal effect. *T. hemsleyanum* is often used to treat febrile convulsions in children and snakebites and it exerts its effects by activating blood components involved in coagulation, dispersing nodules, dispelling weathering

and phlegm (Shubin *et al*., 2020; Sun, 2018). The root extract of *T. hemsleyanum* contains flavonoids, phenolic acids, amino acids, polysaccharides, and other components that have been widely studied (Hongtao, 2022; Ji *et al*., 2021). In addition to antitumor effects, flavonoids and phenolic compounds have strong physiological functions such as hypoglycemic, hypolipidemic, and antioxidative effects (Zhu *et al*., 2020; Hu *et al*., 2021).

In China, few studies have been conducted on the different sources of *T. hemsleyanum*. Yongwei *et al*. (1998) reported that the roots of *T. hemsleyanum* collected from Zhejiang Province (Lishui, Jiande, and Ningbo) had an irregular swelling shape like a spindle, a smooth tangent plane, the off-white or pink color of the section, rare ducts, a narrow phloem and several fiber groups around the vascular bundles. The roots of *T. hemsleyanum* collected from Guangxi (purchased from a medicine supplier) had a round shape, smooth surface, wider phloem, numerous conduits, and few fiber groups between the vascular bundles (Huang *et al*., 2006). The roots of *T. hemsleyanum* collected from Jiangxi (Huaiyu Mountain) were oval or gourd-shaped and brown with a relatively smooth surface. Their cross-section was off-white and powdery and the powder was grayish-brown after drying (Ping *et al*., 2018). Phytochemical evaluations showed that *T. hemsleyanum* roots from Zhejiang and Guangxi contained flavonoids, polysaccharides, and amino acids, while *T. hemsleyanum* roots from Jiangxi contained only flavonoids and amino acids.

These findings reveal that *T. hemsleyanum* roots from different regions show variations in their macro and micro appearance and active ingredient contents. However, studies on the abovementioned parameters, especially on pharmacogenetic and antioxidant activities, in root samples of *T. hemsleyanum* plants are currently sporadic. To control the quality, efficacy, and clinical application of *T. hemsleyanum*, it is essential to conduct a comprehensive pharmacogenetic study on *T. hemsleyanum* plants from different regions.

In this study, firstly, macroscopic, microscopic, and phytochemical analyses were carried out on the roots of *T. hemsleyanum* from different origins. Then the content of flavonoids, polyphenols, rutin, quercetin, kaempferol, catechin in the root extracts was determined. Finally, the antioxidant activity of *T. hemsleyanum* root from different origins was determined and the Pearson correlation coefficient was analyzed.

Materials and Methods

Plant Materials

T. hemsleyanum plants were identified and authenticated by associate professor Ge Jian of China Jiliang University. Molecular identification was performed using the universal primer 5′-GGCAAGTCTGGTGCC-3′/5′-ACGGTATCTRATCRTC-3′. Representative samples of *T. hemsleyanum* roots were collected from the following seven sites: Hangzhou, Zhejiang Province (ZJ-HZ); Taizhou, Zhejiang Province (ZJ-TZ); Wenzhou, Zhejiang Province (ZJ-WZ); Fenghuang, Hunan Province (HN-FH); Baise, Guangxi Province (GX-BS); Guilin, Guangxi Province (GX-GL); and Hechi, Guangxi Province (GX-HC). All samples were confirmed to be *T. hemsleyanum*.

Macroscopic Study of the Roots

Macroscopic characteristics such as consistency, color, texture, size, and odor of the samples of raw *T. hemsleyanum* root powder and freshly collected *T. hemsleyanum* roots were observed through sensory organ evaluation by the researchers and experts (Nathulal *et al*., 2022).

Microscopic Study of the Roots

Microscopic studies were performed on the cross section of *T. hemsleyanum* roots. The roots were manually cut to obtain 4 to 7 μm thick cross sections, which were then stained with Safranin O-Fast green to confirm lignification. Microphotographs along with appropriate scale bars were used to describe the microscopic observations of tissues. All photographs were acquired using the ZEISS Axio Vert A1 microscope.

Microscopic Study of Root Powder

The collected roots were washed, dried at 60°C, and pulverized. The pulverized roots were then passed through a #60 mesh and the root powder was collected and used for microscopic examination. A small amount of stem powder was placed on the slides. Each slide was then covered with a cover slip, mounted with 2-3 drops of chloral hydrate, and examined under a microscope. Iodine was used to observe starch grains, while concentrated Hydrochloric acid (HCl) and phloroglucinol were used to observe lignified tissues. Glycerin was used as a mounting compound and sulfuric acid (H_2SO_4) was used to detect calcium oxalate crystals (Mohan *et al*., 2012).

Phytochemical Evaluations

Based on the methods of (Shagufta and Al-Taweel, 2019) the reaction of the *T. hemsleyanum* root powder with different chemical reagents was studied to detect the presence of phytoconstituents such as polysaccharides, phenols, flavonoids, and amino acids based on color changes.

