Phenolic Acids of *Rhaponticum carthamoides*

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Abstract

The relation between the age of plants and the accumulation of phenolic acids, as well as their composition in particular organs of *Rhaponticum carthamoides* (Willd.) Iljin [*Leuzea carthamoides* D.C.] was investigated. The content of phenolic acids in rhizomes was higher than in roots (1.40% and 0.85% respectively). The content of these compounds in basal leaves was higher than in stem leaves. Underground organs and leaves were characterised by different compositions of phenolic acids. The dominant compound in the fraction of free phenolic acids from underground organs (rhizomes and roots) appeared to be gentisic acid and in the fractions of bound acids – o-hydroxyphenylacetic acid. The dominant compound in the fraction of free phenolic acids from basal leaves was protocatechuic acid. The biggest share in the fraction of free phenolic acids from stem leaves had o-hydroxyphenylacetic and m-hydroxybenzoic acids. It is worth stressing that both gentisic acid (present in underground organs) and protocatechuic acid (in leaves) are known to reveal an immunotropic activity.

INTRODUCTION

Rhaponticum carthamoides (Willd.) Iljin [*Leuzea carthamoides* D.C.] – a perennial originating from Siberia – is considered to be one of the adaptogenic plants. Its rhizomes with roots have been used for many years as a stimulant, roborant and tonic agent. Recently, also *Rhaponticum*'s leaves have been taken into consideration as a medicinal raw material, especially in respect of their immunotropic properties. So far, it has been stated that immunomodulatory activity of *Rhaponticum* extracts could be connected with the presence of polysaccharides (Lamer-Zarawska et al., 1996). However, it is known that one of the important groups of plant constituents of immunotropic activity are phenolic compounds, including phenolic acids. They are responsible for many other pharmacological effects, too. Some of them reveal e.g. biligenic, antibacterial and antiviral activity (Borkowski, 1993; Borkowski and Miłkowska, 1996). Phenolic acids have not been an object of thorough research in *Rhaponticum* as yet.

The aim of the present study was to determine the content and composition of phenolic acids in underground and above ground organs of *Rhaponticum*, obtained from plants of different age.

MATERIALS AND METHODS

Plant Material

Plant material for analysis was obtained from the experimental field of Dept. of Vegetable and Medicinal Plants.

Rhizomes and roots of one-, two- and three-year-old plants were collected in October. Basal and stem leaves of two-, three- and four-year-old plants were harvested in May (at the stage of plant blooming). The raw materials were dried at 40°C.

Quantitative Determination of Phenolic Acids

The total polyphenolic acid content (converted into caffeic acid) was determined according to Polish Standard (PN-91/R-87019).

Qualitative Determination of Phenolic Acids

The isolation of phenolic acid fractions was made according to Krzaczek (1984). Free phenolic acids and those revealed after alkaline and acid hydrolysis were sililated (with BSTFA, Fluck) and identified by a GC method. Gas chromatography was performed on a Hewlett Packard 6890 as follows: capillary column HP-5, length 30 m, diameter 0.32 mm; detector FID temperature 250ºC; injector temperature 220ºC; carrier gas–helium with the flow rate 2 ml/min. Column temperature was programmed as follows: 60ºC (2 min.), temperature increment 8ºC/min. and finally 280ºC (5 min.). Particular compounds were identified on the ground of retention times of the standard phenolic acids.

RESULTS

The content of phenolic acids in rhizomes was higher than in roots, regardless of the age of plants (Fig. 1). On the basis of GC, 17 phenolic acids were identified. They were present especially in a free form and some of them also in a bound form. The composition of these compounds in rhizomes and roots of one- and two-year-old plants was similar (in the fractions of free phenolic acids the dominant was gentisic acid and in the fractions of bound acids – o-hydroxyphenylacetic acid). Three-year-old plants were characterised by different composition of phenolic acids. There was no clearly dominant compound in the fractions of free phenolic acids, however in rhizomes a little bigger share in this fraction had protocatechuic acid and in roots - p-hydroxybenzoic, protocatechuic and p-coumaric acids. In the fractions obtained after alkaline and acid hydrolysis of extracts, of both rhizomes and roots, the main compound was p-coumaric acid (Table 1).

The content of phenolic acids in basal leaves of two- and three-year-old plants was higher than in stem leaves. In four-year-old plants there was no difference in the content of these compounds between basal and stem leaves (Fig. 2). The dominant compound in the fraction of free phenolic acids from basal leaves was protocatechuic acid. In the fractions of bounded acids the main compounds were protocatechuic and p-hydroxybenzoic acids. In stem leaves, the biggest share in the fraction of free phenolic acid had o-hydroxyphenylacetic and m-hydroxybenzoic acids (Table 2).

