

DIFFERENTIAL ANALYSIS OF QUALITY TRAITS BETWEEN FUPU AND VIETNAMESE *CAMELLIA CHRYSANTHA* IN CHINA

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(Received 8th Jan 2025; accepted 22nd Apr 2025)

Abstract. This study aims to analyze the phenotypic traits and multi-index chemical composition of Fupu and Vietnamese *Camellia chrysantha* in China to clarify their quality trait differences, and provide a basis for identifying and classifying *C. chrysantha* germplasm and selecting superior varieties. Ecological and statistical methods were used to evaluate the quality trait differences of the two varieties of *C. chrysantha*. Fupu and Vietnamese *C. chrysantha* exhibit distinct phenotypic traits and significant differences in chemical composition. According to the cluster analysis results, both varieties of *C. chrysantha* can be divided into six clusters. Cluster YN04, YN23, YN28, YN01, YN19, YN34, YN20, YN25, YN27, YN30, YN32, YN03, YN12, YN05, YN35, and YN39 are characterized” and “Cluster YN13, YN09, YN29, YN16, YN21, YN07, YN40, YN10, YN24, YN08, YN31, YN14, and YN36 are characterized by linear leaves, small leaf area, short petioles, light leaves, and high content of total flavonoids and polysaccharides. Compared to Fupu *C. chrysantha*, Vietnamese *C. chrysantha* has shorter petioles, a distinct leaf area classification, and higher contents of total flavonoids, polyphenols, and polysaccharides. Fupu and Vietnamese *C. chrysantha* exhibit significant differences in quality traits, with Vietnamese *C. chrysantha* being superior in quality.

Keywords: *Camellia chrysantha*, phenotypic traits, quality traits, cluster analysis, chemical composition

Introduction

Camellia chrysantha, known as *C. nitidissima*, is classified as a nationally first-class rare and endangered plant in China, celebrated as the “giant panda of the plant kingdom” and the “queen of the tea family” (Gao et al., 2022). Its leaves contain various active compounds such as flavonoids, tea polyphenols, and tea polysaccharides, which exhibit pharmacological effects including lowering blood pressure, reducing blood sugar, and providing anti-tumor, anti-allergic, and antioxidant properties. (Yoshikawa et al., 2008; Song et al., 2011; Matsuda et al., 2012; Xing et al., 2013; Wei et al., 2015; Dai et al., 2016; and Zhang et al., 2020). Natural populations of *C. chrysantha* are primarily found in the Fangpu District of Fangchenggang City, Guangxi Province, China, and northern Vietnam (Liang, 1993; Lu et al., 2005; Zhao et al., 2021). Fangchenggang, Guangxi, features a humid subtropical monsoon climate that is hot and humid (Chen, 2007). Northern Vietnam experiences a tropical monsoon climate characterized by cool, dry winters and warm, humid summers (Tran et al., 2005; Bui-Minh et al., 2024). The climatic disparities in temperature and humidity between these regions are crucial factors influencing the growth of *C. chrysantha*. Current research on

C. chrysantha predominantly focuses on extraction techniques, pharmacological effects, cultivation methods, leaf epidermal characteristics, and stress resistance (Xie et al., 2006; Zhou et al., 2019; Beghin et al., 2010; Wang et al., 2020; Qin et al., 2020). There is limited research specifically addressing variations in the quality of *C. chrysantha*. Phenotypic traits are often utilized as the basis for plant classification (Chen et al., 2019), while chemical composition reflects the quality and characteristics of medicinal herbs. The correlation between the phenotypes and characteristics of medicinal herbs enables the classification and identification of *C. chrysantha* Germplasm based on phenotypes (Yang, 2021). Therefore, the present study aims to deal with the differences in leaf phenotypic traits and chemical composition among various varieties of Fangpu *C. chrysantha* and Vietnamese *C. chrysantha* in China. The objective of the study was carried out by correlation and cluster analyses methods and it aims to provide a foundation and guidance for evaluating quality, classifying varieties, and selecting superior Germplasm of *C. chrysantha*.

Materials and methods

Collection of plant materials

In January 2019, samples of Fangpu *C. chrysantha* and Vietnamese *C. chrysantha* were collected from the Chongfeng *C. chrysantha* plantation base in Dalu Village, Jiangping Town, and Dongxing City (21.640619, 108.132728, 23.51) (Fig. A1), Fangchenggang City, and Guangxi Province in China (Fig. 1). Each plant was coded as YN04, YN23, YN28, YN01, YN19, YN34, YN20, YN25, YN27, YN30, YN32, YN03, YN12, YN05, YN35, YN39” and YN13, YN09, YN29, YN16, YN21, YN07, YN40, YN10, YN24, YN08, YN31, YN14, and YN36. Forty plants of each variety were collected, totaling 80 plants. The collected samples were transported to the production workshop of Dongxing Tianzhi Agricultural Technology Co., Ltd., frozen at -40°C for 2 h. and then freeze-dried at -45°C. After drying, the leaves were crushed and sieved through a No. 3 mesh sieve, stored in dry-sealed kraft paper bags, and kept in the refrigerator until further experimental use.

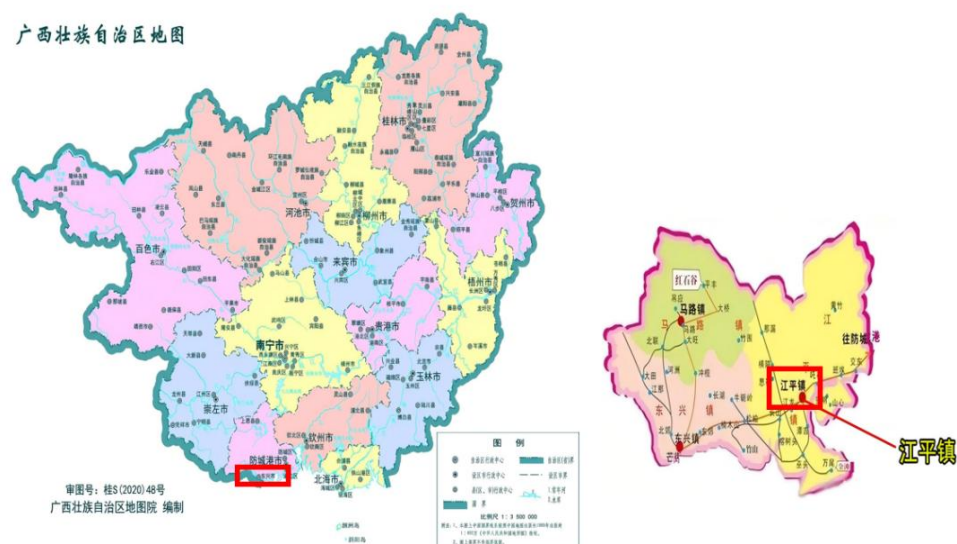


Figure 1. Map showing the locations of the sampling site

Methods

The preparation of rutin (China National Institutes for Food and Drug Control, Batch No.: B20771-20 mg), gallic acid (China National Institutes for Food and Drug Control, Batch No.: B20103-20 mg), and glucose (110833-200904-100 mg) was used as reference standard solutions, and the determination of total flavonoids, total polyphenols, and polysaccharide contents followed the methods outlined in the 2015 edition of the Chinese Pharmacopoeia.

