Accumulation of phenolic and sterol compounds in *Euphorbia hirta* (L.)

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Summary

Euphorbia hirta is an annual plant grownig commonly in the tropical and subtropical regions of Asia, Africa as well as Central and South America. Extracts from herb reveal diastolic, anti-inflammatory, diuretic and anthelminthic effects. Biologically active compounds of this plant are sterols, saponins, flavonoids and phenolic acids. The results of this study indicate that obtaining relatively high yield of herb and well germinating seeds which retain their germinability after two years of storage is possible in Poladn. Chemical analysis of the herb and callus obtained *in vitro* has shown that these raw materials accumulate phenolic compounds and sterols in high quantities.

Key words: *seeds, herb, callus, flavonoids, phenolic acids, sterols*

INTRODUCTION

Euphorbia hirta L. is an annual plant from the *Euphorbiaceae* family native to tropical and subtropical regions of Asia, Africa, and Central as well as South America. Extracts from the herb of this plant reveal diastolic, anti-inflammatory, diuretic, antibacterial and anthelminthic effects [1, 2]. It is used to treat asthma (it is known as "asthma weed"), respiratory tract infections, malaria, jaundice, hypertension

[2-4]. The milky sap of the stalks is used treatment of wounds and warts [2]. The biologically active compounds responsible for these activities are sterols, saponins, flavonoids and phenolic acids [2-5].

The aim of the work was a preliminary study on *E. hirta* cultivation in Poland, with a special emphasis on the yield of raw material rich in active compounds for the production of drugs i the treatment of prostate disorders. The production of biologically active compounds in callus was investigated as well. The reproductive potential of this plant was also taken into consideration.

MATERIALS AND METHODS

The study was carried out in 2007–2010. *E. hirta* seeds originated from the Democratic Republic of the Congo and were collected by W. Prandota, professor of ISDR in Bukavu. The voucher specimen of herb and seeds of *E. hirta* is deposited in the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences. The plants were cultivated in a greenhouse and in an experimental field of the Warsaw University of Life Sciences (SGGW). The seedlings were produced in greenhouse. In mid-May, 100 three-month-old seedlings were planted out into containers filled with peat substrate (3 plants per one container) and grown in a greenhouse. In the same period 100 seedlings were planted out to the field at 50×20 cm distance.

Seeds were collected both from plants growing in the greenhouse and in the field after their ripening and tested for their germinability immediately after collection, next after a year and two years of storage (at 10°C). The tests were carried out on filter paper in Petri dishes, in an air-conditioned chamber, at 25°C, PPFD=150 μE m^2s (for 24 hours). The first count was carried out following 10 days after seeding, and the second after 21 days. Test results were expressed as a percentage of normal seedlings [6].

The raw material for chemical analysis was the herb of plants grown both in the greenhouse and in the field, collected at the stage of blooming (in the beginning of July and in the beginning of August, respectively). The plant material was obtained from 20 randomly chosen plants. The herb was dried at 35°C and subjected for chemical analysis.

Another raw material for chemical analysis was callus obtained from seedling explants. For callus production the *E. hirta* seeds were cultured on half-strength LS [7] (agar 6.5 g/dm^3, sucrose 20 g/dm^3) in Petri dishes (25 seeds per one dish). Seeds (were sterilized for 10 minutes in 10% solution of a chemical bleaching containing sodium hypochlorite) started to germinate 5–6 days after sowing. Seedlings were transferred on half-strength LS, on Petri dishes, and then after producing leaves, were transferred on MS in small jars with polypropylene caps. 2 cm long seedlings were used as a source of explants. Shoot tips (1 mm long) were placed

on MS [8] (agar 6.5 g/dm^3, sucrose 30 g/dm^3) supplemented with 1 mg/dm^3 2,4-D, in Petri dishes filled with 30 ml of medium. The medium was adjusted to pH 5.7 before autoclaving. The culture room was maintained at 24°C, 16 h photoperiod, PPFD=30 μmol/m^2/s (white fluorescent tubes). Light-green, loose callus was subcultured every 4 weeks on fresh medium containing 2,4-D. For chemical analysis the callus obtained from the 6th subculture was used.