DISCUSSION

Previous studies on *Rhaponticum carthamoides* dealt with such groups of plant constituents as ecdysteroids (Varga et al., 1986; Girault et al., 1988), flavonoids (Varga et al. 1990), triterpenoid saponins (Vereskovskij et al., 1977) and polyacetylenes (Szendrei et. al., 1984). There was no report on the presence and composition of phenolic acids in *Rhaponticum* plants. Our preliminary investigation indicated that they are present in considerable amount both in underground and above ground organs of plants. That study showed also differences in the composition of this group of compounds between particular organs of four-year-old plants (Skiba and Węglarz, 1999). The results of the present study confirm that the content of phenolic acids in rhizomes is higher than in roots, with no respect to the age of plants. The composition of this group of plant constituents in rhizomes and roots appeared to be dependent on the age of plants. Three-year-old plants differed markedly in this respect from one- and two-year-old plants. It is worth emphasising the remarkable dominance of gentisic acid in the fraction of free phenolic acids isolated from rhizomes and roots of one- and two-year-old plants. According to Borkowski (1993), gentisic acid has immunotropic activity – it affects lymphocyte-induced angiogenesis and enhances IgG antibodies production. The other phenolic acid, which seems to be important from the pharmacological point of view, is p-coumaric acid. It is known to have an immunomodulatory and antibacterial activity (Świątek et al., 1984; Borkowski and Miłkowska, 1996) and according to our results, it is present in rhizomes and roots of one-, two- and three-year-old plants, both in a free and bound form.

The results of our previous and present study indicate that the dominant phenolic

acid in *Rhaponticum* leaves (especially in the basal ones) is protocatechuic acid. This is an important finding, taking into consideration that this acid also affects the humoral response of organisms by enhancing IgG antibodies production (Borkowski, 1993).

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Table 1. Composition of phenolic acids in rhizomes and roots [% in particular fraction]

Acid	1-year-old plants						2-year-old plants						3-year-old plants					
	Rhizomes			Roots			Rhizomes			Roots			Rhizomes			Roots		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
benzoic	0.13	-	-	0.63	-	-	0.17	-	-	0.58	-	-	1.82	1.16	-	0.87	-	-
elagic	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-	-
chlorogenic	-	-	-	1.42	-	-	-	-	-	1.02	-	-	-	-	-	0.36	-	-
salicylic	0.73	-	-	0.29	-	-	0.48	-	-	0.23	-	-	1.94	-	-	0.94	-	-
m-hydroxy-benzoic	0.34	-	-	0.47	-	-	0.35	-	-	0.23	-	-	-	1.41	-	0.43	-	-
o-hydroxy-phenylacetic	10.27	37.34	62.94	13.02	54.70	10.46	10.73	37.26	50.79	14.74	41.05	56.20	-	-	-	0.45	-	-
p-hydroxy-benzoic	3.42	0.83	-	2.19	-	-	2.67	-	-	2.04	-	-	-	-	-	5.29	-	-
p-hydroxy-phenylacetic	0.16	3.32	-	0.93	3.74	0.30	0.33	2.87	-	2.06	2.30	-	0.33	-	-	1.98	-	-
vanillic	0.61	1.82	-	4.53	1.66	-	0.37	1.70	-	9.44	1.24	-	0.30	-	-	1.28	-	-
gentisic	23.53	1.85	-	21.35	2.52	-	16.61	1.63	-	16.91	1.54	-	0.34	-	-	0.70	-	-
o-coumaric	-	-	-		-	-		-	-	-	-	-	-	-	-	-	-	-
protocatechuic	5.34	-	-	3.44	1.10	-	4.47	0.92	-	4.23	0.57	-	2.89	-	-	4.73	1.06	0.84
syringic	0.37	-	-	1.11	-	-	0.29	-	-	0.92	-	-	1.89	1.91	-	0.83	-	-
p-coumaric	2.51	3.93	7.34	3.68	4.01	1.57	2.18	5.37	10.34	4.08	3.07	10.05	1.03	4.27	4.00	3.59	4.38	5.72
gallic	1.13	2.96	-	1.17	3.42	-	0.18	2.79	-	0.24	2.70	-	0.49	2.81	-	0.31	3.42	-
ferulic	0.20	-	-	0.26	-	0.46	0.21	-	-	0.21	-	2.28	-	1.07	-	-	1.28	-
caffeic	0.25	-	-	1.10	-	-	0.19	-	-	0.86	-	-	1.15	-	-	0.47	-	-
synapic	0.29	0.64	-	0.28	2.14	0.49	0.61	1.35	-	0.34	1.11	-	0.45	0.99	-	0.60	1.36	1.20

Fractions of phenolic acids: A - free, B – after alkaline hydrolysis, C – after acid hydrolysis

Table 2. Composition of phenolic acids in basal and stem leaves of 2-year-old plants [% in particular fraction]