Construction of standard curves

Standard solutions of rutin ($0.2 \text{ mg}\cdot\text{mL}^{-1}$), gallic acid ($0.01 \text{ mg}\cdot\text{mL}^{-1}$), and glucose ($0.1 \text{ mg}\cdot\text{mL}^{-1}$) were prepared. The coloration and measurement processes were the same as those used for determining total flavonoids, total polyphenols, and polysaccharide contents. Their absorbance was measured using UV-Vis spectrophotometry (Model 521801007, Shanghai Jinghua Technology Instrument Co., Ltd.). A standard curve was plotted with the concentration of standard solutions (x-axis) and absorbance (y-axis), resulting in regression equations, correlation coefficients, and linear ranges (Table 1).

Table 1. Construction of standard curves

Compound	Wavelength	Regression equation	Correlation coefficient	Linear range
Total flavonoids	510 nm	$y = 0.266x - 0.003$	0.9999	0-2.4
Total polyphenols	765 nm	$y = 0.0683x + 0.0031$	0.9925	0-2.8
Polysaccharides	490 nm	$y = 0.06236x + 0.0096$	0.9995	0.05-0.4

Phenotypic characterization:

Leaf morphology, including leaf shape, leaf apex, leaf base morphology, and vein count, was observed visually. Leaf length, leaf width, and petiole length were measured using a ruler, and leaf area was calculated based on leaf length and width (Figures A2–A7 in the Appendix). Fresh and dry weights of leaves were measured using an electronic balance. For analysis, non-numerical traits such as leaf shape, leaf apex, leaf base, and vein count were converted into dummy variables, with conversion values detailed in Table 2. The converted data, along with leaf area, length-to-width ratio, petiole length, leaf fresh weight, leaf dry weight, and component contents, were recorded in the overall table for subsequent statistical analysis.

Table 2. Dummy variable conversion table for phenotypic traits

Dummy variables	Leaf shape				Leaf apex				Leaf base		Vein count	
	Lanceolate	Elliptic	Linear	Oblong	Acuminate	Acute	Tapered	Obtuse	Decurrent	Rounded	High	Moderate
X1	1	0	0	0	1	0	0	0	0	1	0	1
X2	0	1	0	0	0	1	0	0				
X3	0	0	1	0	0	0	1	0				

Data statistical analysis

After standardizing all data, hierarchical clustering based on Euclidean distance and Ward's method was performed using R software (version R×64 3.6.2) (Chan and Chan,

2018). Single-factor analysis of variance (ANOVA), independent samples t-test, and Pearson correlation analysis were conducted using SPSS 23.0 software.

Results and analysis

Analysis of phenotypic differences between Fangpu C. chrysantha and Vietnamese C. chrysantha

After measuring leaf phenotypes, leaf area, petiole length, length-to-width ratio, leaf fresh weight, and dry weight of the two varieties of *C. chrysantha*, significant differences were observed in morphological characteristics at the individual plant level (Tables 3 and 4). Table 3 shows that leaf shapes mainly include four types: lanceolate, elliptic, linear, and oblong. Leaf apices are represented by four shapes: acuminate, acute, tapered, and obtuse, and leaf bases by two shapes: decurrent and rounded. Differences in leaf veins primarily manifest in the number and depth of veins. Fangpu *C. chrysantha* is predominantly linear, accounting for 62.5%, while Vietnamese *C. chrysantha* is mostly oblong, at 35%. Both Fangpu and Vietnamese *C. chrysantha* have predominantly acute leaf apices, accounting for 37.5% each. Decurrent leaf bases are more common in Fangpu *C. chrysantha*, with a proportion of 77.5%, while rounded leaf bases are more common in Vietnamese *C. chrysantha*, with a proportion of 62.5%. Fangpu *C. chrysantha* has mostly medium-sized veins, accounting for 95%, whereas Vietnamese *C. chrysantha* has a majority of veins of varying sizes, accounting for 60%. From Table 4, it is evident that Vietnamese *C. chrysantha* and Fangpu *C. chrysantha* exhibit significant variations and relatively high coefficients of variation (RSD values) in quantitative phenotypic traits such as leaf area, petiole length, length-to-width ratio, leaf fresh weight, and dry weight, indicating considerable differences among individual strains of the two varieties.

From the independent samples t-test results of Fangpu and Vietnamese *C. chrysantha*, significant differences between the two varieties were observed in leaf area, leaf fresh weight, and leaf dry weight ($P < 0.05$), while differences in length-to-width ratio and petiole length were not statistically significant. Please refer to Table 5 for specific details of the independent samples t-test results.

Table 3. Frequency of non-numerical phenotypic variables

Phenotypic	Variable	Fangpu <i>C. chrysantha</i> percentage (%)	Vietnamese <i>C. chrysantha</i> percentage (%)
Leaf shape	Lanceolate	10	27.5
	Elliptic	20	12.5
	Linear	62.5	25
	Oblong	7.5	35
Leaf apex	Acuminate	27.5	12.5
	Acute	37.5	37.5
	Tapered	35	32.5
	Obtuse	0	17.5
Leaf base	Decurrent	77.5	37.5
	Rounded	22.5	62.5
Number of fine veins	High	0	60
	Moderate	95	40
	Low	5	0

Table 4. Basic information of numerical phenotypic variables

Phenotypic	Variety	Minimum value	Maximum value	Range	Average	Standard deviation	RSD (%)
Leaf area (cm ²)	Fangpu	42.13	113.36	71.23	77.80	16.26	21.00
	Vietnamese	34.26	137.51	103.25	78.42	27.34	35.00
Petiole length (cm)	Fangpu	1.03	2.17	1.14	1.45	0.23	16.00
	Vietnamese	0.47	1.33	0.86	0.87	0.21	24.00
Length-to-width ratio of leaf (cm)	Fangpu	2.01	5.37	3.36	2.61	0.51	20.00
	Vietnamese	1.82	2.93	1.11	2.27	0.25	11.00
Leaf fresh weight (g)	Fangpu	0.87	1.98	1.11	1.34	0.28	21.00
	Vietnamese	0.33	2.62	2.29	1.29	0.48	37.00
Leaf dry weight (g)	Fangpu	0.30	1.49	1.19	0.64	0.19	30.00
	Vietnamese	0.16	1.41	1.25	0.66	0.26	39.00

