ISSN 1644-0692 www.acta.media.pl

UM POLONO TAE Acta Sci. Pol. Hortorum Cultus, 15(4) 2016, 161-172

# CONTENT OF ALKALOIDS AND FLAVONOIDS IN CELANDINE (Chelidonium majus L.) HERB AT THE SELECTED DEVELOPMENTAL PHASES

Katarzyna Seidler-Łożykowska<sup>1</sup>, Bogdan Kędzia<sup>1</sup>, Jan Bocianowski<sup>2</sup>, Agnieszka Gryszczyńska<sup>1</sup>, Zdzisław Łowicki<sup>1</sup>, Bogna Opala<sup>1</sup>, Aurelia Pietrowiak<sup>1</sup>

<sup>1</sup> Institute of Natural Fibres and Medicinal Plants, Poznań, Poland

<sup>2</sup> Poznań University of Life Sciences, Poznań, Poland

Abstract. The content of alkaloids and flavonoids and the yield of herb were analyzed in greater celandine cultivar 'Cynober' during six following phases: spring rosette formation, the beginning of flowering, full bloom, green fruit, seed harvest, fall rosette formation. Yield of celandine herb was different at the investigated phases and in years of cultivation. The highest yield of herb was observed at the beginning of flowering, then a decrease was noticed, up to the phase of seed harvest, when the yield grown up. The lowest yield of celandine herb was obtained in last phase - fall rosette formation, except 2011 when the lowest yield was in spring rosette formation. The average content of alkaloids was the highest in phase of green fruit (1.097%), while in 2012, the highest content was reached in phase of fall rosette formation - 1.200%. The lowest content of alkaloids was obtained in herb of the beginning of flowering (0.608%) in both years. The mean content of flavonoids was from 0.310% (the beginning of flowering) to 0.522% (seed harvest) and was the same in both years. The stable high content of total alkaloids and flavonoids and individual alkaloids was noticed in phase of fall rosette. Our results suggest that seed maturity is the best time for celandine herb harvest regarding the herb yield and content of alkaloids and flavonoids.

Key words: medicinal plants, yield of herb, phenologic phase, active substances

Corresponding author: Katarzyna Seidler-Łożykowska, Institute of Natural Fibres and Medicinal Plants, 60-630 Poznań, Wojska Polskiego 71B, Poland, e-mail: katarzyna.lozykowska@iwnirz.pl

<sup>©</sup> Copyright by Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie, Lublin 2016

### INTRODUCTION

Greater celandine (Chelidonium majus L.) is the interesting species of Papaveraceae family, which commonly grows in Europe and Western Asia, mainly as a ruderal plant [Colombo and Bosisio 1996]. Celandine herb (Chelidonii herba) harvested when blooming and celandine roots (Chelidonii radix) are used in phytotherapy for their spasmolytic effect on muscles of digestive tract and bile duct. Celandine extracts have also fungistatic [Matos et al. 1999, Kedzia et al. 2013] and antiviral effects [Kèry et al. 1987, Tan et al. 1991, Gerenčer et al. 2006]. In folk medicine, the juice or orange latex of celandine has been used to remove verrucae, papillomas, condylomas and malignant tumors of the skin [Kèry et al. 1987]. Celandine alkaloids are regarded as tumor inhibitors [Colombo and Bosisio 1996, Garcia et al. 2006, Nawrot et al. 2007]. Celandine raw material contains mainly alkaloids but also flavonoids, phenolic acids, carotenoids and proteins. The others active substances are organic acids, choline, amins, saponins, triterpens, vitamins, tannins and essential oil, lipids and carbohydrates [Gertig et al. 1957, Kwasniewski 1958, Hahn and Nahrstedt 1993, Fik et al. 2000, Stancic-Rotaru et al. 2003, Kopytko et al. 2005, Horváth et al. 2010]. Celandine raw material contains isoginoline alkaloids with different structures: benzophenanthridine (chelidonine, chelerythrine, sanguinarine) and protoberberine (coptisine, berberine) [Slavik and Slavikova 1977, De Rosa and di Vincenzo 1992, Sárközi et al. 2006]. Chelidonine, berberine and coptisine are the main alkaloids in the herb, while sanguinarine and chelerythrine are dominant in the roots of celandine [Colombo and Bosisio 1996].