Preparation of Standard Solutions

A gallic acid standard solution was prepared by dissolving gallic acid in diluted 70% aqueous ethanol. Standard solutions of catechin, quercetin, kaempferol, and rutin were also prepared with diluted 60% aqueous

ethanol. An ascorbic acid standard solution was prepared with ultrapure water (Table 1).

Root Extract Preparation

To obtain total flavonoids, the dried and ground powdered root sample (1.0 g) was extracted with 20 mL of 60% aqueous ethanol (v/v) by using an ultrasound extractor at 60°C for 44 min (Weihua *et al*., 2016). To obtain total polyphenols, the powdered root sample was extracted following the method of Qiu with slight modifications (Mengyu *et al*., 2022). The dried and ground powdered root sample (1.0 g) was extracted with 40 mL of 70% aqueous ethanol (v/v) using an ultrasound extractor at 60°C for 40 min. The supernatant of the extract was then centrifuged at 3000 rpm and its volume was adjusted with ethanol. Finally, the supernatant was maintained in an airtight glass container at 4°C until further analysis.

Determination of Total Flavonoids Content

Total flavonoids content was determined using the sodium nitrite-aluminum nitrate colorimetric method according to (Tang *et al*., 2020). Rutin was used as the standard compound and the standard curve was constructed from the standard concentration and the corresponding absorbance value. The total flavonoids content of the samples was expressed as mg rutin equivalent per gram of the sample (mg RE/g). Each sample was analyzed three times.

Determination of Total Polyphenolics Content

The total phenolic content in *T. hemsleyanum* root extracts was determined by Folin-Ciocalteu assay with gallic acid as the standard compound and the corresponding standard absorbance curve constructed for gallic acid (Limam *et al*., 2021; Chilanti *et al*., 2021). The content of phenolic compounds in the extract was expressed as Gallic Acid Equivalent (GAE) per gram of the sample (mg GAE/g). Each sample was analyzed three times.

Determination of Catechin, Quercetin, Kaempferol, and Rutin Contents

High-Performance Liquid Chromatography (HPLC) was performed according to the guideline of a previous study (Wang *et al*., 2019). Fresh *T. hemsleyanum* root extracts were dissolved in diluted 60% aqueous ethanol and subjected to chromatography using a wakosil 5C18 column (4.6 × 150 mm, 5 μm) operated at 30°C with an HPLC instrument (Shimadzu LC-20AT). The flow rate was maintained at 1 mL/min and the injection amount was 20 μL. Table 2 the HPLC conditions for the determination of catechin, quercetin, kaempferol, and rutin contents. For quantitative analysis, the chemical standards of the four main components (catechin, quercetin, kaempferol, and rutin) of *T. hemsleyanum* root extracts were freshly dissolved at different concentrations ranging from 50 to 500 μg/mL and the standard curve was constructed based on the standard concentration and the corresponding peak area. The standard margin of error was<5%.

Determination of Antioxidant Capacity of Root Extracts

2, 2-Diphenylpicrylhydrazyl (DPPH) radicals are stable free radicals with a maximum absorption wavelength of 517 nm. In the presence of a radical scavenger, the single electron of the DPPH radical is paired, the concentration of DPPH radicals is reduced and its color becomes lighter. Consequently, the absorbance value at the maximum absorption wavelength becomes smaller and the color change is stoichiometrically related to the number of paired electrons. As a simple, sensitive, and reliable method, the DPPH assay has been widely accepted in China and abroad for studying free radical scavenging activities of antioxidants.

The antioxidant capacity of *T. hemsleyanum* root extracts was determined by the DPPH assay (Ayele *et al*., 2022). A solution of 0.4 mmol/L DPPH was prepared by dissolving DPPH in ethanol. Next, 0.8 mL of DPPH was added to 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the sample extracts, and 60% aqueous ethanol was used to adjust the final volume of each solution to 2 mL. To prepare the control solution, 250 μL of DPPH solution was added to 50 μL of 60% aqueous ethanol. The mixture was kept in the dark for 30 min. Finally, the absorbance value was measured at 517 nm. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$Inhibition(\%) = \left[1 - \left(A_{sample} - A_{background}\right) / A_{control}\right] \times 100$$

where, *Inhibition*(%) is the *DPPH* radical scavenging activity, $A_{background}$ is the absorbance value of the sample without DPPH, and A_{sample} and $A_{control}$ are the absorbance values of the sample and control, respectively.

The ability of ascorbic acid (VC) to scavenge DPPH radicals was also determined at different concentrations (100, 200, 300, 400, and 500 mg/L) with a standard curve. The VC equivalent antioxidant capacity of *T. hemsleyanum* was calculated based on the obtained curve. The measurements were performed three times and the results are expressed as ascorbic acid equivalent (mg AAE/g sample).

Statistical Analysis

All experiments were repeated 3 times and the data were expressed as mean ± SD from three replicates. The experimental data were plotted and subjected to regression analysis by using Excel 2007. Significance analysis (Duncan's method) and correlation analysis (Pearson's method) were performed using graph pad prism 9 statistical software. P<0.05 and P<0.01 indicated significant and very significant differences, respectively.