Table 5. Independent samples *t*-test results for phenotypic variables

Pairwise differences	Mean difference	Standard deviation of difference	95% Confidence interval		F Value	Degrees of freedom	Sig. value
			Lower limit	Upper limit			
FP leaf area - YN leaf area	-0.617	5.030	-10.631	9.396	16.381	78.000	0.000
FP length-to-width ratio - YN length-to-width ratio	0.338	0.090	0.159	0.517	1.432	78.000	0.237
FP petiole length - YN petiole length	0.575	0.049	0.477	0.673	0.020	78.000	0.887
FP leaf fresh weight - YN leaf fresh weight	0.045	0.088	-0.130	0.221	8.156	78.000	0.005
FP leaf dry weight - YN leaf dry weight	-0.018	0.051	-0.120	0.084	5.821	78.000	0.018

Differences in total flavonoid, total polyphenol, and polysaccharide content between Fangpu C. chrysantha and Vietnamese C. chrysantha

Single-factor analysis of variance was performed on the total flavonoid, total polyphenol, and polysaccharide content of the two varieties of *C. chrysantha*. The results are shown in *Table A1 (Appendix)*. In terms of total flavonoid content, FP06 and FP24, FP30 and FP29 exhibited extremely significant differences compared to other genotypes ($P < 0.01$). FP04 showed significant differences compared to FP32 ($P < 0.05$) and extremely significant differences compared to other genotypes ($P < 0.01$). FP27 and FP28 showed significance ($P < 0.05$) and extremely significant differences compared to other genotypes ($P < 0.01$). FP26 differed significantly from FP10, 12, 21, 22, 23, 37, and 31 ($P < 0.05$), with differences reaching extremely significant levels compared to the remaining genotypes ($P < 0.01$). YN15, 21, 24, and YN26 exhibited extremely significant differences compared to other genotypes ($P < 0.05$). YN04, 22, 24, and 27 differed significantly from YN23 and 37 ($P < 0.05$), with extremely significant differences compared to other genotypes ($P < 0.01$). YN06 and 32 differed significantly ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN11, 20 showed no significant differences compared to YN13, 19, 30, 33, 39, but differed significantly from other genotypes ($P < 0.05$).

In terms of total polyphenol content, FP16, 21, 30, FP26, 27, and FP37 exhibited extremely significant differences compared to other genotypes ($P < 0.01$). FP08, 10, 36 differed significantly from other genotypes ($P < 0.05$). FP04, 13 showed no significant differences compared to FP06, 19, 22, but differed significantly from FP35 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). FP03, 28 showed no significant differences compared to FP01, 02, 05, 07, 11, 18, 20, 23, 24, 31, 32, 38, 40, but differed significantly from FP17, 39 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN38 exhibited extremely significant differences compared to other genotypes ($P < 0.01$). YN24 showed no significant differences compared to YN02, 18, and 22 but differed significantly from other genotypes ($P < 0.05$). YN23 showed significant differences compared to YN22, 24 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN09, 11 exhibited significant differences compared to YN07 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN07 exhibited significant differences compared to YN11 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN33, 34, 37 showed no significant differences compared to other genotypes ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$).

In terms of polysaccharide content, FP14, 34, 04, FP35, 20, and FP36 exhibited extremely significant differences compared to other genotypes ($P < 0.01$). FP01, 03, 06 exhibited extremely significant differences compared to other genotypes ($P < 0.01$). FP08 showed no significant differences compared to FP10 and showed significant differences compared to FP23 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). FP09, 16, 18 showed no significant differences compared to FP17 and showed significant differences compared to FP07 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). FP32 showed no significant differences compared to FP28 and showed significant differences compared to FP15 ($P < 0.05$). FP19 showed no significant differences compared to FP02, 05, 13, 22, 31, 33, 38 and showed significant differences compared to FP09, 38 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN01, 08, 14, 24 showed no significant differences compared to YN23, 31 and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN28 showed no significant differences compared to YN23, 31 and showed significant differences compared to YN29 ($P < 0.05$). YN29 showed no significant differences compared to YN02, 05, 07, 17 and showed extremely significant differences compared to other genotypes ($P < 0.01$). YN34 showed no significant differences compared to YN03, 06, 10, 18, 19, 33 and showed significant differences compared to YN30 ($P < 0.05$) and showed extremely significant differences compared to other genotypes ($P < 0.01$).

Based on the independent samples t-test results for the components of Fupu and Vietnamese *C. chrysantha*, significant differences between the two varieties were found in total flavonoid and total polyphenol content ($P < 0.05$), while the difference in polysaccharide content was not significant. Refer to *Table 6* for details.

Correlation analysis of the content of three chemical components and leaf phenotypic traits

A correlation analysis was conducted between the content of total flavonoids, total polyphenols, and polysaccharides of the two varieties of *C. chrysantha* and their leaf phenotypic traits. The results are shown in *Table 7*. The selected phenotypic traits all

showed certain correlations with the three chemical components. Among them, there is a highly significant correlation between the three chemical components of Fangpu *C. chrysantha* and Vietnamese *C. chrysantha*. Lanceolate leaves are positively correlated with total flavonoids, total polyphenols, and polysaccharides, while linear leaves have a highly significant negative correlation with the three chemical components. Gradually tapering and acuminate leaf tips are negatively correlated with total flavonoids, total polyphenols, and polysaccharides, whereas sharply pointed leaf tips are positively correlated with them. Round blunt leaf bases have a highly significant correlation with total flavonoids and total polyphenols. Strains with a higher number of fine veins show a highly significant correlation with all three chemical components. Leaf petiole length has a highly significant negative correlation with them. The leaf length-to-width ratio has a highly significant negative correlation with total flavonoids and total polyphenols, and a significant negative correlation with polysaccharides. Leaf fresh weight, leaf dry weight, and leaf water content do not show a significant correlation with any of the three chemical components. In conclusion, to select strains with high contents of total flavonoids, total polyphenols, and polysaccharides, it is advisable to choose strains with lanceolate leaves, round blunt leaf bases, a higher number of fine veins, and short leaf petioles.