Celandine raw material show significant differences in alkaloids content in different parts of plant. Tomé and Colombo [1995] observed 3.5 times higher total amount of alkaloids in roots compare to herb. They also found the differences in alkaloids content in raw material collected in different parts of day – the highest content was determined in late evening while the lowest in the morning. The average content of alkaloids in celandine raw material is reported to vary for different part of plant from 0.84% (stems), 0.96% (leaves), 1.20% (herb), 1.71% (roots) to 18.40% (milky sap) [Kustrak et al. 1982, Bugatti et al. 1987, Tamas et al. 1987, Tomé and Colombo 1995, Sárközi et al. 2006]. Probably, the differences depend on genotype (chemotype), plant age, plant part, developmental stage or environmental conditions. According to Polish Pharmacopoeia IX [2011] dried herb of celandine should contain no less than 0.6% of total alkaloids recalculated on chelidonine. The herb harvesting at the flowering phase is also recommended. Evaluation of total alkaloids content in celandine raw material during every stage of production process is a subject of standardization following Polish Pharmacopoeia IX [2011].

Wide variation of the content of active substances in celandine herb has inspired the authors to discover the correlation between total content, the selected alkaloids and total flavonoids with plant age and developmental phases, which can help recognized the optimum time for herb harvest.

162

#### MATERIAL AND METHODS

Plant material. Between 2011–2012, in the experimental field of Institute of Natural Fibres and Medicinal Plants of Poznan, the experiment was established on the loamy soil of medium fertility. The mean temperature of vegetation period (March-October) was 12.2°C (2011) and 16.4°C (2012) and the amount of rainfall was 299.7 mm (2011) and 364.0 mm (2012). Celandine plants of cultivar 'Cynober' were investigated. The experimental design was completely random. Plants were harvested randomly in the second and third year of the experiment. Ten plants from each of the six following phases were collected: spring rosette formation, the beginning of flowering, full bloom, green fruit, seed harvest, fall rosette formation. The collected herbs from each harvesting phase were dried in dark, well ventilated place, then stored at room temperature. The yield of herb was measured in fresh and dried samples as well.

Determination of alkaloids. Total alkaloids expressed as chelidonine were analyzed according to the European Pharmacopoeia VI [2008] (monography of greater celandine).

**Sample:** 0.75 g of dry greater celandine herb was placed in a 250 ml roundbottomed flask and heated under reflux condenser for 30 min in 200 ml of 12% V/V glacial acetic acid. The sample was cooled down and diluted with the same solution up to 250 ml. The first 20 ml of the filtrate was rejected. To 30.0 ml of filtrate was added 6.0 ml of concentrated ammonium and 100.0 ml of methylene chloride. The solution was shaken for 30 min. 50.0 ml of the organic fraction was evaporated to dryness in vacuum at a temperature not higher then 40°C. The residue was dissolved in 3 ml of 96% ethanol, transferred to a 25 ml volumetric flask by rinsing with sulphuric acid (98 g·L) and diluted with the same solution to 25.0 ml.

**Test solution:** to 5.0 ml of the sample was added 5.0 ml of 10g·L chromotropic acid sodium salt in sulphuric acid and diluted to 25.0 ml by the sulphuric acid.

**Compensation liquid:** to 5.0 ml of the sulphuric acid (98 g·L<sup>-1</sup>) was added 5.0 ml of 10 g·L chromotropic acid sodium salt in sulphuric acid and diluted to 25.0 ml by the sulphuric acid.

All samples were placed in water-bath for 10 min and cooled down to 20°C, diluted if necessary to 25.0 ml with sulphuric acid. The absorbance was measured at 570 nm by comparison with the compensation liquid.

Determination of alkaloids by HPLC-DAD. Alkaloids were analyzed by means of modified methods of: European Pharmacopea VI [2008] (monography of greater celandine) and Sárközi et al. [2006].

**Sample:** 0.5-1.0 g of dry greater celandine herb was placed in a 250 ml roundbottomed flask and heated under reflux condenser for 30 min in 150 ml of 12% V/V glacial acetic acid. The sample was cooled down and diluted with the same solution to 200 ml. To 60.0 ml of filtrate was added 6.0 ml of concentrated ammonium and 200.0 ml of methylene chloride. The solution was shaken for 30 min. The organic fraction was evaporated to dryness in vacuum at a temperature not exceeding 40°C. The residue was dissolved in 5.0 ml of methanol.

**HPLC-DAD analysis.** HPLC-DAD analysis was performed on a Agilent 1100. Separation of methanolic sample was prepared on ZORBAX Poroshell 120 SB-C18 (Agilent)  $3 \times 100$  nm (2.7 µm). Column temperature was: 40°C. The volume of injection was 50 µl. A gradient mixture of: phase A: 30 mM ammonium formate (pH = 2.8) and phase B: acetonitrile:methanol 14.7:18.0 (V:V) were used as eluent, starting from 20% phase B to 60% phase B in 16 min. The flow-rate was: 0.50 mL·min. Peaks were identified by the addition of standards solutions and by UV-VIS spectra.

