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Red Pigment of *Camellia japonica* "Margaret Walker" Flower

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Abstract

The variety of *Japanese camellia* is to perform qualitative and quantitative analyzes of anthocyanins pigments in the flowers of various colors are reviewed. Four variety of Japanese camellias of different colors were taken to analyze from the Batumi Botanic Garden *Camellia japonica* ,,Margaret Walker" based on the research results it could be concluded that anthocyanin's composition plays dominant role in flower coloration of *Japanese camellia*. The highest amount of them is detected in Camellia variety "Margaret Walker" - G (410 mg / kg); The lowest amount is found in variety "Margaret Walker" - A (30 mg / kg) The anthocyanins cyanidin-3-diglucosideis dominant one in both variation (A) (85 and 75 %, respectively).

Keywords: Margaret Walker, flavonoid, flower, cyanidin-3-glucoside, cyanidin-3-galactoside

Introduction

The species and forms of *Japanese camellia* **"Margaret Walker"** are characterized with red, pink, white, purple and other colors. A combination of various colors is also genetically peculiar for them.

Study of a flower pigmentation is very significant in vegetation breeding and is the key in the flower color variation in Genetic engineering (Tanakaetal, 2008).

It is well known that the pigments and flavonoids determine the flower pigmentation. They are characterized with diverse and universal function in the plant world and have the main input in synthesis of anthocyanins, playing the key role in coloration (Bueretal, 2010).

Anthocyanin's or colored glycosides are found in petals, stamens, fruit and other tissues of other organs.

The review of the scientific literature on Camellias revealed information about high content of flavonoids in different types of camellias (*Camellia reticulate, C. Japonica, C. Nitidissima, C.salienensis, C.hongkongensis, Camellia sinensis*). Its composition is in peak when the flower budis fully open (Xing-Wen Zhou et al., 2013; Ikuo Miyajima et al., 1985; Terahara et al.,2001, Yueh-Jiang Hwang et al., 1992).

Two basic anthocyanins were identified in the petals of the wild ancestor of Japanese camellia, mainly cyanidin-3-glucoside and cyanidin-3-galactoside, which participate in the formation of the red color of the flower (Nobumine et. Al., 2010).

It is to be an interesting challenge to identify the chemical composition of the Japanese Camellia variety flower of intense coloration and to study biological peculiarities of coloration formation. Based on the above stated information, we decided to experimentally identify the color formation and biological features of the most interesting flowers in the Japanese camellia variety.

Experimental

Four variety of *Japanese camellias* of different colors were taken to analyze from the Batumi Botanic Garden *Camellia japonica* "Margaret Walker".

The following color variations of the research species were included in the study: The variety "Margaret Walker" (four variations):

A- White Petals with light red spots;

B-White Petals with dark red spots;

C- Light red petals;

D- Red petals;

Frozen samplers were blended to a puree using commercial blender. Subsamples (5 g) of puree were then homogenized for 1 min in 20 ml of extraction solution containing by 70 %-ethanol (the ratio

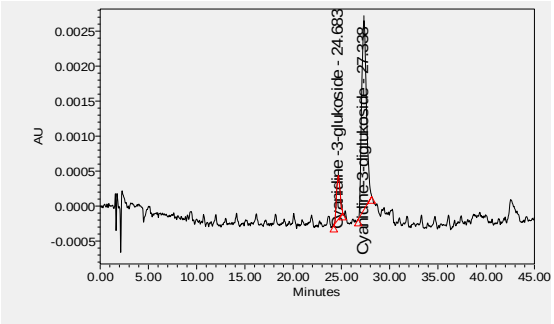
of sample and extracting agent is 1:15), first at room temperature, and then in the boiling chamber with the same solvent. Extracts were filtered and the filtrates were centrifuged for 5 min at 5000 rpm. All samples were passed through o,45 µm filters (Acrodist LC PVDF Syringe Filters Waters) prior to HPLC analysis.

In cases of flavonols extraction of samples was carried out with 70 % ethanol. For qualitative analysis following reagents have been used, and their composition has been defined by spectric method by setting standard caliber curve. Flavonols quantified as - rutin (400 nm), anthocyanins quantified as – cyanidine-3-glucoside (510 nm). The results are shown in tables 1. HPLC analysis of flavonoids Samples (10 µL) were analyzed using a Waters HPLC system equipped with a model 525 pump, UV/Vis detector. Separation was carried out using a 4,6 X 150 Symmetry C 18 column (Waters Corp, Milford, MA, USA) with a 3,9 mm X 20 mm C 18 guard column. The mobile phase was a linear gradient of 1 % Phosphoric acid (A) and Acetonitrile (B) from 0 % to 10 % B for 0-10 min, 10 % -14B for 10-25 min, 14-20 % B for 25-40 min, 20-0 % B for 40-45 min, , at 1,0 ml min-1. The system was equilibrated for 10 min at the initial gradient prior to each injection. Detection wavelengths used were 510 nm for anthocyanins and 370 nm for flavonols. Total anthocyanin derivatives were calculated as the sum of individual anthocyanin. Flavonols were quantified as rutin equivalents.

