Sterols and fatty acids in the seeds of evening primrose (*Oenothera* sp.) and willow herb (*Epilobium* sp.)

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Summary

The content and composition of fatty acids and sterols in the seed oil of two cultivated evening primrose species (*Oenothera paradoxa* Hudziok, *O. glazioviana* Micheli in Mart.) and two willow herb species (*Epilobium tetragonum* L., *E. hirsutum* L.) were compared. A GC analysis of the evening primrose seed oil resulted in identifying five fatty acids (palmitic, stearic, oleic, linoleic, and γ-linolenic acids), with linoleic and γ-linolenic acids as dominant compounds. In the willow herb seed oil six fatty acids were identified (palmitic, stearic, oleic, linoleic, γ-linolenic and α-linolenic acids) with palmitic and linoleic acids as dominant compounds. An HPLC analysis indicated the presence of four free sterols in the evening primrose seed oil and five in the willow herb seed oil. β-sitosterol and brassicasterol appeared to be the main sterols in seed oils of the investigated species.

Key words: evening primrose, willow herb, β-sitosterol, linoleic acid, linolenic acid, GC, HPLC

Evening primrose and willow herb belong to the *Oenotheraceae* family. They are wild growing plants, commonly occurring in Poland. In the 1970s evening primrose became the object of interest of medicine because of the composition of its seed oil, which is rich in biologically active unsaturated fatty acids (especially linoleic and γ-linolenic) [1, 2, 3].

The extracts from willow herb have been used in folk medicine for many years as analgesic, anti-inflammatory and anti-contractive medicaments [4, 5, 6]. Unlike in the case of evening primrose, there is almost no information about the chemical composition and pharmacological activity of willow herb [7]. The studies carried out hitherto indicated the presence of sterols in the seeds of evening primrose and willow herb, however there is no data on composition of these group of substances. Both unsaturated fatty acids and sterols reveal distinct pharmacological

activity. Linoleic and γ-linolenic acids are very important in synthesis of tissue hormones, which regulate blood pressure and take part in immunological response [2]. Sterols show anti-inflammatory and immunostimulatory activity [8, 9].

The aim of the study was to compare the composition of fatty acids and sterols in the seed oils of two species of evening primrose and two species of willow herb.

MATERIAL AND METHODS

The object of the study were seeds of two cultivated species of evening primrose (*Oenothera paradoxa* Hudziok and *O. glazioviana* Micheli in Mart.) and two species of willow herb (*Epilobium tetragonum* L. and *E. hirsutum* L.). The seeds originated from a plant collection of the Department of Vegetable and Medicinal Plants. The experiment was carried out in the years 2003 and 2004. The phytochemical analyses were done at the Department's lab. The presented results are the mean values from two years.

Separation and identification of fatty acids

Powdered seeds were extracted for 30 min. with hexane in ultrasound bath at room temperature. After evaporation of solvent, five drops of oil were collected in the ampoule. Saponification with the methanol solution of sodium hydroxide followed by estrification with methanol solution of boric trifluoride were carried out at 75°C.

Gas chromatography was performed on the Anglia Instrument Chromatograph equipped with the capillary column Carbowax 20M (length 25 m, diameter 0.32 mm). The following conditions of analysis were applied: detector temperature 250°C, injector temperature 220°C, and helium as a carrier gas, with the flow rate 1.7 ml/min. Column temperature was programmed as follows: 100°C (2 min.), temperature rise 4°C per minute, and finally 220°C (5 min.).

Retention times of the standard fatty acids: palmitic, stearic, linoleic, γ-linolenic, and α-linolenic acids were 31.8, 36.3, 37.3, 37.7 and 38.3 min, respectively.

Separation and identification of free sterols

5 g of powdered seeds were extracted for four hours with hexane. After evaporation of solvent, the residue was dissolved in 2 ml of methanol and subjected to a chromatographic analysis. A reversed-phase HPLC was carried out using a Shimadzu Chromatograph equipped with a UV detector and a C8 column (Phenomenex, 250x4,6 mm). The gradient of acetonitrile in methanol (92-100%) was applied. The column temperature was 25°C. The detection was carried out at 210 nm. Retention times of the standard sterols: cholesterol, brassicasterol, campesterol, stigmasterol, and sitosterol were 11.46, 12.14, 13.68, and 14.64 min., respectively.

RESULTS AND DISCUSSION

The total oil content in the seeds of *O. paradoxa* was 24.8%, *O. glazioviana* – 21.2%, *E. tetragonum* – 22.3% and *E. hirsutum* – 28.1%.