Quantitative determination for coptisine and chelidonine were performed at 240 nm, for sanguinarine and chelerithrine -280 nm and for berberine -345 nm by an external standard method. Standard solutions were prepared in methanol.

**Determination of flavonoids.** Total flavonoids, expressed as quercetine, were analyzed according to the European Pharmacopoeia VI [2008] (monography of birch leaf).

**Sample:** 0.5–1.0 g of dry greater celandine herb was placed in 100 ml roundbottomed flask. 1 ml of hexamethylenetetraamine solution (5 g·L<sup>-1</sup>), 20 ml of acetone and 2 ml of hydrochloric acid (250 g·L<sup>-1</sup>) were added and heated under reflux condenser for 30 min. The liquid was cooled down and filtered into 100 ml flask. The absorbent cotton was added, to remove residue, in the round-bottomed flask and extract two times, each of 20 ml of acetone for 10 min. All liquid extracts were combined and diluted to 100.0 ml with acetone. 20.0 ml of this solution was transferred to a separating funnel, 20 ml of water and extracted the mixture with 1 quantity of 15 ml and 3 quantities, each of 10 ml, of ethyl acetate. Combined ethyl acetate fractions were rinsed with 2 quantities, each of 50 ml of water. Organic fraction was filtrated over anhydrous sodium sulphate and diluted to 50.0 ml with ethyl acetate.

**Test solution:** to 10.0 ml of ethyl acetate solution was added 1 ml of aluminum chloride reagent (2 g $\cdot$ 100 ml<sup>-1</sup> of a 5% V/V glacial acetic acid in methanol) and diluted to 25.0 ml with 5% V/V glacial acetic acid in methanol.

**Compensation liquid**: a 10.0 ml of ethyl acetate solution was diluted to 25.0 ml with 5% V/V glacial acetic acid in methanol.

The absorbance was measured after 30 min, by comparison with the compensation liquid at 425 nm.

**Statistical analysis.** The normality of distribution of studied traits was tested using Shapiro-Wilk's *W* test (P < 0.05) [Shapiro and Wilk 1965]. A two-way analysis of variance (ANOVA) was used to analyze content of: chelidonine, coptisine, sanguinarine, berberine, chelerythrine, flavonoids and alkaloids as well as fresh and dried herb yield with year and phase as the two fixed factors. The mean value and standard deviations were calculated. The least significant differences (LSDs) *post hoc* test was used to distinguish significant treatments. The relationships between observed traits were estimated using Pearson correlation coefficients [Kozak et al. 2013]. Analysis of the data was performed using the GenStat v. 17 statistical package [VSN International 2014].

## **RESULTS AND DISCUSSION**

The main effects of year were significant for alkaloids and flavonoids content (tab. 1) as well as for content of: chelidonine, coptisine, sanguinarine, berberine and chelerythrine (tab. 2). The main effect of phase were significant for fresh and dried herb yield (tab. 1) as well as for all alkaloids content of celandine in herb, except chel-

164

erythrine content (tabs 1 and 2). The effects of year  $\times$  phase interaction were significant for all observed traits, except fresh and dried herb yield (tab. 1) and chelerythrine content (tab. 2).

Table 1. Mean squares from two-way analysis of variance for quantitative traits of celandine herb (Poznan 2011–2012)

Source of variation	d.f.	Fresh herb yield	Dried herb yield	Alkaloids content	Flavonoids content
Year	1	18330	158	0.0168***	0.0990***
Phase	5	148713**	3892.1*	0.2818***	0.0414***
Year × phase	5	10847	547.3	0.1560***	0.0347***
Residual	36	10958	676.8	0.0009	0.0001

\* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001

Table 2. Mean squares from two-way analysis of variance for alkaloids content of celandine herb (Poznan 2011–2012)

Source of variation	d.f.	Chelidonine content	Coptisine content	Sanguinarine content	Berberine content	Chelerythrine content
Year	1	2.37675***	1.75418***	0.0005243***	0.0008551***	0.0002938***
Phase	5	0.21431***	0.03251***	0.0001221***	0.0001454***	2.26E-05
Year $\times$ phase	5	0.21377***	0.01528***	0.00009117***	0.00002284***	2.17E-05
Residual	12	0.00009	0.00002	3.07.10-8	5.12.10-8	9.33.10-6

