International Journal of Horticulture, 2023, Vol.13, No.10, 1-9
http://hortherbpublisher.com/index.php/ijh

Research Article Open Access

Determination and Pharmacological Analysis of the Color Related Secondary Metabolites in *Clematis Hybridas*

Yuan Mingxia [1], Song Wei [1] ✉, Liu Huanhuan [1], Ma Tao [1], Wang Haoyang [1], Liu Lanxiang[1], Ding Yi [2]
1 Jiangsu Vocational College of Agriculture and Forestry, Jurong, 212400, China
2 Jiangsu Jichuanglanhai Technology Co. Ltd, Suzhou, 215000, China
✉ Corresponding email: songwei@jsafc.edu.cn
International Journal of Horticulture, 2023, Vol.13, No.10 doi: 10.5376/ijh.2023.13.0010
Received: 14 Sep., 2023
Accepted: 18 Sep., 2023
Published: 23 Sep., 2023

Preferred citation for this article:
Yuan M.X., Song W., Liu H.H., Mao T., Wang H.Y., Liu L.X., and Ding Y., 2023, Determination and pharmacological analysis of the color related secondary metabolites in *Clematis Hybridas*, International Journal of Horticulture, 13(10): 1-9 (doi: 10.5376/ijh.2023.13.0010)

Abstract In order to find out the content and pharmacological effects of the color related secondary metabolites in clematis hybridas, methods such as Folin-phenol method, vanillin-hydrochloric acid method, spectrophotometry were used to determine the content of total phenols, tannins, proanthocyanidins, flavonoids and anthocyanins in the calyx petals of 7 clematis hybridas varieties, and their pharmacological effects were studied and analyzed one by one. The results showed that 7 clematis hybridas varieties all accumulated abundant phenols and flavonoids in their calyx petals, with 6.51~8.50 mg/g and 1.15~3.04 mg/g, respectively, and also all accumulated a certain amount of tannins, proanthocyanidins and anthocyanins. The pharmacological analysis showed that the color related secondary metabolites (total phenols, tannins, proanthocyanidins, flavonoids and anthocyanins) in clematis hybridas all have good antioxidant activity and the ability to remove free radicals, which can effectively resist oxidation, anti-aging, remove free radicals in the body, protect cardiovascular and cerebrovascular systems, alleviate inflammation, inhibit cancer, etc. The implementation of this study has laid a foundation for ascertaining the medicinal value of the color related secondary metabolites in clematis hybridas, and also provided a theoretical basis for pharmaceutical development of secondary metabolites in the future.
Keywords *Clematis* L.; Secondary metabolites; Pharmacological effects

The *Clematis florida* Thunb., belonging to the Ranunculaceae family, is a perennial woody or herbaceous vine of the *Clematis* L. genus. There are numerous varieties of this plant, with approximately 355 species found globally, and over 150 species found in China alone, with a wide distribution range. The *Clematis florida* Thunb. is rich in active ingredients such as oleanolic acid, triterpenoid saponins, and protoanemonin, and has significant medicinal value (Chen and Pu, 2006, Journal of Yunnan College of Traditional Chinese Medicine, 29(1): 31-33). The root and whole plant of the *Clematis florida* Thunb. can be used in medicine, and there are relevant discussions on the medicinal efficacy of this plant in pharmacopoeias such as *Herbal Medicines of Southern Yunnan*, *Pharmacology of Traditional Chinese Medicine*, and *Chinese Medicinal Plant Atlas*. As a traditional Chinese medicinal material, the *Clematis florida* Thunb. has been widely used, and there have been relatively comprehensive studies on its chemical components (Zhang et al., 2008, Traditional Chinese Medicinal Research, (3): 22-24; Sun et al., 2009) and trace elements (Wu et al., 2008). However, currently, there is a relative lack of pharmacological research on the sepals of the *Clematis florida* Thunb.

Secondary metabolites are various non-essential small-molecule organic compounds produced by plant secondary metabolism. Although secondary metabolites are byproducts of metabolic processes, they have a variety of pharmacological activities. The active ingredients of many herbal medicines are secondary metabolites contained in them, and plant secondary metabolites are an important source of herbal medicines. It can be seen that the pharmacological effects of plants are closely related to the pharmacological activity of their secondary metabolites. Therefore, the determination and analysis of plant secondary metabolites are effective ways to clarify the pharmacological effects of related plants.

