Intraspecific variability of fireweed (*Chamaenerion angustifolium* /L./ Scop.) and evening primrose (*Oenothera biennis* L.) in respect of sterol content

ZENON WĘGLARZ*, OLGA KOSAKOWSKA, MAŁGORZATA PELC, ANNA GESZPRYCH, JAROSŁAW L. PRZYBYŁ, KATARZYNA BĄCZEK

Department of Vegetable and Medicinal Plants
Warsaw University of Life Sciences – SGGW
Nowoursynowska 159
02-776 Warsaw, Poland

* corresponding author: zenon_weglarz@sggw.pl

S u m m a r y

Composition of sterol fraction and content of identified compounds in herb of fireweed (*Chamaenerion angustifolium* /L./ Scop.) and leaves of evening primrose (*Oenothera biennis* L.) obtained from several populations of these species growing wild in Poland were determined by HPLC. The investigated species differed in respect of the composition and content of identified sterols. High intraspecific variability concerning accumulation of sterols was also observed. Herb of fireweed was characterised by relatively high content of β-sitosterol (85.80–171.18 mg/100 g), campesterol (24.24–334.49 mg/100 g), and β-sitosterol D-glucoside (26.33–86.32 mg/100 g). Leaves of evening primrose appeared to be much poorer source of sterols. Content of β-sitosterol (the dominant compound in the sterol fraction) in this raw material ranged from 5.21 to 34.66 mg/100 g.

Key words: *wild growing medicinal plants, populations, β-sitosterol, HPLC*

INTRODUCTION

Plant sterols reveal anti-inflammatory, anticoagulant and cholesterol-lowering activity, which contributes to their anti-atherogenic effect. They are also considered to play an essential role in the prophylaxis and treatment of benign prostatic hyperplasia and prostate cancer [1-4]. There are few plant raw materials rich in

sterols. In Poland and in many other European countries rhizomes and runners of stinging nettle (*Urtica dioica* L.) are an important sterol-containing raw material used in the treatment of prostate disorders [5, 6]. In folk medicine herb of fireweed (*Chamaenerion angustifolium* /L./ Scop., syn. *Epilobium angustifolium* L.) has been also used to cure such disorders. Evening primrose (*Oenothera biennis* L.) belongs to the same family as fireweed (*Oenotheraceae*). It is widely known for its valuable seed oil containing polyunsaturated fatty acids. Our previous studies [7] indicated that seeds of evening primrose are also a rich source of sterols which often accompany fatty oils in plant materials. However, the presence of sterols in other organs of this species has not been reported yet.

The aim of the present study was to determine the content and composition of sterols in herb of fireweed and leaves of evening primrose and to define the range of intraspecific variability concerning accumulation of these compounds.

MATERIALS AND METHODS

Herb of fireweed (*Chamaenerion angustifolium* /L./ Scop.) was obtained from 20 populations of this species growing wild in the southern part of Poland (Bieszczady) and the eastern one (Podlasie, middle part of Bug river valley). Leaves of evening primrose (*Oenothera biennis* L.) were obtained from 16 populations growing in eastern Poland (tab. 1, 2, fig. 1). The raw materials were collected in 2005, at the stage of plant blooming, from 20 randomly selected plants from each site and dried at 40°C.

Table 1.

Geographical origin of the investigated populations of fireweed

Area (region)	Population	Co-ordinates of natural site
I – Bieszczady	E1	N 49º 14.707' E 022º 23.637'
	E2	N 49º 18.198' E 022º 16.482'
	E3	N 49º 13.663' E 022º 22.034'
	E4	N 49º 19.090' E 022º 06.803'
	E5	N 49º 16.815' E 022º 16.887'
	E6	N 49º 11.312' E 022º 26.259'
	E7	N 49º 18.992' E 022º 03.806'
	E8	N 49º 15.355' E 022º 42.984'
	E9	N 49º 20.834' E 022º 24.674'
	E10	N 49º 27.157' E 022º 16.482'

Area (region)	Population	Co-ordinates of natural site
II – Podlasie	E11	N 52º 30.632' E 021º 06.734'
	E12	N 52º 27.016' E 021º 04.007'
	E13	N 52º 24.745' E 022º 33.399'
	E14	N 52º 05.028' E 023º 34.685'
	E15	N 52º 23.678' E 022º 53.178'
	E16	N 52º 31.779' E 022º 16.482'
	E17	N 52º 43.277' E 023º 48.363'
	E18	N 52º 38.247' E 022º 47.911'
	E19	N 52º 30.888' E 021º 21.563'
	E20	N 52º 41.908' E 022º 13.857'

Table 2.

