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A study of the component composition of phenolic compounds obtained from Dahlia varieties Ken's Flame herb

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ABSTRACT

The phenolic compounds of Dahlia varieties Ken's Flame herb was analyzed by HPLC. 42 components were found, 11 of which are identified. Components of phenolic compounds were represented by the following groups of substances: hydroxycinnamic acids, flavonols, flavan-3ol, flavones, catechins, cumarin, tannins and anthocyans. Among the identified substances dominated rutin, apigenin-7-glycoside, apigenin.

Keywords: Dahlia, HPLC, phenolic compounds.

INTRODUCTION

Among the various sources of natural vegetable inulin most promising raw materials are tubers of dahlia which posses valuable properties for the prevention and treatment of diseases of the digestive and endocrine system, disorders of lipid metabolism [1-3, 9, 10].

Among the various sources of natural vegetable inulin most promising raw materials are tubers of dahlia который inulin possesses valuable properties for the prevention and treatment of diseases of the digestive and endocrine system, disorders of lipid metabolism [4-8]. Interest deepens to the cultivated species of the genus Dahlia plants but complete phytochemical study of herba has not been. Accordingly, the purpose of our research was studying the qualitative structure and the quantitative content of phenolic compounds of herba Ken's Flame grade.

MATERIALS AND METHODS

2.1 Plant Material and Chemicals

The plant material of Dahlia Waterlily varieties Ken's Flame (herb) harvested during the plant's flowering was collected in August-September 2012 from Kharkivskiy region, Ukraine. The products were naturally dried in the shadow and stored in controlled laboratory conditions. The grounded dried samples (10.0 g / 40 meshes) of Dahlia herb were extracted with 100 ml of solvent - 70 % ethanol for 30 minutes. Extraction was carried out for three times by extraction in water bath. Extracts were adjusted to the volume 100 ml and filtered through a 0.45 mm Millipore membrane filter before direct injection into the HPLC system.

Identification of phenolic compounds in dahlia extracts was achieved by comparing their retention times with those of standards. The reference standards of rosmarinic acid, luteolin, quercetin, apigenin, gallic acid, chlorogenic acid, caffeic acid, rutin, ferulic acid was purchased from Ukrainian pharmacopoeia committee.

The purity for all reference standards was over 98 %.

2.2 Chromatographic conditions

HPLC was performed on Shimadzu LC-20 Prominence module system equipped with a LC-20AD quaternary pump, a CTO-20A column oven, a SIL-20A autosampler, a SPD-M20A diode array detector and LC-20 chemstation for data analysis was used.

A Phenomenex® Luna C18 (250 x 4.6 mm I.D., 5 µm) column at 35 °C was used. The flow rate was 1ml/min; injection volume was 5 µl. UV–VIS absorption spectra were recorded on-line during HPLC analysis and the spectral measurements were made over the wavelength range 300 nm.

Solvent system for elution: A: 0.01 % trifluoroacetic acid in acetonitrile and B: 0.01 % trifluoroacetic acid in water in gradient elution was used.

Phenolic compounds of Dahlia waterlily var. Ken's Flame extracts were identified by comparison of their retention times with those of reference standards.

UV–VIS absorption spectra were recorded on-line during HPLC analysis. The spectral measurements were made over the wavelength range 180 – 800 nm in steps of 2 nm. The purity of each peak was checked by DAD software. The content of each compound was established by external standard calibration curves.

Table 1 The Program of Gradient Elution

Time, min	Solvent A, %	Solvent B, %
0–5	95	5
5–35	95 → 75	5 → 25
35–40	75	25
40–60	75 → 50	25 → 50
60–65	50 → 20	50 → 80
65–70	20	80
70–85	95	5

Phenolic acids and flavonols were identified at the wavelengths of 330 nm, catechins and procyanidins – at the 280 nm.

Calculations:

$$Assay\ (\%) = \frac{A_{pr} \times m_{st} \times V_{pr} \times P \times 100}{A_{st} \times V_{st} \times m_{pr} \times 100}, \text{ where}$$

Assay (%) – phenolic compound content in dahlia liquid extract.
A_{pr} – area of phenolic compound peak in the chromatogram recorded for the test solution;
A_{st} – area of phenolic compound in the chromatogram recorded for the standard solution;
m_{st} – mass of phenolic compound used to prepare the standard solution, mg;
m_{pr} – mass of dahlia herb used to prepare the extract, mg;
V_{pr} – dilution of the test solution, ml;
V_{st} - dilution of phenolic compound for the standard solution, ml;
P – purity of phenolic compound, %.
All analyses were carried out three times to confirm reproducibility.