Plant secondary metabolites are diverse and can be classified into different categories according to different classification criteria. Each known compound in each category can number in the thousands or even tens of

thousands. Common flower-related secondary metabolites include total phenols, tannins, anthocyanins, flavonoids, and glycosides. Some studies have been conducted on the presence of flower-related secondary metabolites in some plants such as *Paeonia suffruticosa* Andr. (Gan et al., 2020), *Rhododendron pulchrum* (Xia et al., 2022), and *Brassica campestris* (Liu et al., 2021). However, the content of flower-related secondary metabolites in the sepals of *Clematis florida* Thunb. is still unclear, and previous studies have not analyzed their pharmacological effects. Research on plant secondary metabolites needs to be combined with pharmacological analysis to understand their pharmacological effects and to effectively help develop their medicinal applications.

In this study, the sepals of seven *Clematis* varieties were selected as research objects, and the content of flower-related secondary metabolites (total phenols, tannins, anthocyanins, flavonoids, and glycosides) was measured. Furthermore, the pharmacological effects of each of the secondary metabolites were studied and analyzed, with the aim of laying the foundation for exploring the medicinal value of flower-related secondary metabolites in *Clematis florida* Thunb. and providing a theoretical basis for future medicinal development.

1 Results and Analysis

1.1 Content and pharmacology of total phenols in calyx of *Clematis florida*

1.1.1 Determination of total phenol content

A certain amount of monohydrate gallic acid ($C_7H_6O_5.H_2O$) was dissolved in distilled water and made up to a fixed volume to prepare standard solutions of monohydrate gallic acid at different concentrations. These solutions were then mixed with 1 mL of Folin-Ciocalteu reagent (FC) and 3 mL of 7.5% sodium carbonate solution, and the absorbance was measured at 765 nm wavelength. A standard curve was plotted with the monohydrate gallic acid content as the abscissa and the absorbance as the ordinate, and the equation of the regression line was Y=0.0065X+0.0071, with an R^2 value of 0.9 962. The results showed that there was a good linear relationship between the monohydrate gallic acid content and the absorbance (Figure 1), which met the detection requirements within the measurement range.

Take 0.1 g of *Clematis florida* Thunb. sepals samples to prepare a 25 mL of filtrate, add 1 mL of Folin-Ciocalteu reagent (FC) and 3 mL of 7.5% sodium carbonate solution to make a final volume of 500 mL. After the color reagent had fully developed, the absorbance was measured at 765 nm wavelength, and the total phenol content of the *Clematis florida* Thunb. sepals was calculated using the regression equation of the standard curve (Table 1). The results showed that the tested *Clematis florida* Thunb. sepals samples accumulated rich phenolic substances, ranging from 6.51 to 8.50 mg/g.

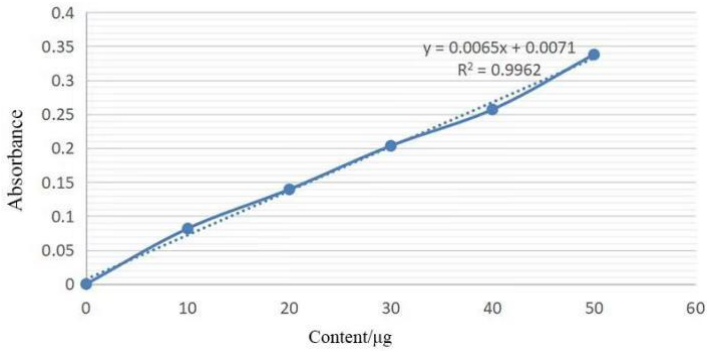

Figure 1 Standard curve of total phenols

1.1.2 Pharmacological effects of total phenols

Total phenols are a general term for molecules containing multiple hydroxyphenolic substances, which are a type of secondary metabolite widely present in plants. Their characteristic feature is the presence of a large number of phenolic structural units, and the properties of these phenolic structures constitute the unique pharmacological activity of phenolic substances. The phenolic hydroxyl groups in the phenolic structure are prone to substitution reactions and can scavenge free radicals, making them have excellent antioxidant activity and the ability to

eliminate free radicals. These properties allow phenolic substances to effectively resist oxidation, aging, remove free radicals in the body, protect the cardiovascular system, prevent arteriosclerosis and thrombosis, inhibit bacterial and cancer cell growth, etc.

