



## RESEARCH ARTICLE

### CONSISTENCY OF ANTHOCYANINS AND PHLAVANOIDIC GLYCOSIDES IN THE DECORATIVE BUSHES OF ABELIA GRANDIFLORA, FORSYTHIA VIRIDISSIMA AND PIERIS JAPONICA ANTHOCYANINS AND FLAVONOID GLUCOSIDES

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#### ARTICLE INFO

##### Article History:

Received 26<sup>th</sup> October, 2015

Received in revised form

12<sup>th</sup> November, 2015

Accepted 31<sup>st</sup> December, 2015

Published online 31<sup>st</sup> January, 2016

##### Key words:

Abelia, Forsythia, Pieris, Anthocyanins, Flavonoid, Flower.

#### ABSTRACT

Decorative bushes introduced in Batumi Botanical Garden: *Abelia grandiflora* (Andre) Rhed, *Forsythia viridissima* Lindl and *Pieris japonica* Thunb. D. in the humid subtropical zone of the Black Sea are characterized with high growth rhythm and abundant blossom. Therefore, these plants are widely used in greening and phitodesign of sea coast parks and orchards. The aim of our research was to determine the consistency of the anthocyanins and phlavanoidic glycosides in the introduced decorative bushes for the medical and cosmetic purposes.

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**Citation:** Nino Lomtadze, EterMachutadze, Nargiza Alasania and Aleko Kalandia, 2016. "Consistency of anthocyanins and phlavanoidic glycosides in the decorative bushes of *Abelia grandiflora*, *Forsythia viridissima* and *Pieris japonica* anthocyanins and flavonoid glucosides", *International Journal of Current Research*, 8, (01), 24846-24851.

## INTRODUCTION

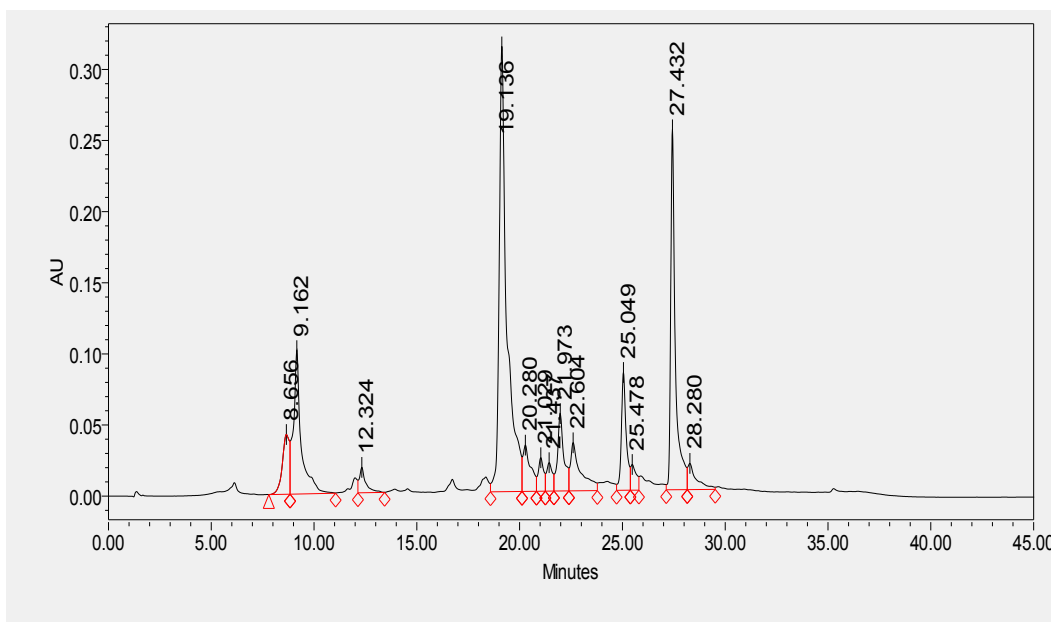
***Abelia grandiflora* /Andre/ Rhed:** Originally from China and Japan. It's a half evergreen decorative bush from the family of Caprifoliaceae; introduced in Batumi Botanical Garden in 1913. It is leafy, blossoming 2 m. high bush with reddish fluffy sprouts. The leaf is silverfish, dark green, shiny, oval shaped, with a pointed tip of about 1.5 – 3.5 m. long. It's characterized with abundant and long blossom, quick growth and aromatic flowers; blooms and is fruitful regularly. These are the factors that determined its wide spread and usage in the decorative gardening (*Abelia*, 2006).