\*\*\* P < 0.001

Fresh and dried yields of celandine herb were different at the investigated phases and years (tab. 3). Dynamics of herb development and yield were similar for both years of investigation. The highest yield of herb was observed at the beginning of flowering, then a decrease was noticed, up to the phase of seed harvest, when the yield grown up. The lowest yield of celandine herb was obtained in last phase – fall rosette formation, except 2011 when the lowest yield was in spring rosette formation (data not shown). At the full bloom, green fruit and seed harvest the herb yields were similar, but the ratio between fresh and dried herb was different because of different drying rate. The herb yield collected in the fall reached the quantity of that at the beginning of vegetation.

According to Polish Pharmacopoeia IX [2011] the recommended time for celandine herb harvest is plant flowering. Kucharski [2010] claimed that the dried yield of celandine herb oscillated from 15 to 20 dt ha<sup>-1</sup> and the highest yield is usually obtained in the second year of cultivation. The herb should be harvested during plant flowering, then in autumn, before root harvest. Harvesting of herb decreases the yield of roots. Similar yields of celandine herb (6–16 dt ha<sup>-1</sup>) were obtained in our experiment. The plant phase affected differences in the herb yield.

			Yiel	d of herb	o (g·m⁻²)			Alkaloids (%)		Flavonoids (%)			
Phase	20	2011		12	mean		2011	2012		2011	2012		
	fresh	dried	fresh	dried	fresh	dried	2011	2012	mean	2011	2012	mean	
Spring rosette formation	287.5	48.3	426.5	73.5	357.0 ±98.29	$60.9 \pm \! 17.82$	$0.712\pm\!\!0.01$	$0.620\pm\!\!0.01$	$0.681 \pm 0.05$	$0.253 \pm 0.01$	$0.468 \pm 0.02$	$0.361\pm\!\!0.14$	
Beginning of flowering	1020.8	174.2	1075.0	150.8	1047.9 ±38.33	$162.5 \pm 16.55$	$0.604\pm\!0.05$	$0.616\pm\!\!0.03$	$0.608 \pm 0.04$	0.189 ±0.0034	$0.432 \pm 0.01$	$0.310\pm\!\!0.13$	
Full bloom	590.1	114.1	541.7	83.4	$565.9\pm\!\!34.22$	$98.8\pm\!\!21.71$	$0.941 \pm 0.01$	$0.858 \pm 0.03$	$0.913 \pm 0.05$	$0.403 \pm \! 0.01$	$0.424 \pm \! 0.01$	$0.414 \pm 0.01$	
Green fruit	458.3	100.2	676.5	117.6	$567.4 \pm 154.3$	$108.9\pm\!\!12.30$	$1.265\pm\!\!0.05$	$0.760 \pm 0.01$	$1.097 \pm 0.26$	$0.334 \pm 0.01$	$0.396 \pm 0.003$	$0.365\pm\!\!0.03$	
Seed harvest	533.3	143.7	750.1	178.6	$641.7 \pm \! 153.3$	$161.2\pm\!\!24.68$	0.622±0.02	$0.726 \pm 0.01$	$0.657 \pm 0.06$	$0.528 \pm 0.01$	$0.516 \pm 0.03$	$0.522 \pm 0.03$	
Fall rosette formation	320.5	100.8	209.7	33.9	$265.1\pm\!78.35$	67.4 ±47.31	$0.858 \pm 0.004$	$1.200\pm\!\!0.06$	$0.972 \pm 0.18$	$0.476\pm\!0.002$	$0.493 \pm 0.004$	$0.485 \pm 0.01$	
Mean	535.08	113.5	613.25	106.30	574.2 ±271.2	$109.9{\scriptstyle\pm}46.24$	$0.834 \pm \! 0.23$	$0.797 \pm \! 0.80$	-	$0.364 \pm 0.12$	$0.455 \pm 0.04$	_	
LSD $\alpha = 0.05$							Y = 0.0177 PH = 0.0307 $Y \times PH = 0.0434$	Y = 0.00552 PH = 0.00956 Y × PH = 0.0135					

Table 3. Yield of fresh and dried herb and total alkaloids and flavonoids content (± standard deviation) in celandine cultivar 'Cynober' herb at the selected developmental phases (Poznan 2011–2012)