Table 1 Determination of total phenol content of calyx petals of *clematis* hybridas varieties by Folin-phenol methods

Sample color	Sample name	Weighing (g)	Extraction (mL)	Liquid extraction (mL)	Abs	Total phenol (mg/g)
Pale purple-red	LadyKyoko	0.1 089	25	1	0.2 264	7.73
Dark pink	Josephine	0.1 097	25	1	0.2 501	8.50
Blue purple	Taiga	0.1 058	25	1	0.2 014	7.05
Purple blue	Sodertalje	0.1 068	25	1	0.1 983	6.87
Pale blue-purple	MultiBlue	0.1 074	25	1	0.1 954	6.73
Light blue	BlueLight	0.1 049	25	1	0.1 967	6.94
White	Yukiokoshi	0.1 029	25	1	0.2 041	6.51

1.2 Tannin content of calyx petal of *Clematis florida* Thunb. and its pharmacology

1.2.1 Determination of tannin content

Standard solutions of tannic acid at different concentrations were prepared using a 75% dimethylformamide solution. 5 mL of water and 1 mL of ferric ammonium citrate solution were added to each solution, and the absorbance was measured at 525 nm wavelength. A standard curve was plotted with the tannic acid content as the abscissa and the absorbance as the ordinate, and the equation of the regression line was Y=1.3734X+0.0816, with an R^2 value of 0.999 7. The linearity was good within the measurement range (Figure 2), which met the detection requirements.

Wash 1 g of *Clematis* sepal sample with 20 mL of 75% dimethylformamide solution into a centrifuge tube to obtain the supernatant. 1 mL of the supernatant was shaked by oscillator with 6 mL of water and 1 mL of 8.0 g/L ammonia solution to form the control tube. 1 mL of the supernatant was shaked by oscillator with 5 mL of water and 1 mL of ferric ammonium citrate solution to form the test tube. Water was used as the blank tube, and the absorbance value at 525 nm wavelength was measured. The tannin content in the sample was calculated using the standard curve (Table 2). The results showed that the tested *Clematis florida* Thunb. sepals samples contained a certain amount of tannin substance.

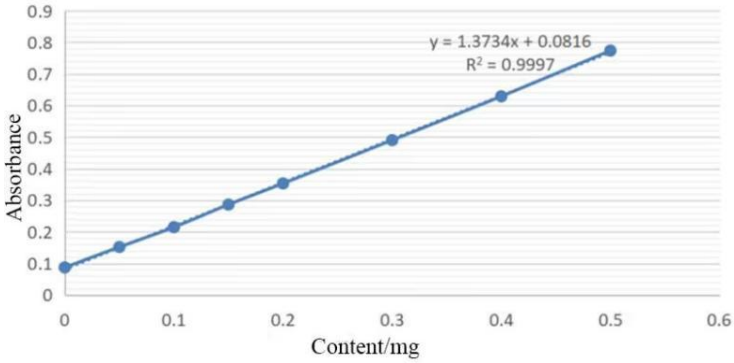

Figure 2 Standard curve of tannin

1.2.2 Pharmacological effects of tannins

Tannin is a class of complex polyphenolic compounds formed by the condensation or polymerization of some highly active basic molecules. They are important secondary metabolites, and their molecular structure is relatively complex, with a molecular formula of $C_{76}H_{52}O_{46}$. The pharmacological activity of tannins is the ultimate manifestation of their interaction with proteins, polysaccharides, nucleic acids, and other substances in plants. Their main effects are antioxidant, anti-aging, anti-allergic, anti-mutagenic, anti-inflammatory, hemostatic, and protective for the cardiovascular system and neural cells. These effects mainly depend on their molecular structure.