Table 6. Independent sample *t*-test results for component content

Pairwise differences	Mean difference	Standard deviation of difference	95% Confidence interval		F Value	Degrees of freedom	Sig. value
			Lower limit	Upper limit			
FP total flavonoids - YN total flavonoids	-14.212	0.629	-15.465	-12.959	52.589	78.000	0.000
FP total polyphenols - YN total polyphenols	-0.127	0.010	-0.147	-0.108	17.443	78.000	0.000
FP Polysaccharides - YN Polysaccharides	-0.652	0.122	-0.895	-0.409	0.840	78.000	0.362

Table 7. Correlation analysis of the content of three components and leaf phenotypic traits

	Total flavonoids	Total polyphenols	Polysaccharides
Total flavonoids	1	0.837**	0.558**
Total polyphenols	0.837**	1	0.542**
Polysaccharides	0.558**	0.542**	1
Leaf shape 1	0.191	0.183	0.028
Leaf shape 2	-0.060	-0.125	0.103
Leaf shape 3	-0.344**	-0.325**	-0.291**
Leaf apex 1	-0.147	-0.175	-0.164
Leaf apex 2	0.037	0.077	0.033
Leaf apex 3	-0.068	-0.073	-0.004
Leaf base	0.387**	0.381**	0.101
Number of fine veins	-0.560**	-0.570**	-0.309**
Leaf area	-0.030	0.120	0.050
Petiole length	-0.763**	-0.618**	-0.384**
Leaf length-to-width ratio	-0.397**	-0.367**	-0.247*
Leaf fresh weight	-0.036	0.096	0.016
Leaf dry weight	0.070	0.195	0.018
Leaf water content	-0.175	-0.202	0.004

**Correlation is significant at the 0.01 level (two-tailed)

*Correlation is significant at the 0.05 level (two-tailed)

Cluster analysis of Fangpu *C. chrysantha* and Vietnamese *C. chrysantha*

Based on leaf phenotypic traits and the content of three chemical components, hierarchical clustering using the Euclidean distance squared sum method was performed on 80 plants of the two varieties of *C. chrysantha*. The results are shown in *Figure 2*. The frequency of phenotypic traits and the average content of total flavonoids, total polyphenols, and polysaccharides for each cluster are shown in *Table 8*. From the figure and tables, it can be seen that the 80 plants of the two varieties of *C. chrysantha* can be divided into six major clusters. Cluster 1 contains 12 plants, including 11 from Fangpu and 1 from Vietnam, mostly with oval and linear leaf shapes, all with gradually tapering tips, decurrent bases, moderate fine vein count, medium leaf area, long petioles, and the lowest total polyphenol content. Cluster 2 contains 8 plants, all from Fangpu *C. chrysantha*, with all oval leaves, mostly acuminate and sharp tips, equal proportions of decurrent and round blunt bases, moderate fine vein count, medium leaf area and petiole length, second heaviest dry leaf weight, and low total flavonoid content. Cluster 3 contains 19 plants, all from Fangpu *C. chrysantha*, with all linear leaf shapes, mostly acuminate and sharp tips, and equal proportions of decurrent and round blunt bases, moderate leaf area, the longest petioles, the highest leaf length-to-width ratio, and the lowest total flavonoid content. Cluster 4 contains 16 plants, all from Vietnamese *C. chrysantha*, with long oval leaves, mostly acuminate and convex tips, round blunt bases, a high number of fine veins, the largest leaf area, short petioles, the smallest leaf length-to-width ratio, the heaviest leaves, the highest total polyphenol content, and the second highest content of total flavonoids and polysaccharides. This cluster represents the large-leaf short-petiole type with high component content. Cluster 5 contains 12 plants, including 10 from Vietnamese *C. chrysantha* and 2 from Fangpu *C. chrysantha*, with lanceolate leaves, mostly acuminate and sharp tips, and equal proportions of decurrent and round blunt bases, moderate to high fine vein count, the smallest leaf area, short petioles, and the lightest leaves. Cluster 6 contains 13 plants, all from Vietnamese *C. chrysantha*, with linear leaves, mostly acuminate and sharp tips, equal proportions of decurrent and round blunt bases, moderate to high fine vein count, small leaf area, the shortest petioles, a small leaf length-to-width ratio, light leaves, and the highest content of total flavonoids and polysaccharides, representing the small-leaf short-petiole type with high component content. Overall, based on the clusters obtained from the analysis, Fangpu and Vietnamese *C. chrysantha* each have their own phenotypic characteristics, which are generally consistent with the correlation analysis results. These traits can be used to distinguish clusters and predict chemical components. Compared to Fangpu *C. chrysantha*, Vietnamese *C. chrysantha* leaves are short-petioled with distinct leaf area classifications, lighter leaves, smaller leaf length-to-width ratios, and higher content of the three components, making them a high-quality variety.

Discussion

C. chrysantha, with its long history and numerous varieties, is predominantly found in Fangchenggang City, Guangxi Province, particularly in Fangcheng District. The leaves of this species have been classified as a national new resource food product in China (Hou et al., 2018). Vietnamese *C. chrysantha* is mainly distributed in northern Vietnam, which borders Fangchenggang City in China. There is a concept in traditional Chinese medicine called “authentic medicinal materials,” which emphasizes the regional characteristics and quality differences of medicinal materials from different

geographical origins (Zhang et al., 2021). The types and amounts of chemical compounds in these medicinal materials can vary, making it crucial to comprehensively evaluate the quality, select superior Germplasm, and identify advantageous production areas.

Table 8. Trait characteristics of each cluster of Fangpu and Vietnamese *C. chrysantha*

Items	Population					
	1	2	3	4	5	6
Number of individuals	12	8	19	16	12	13
Variety	Fangpu	Fangpu	Fangpu	Vietnamese	Vietnamese	Vietnamese
Leaf shape	Elliptic/linear	Elliptic	Linear	Oblong	Lanceolate	Linear
Leaf apex shape	Acuminate	Acute/Tapered	Acute/Tapered	Acute/Obtuse	Acute/tapered	Acute/Tapered
Leaf base shape	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
Number of fine veins	Moderate	Moderate	Moderate	High	High/Moderate	High/Moderate
Leaf area (cm ²)	77.76	79.22	79.05	105.09	58.69	61.08
Petiole length (cm)	1.41	1.35	1.53	0.99	0.86	0.75
Leaf length/width ratio (cm)	2.53	2.35	2.74	2.20	2.39	2.31
Fresh leaf weight (g)	1.43	1.40	1.25	1.68	0.98	1.09
Dry leaf weight (g)	0.65	0.73	0.62	0.89	0.46	0.53
Total flavonoids (%)	3.953	3.396	0.071	17.226	14.929	18.494
Total polyphenols (%)	0.124	0.129	0.126	0.263	0.231	0.251
Polysaccharides (%)	1.857	1.958	1.676	2.368	2.185	2.652