Y = year, PH = phase

Phase	Chelidonine content (%)			Coptisine content (%)			Sanguinarine contet (%)			Berberine content (%)			Chelerythrine content (%)		
	2011	2012	mean	2011	2012	mean	2011	2012	mean	2011	2012	mean	2011	2012	mean
Spring rosette formation	0.053	0.483	0.268	0.068	0.343	0.206	0.005	0.006	0.006	0.005	0.016	0.011	0.0001	0.001	0.001
s.d.	0.008	0.001	0.243	0.002	0.004	0.160	0.001	0.001	0.001	0.001	0.001	0.006	0.000	0.000	0.001
Beginning of flowering	0.082	0.179	0.131	0.057	0.340	0.199	0.006	0.007	0.006	0.005	0.014	0.010	0.0001	0.002	0.001
s.d.	0.007	0.005	0.057	0.001	0.009	0.165	0.001	0.001	0.001	0.001	0.001	0.006	0.000	0.001	0.001
Full bloom	0.059	0.203	0.131	0.056	0.478	0.267	0.005	0.012	0.009	0.004	0.010	0.007	0.0001	0.004	0.002
s.d.	0.006	0.001	0.083	0.002	0.004	0.244	0.001	0.001	0.004	0.001	0.001	0.003	0.000	0.001	0.002
Green fruit	0.085	0.598	0.341	0.058	0.466	0.262	0.006	0.024	0.015	0.005	0.010	0.007	0.0001	0.005	0.003
s.d.	0.001	0.016	0.296	0.001	0.001	0.236	0.001	0.001	0.010	0.001	0.001	0.003	0.000	0.001	0.003
Seed harvest	0.053	0.532	0.293	0.092	0.489	0.291	0.009	0.012	0.010	0.007	0.012	0.009	0.0002	0.007	0.004
s.d.	0.001	0.014	0.277	0.001	0.002	0.229	0.001	0.001	0.002	0.001	0.001	0.003	0.000	0.001	0.004
Fall rosette formation	0.069	1.077	0.573	0.122	0.626	0.374	0.008	0.019	0.014	0.012	0.026	0.019	0.0002	0.010	0.005
s.d.	0.010	0.020	0.582	0.001	0.013	0.292	0.001	0.001	0.006	0.001	0.001	0.008	0.000	0.011	0.009
Mean	0.067	0.512		0.075	0.458		0.007	0.013		0.006	0.15		0.0001	0.005	
s.d.	0.014	0.312		0.025	0.101		0.001	0.007		0.003	0.006		0.000	0.005	
	Y = 0.0061; PH = 0.0106		0.0106	Y = 0.0031; PH = 0.0053		Y = 0.0001; PH = 0.0002			Y = 0.00014; PH = 0.00025			Y = 0.0019; PH = 0.0033			
LSD $\alpha = 0.05$	$Y \times PH = 0.0150$		$Y \times PH = 0.0075$		$\mathbf{Y} \times$	PH = 0.00	0027	$Y \times PH = 0.00035$			$Y \times PH = 0.0047$				

Table 4. Alkaloids content in celandine cultivar 'Cynober' herb at the selected developmental phases (Poznan 2011–2012)

s.d. = standard deviation, Y = year, PH = phase

The total alkaloids content in celandine herb was significantly different at the studied phases, and years (tab. 3). The average content of alkaloids was the highest in phase of green fruit (1.097%), while in 2012, the highest content was reached in phase of fall rosette formation -1.200%. The lowest content of alkaloids was obtained in herb of the beginning of flowering (0.608%) in both years. Alkaloid content increased over the period from the beginning of vegetation up to green fruit, then there was decrease of content in phase of seed harvest, whereas in herb of fall rosette formation the following increase was noticed. Over the two investigated years the process of alkaloids accumulation in the investigated phases was similar. A correlation among alkaloids and other active substance contents was not found (tab. 5).

Table 5. Correlation coefficient among the investigated traits in celandine herb (Poznan 2011–2012)

Content of	Chelidonine	Coptisine	Sanguinarine	Berberine	Chelerythrine	Flavonoids
Coptisine	0.855***					
Sanguinarine	0.745***	0.734***				
Berberine	0.839***	0.794***	0.474**			
Chelerythrine	0.768***	0.760***	0.654***	0.622**		
Flavonoids	0.378	0.450**	0.259	0.578**	0.284	
Alkaloids	0.288	0.109	0.231	0.190	0.272	0.066

\*\* P < 0.01, \*\*\* P < 0.001

168

The content of flavonoids in celandine herb was significantly different depending on the year of cultivation and plant phase. The mean content of flavonoids was from 0.310% (the beginning of flowering) to 0.522% (seed harvest) and was the same in both years (tab. 3). In 2012, the lowest content of flavonoids was noticed in herb of green fruit phase. The tendency of flavonoids content was variable. Over the two investigated years the process of flavonoids accumulation in the investigated phases was similar. A correlation among flavonoids and two of alkaloids coptisine and berberine content was significant (tab. 5).