Table 2 Determination of tannin content in calyx petals of seven tested *clematis* hybridas varieties

Sample color	Sample name	Weighing (g)	Extraction (mL)	Dilution multiple	Liquid (mL)	extraction	Measuring tube	Control tube	Abs	Tannin (%)
Pale purple-red	LadyKyoko	0.8 599	20	2	1		0.4 586	0.1 950	0.2 636	0.62
Dark pink	Josephine	1.0 368	20	2	1		0.4 298	0.1 175	0.3 123	0.65
Blue purple	Taiga	1.0 203	20	2	1		0.4 687	0.0 993	0.3 694	0.82
Purple blue	Sodertalje	1.0 178	20	2	1		0.4 285	0.0 681	0.3 604	0.80
Pale blue-purple	MultiBlue	1.0 012	20	2	1		0.4 675	0.1 151	0.3 524	0.79
Light blue	BlueLight	1.0 651	20	2	1		0.4 956	0.1 748	0.3 208	0.65
White	Yukiokoshi	1.0 213	20	2	1		0.4 423	0.1 227	0.1 796	0.12

1.3 Proanthocyanidin content and pharmacology of calyx petal of *Clematis florida*

1.3.1 Determination of proanthocyanidin content

A certain amount of catechin standard was dissolved in methanol to prepare standard solutions of catechin at different concentrations. 3 mL of 5% vanillin methanol solution and 1 mL of 4% hydrochloric acid methanol solution were added to each solution, and the absorbance was measured at 500 nm wavelength. A standard curve was plotted with the catechin content as the abscissa and the absorbance as the ordinate, and the equation of the regression line was Y=0.0015X+0.001, with an R^2 value of 0.999 6. The results showed that there was a good linear relationship between the catechin content and the absorbance (Figure 3).

A sample of 1 g of *Clematis florida* Thunb. sepals was taken and mixed with 3 mL of 5% vanillin methanol solution and 1 mL of 4% hydrochloric acid methanol solution, and then made up to 5 mL. The absorbance value was measured at 500 nm wavelength, and the mass concentration of proanthocyanidins in the *Clematis florida* Thunb. sepals sample was calculated using the standard curve (Table 3). The results showed that all *Clematis florida* Thunb. sepals samples except for the white variety Yukiokoshi contained a certain amount of proanthocyanidin substance.

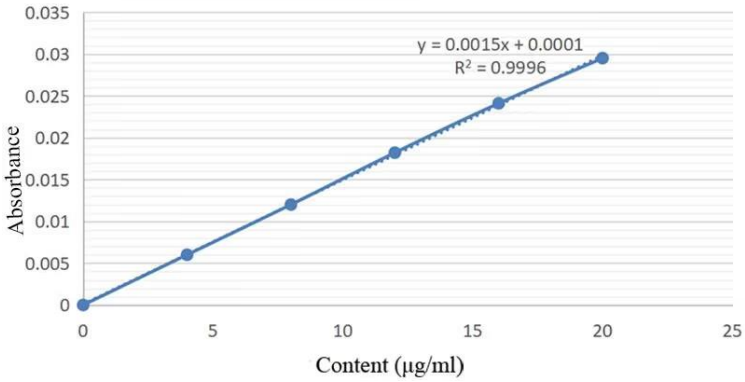

Figure 3 Standard curve of proanthocyanidins

1.3.2 Pharmacological effects of proanthocyanidins

Proanthocyanidins are polymers formed by the condensation of different amounts of catechin or epicatechin through C-C bonds, with a unique molecular structure and a molecular formula of $C_{30}H_{26}O_{12}$. Proanthocyanidins are a widely existing class of secondary metabolites in plants, and they have strong antioxidant and free radical scavenging effects. They can effectively promote blood circulation, improve hypoxia, protect the cardiovascular system, slow down aging, prevent stroke, alleviate inflammation, and inhibit cancer. In addition, proanthocyanidins also have the functions of protecting neural cells, and inhibiting Alzheimer's disease and senile dementia.

Table 3 Determination of proanthocyanidins by vanillin-hydrochloric acid method

Sample name	Sample color	Weighing (g)	Extraction (mL)	Liquid extraction (mL)	Constant volume (mL)	Abs	Proanthocyanidins (mg/kg)
LadyKyoko	Pale purple-red	1.0 167	25	1	5	0.0 954	0.0309
Josephine	Dark pink	1.0 351	25	1	5	0.0 955	0.0304
Taiga	Blue purple	1.0 361	25	1	5	0.0 667	0.0252
Sodertalje	Purple blue	1.0 196	25	1	5	0.0 715	0.0265
MultiBlue	Pale blue-purple	1.0 027	25	1	5	0.0 618	0.0251
BlueLight	Light blue	1.0 023	25	1	5	0.0 698	0.0266
Yukiokoshi	White	1.0 397	25	1	5	--	--