Geographical origin of the investigated populations of evening primrose

Population	Co-ordinates of natural site
O1	N 52º 08.499' E 023º 30.751'
O2	N 52º 10.219' E 023º 25.078'
O3	N 52º 10.180' E 023º 25.603'
O4	N 52º 10.049' E 023º 23.591'
O5	N 52º 11.069' E 023º 18.256'
O6	N 52º 21.656' E 022º 52.162'
O7	N 52º 21.922' E 022º 50.743'
O8	N 52º 22.521' E 022º 48.680'
O9	N 52º 22.549' E 022º 47.402'
O10	N 52º 22.054' E 022º 46.217'
O11	N 52º 21.803' E 022º 43.011'
O12	N 52º 23.508' E 022º 36.608'
O13	N 52º 24.683' E 022º 33.099'
O14	N 52º 23.646' E 022º 34.255'
O15	N 52º 24.883' E 022º 33.029'
O16	N 52º 29.011' E 022º 15.585'

The powdered raw materials (5 g) were extracted with 100 ml of hexane in Büchi B-811 extraction system for 2 hours. After evaporation of solvent, the residue was dissolved in 5 ml of chloroform-methanol mixture (4:1), filtered through a Supelco IsoDisc PTFE 25 mm $\times$ 0.45 μm filter, and subjected to HPLC. The raw material obtained from each population was extracted and analysed in 3 replications.

Figure 1.
Geographical origin of the investigated populations of fireweed (area I and II) and evening primrose (area II)

Separation and identification of free sterols

The analysis was carried out using the Shimadzu chromatograph equipped with SPD-M10A VP DAD detector and Luna RP8 5 μm 250 mm × 4.6 mm column (Phenomenex). The gradient of methanol and acetonitrile was used; flow rate 1.0 ml/min. The following analysis parameters were used: injection volume: 20 μl, oven temperature 28ºC, recorded wave range: 190-300 nm, detection wave length: 210 nm. Peaks were identified by comparison of retention time and spectral data with adequate parameters of standards (Chromadex). Quantification was based on the peak area. The content of the determined compounds was calculated in mg/100 g dry matter. The results were analysed with ANOVA Tukey's HSD test at the 0.05 significance level in Statgraphics Plus for Windows v. 4.1.

RESULTS AND DISCUSSION

As they are the constituents of plant cell membranes, sterols are widely distributed in plant kingdom. The most commonly occurring compounds are Δ5-sterols:

C_{29} (β-sitosterol and stigmasterol) and C_{28} (campesterol and brassicasterol). Δ7-sterols are usually present in much lower amount. Cholesterol (C_{27}) is found mainly in animal tissues, although trace amounts of this compound are also present in some plant materials [8, 9]. Interest in sterol content and composition in food of plant origin and medicinal plant raw materials results from their multidirectional biological activity and health promoting effects [1-3].

In the present study the raw materials obtained from two species of medicinal plants belonging to the family *Oenotheraceae*, i.e. fireweed and evening primrose, were evaluated as potential sources of sterol compounds. The composition and content of sterols in raw materials collected from different natural sites in Poland was compared in order to determine intraspecific variability in accumulation of these compounds.