Table 1: Preparation methods for standard solutions

Standard solutions	Methods
Total flavonoids	Precision weighed rutin standard 20.0 mg, added diluted 60% aqueous ethanol to completely dissolve, prepared as 0.200 mg/mL rutin standard solution
Total phenolics	Precision weighed gallic acid standard 12.4 mg, added diluted 70% aqueous ethanol to completely dissolve, prepared as 0.124 mg/mL gallic acid standard solution
Catechin	Precision weighed catechin standard 10.0 mg, added diluted 60% aqueous ethanol to completely dissolve, prepared as 0.500 mg/mL catechin standard solution
Quercetin	Precision weighed quercetin standard 10.0 mg, added diluted 60% aqueous ethanol to completely dissolve, prepared as 0.525 mg/mL quercetin standard solution
Kaempferol	Precision weighed kaempferol standard 10.0 mg, added diluted 60% aqueous ethanol to completely dissolve, prepared as 0.413 mg/mL kaempferol standard solution
Rutin	Precision weighed rutin standard 10.0 mg, added diluted 60% aqueous ethanol to completely dissolve, prepared as 1.000 mg/mL rutin standard solution
Ascorbic acid	Precision weighed ascorbic acid standard 10.0 mg, added ultrapure water to completely dissolve, prepared as 0.500 mg/mL ascorbic acid standard solution

Table 2: High-performance liquid chromatography conditions for estimating catechin, quercetin, kaempferol, and rutin contents

Name	Mobile phase (V/V)	Detector wavelength (nm)
Catechin (Na *et al*., 2022)	Methanol-0.1% aqueous phosphoric acid (20: 80)	280
Kaempferol, quercetin (Pan *et al*., 2021)	Methano-0.2% aqueous phosphoric acid (60: 40)	360
Rutin (Zhu *et al*., 2022)	Acetonitrile-0.2% aqueous phosphoric acid (20: 80)	256

Results

Macroscopic Characteristics

No differences were observed in the texture and smell of *T. hemsleyanum* roots from the different sites; however, the roots varied in appearance, section, and color of dry powder (Fig. 1). *T. hemsleyanum* roots from Guangxi and Hunan were round with light brown skin. The roots from Hunan were larger with a 3-5 cm length and 2-4 cm diameter; furthermore, their texture was rough with transverse rings and punctate protruding root marks on the surface. *T. hemsleyanum* roots from Zhejiang were elongated, fusiform, and gourd-shaped with light brown skin. They measured 2-7 cm in length and 0.5-2 cm in diameter and had a hard and rough surface. The cross-section of the root was flat and smooth with a powdery appearance. The color of the cross-section of the roots, however, varied between different sites; for example, Guangxi roots had a pink or light yellow cross-section, Hunan roots had a mainly light yellow cross section and Zhejiang roots had a mainly white cross-section. The results of the water test for *T. hemsleyanum* roots from the seven sites showed that the water infusion was viscous; the viscosity of the water infusion of Zhejiang *T. hemsleyanum* roots was higher than those of Guangxi and Hunan roots. Figure 1 the color of the powder after drying and crushing. There were three categories of dry powder color: Brown (GX-BS, ZJ-TZ, and HN-FH), light brown (GX-GL, ZJ-HC, and ZJ-WZ), and gray (ZJ-HZ).

Microscopic Characteristics

Microscopic Observations of the Cross-Section of the Root

Tissue slices showed ring formation for the *T. hemsleyanum* root cambium. The cork layer was thin with approximately 5 layers of cork cells and contained mucous cells and parenchymal cells. The mucous cells were round or square with 50-120 μm diameter and the parenchymal cells showed the presence of abundant single starch granules. *T. hemsleyanum* starch grains showed significant differences in diameter, crystal shape, and vessel arrangement (Fig. 2). The starch grains of Guangxi roots were 5-25 μm in diameter and contained calcium oxalate needle crystal bundles (80-120 μm diameter) with lupus like or scattered distribution and rare calcium oxalate cluster crystals (10-20 μm diameter). However, the starch granules of Hunan and Zhejiang roots were 10-25 μm in diameter and contained many calcium oxalate clusters (20-100 μm in diameter) and few needle crystals (50-120 μm in diameter), which were mostly scattered distribution. Moreover, in roots from some sites, the vessels were arranged radially (such as GX-BS, GX-HC, ZJ-TZ, and ZJ-WZ), while in roots from other sites, the vessels were arranged in a “V” shape (such as GX-GL and HN-FH).