1.4 Flavonoids content in calyx petal of *Clematis florida* and its pharmacology

1.4.1 Determination of flavonoid content

A certain amount of rutin standard was used to prepare standard solutions of rutin at different concentrations. 0.15 mL of 5% (mass ratio) $NaNO_2$, 0.15 mL of 10% (mass ratio) Al $(NO_3)_3$, and 2 mL of 4% (mass ratio) NaOH reagent solution were added sequentially to each solution, and the absorbance was measured at 510 nm wavelength. A standard curve was plotted with the rutin content as the abscissa and the absorbance as the ordinate, and the equation of the regression line was Y=0.0007X+0.0046, with an R^2 value of 0.999 7. The linearity was good within the measurement range (Figure 4), which met the detection requirements.

A 0.5 g sample of *Clematis florida* Thunb. sepals was taken, and 0.15 mL of 5% (mass ratio) $NaNO_2$, 0.15 mL of 10% (mass ratio) $Al(NO_3)_3$, and 2 mL of 4% (mass ratio) NaOH reagent solution were added sequentially, and then the solution was diluted to 4.5 mL. The absorbance was measured at 510 nm wavelength, and the flavonoid content in the *Clematis florida* Thunb. sepals sample was calculated according to the regression equation of the standard curve (Table 4). The results showed that all tested *Clematis florida* Thunb. sepals samples had rich flavonoid substances, ranging from 1.15 to 3.04 mg/g.

1.4.2 Pharmacological effects of flavonoids

Flavonoids are a series of compounds that consist of two benzene rings with hydroxyl groups connected by a central three-carbon atom. They exist in most plants in the form of conjugates (flavonoid glycosides) or aglycones (flavonoids), with a molecular formula of $C_{15}H_{10}O_8$. Flavonoids can achieve pharmacological effects such as inhibiting lipid peroxidation, inhibiting enzyme activity, antibacterial, antiviral, anti-allergic, anti-inflammatory, protecting the cardiovascular system, and reducing aging of the body, by exerting their antioxidant, free radical scavenging, and chelating effects on divalent anions.

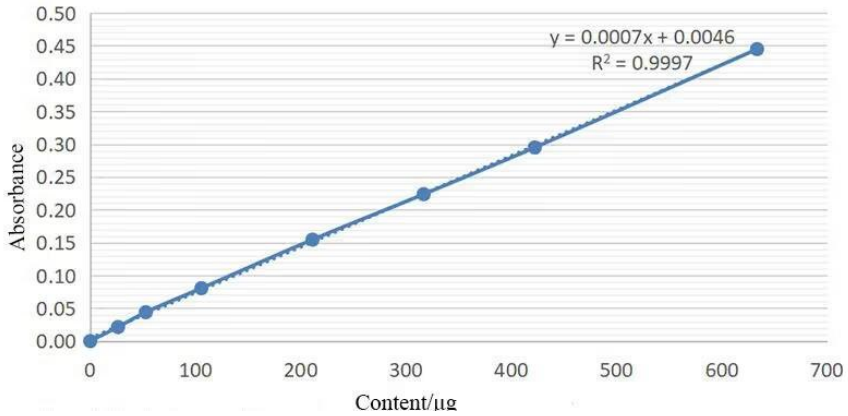

Figure 4 Standard curve of flavonoids

Table 4 Determination of flavonoid content by spectrophotometry

Sample color	Sample name	Weigh (g)	Extractiom (mL)	Sample (mL)	Constant volume (mL)	Abs	Flavonoid (mg/g)
Pale purple-red	LadyKyoko	0.5 024	10	0.2	4.5	0.0 257	3.04
Dark pink	Josephine	0.5 134	10	0.2	4.5	0.0 204	2.23
Blue purple	Taiga	0.5 120	10	0.2	4.5	0.0 234	2.66
Purple blue	Sodertalje	0.5 041	10	0.2	4.5	0.0 196	2.16
Pale blue-purple	MultiBlue	0.5 034	10	0.2	4.5	0.0 241	2.80
Light blue	BlueLight	0.5 069	10	0.2	4.5	0.0 181	1.93
White	Yukiokoshi	0.5 122	10	0.2	4.5	0.0 199	1.15