Following the recommendation for Polish celandine growers [Kucharski 2010] herb should be harvested during plant flowering in the second year. At that time herb yield is high, but alkaloid and flavonoids contents are low. Results presented by Kustrak et al. [1982] showed that the highest content of total alkaloids (1.17–1.74%) in herb collected in three location was obtained in summer (end of August) and in the early autumn (beginning of October) which is almost similar to our results. The authors recommend the best time for harvest period from early August to late October. Migas and Heyka [2011] reported that the total content of alkaloids is from 0.1 to 1.0% in herb of celandine and in roots could even reached 3.0%. These data are corresponded with our results. These authors obtained 2.0% of total flavonoids content in herb, which is much more then results presented in this paper. According to Kohlmünzer [2000] the content of total alkaloids in celandine herb is 0.3% which is very low compare to results obtained in our investigation and recommendation given in Polish Pharmacopoeia IX [2011]. Similar

results of total content of alkaloids in celandine herb (0.351–0.938%) was presented by Tamas et al. [1987] who used volumetric method of analysis. The stem, leaves and flowers of celandine contain a single flavonoid – flavonol in aglyconic form which was found by Stancic-Rotaru et al. [2003], but these authors have not presented any quantity data. They also claimed that fruit and seeds of celandine did not contain flavonoids at all.

In 2012, the content of chelidonine, coptisine, sanguinarine, berberine and chelerythrine was higher in all analyzed phases then in 2011 (tab. 4). The high differences were caused probably by plant age or higher mean temperature during vegetation. The average amount of chelidonine oscillated from 0.131% (the beginning of flowering, full flowering) to 0.573% (fall rosette formation). In 2012 the highest content of chelidonine was noticed in fall rosette formation, while in 2011 in green fruit phase. However, the fluctuation of chelidonine content in celandine herb during vegetation was similar in the both analyzed years. The content of chelidonine was positively correlated with others analyzed alkaloids (tab. 5). The average content of coptisine was from 0.199% (beginning of flowering) to 0.374% (fall rosette) (tab. 4). In both years the highest amount of coptisine was noticed in fall rosette. During vegetation the celandine herb slightly increased in content of coptisine. There were no big differences among sanguinarine content in the evaluated phases, but the highest content was obtained in the green fruit phase (average 0.015%, and 0.024% in 2012). While in 2011, the highest amount (0.009%) was in seed harvest phase. At the green fruit and the fall rosette phases an increase in sanguinarine content in celandine herb was noticed, after a slight decrease during the seed harvest phase. The highest content of berberine was in herb of fall rosette phase in the both years of investigation. The average content was from 0.007% (full flowering and green fruit) to 0.019 (fall rosette). Also, the relatively high content of berberine was noticed in the first two phases: spring rosette and beginning of flowering (0.010–0.011%). The highest content of chelerythrine in celandine herb of fall rosette was also indicated. The average content was from 0.007% (spring rosette) to 0.005% (fall rosette). Over the two investigated years the process of chelethrine accumulation was similar with growing tendency from beginning of vegetation. All alkaloids had a positive correlation among each other (tab. 5).

The investigation done by Fulde and Wichtl [1994] and Colombo and Bosisio [1996] showed that celandine herb contains mainly chelidonine, berberine and coptisine, while sanguinarine and chelerythrine are dominant in the roots. Our results confirmed these observation. The results of the study of alkaloid composition of celandine done by Sárközi et al. [2006] showed that the main component of herb (leaf, stem, generative part) was coptisine which varied from 275.3 to 970.7 mg·100 g, chelidonine (17.7–78.27 mg·100 g), sanguinarine (18.4–107.9 mg·100 g), berberine (20.3–61.37 mg·100 g) and chelerythrine was not detected. The authors indicated that alkaloid distribution showed significant differences. Therefore, the adequate harvesting time of herb was of greatest importance. Kustrak et al. [1982] who analyzed seasonal changes of alkaloids content in celandine reported that chelidonine, chelerythrine, sanguinarine and berberine were found in both herb and roots, but they did not show any quantity of these alkaloid.

### CONCLUSION

1. The highest yield of celandine herb was obtained in the beginning of flowering, but at that time the herb contained the lowest amount of alkaloids and flavonoids.