The presence of sterols (β-sitosterol and β-sitosterol-D-glucoside) in herb of fireweed was reported for the first time by Hiermann and Mayr [10]. In our study in herb of fireweed six sterols were identified, namely β-sitosterol and its D-glucoside, campesterol, stigmasterol, brassicasterol, and cholesterol. The investigated populations differed in respect of the content of individual compounds. The content of β-sitosterol ranged from 85.80 to171.18 mg/100 g. In 14 populations it was the main sterol compound, whereas in 6 other populations the dominant compound was campesterol. Its content (24.24–334.49 mg/100 g) was even more diversified as compared to β-sitosterol content, which was confirmed by higher coefficient of variation. The highest variability concerned accumulation of cholesterol, but the content of this compound was generally low (tab. 3). Mean content of campesterol in herb originating from the populations from Podlasie area was significantly higher than in herb collected in Bieszczady area. At the same time, variation between the populations from Podlasie in respect of campesterol content was higher than between the populations from Bieszczady area. The content of other sterols was not specially related to the geographical origin of the populations. The investigated populations from Podlasie were more uniform in respect of β-sitosterol content (tab. 4).

Up to now, there was no data on the content of sterols in leaves of evening primrose. According to Hudson [11], the predominant constituent of free sterol fraction of evening primrose seeds is β-sitosterol. The presence of campesterol [12] and stigmasterol [13] was also reported. In our previous study [7] the main constituent of sterol fraction in seeds of evening primrose was stigmasterol (84.5–298.6 mg/100 g), followed by β-sitosterol (68.8–216.8 mg/100 g), brassicasterol (35.6–117.2 mg/100 g), campesterol (21.9–77.1 mg/100 g), and cholesterol (0-9.7 mg/100 g). The results of the present study indicate considerable differences in accumulation of sterols between seeds and leaves of this plant. The content of sterols in leaves appeared to be much lower than in seeds. The content of dominant sterol compound in leaves – β-sitosterol – ranged from 5.21 to 34.66 mg/100 g. Other sterols, i.e. brassicasterol, cholesterol,

stigmasterol, and campesterol, were detected only in several investigated leaf samples and their content did not exceed 10 mg/100 g. The highest variability between populations concerned campesterol content (tab. 5).

Table 3.

Sterols in herb of fireweed (mg/100 g)

P	SITO-D-glu		CHOL		BRASS		CAMP		STIG		SITO	
E1	40.09	c[1]	6.75	fg	11.14	bc	47.21	b	17.58	bc	94.70	b
E2	49.34	e	5.63	de	24.04	gh	60.20	d	33.91	f	164.28	n
E3	40.80	c	21.47	j	0.00	a	89.74	h	21.33	cd	133.61	j
E4	48.60	e	7.66	gh	13.66	cd	84.80	g	19.96	cd	93.42	b
E5	41.62	c	5.31	de	20.91	ef	74.88	e	19.72	cd	137.77	k
E6	46.37	de	5.05	de	25.84	h	102.10	i	14.16	b	152.72	l
E7	31.21	b	3.01	bc	2.40	a	24.24	a	4.90	a	94.95	b
E8	66.10	g	5.01	de	24.02	gh	137.84	k	20.39	cd	171.18	o
E9	31.09	b	5.10	de	9.20	b	53.04	c	17.44	bc	85.80	a
E10	31.54	b	6.90	fg	10.74	bc	79.69	f	28.17	e	105.42	d
E11	86.32	i	10.63	i	21.82	fg	157.71	l	23.65	d	108.95	e
E12	41.35	c	8.70	h	33.05	i	120.27	j	27.95	e	112.87	f
E13	48.78	e	2.76	ab	13.84	cd	80.28	f	16.78	bc	120.69	hi
E14	59.71	f	6.07	ef	11.01	bc	136.02	k	13.76	b	122.31	i
E15	58.44	f	3.05	bc	25.47	h	334.49	o	24.16	d	115.65	fg
E16	44.95	d	2.31	ab	14.87	d	78.15	f	20.38	cd	132.02	j
E17	38.83	c	1.88	ab	18.78	ef	24.44	a	15.04	b	100.59	c
E18	72.66	h	1.33	a	26.14	h	234.48	n	36.69	g	159.00	m
E19	58.88	f	8.04	h	21.05	ef	163.03	m	21.82	cd	98.81	c
E20	26.33	a	4.22	cd	18.32	e	73.53	e	21.35	cd	118.45	gh
Mean	48.15		6.04		17.31		107.81		20.96		121.16	
CV	0.32		0.72		0.49		0.68		0.34		0.21	

P – population; SITO-D-glu – β-sitosterol-D-glucoside, CHOL – cholesterol,
BRASS – brassicasterol, CAMP – campesterol, STIG – stigmasterol, SITO – β-sitosterol;
CV – coefficient of variation

[1] *Values in columns marked with the same letter do not differ significantly at* $\alpha=0.05$

Table 4.