1.5 Anthocyanin content of calyx petal of *Clematis florida* and its pharmacology

1.5.1 Determination of anthocyanin content

A 1 g sample of *Clematis florida* Thunb. sepals was taken, accurate to 0.000 1 g, and extracted with a 1% hydrochloric acid-ethanol solution in a 1:4 (sample:extractant) ratio at 25 °C for 60 minutes, twice with the second extraction volume being half of the first. The extract was then made up to 20 mL for anthocyanin content determination. A standard curve was plotted with the anthocyanin content as the abscissa and the absorbance as the ordinate, and the equation of the regression line was Y=397.05X+0.0575, with an R^2 value of 0.999 4. The linearity was good within the measurement range (Figure 5), which met the detection requirements.

Using pH differential method with pH 1.0 and pH 4.5 buffer solutions, the *Clematis florida* Thunb. sepals sample was diluted to an appropriate multiple, and the absorbance values were measured twice at 510 nm and 700 nm wavelengths,respectively, with distilled water as a blank control (Table 5). The results showed that, except for the white *Clematis florida* Thunb. Yukiokoshi sample, all other *Clematis florida* Thunb. sepals samples contained a certain amount of anthocyanin.

1.5.1 Pharmacological effects of anthocyanins

Anthocyanins are compounds formed by the combination of flavonoids and sugars through glycosidic bonds, with a molecular formula of $C_{20}H_{38}O_2$. The molecular structure of anthocyanins also contains multiple phenolic hydroxyl groups, which act as hydroxyl donors and therefore have good antioxidant activity and free radical scavenging ability. Anthocyanins can effectively exert pharmacological effects such as antioxidant, anti-aging, free radical scavenging, protecting the cardiovascular system, relieving inflammation, and inhibiting cancer. At the same time, the antioxidant activity of anthocyanins and their ability to prevent βA4 formation can effectively inhibit Alzheimer's disease and senile dementia.

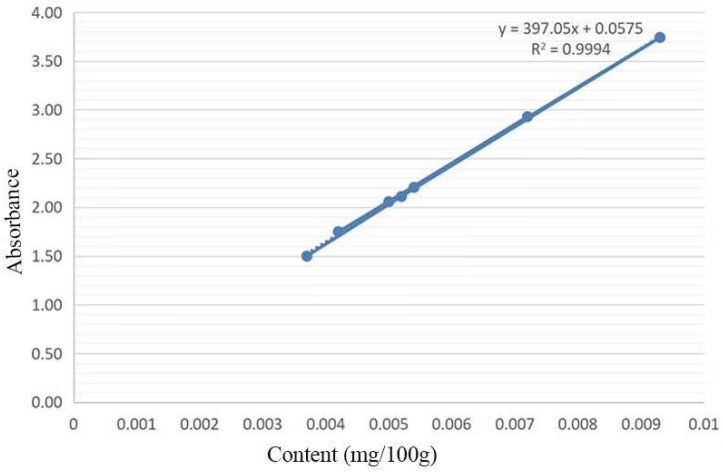

Figure 5 Standard curve of anthocyanin

Table 5 Determination of anthocyanin content by pH differential method

Sample color	Sample name	Weigh (g)	Extractiom (mL)	Sample (mL)	Constant volume (mL)	PH1.0 Abs510	PH1.0 Abs700	PH4.5 Abs510	PH4.5 Abs700	ΔAbs	Anthocyanin (mg/kg)
Pale purple-red	LadyKyoko	1.0 150	25	2	20	0.0 847	0.0 609	0.0 744	0.0556	0.005	0.206
Dark pink	Josephine	1.0 264	25	2	20	0.0 918	0.0 616	0.0 817	0.0587	0.0 072	0.293
Blue purple	Taiga	1.0 313	25	2	20	0.0 823	0.0 597	0.0 759	0.057	0.0 037	0.150
Purple blue	Sodertalje	1.0 231	25	2	20	0.0 892	0.0 572	0.0 889	0.0623	0.0 054	0.220
Pale blue-purple	MultiBlue	1.0 291	25	2	20	0.0 893	0.0 626	0.0 749	0.0534	0.0 052	0.211
Light blue	BlueLight	1.0 381	25	2	20	0.0 847	0.0 609	0.0 612	0.0467	0.0 093	0.374
White	Yukiokoshi	1.0 025	25	2	20	-	-	-	-	-	-