RESULTS AND DISCUSSION

For the first time the component composition of the phenolic compounds obtained from Ken’s Flame varieties of Dahlia Nymphaeales (herb) was determined by means of HPLC. 42 components were found, 11 of which are identified. Components of phenolic compounds were represented by the following groups of substances: hydroxycinnamic acids, flavonols, flavan-3ol, flavones, catechins, cumarin, tannins and anthocyans (Figure 1, Table 2).

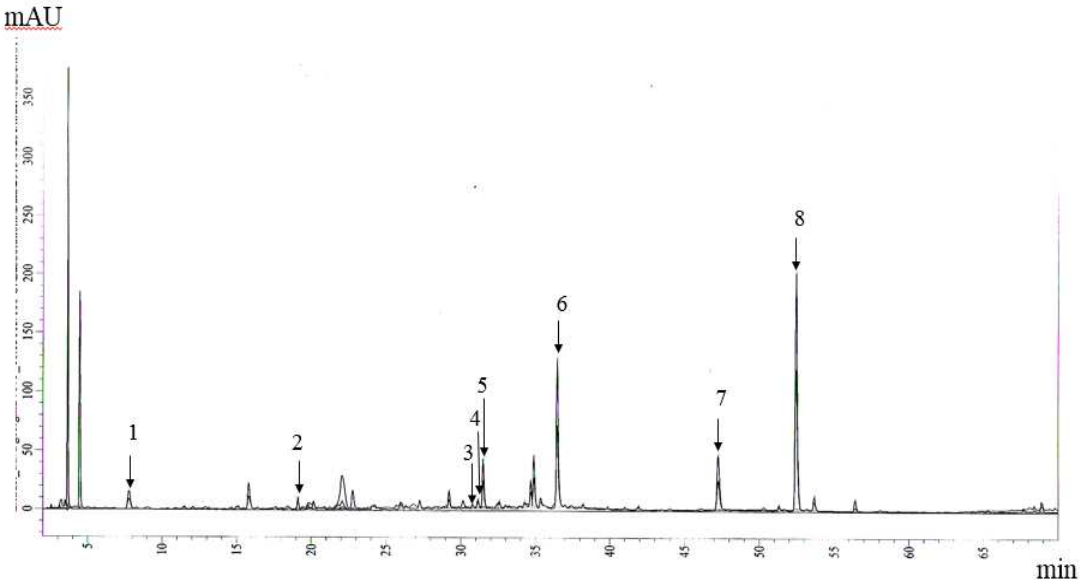

Fig. 1. HPLC Chromatogram of the 70% Ethanol Extract of Dahlia Ken's Flame Herb:
1 – gallic acid, 2 – catechin, 3 – scopoletin, 4 – hyperosid, 5 – rutin, 6 – apigenin-7-glucosid, 7 – luteolin, 8 – apigenin

Four substances belonged to the group of tannins, such as gallic acid identified. Among hydroxycinnamic acids 4 substances were found, 3 of which are identified – neochlorogenic acid, cholorogenic acid and caffeic acid. There are 6 substances belonging to the group of anthocyans. Among the classes of compounds detected flavonoids dominated (21 substances), 6 of which are identified (Table 2).