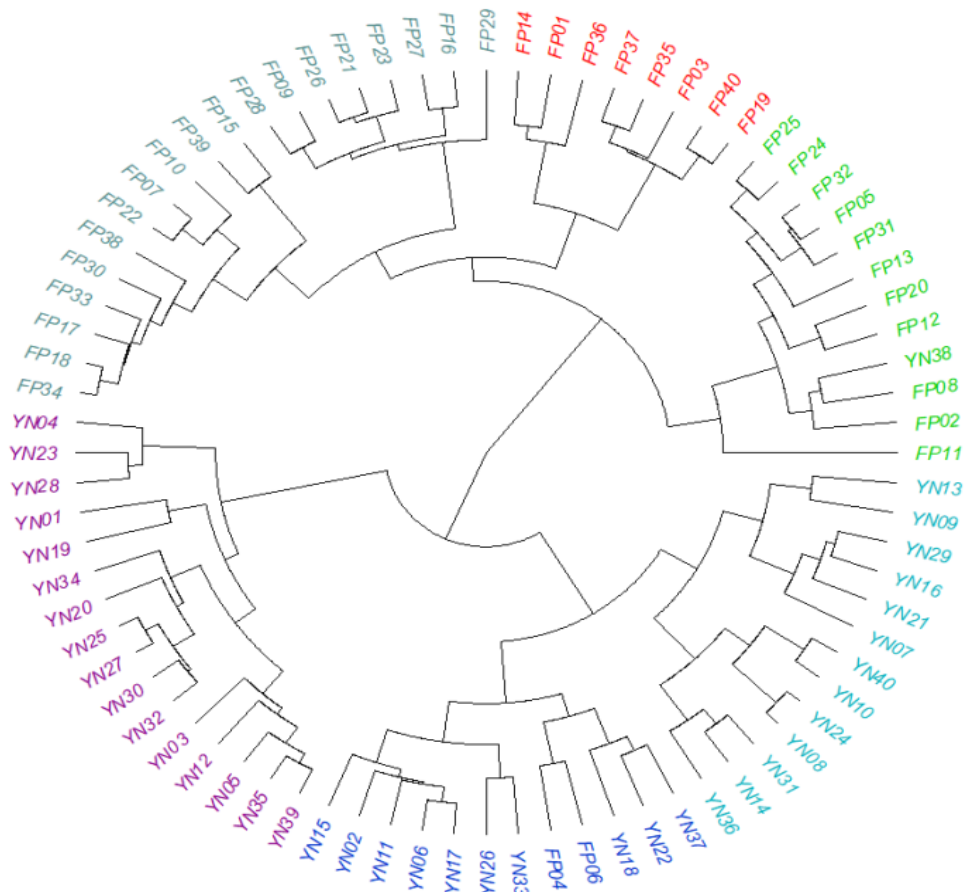


Figure 2. Cluster diagram of Fangpu and Vietnamese *C. chrysantha*

Since the first documented research on *C. chrysantha* in 1975, over forty years of study have accumulated. However, due to the unique growth environment and diverse compound content, a unified quality evaluation standard has not yet been established. Recent years have seen increasing research interest in *C. chrysantha*, with numerous studies reporting the presence of polyphenols, polysaccharides, flavonoids, saponins, and volatile substances in the plant (Wei et al., 2007; Yang, et al., 2008; Zhao et al., 2020 and Wang et al., 2022). Nevertheless, research focusing on its chemical composition remains unstructured. Differences in the chemical composition of medicinal materials are primarily influenced by genetics, while phenotypic traits are the external manifestations of genetic factors. Investigating the relationship between phenotypic traits and chemical compound content can provide a simpler and more effective method for selecting superior Germplasm (Wu et al., 2019; Chen et al., 2023).

This study investigates and compares the phenotypic traits, total flavonoid content, total polyphenol content, and polysaccharide content of Fangpu *C. chrysantha* and Vietnamese *C. chrysantha*. It was found that there are significant differences between the two varieties in both phenotypic traits and chemical compound content, consistent with the findings of Wu Siyu and Yang Rui (Yang, 2019; Li et al., 2019). This indicates a high level of genetic diversity, with most variations occurring within populations (Li et al., 2019). Further research revealed significant differences in leaf phenotypic traits between Fangpu and Vietnamese *C. chrysantha*. Vietnamese *C. chrysantha* leaves have a larger average leaf area but shorter petioles compared to Fangpu *C. chrysantha*. Additionally, the fresh and dry weights of Vietnamese *C. chrysantha* leaves are higher, indicating superior yield traits, consistent with the research of Li et al. (2018) and Wu Siyu (2019). The chemical component analysis showed that Vietnamese *C. chrysantha* has higher contents of the three main compounds, highlighting its greater medicinal value, aligning with the results of Wang et al. (2018). The cluster analysis demonstrated that although there is some overlap between the two varieties, they are distinctly grouped into different clusters. The varieties with higher compound content were primarily found in clusters 4 and 6, all of which are Vietnamese *C. chrysantha*. Therefore, to select varieties with high total flavonoid and polysaccharide content, it is advisable to choose Vietnamese *C. chrysantha* with linear leaves, sharp or pointed leaf tips, descending or rounded leaf bases, small leaf areas, and short petioles. For high total polyphenol content, varieties with long oval leaves, sharp or convex tips, rounded bases, numerous fine veins, large leaf areas, and heavy leaves should be selected.

Conclusion

There are significant differences in the quality traits between Fangpu and Vietnamese *C. chrysantha*. Among the six clusters identified in the cluster analysis, clusters 4 and 6 have the most potential for practical application, and both are composed entirely of Vietnamese *C. chrysantha*. The main characteristics of cluster 4 include long oval leaves, rounded bases, numerous fine veins, the largest leaf area, short petioles, the smallest leaf length-to-width ratio, the heaviest leaves, and the highest total polyphenol content. Cluster 6 is characterized by linear leaves, small leaf areas, the shortest petioles, small leaf length-to-width ratios, light leaves, and the highest total flavonoid and polysaccharide contents. In practical production, the phenotypic traits of the leaves

can be used to predict their quality traits, allowing for targeted selection of desired varieties. The results of this study provide a reference for the classification and selection of superior Germplasm of Fangpu and Vietnamese *C. chrysantha*.

Fund support. Guangxi University of Chinese Medicine Campus Project (XP023070); Key Project of Guangxi Natural Science Foundation (2020GXNSFDA297029); Guangxi First-Class Discipline Construction Project: Guijiao Scientific Research [2022] No. 1); Guangxi Higher Education Key Laboratory for the Research of Du-related Diseases in Zhuang Medicine (Gui Jiao Ke Yan (2022) No.10).

Conflict of interests. All the authors declared that there are no conflicts of interest in this research work.