Microscopic Observation of Dry Root Powder

The characteristic features of a powdered herbal drug are used to identify and detect the drug sample in powder form. Microscopic observation of *T. hemsleyanum* dry root powder showed the presence of starch grains, mucus

cells, and calcium oxalate needle crystal bundles and cluster crystals. The powder contained many calcium oxalate needle crystal bundles (80-100 μm in length), with more aggregated distribution and few cluster crystals. The starch granules were mainly single grains with a circle shape, 4-22 μm in diameter, and punctate umbilical points. The *T. hemsleyanum* root powder from different origins showed variations in the types of vessels. Microscopic observations showed that the vessels of Guangxi *T. hemsleyanum* powder were mainly trapezoidal vessels, occasionally reticulated vessels, and bordered pit vessels with diameters ranging from 20 to 75 μm. The vessels of Zhejiang *T. hemsleyanum* powder were exclusively trapezoidal vessels with 30-90 μm diameter. Microscopic observation of Guangxi and Zhejiang *T. hemsleyanum* root powder showed brown patches and yellowish brown cork cells with a polygonal surface and a slightly thick pendant wall (Fig. 3).

Standard Curve

The regression equation of the curve, correlation coefficients (r) and linear range (μg/ml) were calculated as followed Table 4.

Phytochemical Evaluations

Various chemical tests were performed as described earlier for the extracted samples and the results revealed the presence of polysaccharides, phenols, flavonoids, and amino acids as organic phytoconstituents of the roots (Fig. 4a-d). Table 3 shows the detailed results of the phytochemical analysis.

Flavonoids Content

Flavonoids derivatives show antiviral, anti-inflammatory, and anticancer, particularly radical scavenging, activities (Hossain *et al*., 2011). They ubiquitously occur in plants and provide beneficial health effects. Figure 5 and Table 5, the total flavonoids content of the extracted samples from the seven sites were determined by comparing them with the standard rutin concentration; the results showed that significant differences in the total flavonoids content of *T. hemsleyanum* root extract from all sites, except Guangxi Guilin and Zhejiang Hangzhou.

Polyphenolic Content

Phenolic compounds are important plant constituents because of their redox properties (Aryal *et al*., 2019). The results of the present study showed that the root samples collected from Baise, Guangxi had higher total polyphenolic contents, while the samples collected from Wenzhou, Zhejiang showed the lowest values (Fig. 5). However, no significant differences were observed in the total polyphenol content of *T. hemsleyanum* root extracts from Zhejiang (Wenzhou and Taizhou), Guangxi Guilin and Hunan; moreover, no significant difference was noted in the total polyphenol content in Hechi, Guangxi and Hangzhou, Zhejiang (Table 5).

Catechin, Quercetin, Kaempferol and Rutin Contents

The contents of four monomers, including catechin, quercetin, kaempferol, and rutin in the *T. hemsleyanum* root extracts from different origins, were determined by HPLC (Fig. 6). Based on the results with rutin and catechin as indicators, it was impossible to differentiate between Guangxi Hechi *T. hemsleyanum* and Zhejiang Wenzhou *T. hemsleyanum*; furthermore, with quercetin and kaempferol as indicators, Guangxi Guilin *T. hemsleyanum* could be differentiated from those of Zhejiang, Hunan and other parts of Guangxi.

Table 3: Qualitative phytochemical evaluations of *T. hemsleyanum* root extracts

Phytoconstituents name	Polysaccharide	Phenolic acids	Flavonoids	Amino acids
GX-BS	+	+++++	+++++	+
GX-GL	+	++	++	++++
GX-HC	++	++	++	++++
HN-FH	++	+++++	++++	+
ZJ-HZ	+++	++	++	+++++
ZJ-TZ	+++	++	+++	++
ZJ-WZ	+	+	+	+++

+ present

Table 4: Standard curve for standard solutions

Name	Standard curve	r	Linear range (μg/ml)
Total flavonoids	Y = 0.0110X-0.0108	0.9993	4.14-33.120
Total phenolics	Y = 0.1126X+0.0105	0.9993	2.49-6.2100
Quercetin	Y = 58038X-101119	0.9998	52.50-262.50
Kaempferol	Y = 72875X-110922	0.9998	41.30-206.30
Catechin	Y = 11332X-19845	0.9995	50.00-250.00
Rutin	Y = 39998X-136801	0.9995	100.00-500.00
Ascorbic acid	Y = 1.1864X+0.2535	0.9662	100.00-500.00

Table 5: Analysis of differences in the active ingredients of *T. hemsleyanum* roots from different origins