***Forsythia viridissima* Lindl:** Family of Oleaceae; characterized with shedding leaves; originally from China; introduced in Batumi Botanical Garden in 1913. The reasons it's used in the decorative gardening are early and long-time blossoming. Even when most of the plants have not yet covered with leaves or are just starting the process, forsythia is already covered with beautiful yellowish-goldish bell-shaped flowers. After the end of blooming the bush is covered with light green leaves which turn into blood red during the fall.

***Pieris japonica* Thunb. D.:** Family of Ericaceae; spread in North America, East Asia and Himalayans. It is an evergreen decorative bush. Ericaceae family consists of more than 1500 species of more than 70 names; introduced in Batumi Botanical Garden in 1913. During the vegetation process new leaves are greenish-reddish which makes the plant very beautiful.

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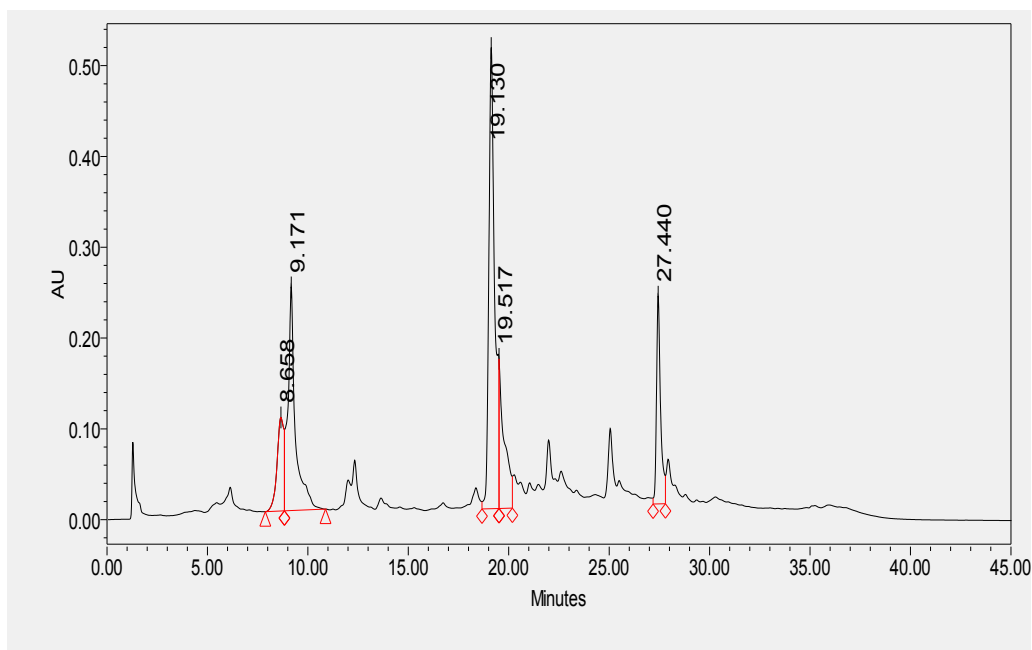
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Picture 1. Flower rainfall is 360 nm. *Abelia grandiflora*