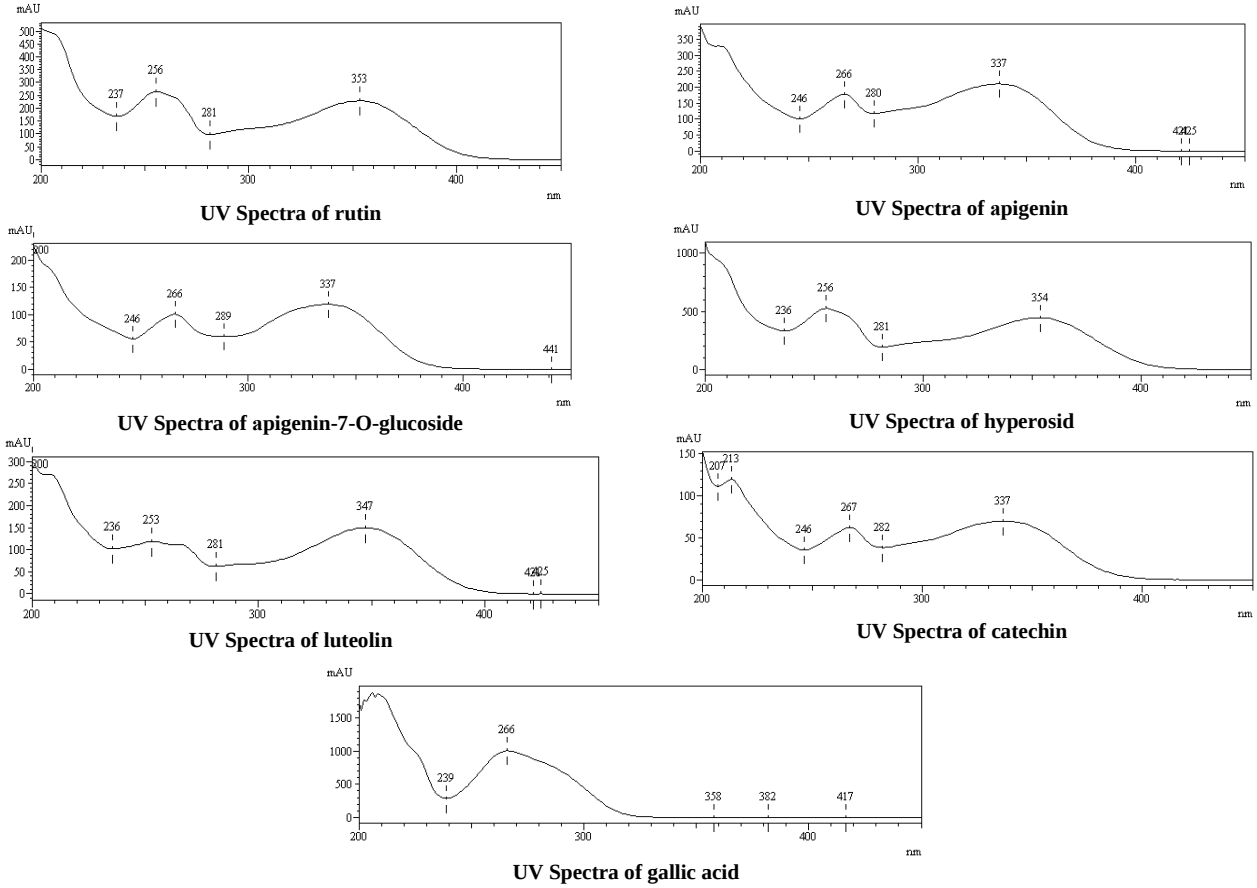

Fig. 2. UV Spectra of Authentically Identified Phenolic Compounds

UV spectra of the major substances presented in Figure 2. For glycosides of flavonoids (rutin and hyperoside) absorption maxima were observed in the shortwave area – 256 nm and long-wavelength – 354 nm. Flavonoids

apigenin, apigenin-7-glycoside and cathechin had a common absorption maxima – 266 nm и 337 nm. For luteolin characteristic absorption maxima 253 nm and 347 nm, for gallic acid – 266 nm

Table 2 Phenolic Compounds Identified in 70% Ethanol Extract of Dahlia Ken's Flame Herb