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APPENDIX

Table A1. Measurement results of the content of three components ($x \pm s$, $n = 40$)

Variety code	Content (%)		
	Total flavonoids	Total polyphenols	Polysaccharides
FP01	3.671 ± 0.038efgFGH	0.111 ± 0.001jklIJK	2.646 ± 0.039cC
FP02	2.531 ± 0.038noMN	0.116 ± 0.001hijklHIJK	1.404 ± 0.024mnNO
FP03	3.551 ± 0.068fghFGH	0.107 ± 0.002IKL	2.729 ± 0.009cC
FP04	4.304 ± 0.001cBC	0.140 ± 0.001dD	1.020 ± 0.010sS
FP05	3.285 ± 0.039hjHI	0.110 ± 0.004klJK	1.373 ± 0.063mnNOP
FP06	6.521 ± 0.003aA	0.134 ± 0.003deDEF	2.656 ± 0.078cC
FP07	3.506 ± 0.076fghGH	0.117 ± 0.002hijklHIJK	1.627 ± 0.041jkJKL
FP08	3.019 ± 0.039jklIJ	0.162 ± 0.007bcBC	2.345 ± 0.078dD
FP09	2.376 ± 0.015oPNO	0.120 ± 0.004ghijklFGHIJK	1.903 ± 0.024gG
FP10	3.642 ± 0.067mnLMN	0.170 ± 0.005bB	2.282 ± 0.016deDE
FP11	3.484 ± 0.054fghGH	0.119 ± 0.005ghijklGHIJK	1.633 ± 0.036jkJKL
FP12	2.708 ± 0.015mnJKLMN	0.125 ± 0.001efghijEFGHIJ	1.679 ± 0.023ijIJKL
FP13	3.462 ± 0.038fghGH	0.138 ± 0.001dDE	1.393 ± 0.027mnNO
FP14	3.240 ± 0.067hjkHI	0.125 ± 0.005efghijEFGHIJ	3.275 ± 0.032aA
FP15	3.462 ± 0.038fghGH	0.123 ± 0.004efghijkFGHIJ	1.207 ± 0.016PqQR
FP16	3.595 ± 0.067fgFGH	0.081 ± 0.003nM	1.867 ± 0.065gG
FP17	3.240 ± 0.067hjkHI	0.121 ± 0.004fghijkFGHIJK	1.825 ± 0.024ghGH
FP18	3.706 ± 0.033efEFG	0.112 ± 0.005jklIJK	1.892 ± 0.081gG
FP19	3.240 ± 0.033hjkHI	0.132 ± 0.014defDEFG	1.456 ± 0.047mMN
FP20	3.617 ± 0.012efgFGH	0.117 ± 0.003hijklGHIJK	3.202 ± 0.054bAB
FP21	2.642 ± 0.067mnLMN	0.098 ± 0.003mL	1.274 ± 0.050PPQ
FP22	2.664 ± 0.077mnKLMN	0.131 ± 0.003defgDEFGH	1.394 ± 0.027mnNO
FP23	2.708 ± 0.015mnJKLMN	0.113 ± 0.005ijklIJK	2.230 ± 0.059eE
FP24	1.068 ± 0.038rQ	0.111 ± 0.002jklIJK	1.555 ± 0.045klLM
FP25	3.950 ± 0.039dDE	0.141 ± 0.008dD	1.633 ± 0.080jkJKL
FP26	2.908 ± 0.067lmJKL	0.086 ± 0.003nM	1.326 ± 0.009noOP
FP27	1.955 ± 0.011qP	0.154 ± 0.006cC	1.591 ± 0.024jkKKL
FP28	4.083 ± 0.068dCD	0.107 ± 0.002IKL	1.144 ± 0.054qrR
FP29	1.134 ± 0.039rQ	0.123 ± 0.006efghijkFGHIJ	1.555 ± 0.009klLM
FP30	6.587 ± 0.039aA	0.210 ± 0.008aA	1.690 ± 0.031ijIJK
FP31	2.730 ± 0.039mnJKLM	0.116 ± 0.002hijklHIJK	1.430 ± 0.024mnNO

FP32	4.526 ± 0.038bB	0.008 ± 0.004hijklGHIJK	1.118 ± 0.009rR
FP33	2.198 ± 0.011POP	0.125 ± 0.006efghiEFGHIJ	1.409 ± 0.016mnNO
FP34	3.285 ± 0.039hjHI	0.125 ± 0.003efghijEFGHIJ	2.022 ± 0.032fF
FP35	3.329 ± 0.038ghHI	0.127 ± 0.004efghDEFGHI	0.963 ± 0.036sS
FP36	3.418 ± 0.068fghGH	0.166 ± 0.005bBC	3.166 ± 0.009bB
FP37	2.686 ± 0.077mnJKLMN	0.155 ± 0.005cC	1.757 ± 0.072hiHI
FP38	1.999 ± 0.038qP	0.114 ± 0.003ijkIJK	1.477 ± 0.009lmMN
FP39	2.996 ± 0.011klIJK	0.121 ± 0.004fghijkFGHIJK	1.269 ± 0.027oPPQ
FP40	3.861 ± 0.067deDEF	0.113 ± 0.001ijklIJK	1.742 ± 0.059hiHIJ