Tukey's multiple comparisons test	Total flavonoids	Total polyphenols	Quercetin	Kaempferol	rutin	Catechin
GX-BS vs. GX-GL	****	****	ns	**	****	ns
GX-BS vs. GX-HC	****	***	ns	ns	****	****
GX-BS vs. HN-FH	****	****	ns	ns	***	****
GX-BS vs. ZJ-HZ	****	****	ns	ns	****	ns
GX-BS vs. ZJ-TZ	****	****	ns	ns	*	****
GX-BS vs. ZJ-WZ	****	****	ns	ns	****	****
GX-GL vs. GX-HC	****	***	*	**	ns	****
GX-GL vs. HN-FH	****	ns	*	**	ns	****
GX-GL vs. ZJ-HZ	ns	*	*	*	**	ns
GX-GL vs. ZJ-TZ	****	ns	*	ns	***	****
GX-GL vs. ZJ-WZ	****	ns	*	*	ns	****
GX-HC vs. HN-FH	****	***	ns	ns	ns	****
GX-HC vs. ZJ-HZ	****	ns	ns	ns	**	****
GX-HC vs. ZJ-TZ	****	****	ns	ns	***	****
GX-HC vs. ZJ-WZ	**	****	ns	ns	ns	ns
HN-FH vs. ZJ-HZ	****	*	ns	ns	****	****
HN-FH vs. ZJ-TZ	****	ns	ns	ns	ns	****
HN-FH vs. ZJ-WZ	****	ns	ns	ns	**	****
ZJ-HZ vs. ZJ-TZ	****	**	ns	ns	****	****
ZJ-HZ vs. ZJ-WZ	****	****	ns	ns	*	****
ZJ-TZ vs. ZJ-WZ	****	ns	ns	ns	****	****

"ns": P>0.05; "*": P<0.05; "**": P<0.01; "***": P<0.001; "****": P<0.0001

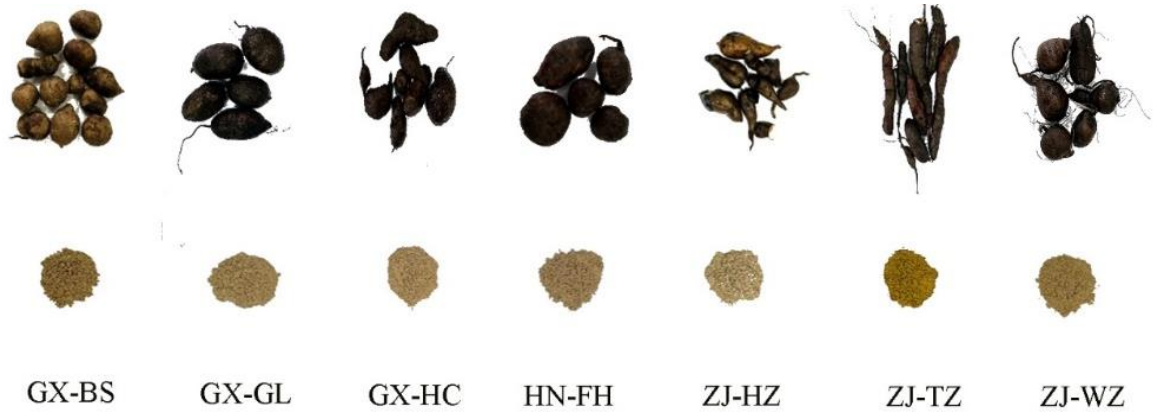

Fig. 1: *T. hemsleyanum* root and dried powder from different sites

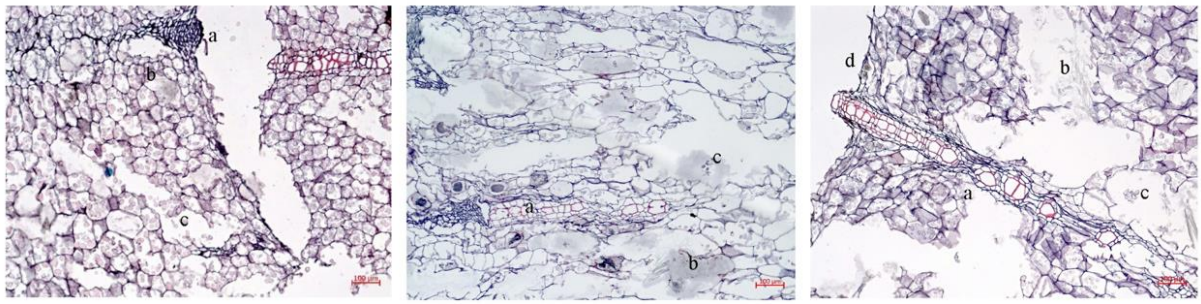

Fig. 2: Microstructure of the cross section of *T. hemsleyanum* roots from different sites; (a) Vessels (b) Calcium oxalate acicular crystals (c) Starch grains (d) Calcium oxalate cluster crystals