Table 1. Flower rainfall rates of 360 nm. *Abelia grandiflora*

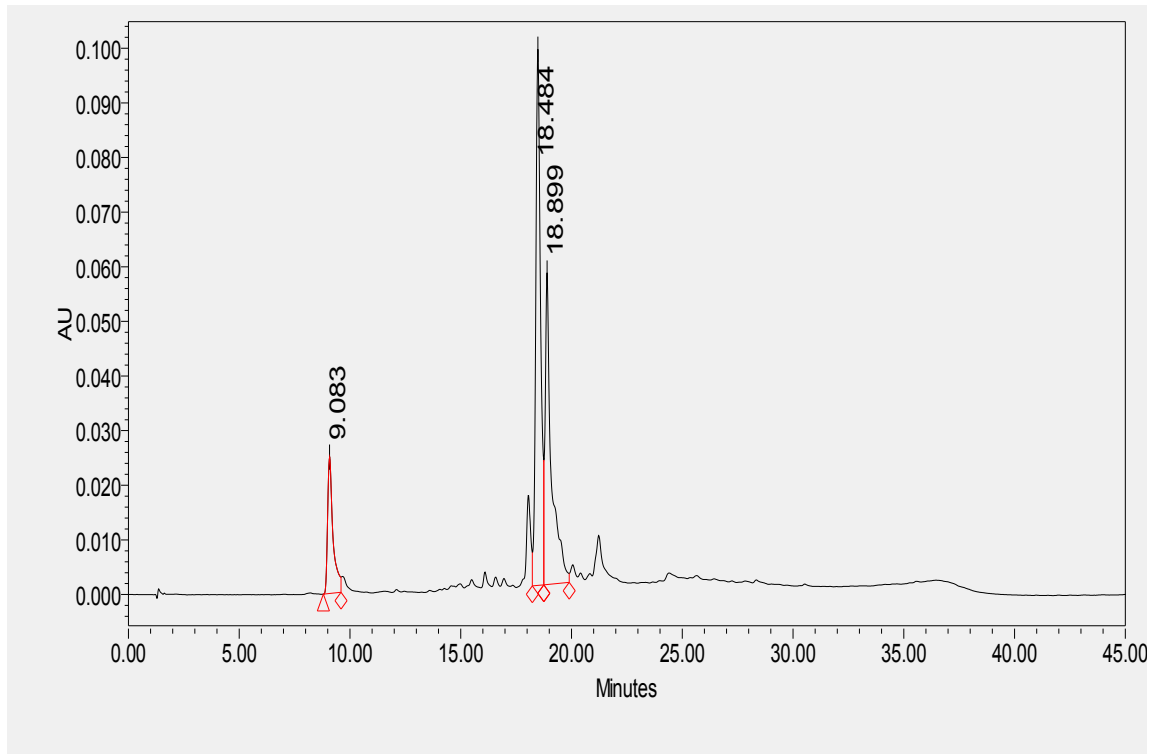
N	Retention Time Minutes	Area	% Area	Height
1	8.656	998522	4.42	41982
2	9.162	2672329	11.84	101086
3	12.324	389232	1.72	17920
4	19.136	8181176	36.24	313094
10	25.049	1361246	6.03	82930
11	25.478	348520	1.54	18117
12	27.432	4037650	17.89	253263
13	28.280	553229	2.45	18441



Picture 2. Flower rainfall is 280 nm. *Abelia grandiflora*

Table 2. Flower rainfall rates of 280 nm. *Abelia grandiflora*

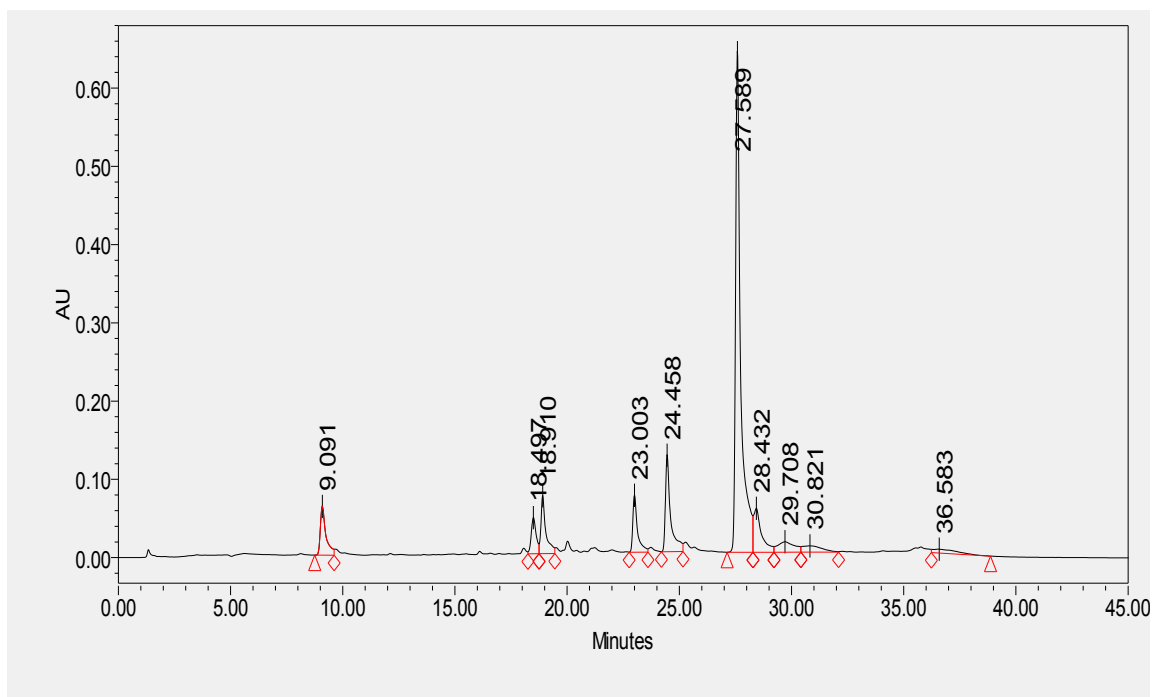
N	Retention Time Minutes	Area	% Area	Height
1	8.658	2393503	9.40	103126
2	9.171	6427420	25.23	246478
3	19.130	10387805	40.78	508058
4	19.517	2988214	11.73	164682
5	27.440	3274548	12.86	229441