2 Discussion

The determination and analysis of plant secondary metabolites are important means for screening the physiological and pharmacological activities of plants (Zhang et al., 2012). In this study, the content of flower-related secondary metabolites in the sepals of seven *Clematis florida* Thunb. varieties was determined by methods such as the Folin-phenol colorimetric method, vanillin-hydrochloric acid method, and spectrophotometry. The results showed that all seven *Clematis florida* Thunb. varieties had rich phenolic and flavonoid substances, as well as a certain amount of tannins, anthocyanins, and anthocyanins. Combined with pharmacological analysis, it can be inferred that phenolic substances, flavonoids, tannins, anthocyanins, and anthocyanins all have good antioxidant activity and free radical scavenging ability. Therefore, it is possible to speculate that *Clematis florida* Thunb. sepals may have good pharmacological effects such as antioxidant, anti-aging, free radical scavenging, protecting the cardiovascular system, relieving inflammation, and inhibiting cancer.

The pharmacological study of plant secondary metabolites is an important way to develop new plant medicines. The continuous analysis and clarification of the pharmacological effects of secondary metabolites in *Lonicera japonica* Thunb. have made it an effective medicine for clearing heat, detoxification, and anti-inflammatory and anti-swelling purposes. Similarly, through the study of secondary metabolites in *Ginkgo biloba* L., the pharmacological effects of its leaves, fruits, and seeds have been continuously recognized. The successful development of traditional Chinese medicine (TCM) *Ginkgo biloba* preparations has become one of the most successful cases of global plant medicine development (Xin et al., 2014). China is the country that uses and exports the most plant medicines in the world and should actively invest in the research and development of new plant medicines. This study quantitatively analyzed the flower-related secondary metabolites in the sepals of *Clematis florida* Thunb. and revealed its pharmacological effects, which may provide a theoretical basis for its full utilization in the field of medicine.

Although this study inferred the physiological and pharmacological effects of *Clematis florida* Thunb. sepals through quantitative analysis of flower-related secondary metabolites and pharmacological research, the complexity of new drug development requires the regulation of key genes related to flower-related secondary metabolites through modern biological technologies such as molecular biology, bioinformatics, and other modern scientific and technological methods to promote their expression. At the same time, by controlling genetic information, the effective medicinal components of *Clematis florida* Thunb. sepals can be analyzed and synthesized, which can contribute to the development of new drugs related to *Clematis florida* Thunb. sepals.

3 Materials and Methods

3.1 Test material

The sepals of seven varieties of *Clematis florida* Thunb. (LadyKyoko, Josephine, Taiga, Sodertalje, MultiBlue, BlueLight, and Yukiokoshi) were used as experimental materials. The experimental materials were obtained from the *Clematis florida* Thunb. breeding base of Jiangsu Vocational College of Agriculture and Forestry (119°16′32″N, 31°55′53″E) in early May 2022, when the tested *Clematis florida* Thunb. varieties entered the

flowering period and more than 75% of the plants had flowered (Liu et al., 2021). During sampling, 10-20 full flower buds were selected from each plant, and the middle part of the fresh sepals was taken with forceps and stored in a centrifuge tube for ultra-low temperature preservation.

3.2 Main reagents

1) Determination of total phenol content: Folin-Ciocalteu reagent (FC), 7.5% sodium carbonate solution, and gallic acid monohydrate standard solution; 2) Determination of tannin content: ammonia (NH_3) solution, 75% dimethylformamide solution, and ferric ammonium citrate solution; 3) Determination of proanthocyanidin content: vanillin-methanol solution, hydrochloric acid-methanol solution, and catechin standard solution; 4) Determination of flavonoid content: 5% (mass ratio) $NaNO_2$, 10% (mass ratio) Al $(NO_3)_3$, 4% (mass ratio) NaOH reagent solution, and rutin standard solution; 5) Determination of anthocyanin content: pH 1.0 and pH 4.5 buffer solutions and distilled water.