The seed oils from the tested plants differed in the content of identified fatty acids (Table 1). In *O. paradoxa* and *O. glazioviana* palmitic, oleic, linoleic and γ-linolenic acids were found. Other authors reported also the presence of erucic, stearic and α-linolenic acids in both *Oenothera* species [7, 10, 11, 12, 13]. In the seed oil of *O. paradoxa* miristic, oleopalmitic, vaccinic, eicosanoic and eicosadienoic acids were found as well [12].

Table 1

The content of fatty acids in the investigated seed oils (mg/g).

species	stearic acid	palmitic acid	oleic acid	linoleic acid	γ-linolenic acid	α-linolenic acid
O. paradoxa	0.0	55.8	76.1	730.0	88.3	0.0
O. glazioviana	0.0	58.9	113.9	695.6	84.7	0.0
E. tetragonum	15.2	94.0	28.4	697.5	43.5	13.9
E. hirsutum	25.2	83.9	51.6	747.3	11.2	1.7

The oil from the seeds of *O. paradoxa* was characterised by a slightly higher content of linoleic and γ-linolenic acids, and lower content of oleic and palmitic acids in comparison with *O. glazioviana* seed oil. The content of linoleic and γ-linolenic acids in *O. paradoxa* seed oil was high (730.0 and 88.3 mg/g, respectively) and in accordance with the standards of FP VI [14].

In the seed oils of both willow herb species six fatty acids were identified. Apart from those found in the evening primrose seed oil, stearic and α-linolenic acids were also present. The latter had not been previously reported in the willow herb seed oil. The content of linoleic acid in the seed oil of willow herb was comparable to that determined in the evening primrose seed oil. The oil from the seeds of *E. tetragonum* was characterised by a higher content of γ-linolenic and α-linolenic acids in comparison with *E. hirsutum* seed oil.

An HPLC analysis of the investigated seed oils resulted in identifying four sterols in *Oenothera* sp. and five in *Epilobium* sp. (Table 2). The content of the most biologically active phytosterol, i.e. β-sitosterol, was significantly higher in the willow herb seed oil. This oil was also characterised by a higher content of brassicasterol and stigmasterol. A high content of β-sitosterol in the willow herb seed oil had been previously reported by Hiermann et al. [15] and Nowak and Krzaczek [16]. The presence of β-sitosterol in the evening primrose seed oil had been reported by Hudson [17].

Table 2

The content of free sterols in the investigated seed oils [mg/g].

species	cholesterol	brassicasterol	campesterol	stigmasterol	β-sitosterol
O. paradoxa	0.00	0.68	0.42	0.13	1.16
O. glazioviana	0.00	0.80	0.66	0.15	2.19
E. tetragonum	1.00	4.39	0.26	0.92	8.65
E. hirsutum	0.12	3.44	0.29	4.02	6.65

The obtained results show that the seed oils of both *Epilobium* species are a rich source of phytosterols and in future may be taken into consideration in the treatment of benign prostatic hyperplasia.

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STEROLE I KWASY TŁUSZCZOWE W NASIONACH WIESIOŁKA (*OENOTHERA* SP.) I WIERZBOWNICY (*EPILOBIUM* SP.)

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Streszczenie

Porównywano zawartość i skład chemiczny steroli i kwasów tłuszczowych w oleju z nasion dwóch uprawnych gatunków wiesiołka (*Oenothera paradoxa, O. glazioviana)* i dwóch gatunków wierzbownicy (*Epilobium tetragonum, E. hirsutum)*. W oleju z nasion wiesiołka zidentyfikowano pięć kwasów tłuszczowych (palmitynowy, stearynowy, oleinowy, linolowy i γ-linolenowy), wśród których dominowały linolowy i γ-linolenowy. W oleju z nasion wierzbownicy zidentyfikowano natomiast sześć kwasów tłuszczowych (palmitynowy, stearynowy, oleinowy, linolowy, γ-linolenowy i α-linolenowy), wśród których dominowały linolowy i palmitynowy. Analiza HPLC wykazała obecność w oleju wiesiołkowym czterech, a w oleju z nasion wierzbownicy pięciu wolnych steroli, wśród których w obu przypadkach dominowały β-sitosterol i brasikasterol.

Słowa kluczowe: wiesiołek, wierzbownica, β-sitosterol, kwas linolowy, kwas linolenowy, GC, HPLC