2. The stable high content of total alkaloids and flavonoids and individual alkaloids was noticed in phase of fall rosette.

3. Seed maturity is the best time for celandine herb harvest regarding the herb yield and content of alkaloids and flavonoids.

#### ACKNOWLEDGEMENTS

The research was financially supported by Polish National Science Centre, found No. N N405 67740.

### REFERENCES

- Bugatti, C., Colombo, M.L., Tome, F. (1987). High-performance liquid chromatographic separation of quaternary alkaloids of *Chelidonium majus* L. roots. J. Chrom., 393, 312–316.
- Colombo, M.L., Bosisio, E. (1996). Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). Pharm. Res., 33(2), 127–134.
- De Rosa, S., di Vincenzo, G. (1992). Isochelidonine, a benzophenanthridine alkaloid from *Chelidonium majus*. Phytochemistry, 31(3), 1085–1086.
- European Pharmacopoeia VI (2008). Monograph of greater celandine. Monograph of birch leaf, vol. 1. Council of Europe, Strasbourg.
- Fik, E., Dalgalarrondo, M., Haertle, T., Goździcka-Józefiak, A. (2000). Comparative biochemical analysis f lecitin and niclease from *Chelidonium majus* L. Acta Biochim. Polon., 47(2), 413–420.
- Fulde, G., Wichtl, M. (1994). Analytik von Schollkraut. Deut. Apoth. Zeit., 135, 1031-1035.
- Garcia, V.P., Valdes, F., Martin, R., Luis, J.C., Afonso, A.M., Ayala, J.H. (2006). Biosynthesis of antitumoral and bactericidal sanguinarine. J. Biomed. Biotech., 24, 1–6.
- Gerenčer, M., Turecek, P.L., Kistner, O., Mitterer, A., Savidis-Dacho, H., Barrett, N.P. (2006). *In vitro* and *in vivo* anti-retroviral activity of the subsance purified from aqueous extract of *Chelidonium majus* L. Antivir. Res., 72(2), 153–156.
- Gertig, H., Frenclowa, I., Alkiewicz, J., Janiakowa, E. (1957). Fungistatical activity of some substances isolated from greater celandine (*Chelidonium majus* L.). Acta Pol. Pharm., 14, 101–108 (in Polish).
- Hahn, R., Nahrstedt, A. (1993). Hydroxycinnamic acid derivatives, caffeoylmalic and new caffeoylaldonic acid esters from *Chelidonium majus*. Planta Med., 59, 71–75.
- Horváth, G., Molnár, P., Farkas, A., Szabó, L., Turcsi, E., Deli, J. (2010). Separation and identification of carotenoids in flowers of *Chelidonium majus* L. and inflorescences of *Solidago canadensis* L. Chromatogr., 67, 1–6.
- Kèry, A., Horváth, J., Nász, L., Verzár-Petri, G., Kulcsár, G., Dán, P. (1987). Antiviral alkaloid in *Chelidonium majus* L. Acta Pharm. Hung., 57(1–2), 19–25.
- Kędzia, B., Hołderna-Kędzia, E., Goździcka-Józefiak, A., Buchwald, W. (2013). The antimicrobial activity of *Chelidonium majus* L. Post. Fitoter., 4, 236–243 (in Polish).
- Kohlmünzer, S. (2000). Farmakognozja. Warszawa PZWL., 450-451.