Picture 3. Flower rainfallis 360 nm. Forsythia viridissima

Table 3. Flower rainfall rates of 360 nm Forsythia viridissima

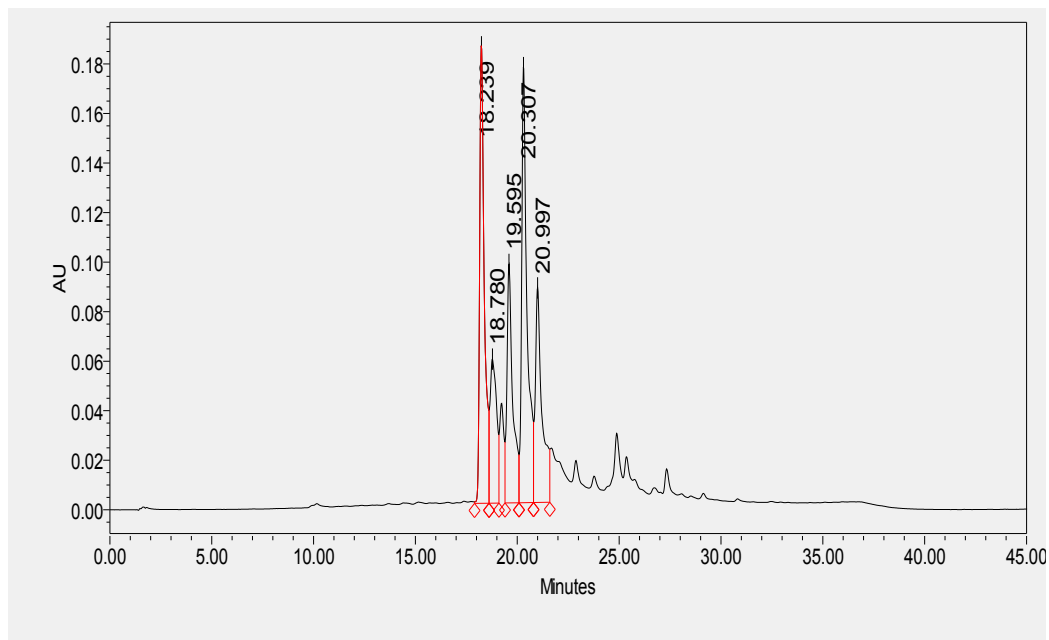
N	Retention Time Minutes	Area	% Area	Rush code
1	9.083	422891	13.86	
2	18.484	1481233	48.55	
3	18.899	1146874	37.59	