Results and discussion

During the study of anthocyanins of the Japanese camellia flowers at least four compounds were recognized in all research species. At this stage of the research two dominant compounds were identified: cyanidin-3-glucoside (retention time 24.6 min) and cyanidin-3-diglucoside(retention time 27.3 min). The composition of these compounds determines the coloration of a flower, which ranges according to the species and the variations. According to coloration of a petal these variations were conditionally subdivided into four groups:

Margaret Walker-A petals are white with red spots. The flower of this variation contains low amount of anthocyanins (30 mg/kg). The cyanidin-3-diglucoside is dominant (up to 85 %) as shown in Picture 1.1; Table 1.1; the petals of the of Margaret Walker-B flower are white with dark read spots. The composition of anthocyanins is higher – 60 mg/kg. The cyanidin-3-diglucoside is still dominant there (up to 75 %) as shown in Picture 1.2 Table 1.2. The composition of anthocyanins in the flower of Margaret Walker-G Crèches 410 mg/kg. The dominant anthocyanin is cyanidin-3-diglucoside - 88 % as shown in Picture 1.3; Table 1.3. Margaret Walker-D contains 560 mg/kg anthocyanin and the dominant anthocyanin is still cyanidin-3-diglucoside - 88 % as shown in Picture 1.4; Table 1.4.

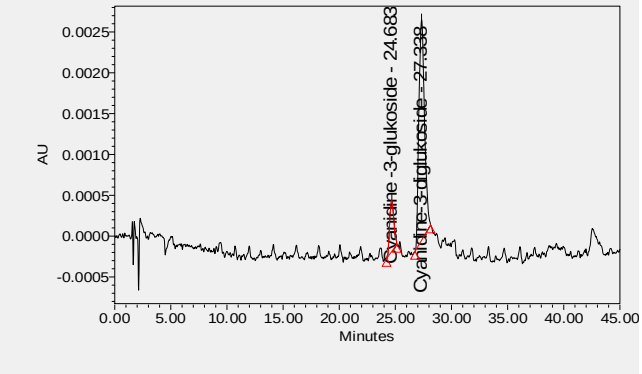


Picture 1.1 – the flower anthocyanins chromatogram of the Margaret Walker-A variety

Table 1.1. The chromatographic characteristics of the Margaret Walker-A flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	Column Type
3	Margaret Walker - ა	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount ნ.მ.	Units
1	Cyanidine -3-glukoside	24.683	15991	14.57	43,8	4,38	მგ/კგ
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	27.338	93738	85.43	256	25,6	მგ/კგ
	Total Anthocyans				310	30	მგ/კგ

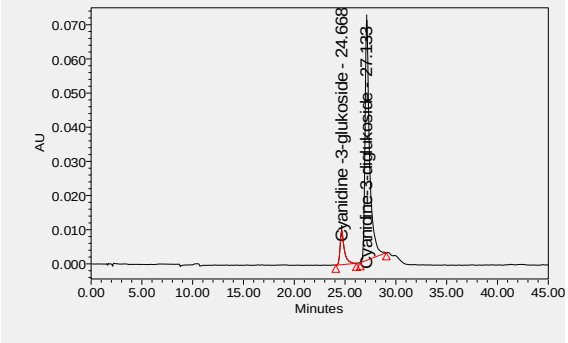


Picture 1.2 – the flower anthocyanins chromatogram of the Margaret Walker-B variety

Table 1.2 -chromatographic characteristics of the Margaret Walker-B flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
3	Margaret Walker -ბ	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount ნ.მ.	Units
1	Cyanidine -3-glukoside	24.356	138723	25.20	150	15	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	27.027	411867	74.80	450	45	mg/kg
	Total Anthocyans				600	60	mg/kg

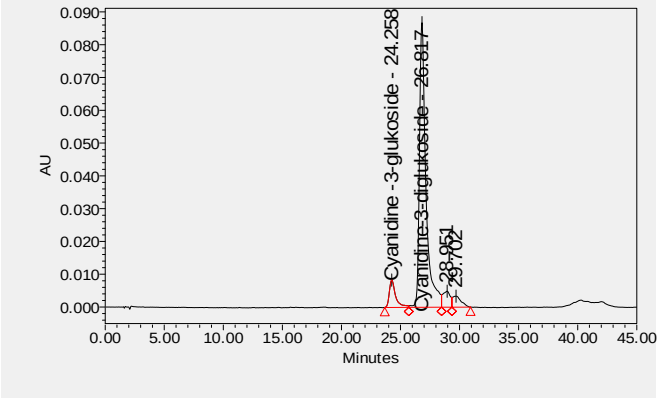


Picture 1.3 .– the flower anthocyanins chromatogram of the Margaret Walker-G variety

Table 1.3- the chromatographic characteristics of the Margaret Walker-G flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
3	Margaret Walker -ბ	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount ნ.მ.	Units
1	Cyanidine -3-glukoside	24.668	289074	11.61	457,8	45,7	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	27.133	2201321	88.39	3624,4	362,4	mg/kg
	Total Anthocyans				4100	410	mg/kg



Picture 1.4 .– the flower anthocyanins chromatogram of the Margaret Walker-D variety

Table 1.4- the chromatographic characteristics of the Margaret Walker-D flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
3	Margaret Walker -ბ	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount ნ.მ.	Units
1	Cyanidine -3-glukoside	24.257	311251	7.72	347,4	43,2	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	26,817	3333439	82.69	3722	463,1	mg/kg
4		28.951	214374	5.32			
5		29.702	172200	4.27			
	Total Anthocyans				**4500**	**560**	**mg/kg**

The highest amount of them is detected in Camellia variety “Margaret Walker” - G (410 mg / kg); The lowest amount is found in variety “Margaret Walker” - A (30 mg / kg) The anthocyanins cyanidin-3- diglucoside is dominant one in both variation (A) (85 and 75 %, respectively Composition and spectrum of anthocyanins in camellia variety is genetically programmed process. Camellia as a cross-pollinating plant and compound heterozygote is highly tend to impact of the environmental factors (temperature, nutrition, chemical and radio toxics, etc). Therefore, as a result of mutation the anthocyaninsare changed as well (both quantitavely and qualitatively) that is finally reflected in the flower coloration.

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