Content of sterols in herb of fireweed originating from two regions of Poland (mg/100 g)

Area (region)	SITO-D-glu	CHOL	BRASS	CAMP	STIG	SITO
I – Bieszczady	42.7	7.2	14.2	75.4	19.8	123.4
II – Podlasie	53.6	4.9	20.4	140.2 *	22.2	118.9
CV I	0.25	0.72	0.65	0.42	0.39	0.26
CV II	0.32	0.67	0.32	0.64	0.30	0.15

SITO-D-glu – β-sitosterol-D-glucoside, CHOL – cholesterol, BRASS – brassicasterol, CAMP – campesterol, STIG – stigmasterol, SITO – β-sitosterol;
CV I – coefficient of variation for area I, CV II – coefficient of variation for area II
* significant difference ($p<0.05$)

Table 5.

Sterols in leaves of evening primrose (mg/100 g)

P	CHOL		BRASS		CAMP		STIG		SITO	
O1	3.26	f[1]	0.83	b	0.00	a	0.00	a	8.66	b
O2	7.18	j	4.39	g	0.00	a	2.32	f	18.42	i
O3	2.54	e	3.03	e	2.46	d	0.33	b	5.21	a
O4	1.00	b	1.39	c	0.00	a	1.48	c	13.22	f
O5	0.00	a	9.86	j	3.35	f	4.17	h	23.12	j
O6	2.05	d	0.00	a	0.00	a	0.00	a	18.45	i
O7	1.45	c	1.40	c	0.00	a	0.00	a	9.25	c
O8	0.00	a	8.89	i	1.01	b	1.88	d	18.62	i
O9	4.71	g	3.48	f	0.00	a	2.33	f	13.50	f
O10	4.98	h	0.00	a	0.00	a	0.00	a	10.90	d
O11	0.00	a	2.50	d	0.00	a	0.00	a	15.56	g
O12	7.09	j	2.89	e	0.00	a	0.00	a	12.83	e
O13	2.41	e	4.50	g	1.81	c	2.05	e	10.64	d
O14	6.60	i	4.52	g	2.62	e	2.16	e	18.09	h
O15	0.00	a	2.99	e	3.47	g	3.03	g	24.99	k
O16	0.00	a	5.86	h	0.00	a	5.08	i	34.66	l
Mean	2.70		3.53		0.92		1.55		16.01	
CV	0.98		0.80		1.46		1.04		0.46	

P – population; CHOL – cholesterol, BRASS – brassicasterol, CAMP – campesterol, STIG – stigmasterol, SITO – β-sitosterol;
CV – coefficient of variation
[1] *Values in columns marked with the same letter do not differ significantly at* $\alpha=0.05$

CONCLUSIONS

1. High intraspecific variability of fireweed and evening primrose concerning accumulation of sterols, especially campesterol and cholesterol, was observed.
2. Among the identified sterol compounds only accumulation of campesterol in fireweed herb was related to the geographical origin of the populations.
3. Fireweed herb is a richer source of sterols in comparison with evening primrose leaves, including β-sitosterol and its glucoside, which are regarded as the most pharmacologically active sterol compounds.