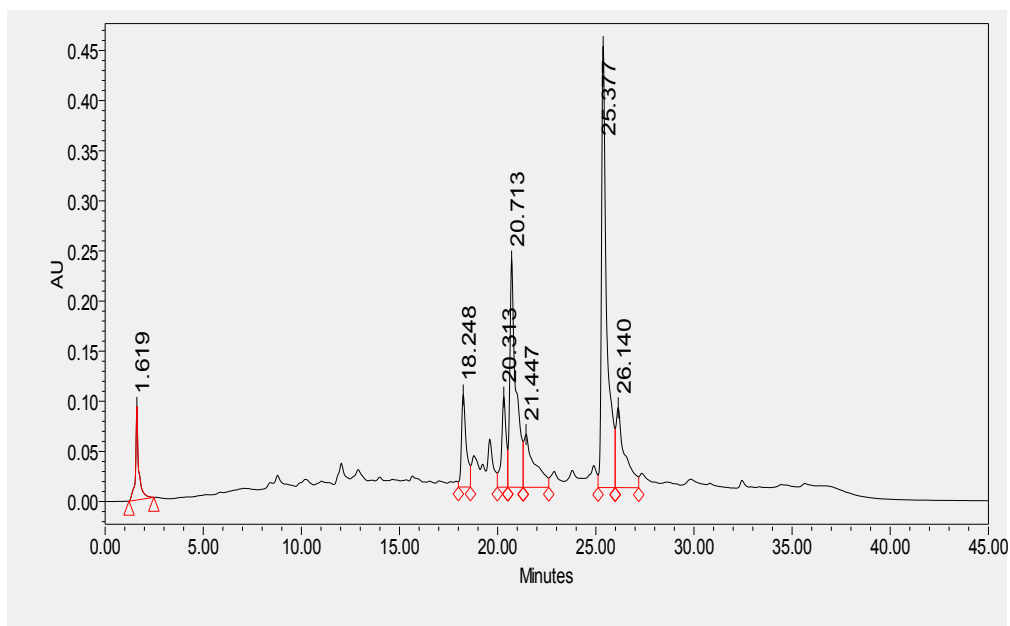
Picture 4. Flower rainfallis 280 nm.Forsythia viridissima

**Table 4. Flower rainfall rates of 280 nm *Forsythia viridissima***

N	Retention Time Minutes	Area	Area	Height	Rush code
1	9.091	1079234	5.62	62444	
2	18.497	708256	3.69	46299	
3	18.910	1127466	5.87	74208	
4	23.003	1059880	5.52	73152	
5	24.458	2001966	10.42	124119	
6	27.589	10244318	53.31	641696	
7	28.432	1393367	7.25	56125	

**Picture 5. Flower rainfallis 360 nm. *Pieris japonica*****Table 5. Flower rainfall rates of 360 nm *Pieris japonica***

N	Retention Time Minutes	Area	% Area	Height	Rush code
1	18.239	2975685	25.88	185105	
2	18.780	1313349	11.42	58149	
3	19.595	1912059	16.63	96485	
4	20.307	3284574	28.57	176480	
5	20.997	2010629	17.49	86714	

**Picture 6. Flower rainfallis 280 nm. *Pieris japonica***

**Table 6. Flower rainfall rates of 280 nm Pieris japonica**

N	Retention Time Minutes	Area	% Area	Height	Rush Code
1	1.619	1023242	4.71	92378	
2	18.248	1565253	7.20	91860	
3	20.313	1479141	6.80	90099	
4	20.713	4919031	22.63	226082	
5	21.447	2021768	9.30	52662	
6	25.377	8220732	37.81	441116	
7	26.140	2512327	11.56	79759	

In GC many of the peaks are yet unknown as at this point we don't have an access to the corresponding authentic compounds. For their further identification and quantitative analysis authentic compounds were used.

**Table 7. Results of the Quantitative Analysis**

Sample Name	Flavonols Mg/kg		Leucoanthocyanins Mg/kg		Catechines Mg/kg	
	Raw material calculation	Dry material calculation	Raw material calculation	Dry material calculation	Raw material calculation	Dry material calculation
AbeliaGrandiflora	170,0	1096,77	13,52	87,23	10,81	69,29
Pieris Japonica	3530,0	4116,52	293,0	341,66	245,4	286,15
Forsythia viridissima Lindl	1865,5	9818,5	143,5	755,26	88,5	466,31
Least essential average 05		284,6		32,18		26,53

Pieris is very pretty in the beginning of blooming (dark hazel-yellowish flower bunches) as well as massive blossoming period when the plant is covered with white abundant flowers around. Flowers and leaves contain Glucoside Andromedotoksin, which belongs to the neurotoxins and can cause the irritation of human nervous system. Usage of pieris in greening is very common (Norihiro Terahara *et al.*, 2001; Mikhalevskaya, 2004).