No	Compound	Retention time, min	Peak area, mAU	Wavelength, nm
1.	tannin 1	3.65	1220524	280
2.	tannin 2	4.44	959307	280
3.	gallic acid	7.78	217159	330
4.	unknown phenolic compound 1	12.92	44115	254
5.	unknown phenolic compound 2	14.64	30243	254
6.	neochlorogenic acid	15.80	241993	330
7.	unknown phenolic compound 3	17.62	24089	330
8.	unknown phenolic compound 4	17.98	9929	330
9.	catechin	19.11	70764	330
10.	antocyan 1	19.92	47198	550
11.	chlorogenic acid	20.15	55099	330
12.	unknown phenolic compound 5	20.87	8199	330
13.	caffeic acid	21.79	60166	330
14.	antocyan 2	22.11	67915	550
15.	tannin 3	22.77	149631	280
16.	antocyan 3	23.95	9001	550
17.	antocyan 4	24.97	6730	550
18.	antocyan 5	26.01	7922	550
19.	antocyan 6	26.86	10749	550
20.	flavonoid 1	27.26	66724	330
21.	hydroxicnnamic acid 1	29.20	123368	330
22.	scopoletin	30.76	35699	330
23.	hyperosid	31.13	67630	330
24.	rutin	31.49	362585	330
25.	quercetin derivative 1	32.41	33118	330
26.	apigenin derivative 1	32.57	58189	330
27.	quercetin derivative 2	34.27	53892	330
28.	quercetin derivative 3 (triglycoside)	34.68	177938	330
29.	flavonoid 2	34.89	404027	330
30.	flavonoid 3	35.35	72683	330
31.	apigenin-7-glucosid	36.46	1185576	330
32.	flavonoid 4	38.19	31568	330
33.	flavonoid 5	39.84	17386	330
34.	flavonoid 6	40.99	26746	330
35.	flavonoid 7	41.90	38215	330
36.	unknown phenolic compound 6	43.97	20080	330
37.	luteolin	47.25	580497	330
38.	flavonoid 8	51.30	38727	330
39.	apigenin	52.45	2066772	330
40.	flavonoid 9	53.18	11792	330
41.	apigenin derivative 2	53.66	124666	330
42.	quercetin derivative 4	56.40	103250	330

The results of determining the quantitative content of the components (Fig. 3) revealed, that the predominant are flavnol – rutin (0,466%). Hyperoside contained in small amounts – 0,021%. Flavones were presented in 3 substances – apigenin (0,081%), apigenin-7-glycoside (0,152%) and luteolin (0,031%). Catechin is flavan-3-ol and contained in an amount 0,014%. Among hydroxycinnamic acids predominant are chlorogenic (0,017%), neochlorogenic and caffeic acids contained in small amounts (0,004% и 0,002% respectively). Gallic acid contained in an amount 0,017%. Among coumarin scopoletin was identified (0,001%).

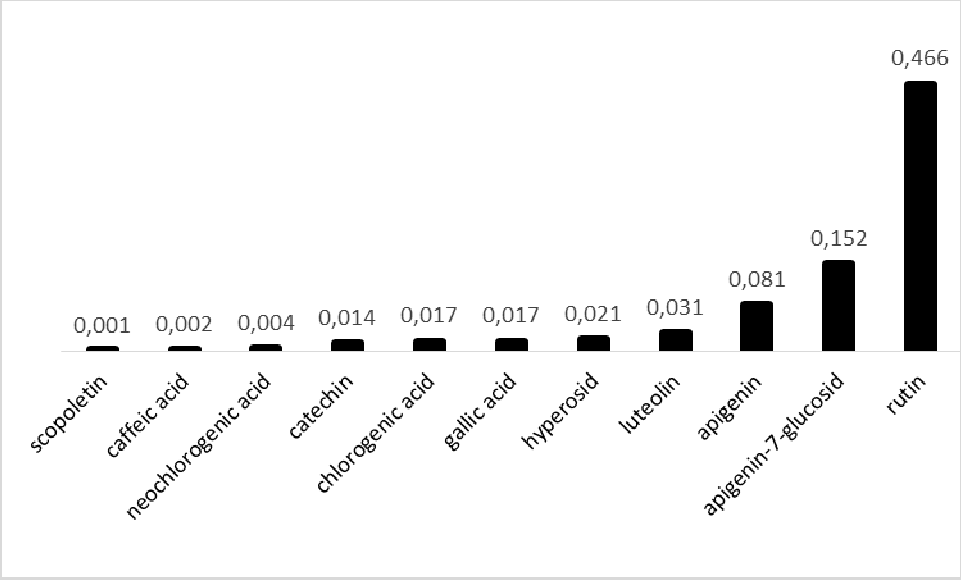

Fig. 3. Content of identified phenolic compounds in 70% Ethanol Extract of Dahlia Ken's Flame Herb (on dry matter)

CONCLUSION

For the first time the component composition of the phenolic compound from Ken's Flame cultivar of Dahlia herb was determined by means of high performance liquid chromatography.

42 components were found, 11 of which are identified. Among the identified substances dominated rutin (0,466%), apigenin-7-glycoside (0,152%) and apigenin (0,081%).

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