YN01	19.045 ± 0.053hiGHIJ	0.272 ± 0.009hijFGH	3.316 ± 0.039aAB
YN02	20.996 ± 0.098defCDEF	0.312 ± 0.002bcdeBCD	2.625 ± 0.056cdeDEF
YN03	19.333 ± 0.021ghiFGHI	0.288 ± 0.009ghEFG	1.991 ± 0.050klmnoPLMNOPQ
YN04	21.727 ± 0.091bcdeBCDE	0.294 ± 0.004efgDEF	2.376 ± 0.054efghiEFGHIJKL
YN05	19.422 ± 0.089ghiFGHI	0.291 ± 0.011fghDEF	2.641 ± 0.054cdeDEF
YN06	12.661 ± 0.028PR	0.246 ± 0.006klmIJKLM	1.861 ± 0.027noPOPQ
YN07	18.535 ± 0.005ijIJK	0.128 ± 0.005qQ	2.615 ± 0.036cdeDEFG
YN08	21.018 ± 0.032defCDEF	0.304 ± 0.007efgCDE	3.483 ± 0.097aA
YN09	17.139 ± 0.035kKLM	0.108 ± 0.002rR	2.511 ± 0.063defgDEFGHI
YN10	20.420 ± 0.077efgEFGH	0.307 ± 0.007defBCDE	1.991 ± 0.050klmnoPLMNOPQ
YN11	16.186 ± 0.069ILMN	0.115 ± 0.006rQR	2.132 ± 0.024hijklmnoIJKLMNQP
YN12	14.058 ± 0.011noPQR	0.252 ± 0.005jklmHIJKL	2.132 ± 0.036hijklmnoIJKLMNQP
YN13	15.166 ± 0.041lmnNOPQ	0.228 ± 0.009nMN	2.121 ± 0.009hijklmnoIJKLMNQP
YN14	13.991 ± 0.032noQR	0.274 ± 0.007hiFGH	3.410 ± 0.100aA
YN15	10.688 ± 0.033qS	0.241 ± 0.007lmnJKLMN	2.340 ± 0.070efghijFGHIJKLM
YN16	20.686 ± 0.083efgDEFG	0.256 ± 0.005ijklHIJKL	2.090 ± 0.024ijklmnoJKLMNQP
YN17	20.486 ± 0.055efgEFGH	0.252 ± 0.005jklmHIJKL	2.745 ± 0.063cdDE
YN18	18.956 ± 0.033hiHIJ	0.322 ± 0.006bcdBC	1.991 ± 0.039klmnoPLMNOPQ
YN19	15.077 ± 0.070lmnNOPQ	0.235 ± 0.006mnLMN	1.929 ± 0.065mnoPNOPQ
YN20	15.964 ± 0.032IMNO	0.264 ± 0.010ijkHIJ	2.449 ± 0.045defghEFGHIJ
YN21	24.809 ± 0.0233aA	0.310 ± 0.010cdeBCDE	2.506 ± 0.050defgDEFGHI
YN22	22.281 ± 0.058bcdBCD	0.326 ± 0.009bcAB	2.532 ± 0.057defDEFGH
YN23	21.483 ± 0.073cdefBCDE	0.343 ± 0.012aA	3.254 ± 0.039abAB
YN24	22.836 ± 0.069bB	0.329 ± 0.006bAB	3.436 ± 0.056aA
YN25	17.582 ± 0.066jkJKL	0.253 ± 0.006jklmHIJKL	2.272 ± 0.045fghijklFGHIJKLMN
YN26	9.048 ± 0.030rT	0.300 ± 0.011efgCDE	2.428 ± 0.059efghEFGHIJ
YN27	22.415 ± 0.017bcBC	0.226 ± 0.011nMN	2.178 ± 0.070hijklmnHIJKLMNQP
YN28	19.577 ± 0.012ghiFGHI	0.222 ± 0.003nN	3.051 ± 0.671bBC
YN29	14.390 ± 0.083mnoOPQ	0.267 ± 0.009ijGHI	2.859 ± 0.047cCD
YN30	15.232 ± 0.039lmnNOPQ	0.239 ± 0.004lmnKLMN	2.028 ± 0.023jklmnoKLMNQP
YN31	17.560 ± 0.054jkJKL	0.263 ± 0.005ijkHIJK	3.213 ± 0.009abAB
YN32	12.794 ± 0.065PR	0.287 ± 0.013ghEFG	2.152 ± 0.033hijklmnoHIJKLMNQP
YN33	15.653 ± 0.035lmNOP	0.188 ± 0.009oO	1.965 ± 0.033lmnoPMNOPQ
YN34	9.513 ± 0.069rST	0.196 ± 0.007oO	1.685 ± 0.009PQ
YN35	17.538 ± 0.080jkJKL	0.289 ± 0.012fghEFG	2.241 ± 0.055fghijklmGHIJKLMNO
YN36	13.658 ± 0.077oPQR	0.223 ± 0.003nMN	1.835 ± 0.039oPPQ
YN37	21.373 ± 0.088cdefBCDE	0.191 ± 0.004oO	1.929 ± 0.047mnoPNOPQ
YN38	13.947 ± 0.015noQR	0.155 ± 0.005PP	2.319 ± 0.074efghijkFGHIJKLMN
YN39	14.856 ± 0.096lmnoNOPQ	0.261 ± 0.006ijkHIJK	2.194 ± 0.050ghijklmnHIJKLMNQP
YN40	20.220 ± 0.033fghEFGH	0.272 ± 0.013hijFGH	2.407 ± 0.083efghiEFGHIJK