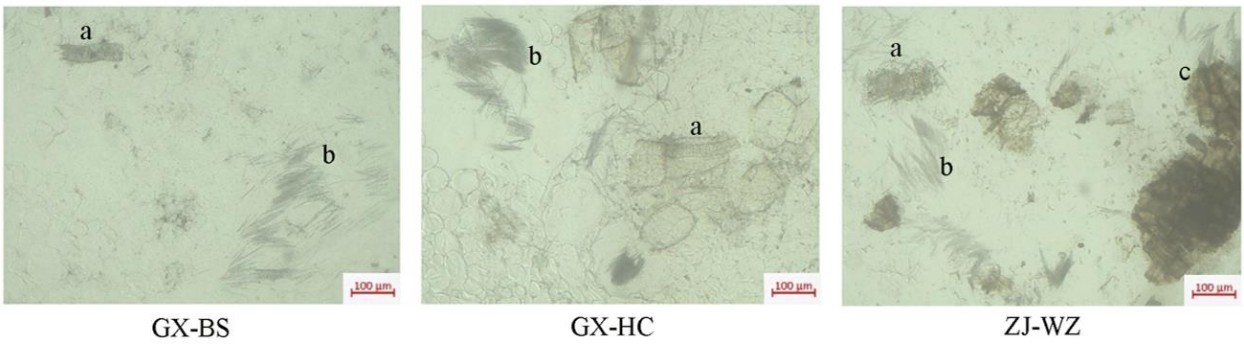

Fig. 3: Micrographs of *T. hemsleyanum* root powder from different origins; (a) Vessels (b) Calcium oxalate acicular crystals (c) Cork cells

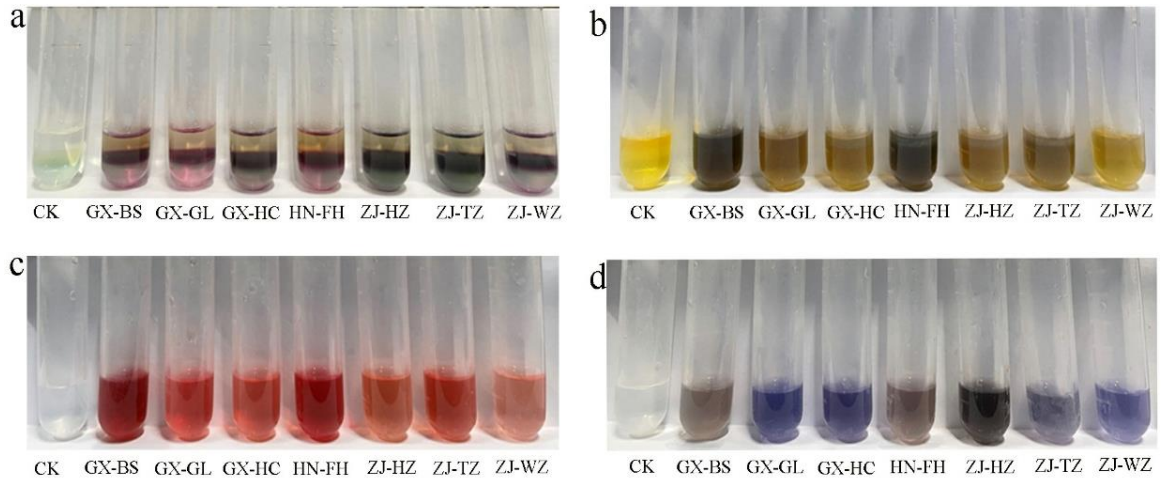

Fig. 4: Phytochemical evaluations results for the extracts of *T. hemsleyanum* roots from different origins; (a) Polysaccharide, (b) Phenolic acids, (c) Flavonoids, (d) Amino acids

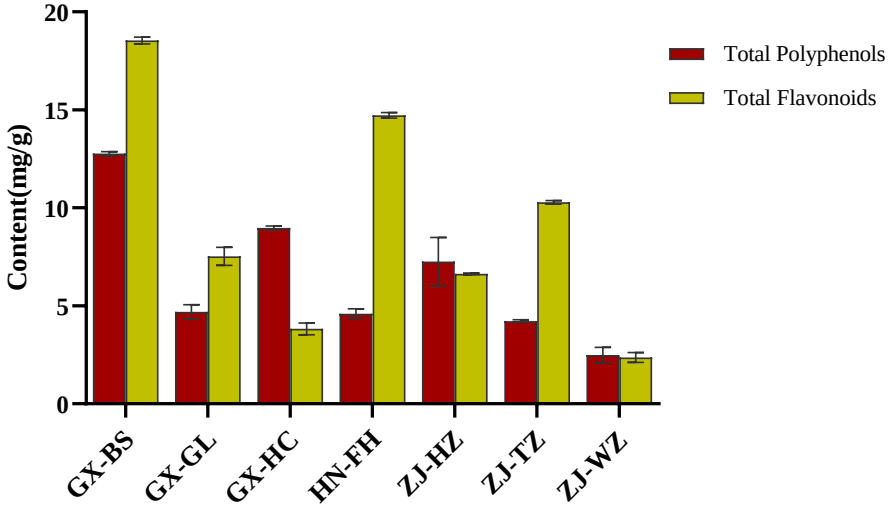

Fig. 5: Total flavonoids and total polyphenols content of *T. hemsleyanum* root extracts from different sites