### Experimental

The purpose of our research was to determine the qualitative and quantitative indicator Anthocyanins end Flavonoid Glucosides in the flowers of Abelia Grandiflora, Forsythia Viridissima and Pieris Japonica. The research was being held with the method of the high pressure liquid chromatographing with the purpose of using them for medical and cosmetic purposes. High pressure liquid chromatographing enables to study qualitative and quantitative characteristics of the various components contained in the flowers of decorative plants and the products received from them. 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 0,45 µm filters (Acrodist LC PVDF Syringe Filters Waters) prior to HPLC analysis (Buer *et al.*, 2010; Gou *et al.*, 2011).

### RESULTS AND DISCUSSION

For the extraction of Anthocyanins 3% hydrochloric acid. Extraction lasted for 24 hours in darkness at a room temperature. As for the extraction of Flavonoid Glucosides 40% ethanol. The duration of extraction was 1 hour and the temperature was 70° C. For the chromatographing gradient HPLC was used Waters (USA), uv/visible Detector 2489, Binary HPLC Pump 1525; detecting on 280 and 360 nm.

Moving phase 5% formic acid (A) and methanol (B), linear gradient, solvent speed 0.7 ml/min; column temperature 40°C, sample number 20 µl; chromatographing duration – 45 mins. For the substance identification and qualitative analysis authentic compounds were used. For each compound calibration curves were built. Main components of the decorative plants' flowers are: cyanidin – 3, Galactoside retention time 13.026 mins; 2.83% of the total amount; delphinidin 3 also was possible to determine - Galactoside retention time 11.565 mins; delphinidin 3- 12.442 mins retention time of Glucoside; Petunidin-3 galactoside 15.729 mins retention time. For identification and quantitative analysis of the Flavonoid Glucosides authentic compounds were used. Flavonoid Glucosides – quercetin 3-glucoside, quercetin 3-rutinozid end quercetin. According to the calibration curves were constructed.

In GC many of the peaks are yet unknown as at this point we don't have an access to the corresponding authentic compounds. For their further identification and quantitative analysis authentic compounds were used. Based on the quantitative analysis of the flowers of decorative bushes: Abelia Grandiflora, Pieris Japonica and Forsythia it has been determined that the consistency of Flavonoids in the flowers of Abelia Grandiflora counting on the raw material equals 170.0 mg/kg and on the dry material – 1096.77 mg/kg. The content of leucoanthocyanins on the raw material equals 13.53 mg/kg and on the dry material – 87.23 mg/kg. As for the Catechins, on the raw material it is 10.81 mg/kg and on the dry material – 69.29 mg/kg. The consistency of Flavonoids in the flowers of Pieris Japonica counting on the raw material equals 3530.0 mg/kg and on the dry material – 4116.52 mg/kg. The content of leucoanthocyanins on the raw material equals 293.0 mg/kg and on the dry material – 341.66 mg/kg. As for the Catechins, on the raw material it is 245.4 mg/kg and on the dry material – 286.15 mg/kg. What concerns Forsythia flowers, The consistency of Flavonoids on the raw material equals 1865.5 mg/kg and on the dry material – 9818.5 mg/kg. The content of leucoanthocyanins on the raw material equals 143.5 mg/kg and on the dry material – 755.26 mg/kg. As for the Catechins, on

the raw material it is 88.5 mg/kg and on the dry material – 466.31 mg/kg. Therefore, the research has shown that the consistency of flavonols in crude plants is the highest in *Pieris Japonica* (3530.0 mg/kg), and in dry plants – flowers of *Forsythia Viridissima* (9818, 5 mg/kg). Leucoanthocyanins' consistency in crude material is the highest in *Pieris Japonica* (293, 0 mg/kg) and in dry material – in flowers of *Forsythia Viridissima* (755, 26 mg/kg). The highest level of Catechins in crude material is included in flowers of *Pieris Japonica* (245,5 mg/kg) and in dry material – the flowers of *Forsythia Viridissima* (466, 31 mg/kg). Based on this research, using these species for medical and perfumery purposes is recommended.

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