Acid	Basal leaves			Stem leaves		
	A	**B**	**C**	**A**	**B**	**C**
benzoic	0.97	-	1.21	1.40	-	-
elagic	-	-	-	-	-	-
chlorogenic	0.27	-	-	0.53	-	-
salicylic	-	-	-	0.61	-	-
m-hydroxybenzoic	0.39	0.79	-	12.67	4.24	-
o-hydroxyphenylacetic	7.03	6.39	3.05	16.37	0.72	-
p-hydroxybenzoic	12.82	19.19	7.72	9.36	-	-
p-hydroxyphenylacetic	0.26	-	-	1.00	-	-
vanillic	7.19	5.30	3.44	9.71	0.79	-
gentisic	-	-	-	7.70	-	-
o-coumaric	1.31	1.48	1.40	0.32	-	-
protocatechuic	55.66	14.43	21.08	9.78	1.12	3.50
syringic	0.15	-	-	0.50	-	-
p-coumaric	0.65	2.41	1.01	0.77	2.74	6.07
gallic	0.25	0.79	1.29	0.56	2.63	-
ferulic	0.09	-	1.24	0.35	-	-
caffeic	0.46	0.70	1.16	2.64	-	-
synapic	-	-	-	0.27	1.31	1.64

Fractions of phenolic acids:
A - free,
B – after alkaline hydrolysis,
C – after acid hydrolysis

Figures

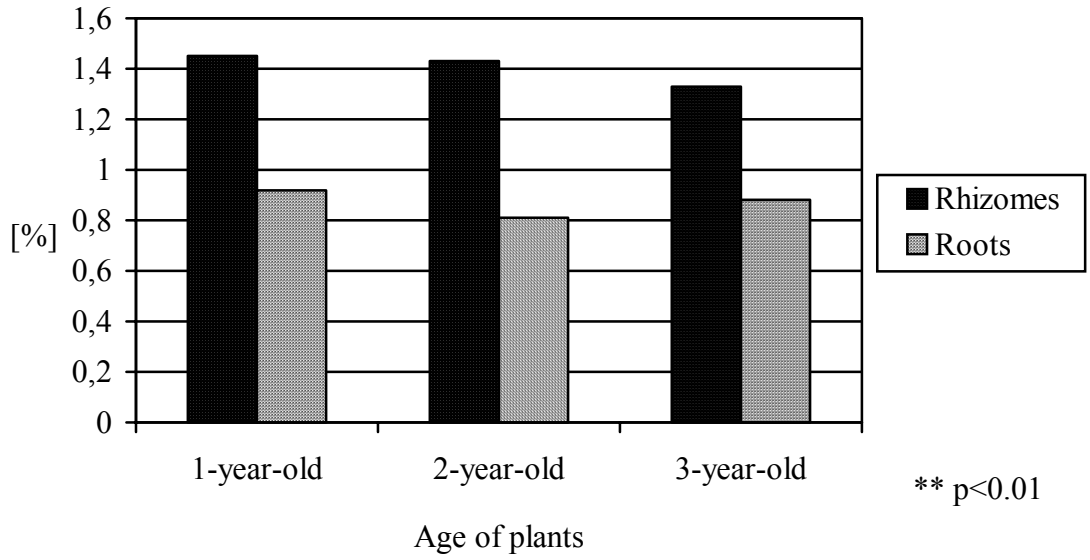

Fig. 1. The content of phenolic acids in rhizomes and roots [%]

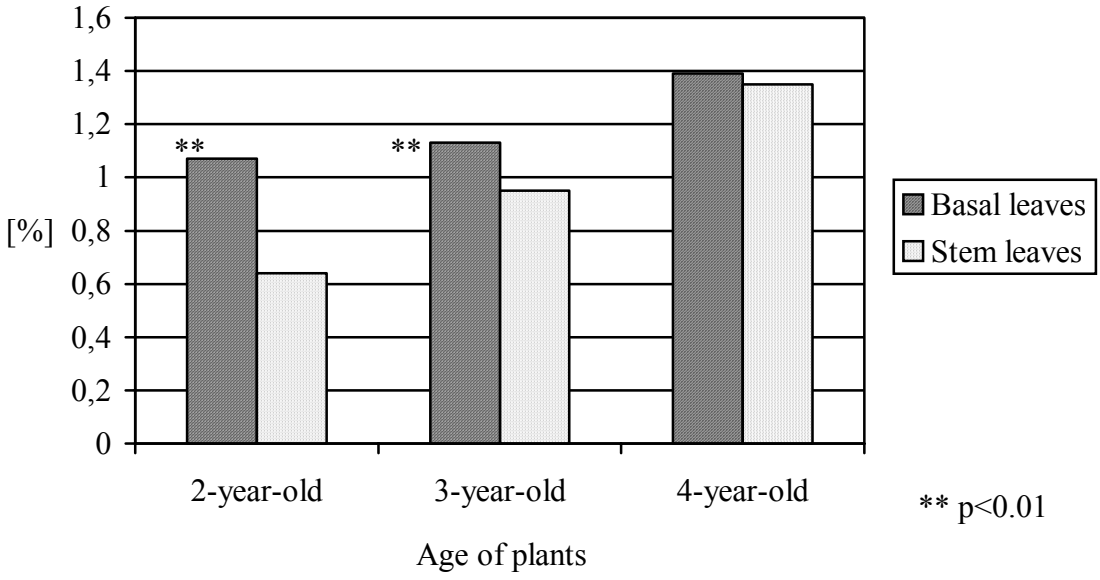

Fig. 2. The content of phenolic acids in basal and stem leaves [%]