The studies on chemical composition of the raw materials were undertaken in the labs of Department of Vegetable and Medicinal Plants. 1 g of dry raw material was extracted with 100 ml of methanol in Büchi B-811 Extraction System. After solvent evaporation, the residue was dissolved in 10 ml of methanol, filtered through a Supelco IsoDisc PTFE 25 mm×0.45 μm filter and subjected to HPLC for determination of phenolic compounds. The analyses were performed using a Shimadzu chromatograph equipped with autosampler SIL-20, photodiode array detector SPD-M10A VP DAD and Class VP 4.3 chromatography software. A reversed-phase C-18 column (Phenomenex Luna® C18(2) RP 18 column, 5 μm particle size, 250×4.6 mm i.d.) was used as a solid phase. Binary gradient of solvents A (10% ACN, LabScan in deionised water adjusted to pH 3 with phosphoric acid) and B (55% ACN, LabScan in deionised water adjusted to pH 3 with phosphoric acid) was used as follows: 0 min, 15% B; 30 min, 75% B; 30.01 min, 100% B; 35 min, 100% B; 35.01 min 15% B; 40 min stop, flow rate 1.0 ml/min. The following conditions were applied: injection volume: 10 μl, column at 30ºC, UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards purchased from ChromaDex. For quantitation of investigated compounds the five-point calibration curve method was used. Detection wave applied: 206 nm ((-)-epigallocatechin galate), (-)-epicatechin gallate), 254 nm (luteolin-7-O-glucoside, isoquercitrin) and 330 nm (syryngic, chlorogenic, and caffeic acids). The content of the determined compounds was calculated in mg/100 g dry matter.

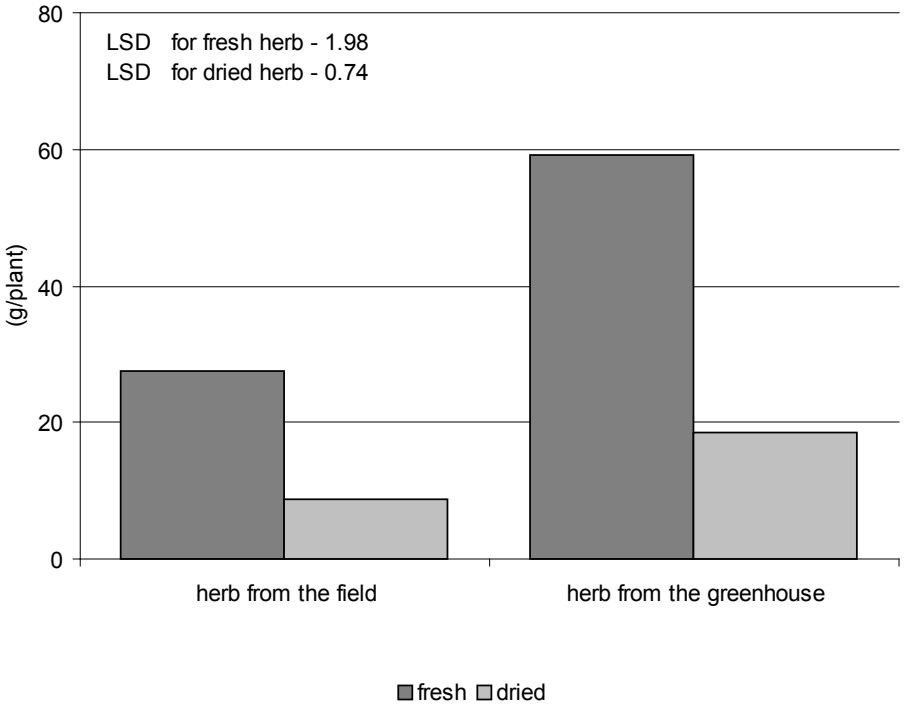
For sterol determination 1g of raw material was extracted with 100 ml of hexane in the Büchi B-811 Extraction System. After evaporation of solvent, the residue was dissolved in 10 ml of chloroform-methanol (4:1) mixture. The obtained extract was filtered through a Supelco IsoDisc PTFE 25 mm×0.45 μm and subjected to HPLC. The analyses were performed using a Shimadzu chromatograph equipped with autosampler SIL-20, photodiode array detector SPD-M10A VP DAD and Class VP 4.3 chromatography software. A reversed-phase C-8 column (Phenomenex Luna® C8(2) RP 18 column, 5 μm particle size, 250×4.6 mm i.d.) was used as a solid phase. Binary gradient of MeOH (LabScan) and ACN (LabScan) was used as follows: 0 min, 92% B; 5 min, 92% B; 20 min, 100% B; 25 min, 100% B; 25.01 min 92% B; 30 min stop, flow rate 1.0 ml/min. The following conditions were applied: injection volume: 20 μl, column at 30ºC, UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards purchased from ChromaDex. For quantitation of investigated compounds the five-point calibration

curve method was used. Quantification was based on the peak area at 210 nm for all substances (β-sitosterol-D-glucoside, β-sitosterol, cholesterol, brassicasterol, campesterol, stigmasterol). The analyses were carried out in four repetitions.