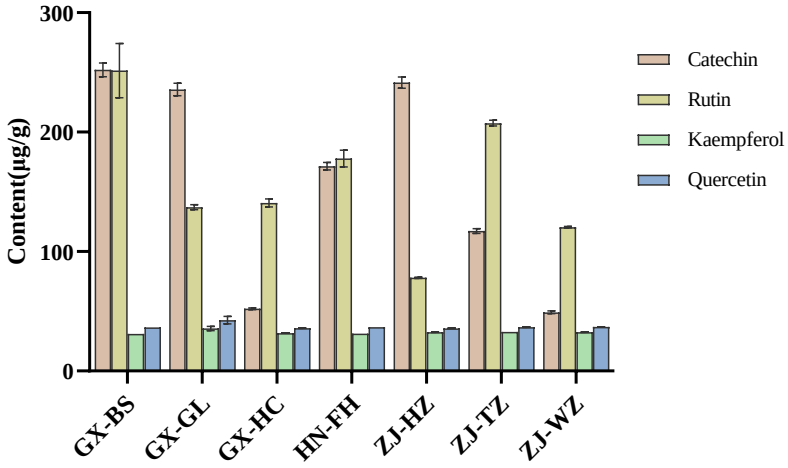

Fig. 6: Catechin, rutin, kaempferol, and quercetin contents in *T. hemsleyanum* root extracts from different origins

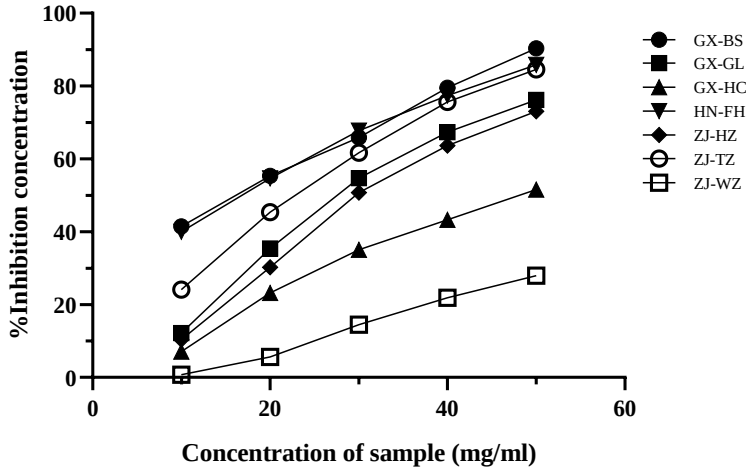

Fig. 7: Inhibition of DPPH radicals by ethanol-water [60:40 (v/v)] extracts of *T. hemsleyanum* roots

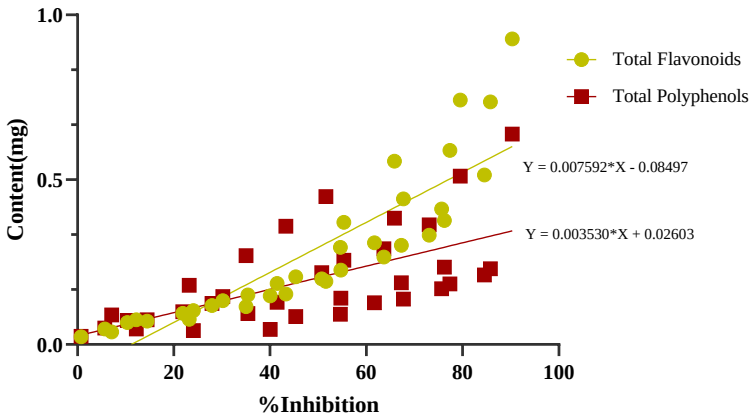

Fig. 8: Results of pearson correlation analysis

Table 6: DPPH radical scavenging activity of *T. hemsleyanum* root extracts ($\bar{x}$ ±s)

Name	IC_{50} / (mg/mL)	AEAC (mg AAE/g sample)
GX-BS	13.844±06.406[ab]	14.655±004.52800
GX-GL	27.089±02.288[a]	6.624±000.30000
GX-HC	47.891±07.185[a]	3.763±000.03500
HN-FH	14.995±02.434[ab]	12.035±000.23700
ZJ-HZ	30.033±02.498[a]	5.975±000.27500
ZJ-TZ	20.950±02.136[ab]	8.570±000.26200
ZJ-WZ	81.690±21.388[a]	2.254±000.24000
VC	0.180±00.025	1000.000±113.40200

"a": P<0.05 vs. ZJ-WZ; "b": P<0.05 vs. GX-HC

Antioxidant Activities and Pearson Correlation Coefficient

To more accurately evaluate the antioxidant activity between samples, the solution mass concentration IC_{50} at the clearance ratio of 50% radicals was commonly compared and a lower IC_{50} value indicated a higher radical scavenging capacity. Figure 7 showed the results of the DPPH clearance ratio for *T. hemsleyanum* root extracts. Table 6 presented the scavenging activities of root extracts that correspond to their concentrations inhibiting 50% of the DPPH radicals (IC_{50}). The IC_{50} values of *T. hemsleyanum* root extracts ranged from 13.844 to 81.69 mg/mL, equivalent to 14.655-2.254 mg AAE/g sample. Although these values were lower than those of the commonly used antioxidant VC (synthetically synthesized), it still indicates a high free radical scavenging capacity. In the present study, the extracts of *T. hemsleyanum* roots from Guangxi, Zhejiang, and Hunan sites showed strong antioxidant activity; moreover, the differences in the antioxidant activity of the root extracts were observed not only for different provinces but also for different regions from the same province. The Pearson correlation analysis revealed that the antioxidant activity of the root extracts and their respective total flavonoids and polyphenols contents were positively correlated ($r = 0.8786$, $P<0.0001$; $r = 0.6391$, $P<0.0001$) (Fig. 8).