170

- Kopytko, Y.F., Dargaeva, T.D., Sokoloskaya, T.A., Grodnitskaya, E.I., Kopnin, A.A. (2005). New methods for the quality control of a homeopathic matrix tincture of greater celandine. Pharm. Chem. J., 39(11), 603–609.
- Kozak, M., Bocianowski, J., Rybiński, W. (2013). Note on the use of coefficient of variation for data from agricultural factorial experiments. Bulgar. J. Agricult. Sci., 19(4), 644–646.
- Kucharski, W. (2010). Glistnik jaskółcze ziele. In: Uprawa ziół poradnik dla plantatorów, Kołodziej, B. (eds.). PWRiL, Poznań, 179–183.
- Kustrak, D., Petricic, J., Kolodera, Z. (1982). Seasonal changes of alkaloid contents in celandine (*Chelidonium majus* L.). Acta Pharm. Jugosl., 32, 225–230.
- Kwasniewski, V. (1958). Untersuchungen über die nichtalkaloidschen Inhaltstoffe des Schöllkrauts (*Chelidonium majus* L.). Pharmazie, 13, 3363–3364.
- Matos, O.C., Baeta, J., Silva, M.J., Pinto Ricardo, C.P. (1999). Sensitivity of *Fusarium* strains to *Chelidonium majus* L. extracts. J. Ethnopharmacol., 66, 151–158.
- Migas, P., Heyka, M. (2011). Celandine (*Chelidonium majus* L.) in modern therapy indications and safety of use. Post. Fitoter., 2, 1–14 (in Polish).
- Nawrot, R., Leśniewicz, K., Pienkowska, J., Goździcka-Józefiak, A. (2007). A novel extracellular peroxidase and necleases from milky sap of *Chelidonium majus*. Fitoter., 78, 496–501.
- Polish Pharmacopea IX (2011). Chelidonii herba, vol. 1. PTF. Warszawa, 1239–1240.
- Sárközi, A., Janicsák, G., Kursinszki, L., Kéry, Á. (2006). Alkaloid composition of *Chelidonium majus* L. studied by different chromatographic techniques. Chromatogr. Suppl., 63, 81–86.
- Shapiro, S.S., Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). Biometrika, 52, 591–611.
- Slavik, J., Slavikova, L. (1977). Minor alkaloids from *Chelidonium majus* L. Collect. Czechoslov. Chem. Commun., 42, 2686–2693.
- Stancic-Rotaru, M., Mititelu, M., Crasmaru, M., Balaban, D. (2003). Spectroanalytical profile of flavonoids from *Chelidonium majus* L. Roum. Biotechnol. Lett., 8, 1093–1100.
- Tamas, M., Chindris, E., Tuia, R.M. (1987). Investigations on the alkaloids in *Chelidonium majus* L. Cluj. Med., 60(3), 256–260 (in Rumanian).
- Tan, G.T., Pezzuto, J.M., Kinghorn, A.D. (1991). Evaluation of natural products as inhibitors of human immunodefieciency virus type 1 (HIV-1) reverse transcriptase. J. Nat. Prod., 54(1), 143–154.
- Tomè, F., Colombo, M.L. (1995). Distribution of alkaloids in Chelidonium majus and factors affecting their accumulation. Phytochem., 40(1), 37–39.
- VSN International (2014). GenStat Reference Manual (Release 17). VSN International, Hemel Hempstead, UK.

# ZAWARTOŚĆ ALKALOIDÓW I FLAWONOIDÓW W ZIELU GLISTNIKA JASKÓŁCZE ZIELE (Chelidonium majus L.) W WYBRANYCH FAZACH ROZWOJOWYCH

Streszczenie. W latach 2011–2012 oceniano plonowanie oraz zawartość alkaloidów i flawonoidów w zielu glistnika jaskółcze ziele odmiany 'Cynober' w sześciu fazach rozwojowych: rozeta wiosenna, początek kwitnienia, pełnia kwitnienia, zielony owoc, dojrzałe nasiona, rozeta jesienna. Plon ziela różnił się zarówno w badanych fazach, jak i w latach. Największy plon ziela zanotowano w fazie początku kwitnienia, a następnie obserwowano spadek aż do fazy zbioru nasion, po czym następował wzrost plonu surowca. Najmniejszy plon uzyskano w fazie rozety jesiennej, jednak w 2011 r. najmniejszy

plon zebrano w fazie rozety wiosennej. Największą zawartość alkaloidów w zielu zanotowano w fazie zielonego owocu (1,097%), a w 2012, w fazie rozety jesiennej – 1,200%. W obu latach najmniej alkaloidów występowało w zielu zebranym w fazie początku kwitnienia (0,608%). Średnia zawartość flawonoidów wynosiła od 0,310% (początek kwitnienia) do 0,522% (zbiór nasion) i była podobna w obu latach. Wysoką zawartość sumy alkaloidów, flawonoidów oraz poszczególnych alkaloidów obserwowano w fazie rozety jesiennej. Uzyskane wyniki sugerują, że najwłaściwszą fazą zbioru surowca glistnika jest faza zbioru nasion ze względu na zawartość substancji czynnych oraz całkowity plon surowca.

Slowa kluczowe: rośliny lecznicze, plon ziela, fazy fenologiczne, substancje aktywne, rośliny lecznicze

Accepted for print: 13.04.2016

For citation: Seidler-Łożykowska, K., Kędzia, B., Bocianowski, J., Gryszczyńska, A., Łowicki, Z., Opala, B., Pietrowiak, A. (2016). Content of alkaloids and flavonoids in celandine (*Chelidonium majus* L.) herb at the selected developmental phases. Acta Sci. Pol. Hortorum Cultus, 15(4), 161–172.