In the same column, uppercase letters indicate statistical significance at the level of $P < 0.01$, while lowercase letters indicate statistical significance at the level of $P < 0.05$



Figure A1. The study site, sampling/experimental sites and habitats

Figures A2–A7. Leaf morphology, such as leaf shape, apex, base, and vein count, was observed visually. Leaf length, leaf width, and petiole length were measured using a ruler. Leaf area was calculated based on leaf length and width

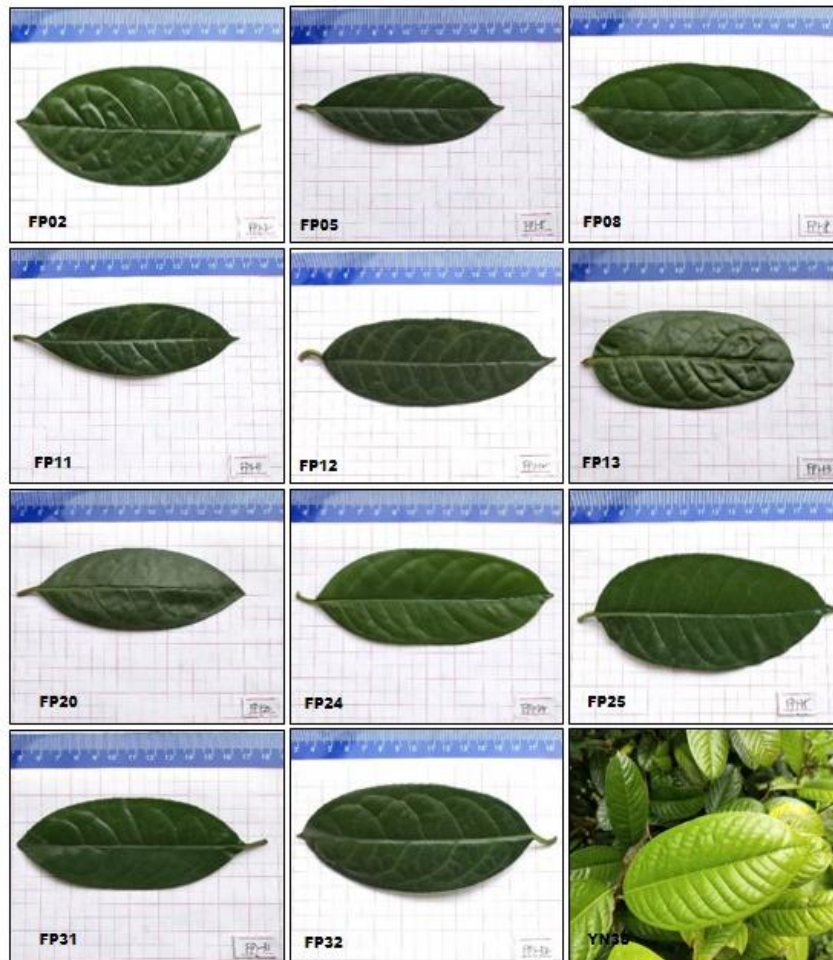


Figure A2

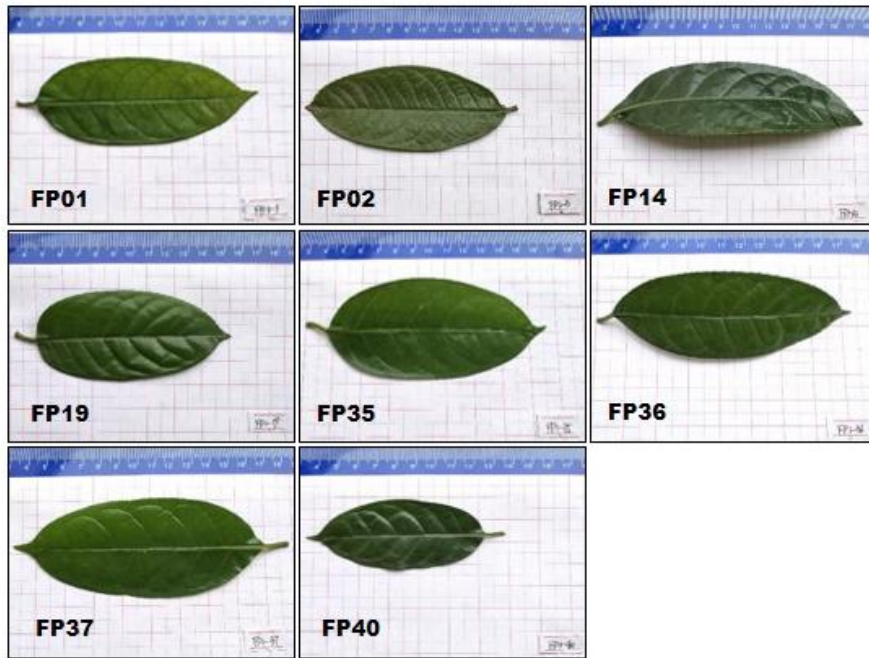


Figure A3

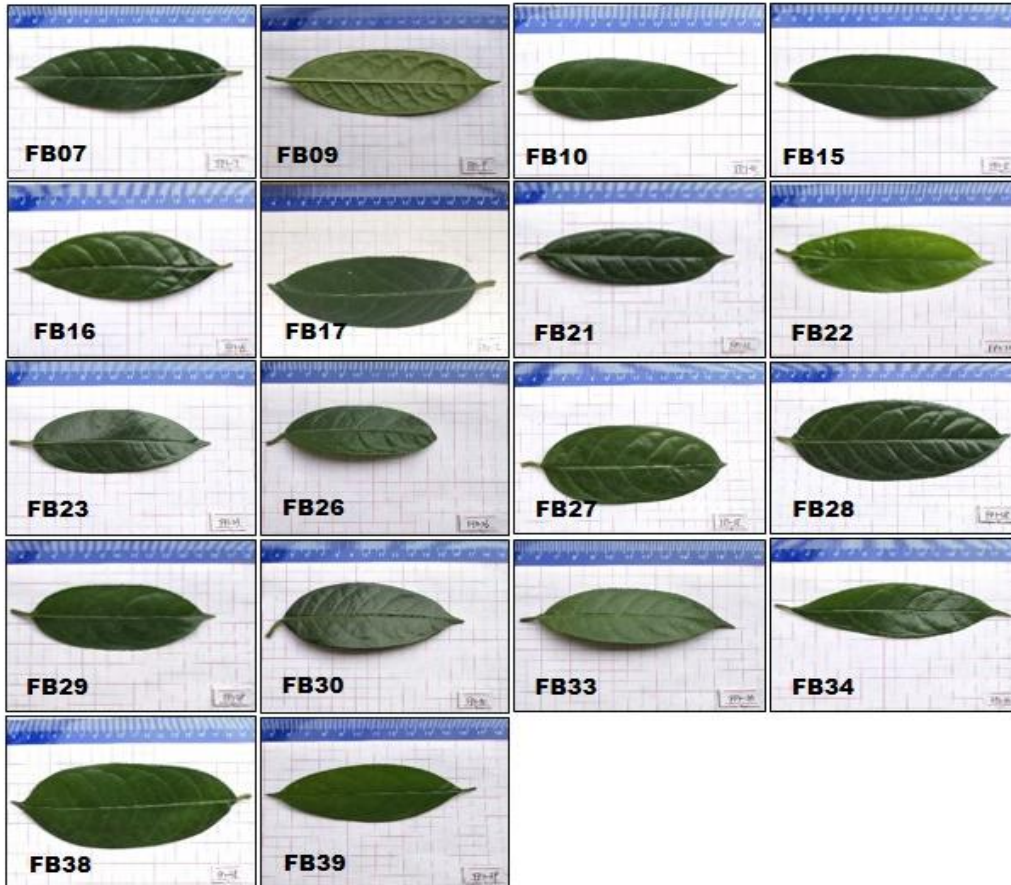


Figure A4

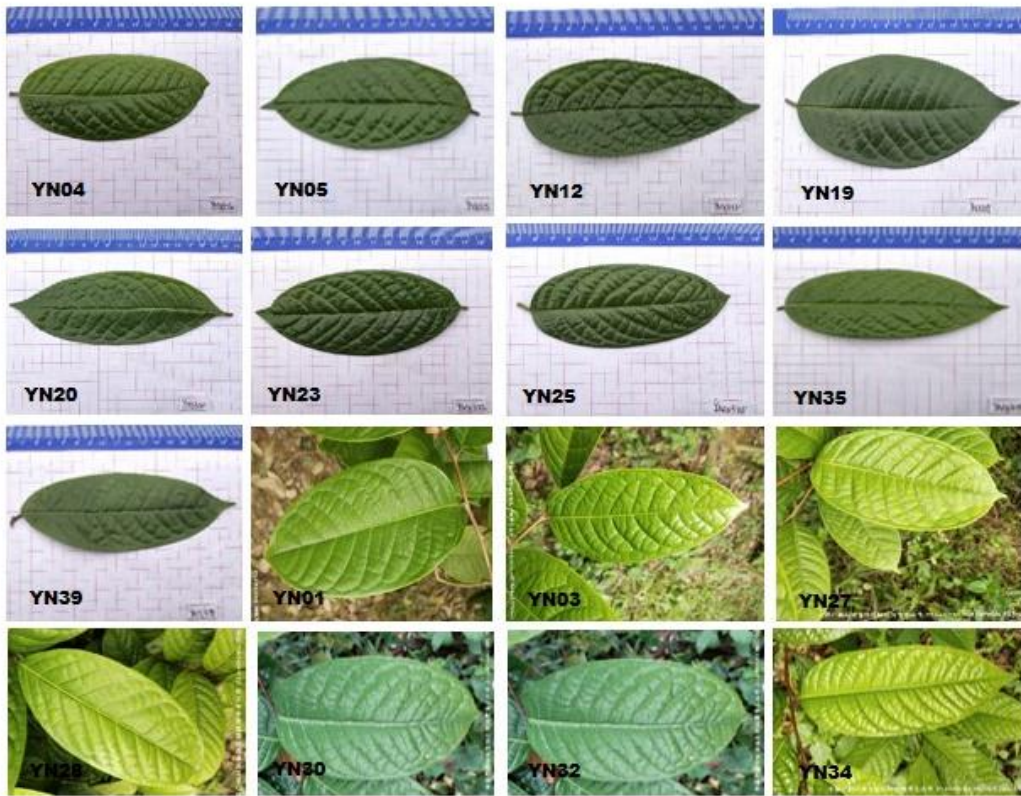


Figure A5



Figure A6



Figure A7