Discussion

Compared to previous studies, the samples collected in the present study were more extensively and comprehensively evaluated. Macroscopic, microscopic, and phytochemical analyses were first performed. Subsequently, the functional components were extracted from the roots and their content was estimated. Finally, the antioxidant activity of *T. hemsleyanum* root extracts from different origins was determined and the correlation between the antioxidant activity and the functional component content of the roots was analyzed based on the Pearson correlation coefficient. The results of the study showed that the root tubers of *T. hemsleyanum* from Guangxi, Hunan, and Zhejiang all contained flavonoids, polysaccharides, polyphenols, and amino acid components, but the active ingredients and contents and antioxidant activities of *T. hemsleyanum* from different regions differed greatly. The quality of *T. hemsleyanum* from different origins varies and the lack of scientific and effective quality evaluation methods was not conducive to the expansion of the scale of the *T. hemsleyanum* industry and the development of subsequent healthy products. However, the results of macroscopic and microscopic studies on *T. hemsleyanum* from different origins in this study provided an effective reference for future quality differentiation of *T. hemsleyanum*.

The functional components of *T. hemsleyanum* roots, particularly the total flavonoids composition, varied greatly among different sites. There were four possible reasons for this difference. The first reason was the altitude, annual average precipitation, and an annual average temperature of the sites. Previous studies had shown that the total flavonoids content of *T. hemsleyanum* was significantly positively correlated with altitude and annual average precipitation, while it shows a significant negative correlation with the annual average temperature of the province (Ji *et al*., 2017). The second reason was a phytogeographic region and plant nutrition. The variation could be attributed to the difference in the phytogeographic region and plant nutrition, which could modify the degree of secondary metabolites produced by the plant (Mekonnen *et al*., 2018). The third reason was the shading area. Under different shading conditions, the total flavonoids content in the roots and leaves of *T. hemsleyanum* first increases rapidly and then slows down with the increase in shading area, thus indicating that proper shading treatment can promote the accumulation of its content (Hu *et al*., 2019). The fourth reason was the number of growth years. Under the same growth environment, the more the number of growth years of the tuberous root of *T. hemsleyanum*, the more the accumulation of total flavonoids (Ying, 2021).

In addition, in the present study, the content of kaempferol and quercetin in the root extracts of *T. hemsleyanum* was generally low; this finding could be related to the sample collection time. The content of these compounds in *T. hemsleyanum* varies from month to month, with the highest content of quercetin and kaempferol in June and April-May, respectively (Li *et al*., 2019). However, because of the COVID-19 pandemic situation, the sample collection time in the present study was from July to August. The antioxidant activity of *T. hemsleyanum* is closely related to its content of total flavonoids and total polyphenols, particularly the content of total flavonoids. Hence, future research studies should isolate and purify total flavonoids from *T. hemsleyanum* roots and investigate theirs *in vivo* antioxidant activity. Finally, although the roots of *T. hemsleyanum* are often used as medicinal herbs, the entire herb can be used for preparing

medicinal formulations. Thus, if conditions permit, the entire *T. hemsleyanum* plant could be collected from different sources for further investigation.

Conclusion

The results of the present study showed that there were differences in appearance, microstructure, constituent content and antioxidant activity among *T. hemsleyanum* from different origins and the differences also occurred in different regions of the same province; it was also found that the root extract of *T. hemsleyanum* had higher antioxidant activity and was a better choice for the treatment of diseases caused by oxidative stress. In addition, a comprehensive evaluation of *T. hemsleyanum* extract based on the content of total flavonoids, polyphenols, rutin, catechin, and its antioxidant activity showed that the quality of *T. hemsleyanum* from Baise, Guangxi was the best, followed by Feng Huang, Hunan, and Wen Zhou, Zhejiang was the worst. This study provided a reference for the quality evaluation of *T. hemsleyanum* from different origins and provided a direction for clinical practice on the antioxidant of *T. hemsleyanum* extract.

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Author's Contributions

Guang Mei Li: Collected the plants; conducted macroscopic and microscopic analyses, determination of polyphenol and flavonoids content and antioxidant activities; and wrote the manuscript.

Jian Ge: Performed sample collection and identification.

Yongli Zheng and Aichun Xu: Performed analysis and interpretation of data and manuscript review.

Qian Li: Provide critical reading and insightful revision of the manuscript.

Ethics

The corresponding author confirms that all the other authors have read and approved the manuscript and that no ethical issues are involved in the present study.

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