REFERENCES

4. Dreikorn K. The role of phytotherapy in treating lower urinary tract symptoms and benign prostatic hyperplasia. World J Urol 2002; 19:426-35.
5. Moreau RA, Whitaker BD, Hicks KB. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis and health-promoting uses. Prog Lipid Res 2002; 41:457-500.
6. Moghadasian MH. Pharmacological properties of plant sterols. *In vivo* and *in vitro* observations. Life Sci 2000; 67:605-15.
7. Wilt TJ, MacDonald R. Sitosterol for the treatment of benign prostatic hyperplasia. Br J Urol 1999; 83:976-83.
8. Chrubasik JE, Roufogalis BD, Wagner H, Chrubasik S. A comprehensive review on the stinging nettle effect and efficacy profiles. Part II: Urticae radix. Phytomedicine 2007; 14(7-8):568-79.
9. Lichius JJ, Muth C. The inhibiting effect of *Urtica dioica* root extracts on experimentally induced prostatic hyperplasia in the mouse. Planta Med 1997; 63(4):307-10.
10. Kosakowska O, Węglarz Z, Przybył J.L. Chemical and genetic diversity of evening primrose (*Oenothera biennis* L.) occurring in the eastern area of Poland. Acta Hort 2008; 765:151-6.
11. Breinhölder P, Mosca L, Lindner W. Concept of sequential analysis of free and conjugated phytosterols in different plant matrices. J Chromatography B 2002; 777:67-82.
12. Wojciechowski ZA. Biochemistry of phytosterol conjugates. In: Patterson GW, Nes WD (eds.). Physiology and Biochemistry of Sterols. Champaign 1991:361-94.
13. Hiermann A, Mayr K. Die Untersuchung potentiller Wirkstoffe in *Epilobium*-Arten. Sci Pharm 1985; 53:39-44.
14. Hudson BJ. Evening primrose (*Oenothera sp.*) oil and seeds. J Am Oil Chem Soc 1984; 61:540-3.
15. Artaud J. Identification d ' huiles riches en acide γ-linolénique. Ann Falsif Expert Chim Toxicol 1992; 85:231-9.
16. Stołyhwo A. 1992. Technologia pozyskiwania i główne składniki oleju z nasion wiesiołka dziwnego (*Oenothera paradoxa* Hudziok). In: Proceedings of the Symposium "Olej z nasion wiesiołka w profilaktyce i terapii"; October 9-10, 1992, Łódź (Poland):9-22.

ZRÓŻNICOWANIE WEWNĄTRZGATUNKOWE WIERZBÓWKI KIPRZYCY (*CHAMAENERION ANGUSTIFOLIUM* L.) I WIESIOŁKA DWULETNIEGO (*OENOTHERA BIENNIS* L.) POD WZGLĘDEM ZAWARTOŚCI ZWIĄZKÓW STEROLOWYCH

ZENON WĘGLARZ*, OLGA KOSAKOWSKA, MAŁGORZATA PELC, ANNA GESZPRYCH, JAROSŁAW L. PRZYBYŁ, KATARZYNA BĄCZEK

Katedra Roślin Warzywnych i Leczniczych
Szkoła Główna Gospodarstwa Wiejskiego
ul. Nowoursynowska 159
02-776 Warszawa

* autor, do którego należy kierować korespondencję: zenon_weglarz@sggw.pl

S t r e s z c z e n i e

Badano skład frakcji steroli oraz zawartość zidentyfikowanych związków sterolowych w zielu wierzbówki kiprzycy (*Chamaenerion angustifolium* /L./ Scop.) i liściach wiesiołka dwuletniego (*Oenothera biennis* L.) pochodzących z kilkunastu populacji tych gatunków dziko rosnących w Polsce. Badane gatunki różniły się pod względem składu i zawartości steroli. Stwierdzono również znaczne zróżnicowanie wewnątrzgatunkowe pod względem gromadzenia się tych związków. Ziele wierzbówki charakteryzowało się wysoką zawartością β-sitosterolu (85,80–171,18 mg/100 g), kampesterolu (24,24–334,49 mg/100 g) i D-glukozydu β-sitosterolu (26,33–86,32 mg/100 g). Liście wiesiołka okazały się znacznie uboższym źródłem steroli. Zawartość β-sitosterolu (dominującego składnika frakcji sterolowej) w tym surowcu wynosiła od 5,21 do 34,66 mg/100 g.

Słowa kluczowe: *dziko rosnące rośliny lecznicze, populacje, β-sitosterol, HPLC*