3.3 Test instruments

UV-Vis spectrophotometer, analytical balance with a precision of 0.0001 g, water bath, centrifuge, shaker, and enzyme-linked immunosorbent assay (ELISA) reader.

3.4 Determination method

1) Determination of total phenol content: modified according to the research method of previous studies (Li et al., 2008); 2) Determination of tannin content: referenced the research method of GB/T 15686-2008; 3) Determination of proanthocyanidin content: modified according to the research method of previous studies (Zhu et al., 2017); 4) Determination of flavonoid content: modified according to the research method of previous studies (Li et al., 2011); 5) Determination of anthocyanin content: modified according to the research method of previous studies (Sun et al., 2009).

3.5 Data analysis

Data analysis was performed using Excel software.

Authors' contributions

YMX was the experimental designer and executor of this study, responsible for writing and revising the paper. SW was the project conceiver and leader, and MT, WHY, and LLX participated in some data statistics and experiments. LHH and DY provided guidance for some experimental operations. All authors read and approved the final manuscript.

Acknowledgements

This study was jointly supported by the Jiangsu Provincial Seed Industry Revitalization Project (JBGS(2021)092), the "Qing Lan Project" for young academic leaders in Jiangsu universities (Su Jiao Shi Han [2020] No.10), and the Science and Technology Plan Project of Jiangsu Vocational College of Agriculture and Forestry (2022kj36).

References

Gan L.X., Li H.H, Yang C., Ming L., Han M.L., and Xin Z.X., 2020, Analysis of the content and composition of peony pigments in different colors, Beifang Yuanyi (Northern Horticulture), 2020(5): 67-73.

Li C.H., Tian J., He B., and Xiao S.H., 2011, Determination of total saponins in astragalus membranaceus by ultraviolet spectroscopy, Chongqing Yike Daxue Xuebao (Journal of Chongqing Medical Univer), 36(8): 954-956.

Liu H., Ren Y., Lin B.G., Zhu J.f., Cheng H., Zhang D.Q., Wang J.W., and Hua S.J., 2021, Dissection of petal pigment components in colored rapeseed (*Brasscia napu* L.) genotypes, Henong Xuebao (Journal of Nuclear Agricultural Sciences), 35(4): 837-845.

Li J., Nie J.Y., Li H.F., XU G.F., Wang X.D., Wu Y. l., and Wang Z.X.,2008, On determination conditions for total polyphenols in fruits and its derivedproducts by Folin- phenol methods, Guoshu Xuebao (Journal of Fruit Science), 25(1): 126-131.

Sun F., and Yang D.B., 2009, Advance in chemical constituents of genus *Clematis*, Zhongguo Zhongyao Zazhi (China Journal of Chinese Materia Medica), 34(20): 2660-2668.

Sun W., Li C.H., Wang L.S., Dai S.l., and Xu Y.j., Anthocyanins presentin flowers of *Senecio cruentus* with different colors, 2009, Yuanyi Xuebao (Acta Horticulturae Sinica), 36(12): 1775-1782.

Wu D.Q., Li C.X., An H.G., and Zhang C.Y., 2008, Determination of microelements in *C. Tangutica* (Maxim.) korsh flower by FAAS, Guangpuxue Yu Guangpu Fenxi(Spectroscopy and Spectral Analysis), 28(1): 228-230.

Xin Q.Q., Liu Y., Yang L., Fu C.G., Chen K.J., 2014, Ginkgo preparations of Chinese medicine and treatment of diabetes:mechanisms and clinical applications, Zhongguo Zhongyao Zazhi (China Journal of Chinese Materia Medica), (23): 39-45.

Xia X., Gong R., and Zhang C.Y., 2022, Anthocyanin composition and coloration mechanism in petals of *Rhododen-dron pulchrum with different colors,* Jiangsu Nongye Xuebao (*Jiangsu J. of Agr.Sci.*), 38(1): 207-213.

Zhang H. F., Niu L.L., and Yang X.H., 2022, Research progress in quantitative assay of plant secondary metabolites, Shiping Gongye Keji(Science and Technology of Food Industry), 33(15): 405-408.

Zhu M.L., Guo H.M., Sun H.T., Li W., and Hong H.S., 2017, Establishment of determination method of proanthocyanidins content in pitaya enzyme, Zhongguo Niangzao (China Brewing), 36(4): 184-187.