

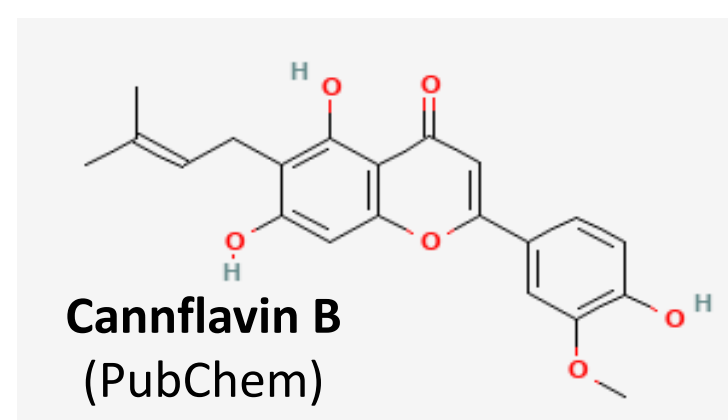
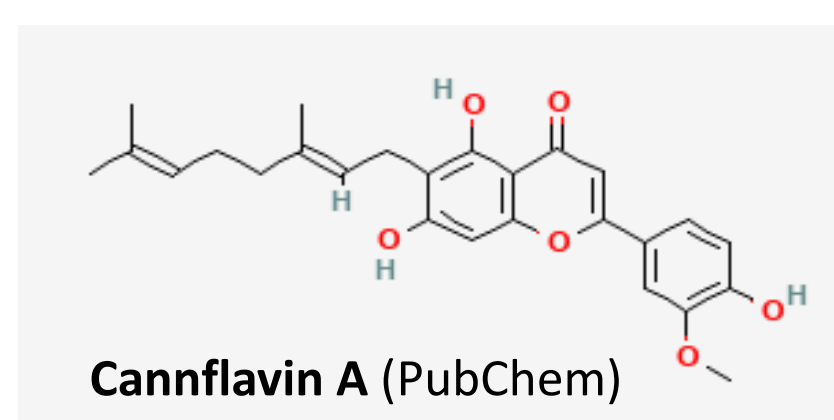
The content of flavonoids in hemp seedlings

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INTRODUCTION & AIM

Hemp (*Cannabis sativa* L.) seedlings do not contain cannabinoids, which are the species' typical active compounds but they synthesize flavonoids, including the anti-inflammatory compounds cannflavin A, B, and C. More than 20 flavonoids have been identified in *C. sativa*, most of which are flavone (apigenin and luteolin) and flavonol (kaempferol and quercetin) aglycones and glycosides (Bautista et al. 2021). There is a need for the development of novel anti-inflammatory medications in the context of rising incidence of inflammatory and auto-immune conditions globally (Erridge et al., 2020). The aim of this study was to analyse the content of flavonoids in different commercial hemp cultivars: Fibrol, Finola, Epsilon 68, Secuieni Jubileum, Fedora, Henola, Uso 31, Earlina 8FC, Kompoliti, and Lipko using the HPLC-MS/MS method.



METHODS



Fot. 1. The Epsilon 68 seedlings growing in pots

Plant material

The plants (Fot. 1) were grown in pots at temperature of 25°C (±2°C) under a 16-hour photoperiod with 80–100 µmol m⁻² s⁻¹ fluorescent daylight. Fifteen days after germination, the aerial parts were collected, weighed, and dried. The flavonoid content was determined in the isopropanol extracts.

The chromatographic analysis was performed using Shimadzu 8040 LC-MS chromatograph and Zorbax SB-C18 column (3.0 mm x 150 mm 3.5 µm). The mobile phase A consisted of acetonitrile and formic acid solution in water 0.1% (V/V), both HPLC grade. The mobile phase B consisted of water and formic acid at concentration 0.1% (V/V). The mobile phase A consisted of acetonitrile and formic acid solution in water 0.1% (V/V), both HPLC grade. The mobile phase B consisted of water and formic acid at concentration 0.1% (V/V). The assay was performed in a gradient elution procedure: 0.0 min—80% of phase B, 5 min—28% of phase B, 5.5 min—0% of phase B, 9 min—0% of phase B, 9.5—80% of phase B. The flow rate was set at 0.8 ml/min, and the injection volume was 2 µl. Detector: MS MRM mode (quantitative and screening). The analysis was performed in negative ion charge, using multiple reaction monitoring (MRM) for qualitative and quantitative analyses.

RESULTS & DISCUSSION

Table 1. The mean flavonoid content in hemp seedlings

Cultivar	Content µg/g				
	Cannflavin A	Cannflavin B	Isovitexin	Vitexin	Apigenin
Fibrol	18.2±4.3 ^b	2.5±0.4 ^b	<n. d.	<n. d.	<n. d.
Finola	8.8±3.8 ^a	1.2±0.1 ^a	16.1±0.0 ^{ab}	12.3±0.0 ^a	<n. d.
Epsilon 68	18.3±1.1 ^b	1.3±0.1 ^a	6.2±0.9 ^a	<n. d.	3.4±0.6 ^{ab}
Secuieni Jubileu	15.6±2.2 ^b	1.3±0.2 ^a	3.3±0.0 ^a	<n. d.	2.1±0.0 ^a
Fedora	6.9±2.6 ^a	1.3±0.0 ^a	2.5±0.6 ^a	<n. d.	2.1±0.0 ^a
Henola	5.9±2.2 ^a	1.2±0.2 ^a	2.2±0.1 ^a	<n. d.	<n. d.
Uso 31	8.6±0.5 ^a	1.3±0.2 ^a	3.5±0.7 ^a	<n. d.	<n. d.
Earlina 8FC	2.0±0.0 ^c	1.2±0.1 ^a	3.7±0.6 ^a	<n. d.	3.2±0.1 ^{ab}
Kompoliti	16.9±1.2 ^b	1.2±0.1 ^a	24.0±15.9 ^b	13.3±9.5 ^a	4.2±1.1 ^b
Lipko	8.8±1.5 ^a	1.2±0.0 ^a	9.4±2.0 ^a	4.3±1.8 ^a	3.7±0.6 ^b

Values represent the mean content (±SD) for three samples per cultivar; < n. d. – below the detection limit <0.002 mg/g. Statistical significance was determined applying Duncan's POST-HOC (p value of 0.05).

- A significant variation in the content of cannflavin A was found among the tested hemp varieties (Table 1).
- The highest concentrations of cannflavin A were recorded for the Epsilon 68, Fibrol (18.2 ± 4.3 µg/g) and Kompolti (16.9 ± 1.2) cultivars.
- The content of cannflavin B was much lower and ranged from 1.2 to 2.5 µg/g.
- The highest level of isovitexin (24.0±15.9 µg/g) and vitexin (13.3±9.5) was determined in the Kompolti seedlings.
- The content of myrcetin, luteolin, narigerin, orientin, quercetin and catechin was below the detection limit <0.010 mg/g in all tested samples.

CONCLUSION

- The Kompolti, Epsilon 68, and Fibrol genotypes are potential sources of valuable flavonoids and can be used as parental material in further breeding work.
- Collecting data for all developmental stages will allow to determine the full profile of flavonoids synthesized and determine when their content reaches maximum level in plants.

REFERENCES

- Erridge et al. (2020). <https://doi.org/10.1016/j.phytochem.2019.05.009>
Bautista et al. (2021). <https://doi.org/10.1021/acsomega.1c00318>
<https://pubchem.ncbi.nlm.nih.gov>

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