The results were shown as mean values from two or three years. They were subjected to analysis of variance (ANOVA). The values of $p<0.05$ were considered as significant. Differences between the means were evaluated using the Tukey test.

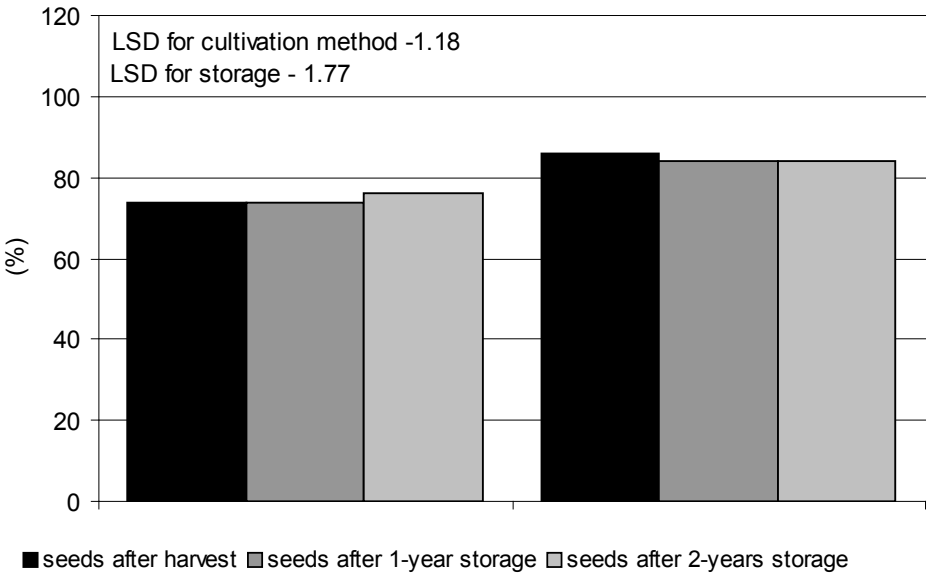
RESULTS AND DISCUSSION

The obtained results indicate that cultivation of *E. hirta* is possible in Poland and gets relatively high yields of the herb. The mass of raw material depended significantly on the cultivation conditions. A significantly higher mass of herb was obtained from the plants cultivated in the greenhouse than those cultivated in the field (fig. 1). In the experiment *E. hirta* produced well-germinating seeds, which retained their germinability after two years of storage (fig. 2).



LSD – Least Significant Difference

Figure 1. Herb mass (g/plant) – mean values from three years



LSD – Least Significant Difference

Figure 2. The germinability of seeds (%) – mean values from two years

Chemical analysis of the herb and callus shows that these raw materials accumulate phenolic compounds and sterols in high quantities. Blanc et al. [9] and Aqil [10] identified quercetin, leucocianidol, camphol and euphorbianin in *E. hirta* herb, whereas Patil et al. [1] gallic acid, 3,4-di-O-galloylquinic acid, myricitrin, 2,4,6-tri-O-galloyl-D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, and phytosterols: β-amyrin, 24-methylenecycloartenol and β-sitosterol.

In present study 7 phenolic compounds ((-)-epigallocatechin gallate, (-)-epicatechin gallate, luteolin-7-O-glucoside, isoquercitrin, syringic, chlorogenic, and caffeic acids – fig. 3) and 6 sterols (β-sitosterol-D-glucoside, β-sitosterol, cholesterol, brassicasterol, campesterol, stigmasterol) were identified. The herb originating from plants grown in the greenhouse was richer in biologically active compounds, especially in epicatechin gallate (41.87 mg/100 g d. m.), luteolin-7-O-glucoside (98.83 mg/100 g d. m.), and β-sitosterol-D-glucoside (45.76 mg/100 g d. m.), as compared to the plants cultivated in the field (tab. 1).

The callus also seems to be interesting in respect of the content of phenolic and sterol compounds showing high content of syringic (68.00 mg/100 g d. m.) and chlorogenic acid (79.67 mg/100 g d. m.) as well as brassicasterol (32.57 mg/100 g d. m.) (tab. 2).

