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Content of sterols in *in vitro* propagated *Chamerion angustifolium* (L.) Holub plants

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Abstract:

Chamerion angustifolium (L.) Holub (syn. *Epilobium angustifolium* L., Onagraceae family) plants are utilized as a component of drugs, nutraceuticals and cosmetic products. The European Medicines Agency (EMA) approved *E. angustifolium* in traditional herbal medicinal products for treatment and alleviating symptoms related to Benign Prostatic Hyperplasia (BPH). Plants are a rich source of ellagitannins, flavonoids and phenolic acids, the herb also contains steroids, triterpenes and fatty acids. Campesterol, cholesterol, stigmasterol and *β*-sitosterol and its derivatives have been identified in plants. Phytosterols are synthesized and accumulated in plant in vitro cultures, in this way in vitro cultures could be an alternative source to produce phytosterols.

The aim of this study was to determine the content of campesterol, *β*-sitosterol and stigmasterol in *Ch. angustifolium* plants cultivated in vitro. The plants after five weeks of culture were subjected to the HPLC-DAD analysis. Additionally, the analysis of phytosterols in the plants regenerated under in vitro conditions and planted in field was performed.

Results of HPLC-DAD analysis have shown that stigmasterol was a dominant compound (382.60–577.77 mg/100 DW) in the plants grown in vitro. Among the tested genotypes, significant variation in the sterol content was found. In contrast to in vitro cultures, plants harvested from field synthesized mainly *β*-sitosterol (103.05 mg/100 g DW), whereas campesterol and stigmasterol were less abundant. Plants cultivated under in vitro conditions contained more sterols than plants grown in field.

Keywords: *Epilobium angustifolium*; HPLC-DAD; in vitro cultures; campesterol; *β*-sitosterol; stigmasterol

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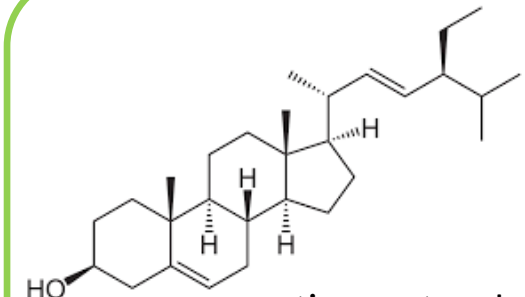
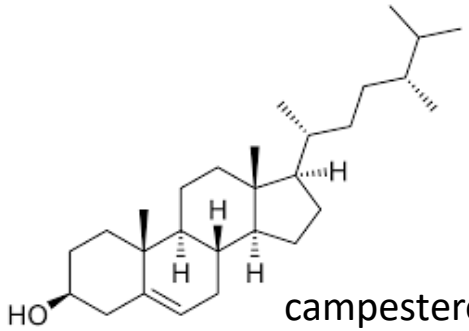
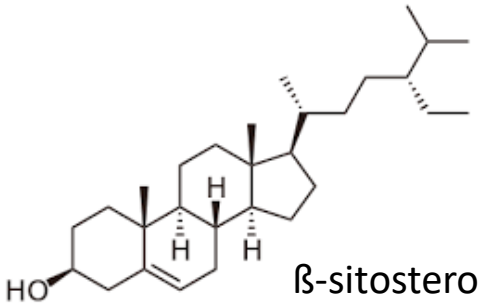


stigmasterol



campesterol



ß-sitosterol

- Phytosterols are essential biomolecules for human health. These compounds have shown hypocholesterolemic, anti-inflammatory, antidiabetic and anticancer activities.
- Phytosterols are added to the cosmetic products for their moisturizing and regenerating skin properties.
- Since the phytosterol extraction methods are based on use of organic solvent that are harmful for human health and toxic for the environment, therefore another alternative source to produce phytosterols are needed.
- Considering, the growing demand for a raw material rich in these compounds, a new alternative source such as in vitro cultures should be considered.

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a

b

Figure 1. (a) Full blooming *Ch. angustifolium* plants grown in field (Plewiska, Poland); (b) In vitro rooted plants (5-weeks-old culture)

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Results and Discussion

Table 1. Variation in sterols content (mg/100g of DW) in *Ch. angustifolium* genotypes derived from *in vitro* culture. Values are mean ± SD of two repetitions for chemical analysis. Mean values in column with the same letter are not significantly different at p = 0.05 (Tukey's multiple range test)

Genotype	Content of sterols mg/100 g [±SD]			Sum of sterols mg/100g
	Campesterol	Stigmasterol	β-sitosterol	
N3	150.87±1.39^{d}	448.08±27.07^{ad}	131.83±5.29^{a}	730.78
N5	200.94±3.27^{e}	501.83±8.55^{ab}	141.64±1.11^{a}	844.41
N6	228.41±9.07^{b}	375.64±21.55^{c}	222.49±9.03^{c}	826.53
N11	255.88±7.88^{a}	525.68±14.88^{be}	105.30±0.61^{e}	886.85
PL37	119.63±5.53^{c}	501.93±11.92^{ab}	69.79±0.43^{d}	691.35
PL38	323.86±10.00^{g}	577.77±6.94^{e}	185.87±2.43^{b}	1087.50
PL44	240.99±2.40^{ab}	389.80±15.78^{cd}	189.34±0.84^{b}	820.13
PL45	255.00±8.62^{a}	382.60±10.92^{c}	211.88±2.07^{c}	849.48
PL58	293.26±6.36^{f}	481.06±4.15^{ab}	146.55±6.74^{a}	920.88
The mean value	229.88±62.99	464.93±69.56	156.08±49.13	850.89

- Results of HPLC-DAD analysis have shown that stigmasterol was a dominant compound among tested sterols.
- The investigated genotypes differed in sterols content, particularly in β-sitosterol.
- The highest content of campesterol was recorded for PL58 genotype
- PL38 genotype was characterized by the highest level of stigmasterol
- N6 line was characterized by high level of β-sitosterol.

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Results and Discussion

Table 2. Content of campesterol, stigmasterol and β-sitosterol (mg/100g of DW) in the *Ch. angustifolium* plants cultivated in field. Values are mean ± SD of four repetitions for chemical analysis.

Plant material	Content mg/100 g [±SD]			Sum of sterols mg/100 g
	Campesterol	Stigmasterol	β-sitosterol	
Above-ground part of plants grown in field	25.06±16.41	7.03±1.50	103.05±16.68	135.14

- Plant harvested from field significantly differ in the composition and content of sterols
- In contrast to in vitro cultures, the plants grown in soil synthesized mainly β-sitosterol (103.05 mg/100 g DW), whereas campesterol and stigmasterol were less abundant.
- Plants cultivated in field contained less sterols than plants cultivated in vitro (135.14 *vs* 850.89 mg/100 g DW).

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Conclusions

- **In vitro cultures of *Ch. angustifolium* are rich source of phytosterols**
- **Genotype has a significant influence on the accumulation of phytosterols under in vitro conditions**

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