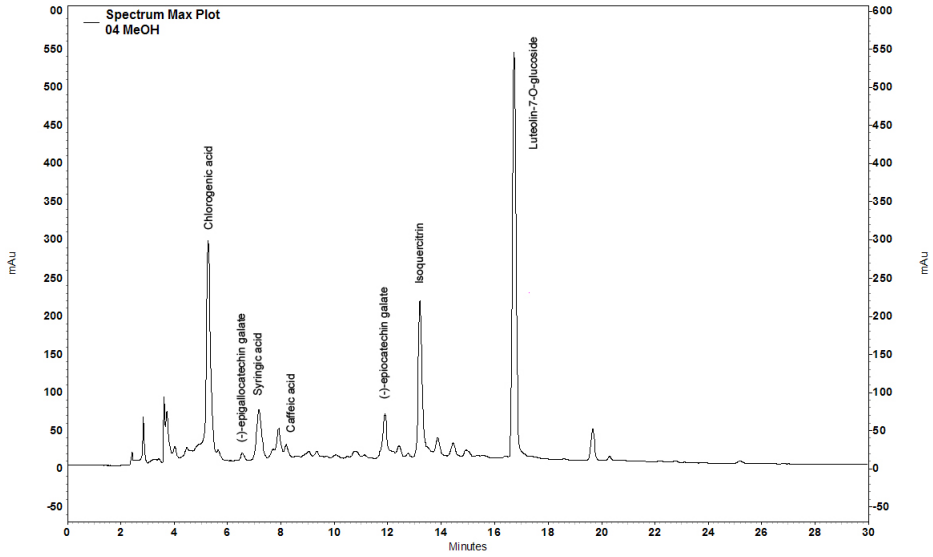


Figure 3. HPLC chromatogram of phenolic compounds (extract of the herb from greenhouse)

Table 1.

The content of phenolic compounds in the herb and in callus (mg/100 g d. m.) – mean values from three years

phenolic compounds	herb from the field	herb from the greenhouse	callus
(-)-epigallocatechin gallate	16.25 b	29.52 a	19.42 b
(-)-epicatechin gallate	16.72 b	41.87 a	19.60 b
luteolin-7-O-glucoside	56.72 b	98.83 a	5.24 c
isoquercitrin	51.87 a	51.21 a	12.30 b
syringic acid	51.14 b	61.93 ab	68.00 a
chlorogenic acid	48.68 b	73.78 a	79.67 a
caffeic acid	0.66 b	1.22 a	0.83 ab

Values in rows marked with the same letter do not differ significantly at $\alpha = 0.05$ (Tukey test).

Table 2.

The content of sterol compounds in the herb and in callus (mg/100 g d. m.) – mean values from three years

sterol compounds	herb from the field	herb from the greenhouse	callus
β-sitosterol-D-glucoside	19.08 b	45.76 a	37.04 a
β-sitosterol	1.20 b	3.56 a	1.96 ab

sterol compounds	herb from the field	herb from the greenhouse	callus
cholesterol	3.36 a	0.41 c	1.57 b
brassicasterol	10.09 c	23.20 b	32.57 a
campesterol	0.51	0.33	nd
stigmasterol	11.69 b	18.19 a	19.66 a

nd – not detected
Values in rows marked with the same letter do not differ significantly at $\alpha=0.05$ (Tukey test).

CONCLUSION

- The seeds originating from the *E. hirta* plants cultivated in Poland are characterised by high, maintained in 2 years, germinability.
- A significantly higher mass of herb was obtained from the plants cultivated in a greenhouse than those cultivated in a field.
- In the herb 7 phenolic compounds and 6 sterols were found with luteolin-7-O-glucoside, chlorogenic acid and β-sitosterol-D-glucoside as a dominant.
- The content of identified compounds was distinctly higher in herb from the plants growing in the greenhouse.
- Preliminary studies indicate that callus obtained *in vitro* contains biologically active compounds, especially chlorogenic and syringic acids.

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GROMADZENIE SIĘ ZWIĄZKÓW FENOLOWYCH I STEROLI W WILCZOMLECZU OWŁOSIONYM (*EsUPHORBIA HIRTA* L.)

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

Wilczomlecz owłosiony jest jednoroczną rośliną dość powszechnie występującą w tropikalnej strefie Azji, Afryki oraz Ameryki Środkowej i Południowej. Wyciągi z ziela wykazują działanie rozkurczowe, przeciwzapalne i diuretyczne. Substancje biologicznie aktywne występujące w tej roślinie to sterole, saponiny, flawonoidy i kwasy fenolowe. Przedstawione w tej pracy wyniki wskazują na możliwość uprawy wilczomlecza owłosionego w Polsce i uzyskania względnie wysokiej masy surowca. Roślina ta wydaje w Polsce dobrze kiełkujące nasiona, które po dwóch latach przechowywania nie tracą zdolności kiełkowania. Analiza chemiczna ziela oraz uzyskanego *in vitro* kalusa wykazała, że w surowcach tych w istotnych ilościach gromadzą się zarówno związki fenolowe, jak i sterole.

Słowa kluczowe: *nasiona, ziele, kalus, flawonoidy, kwasy polifenolowe, sterole*