Impact of polyphenolic fraction and flavone *C*-glycosides on biological activity of red beet leaves (*Beta vulgaris* L. *var. rubra*)

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1. Introduction

Red beet (*Beta vulgaris* L. *subsp. vulgaris;* syn. *B. vulgaris* L. *var. rubra;* Chenopodiaceae) is one of the most popular vegetable plants cultivated in moderate climates worldwide for its roots, young leaves (beet greens), and sprouts. Recent studies have highlighted various health benefits associated with consuming red beets, particularly focusing on the betalain-rich roots and their potential anti-diabetic, anti-inflammatory, and anticancer effects. Additionally, research has shown promising antioxidant and hypoglycemic in vivo effects of polyphenol-rich young beet leaves, suggesting their therapeutic potential in managing metabolic disorders, such as diabetes and related cardiovascular complications. According to the reported activity of young leaves, the investigation into the characteristics of sprouts (dietary product) and autumn leaves (waste of red beet cultivation) seems promising and can provide valuable insights.

2. Aim and goal

Consequently, the main goal of the proposed PhD project is thorough phytochemical characterization (complete structural elucidation and quantitation of individual compounds) and evaluation of the biological activity of red beet sprouts and autumn leaves in the context of diabetes and circulatory system disorders in complementary cellular and non-cellular in vitro and ex vivo models. Moreover, the project will assess the influence of two main types of red beet constituents – polyphenols (primarily flavone *C*-glycosides) and betalains on the investigated activity. According to the objective and hypothesis, the initial stages of the project realized in the academic year 2023/2024 encompassed the acquisition of plant materials and the preparation of extracts for phytochemical and biological studies. To achieve this, sprouting optimization was conducted to determine the most favorable time for harvesting. Additionally, the seasonal variability of leaves was investigated to ascertain the potential of autumn leaves for further studies and functional application.

3. Material and methods

- Plant material collection and preparation of extracts:

Seeds of two varieties of red beet, 'Czerwona Kula' (CK) and 'Egipski' (EG), obtained from commercial source, were germinated using automatic sprouters. The sprouts were collected after the 6th, 9th, 12th, and 15th day of growth. Leaves of CK and EG harvested from June to October 2023 were freeze-dried. Afterward, the sprouts and leaves were used to prepare the extracts (methanol-water solution; 7:3, *v/v*; reflux extraction), which were subjected to phytochemical profiling in the subsequent steps of the project.

- Qualitative phytochemical profiling of sprouts and leaves evaluated by LC-MS/MS (Table 1 and 2, Fig. 5)
- Determination of the total polyphenol content (TPC) in both sprouts and leaves (Fig. 1, 4)
- Determination of the total content of betalains (BC) in leaves (Fig. 2)
- Determination of the content of *C*-flavonoid glycosides in leaves (Fig. 3)
- Evaluation of superoxide anion ($O_2^{\bullet-}$) scavenging capacity of leaves

4. Results

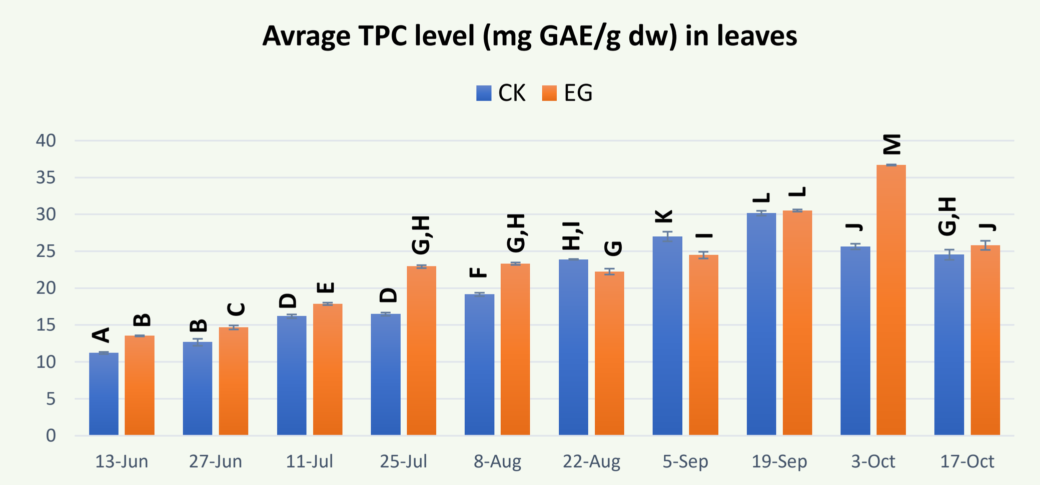

Fig 1. Total polyphenol content (TPC) in beetroot leaves; values marked with various letters indicate significant differences (p<0.05).

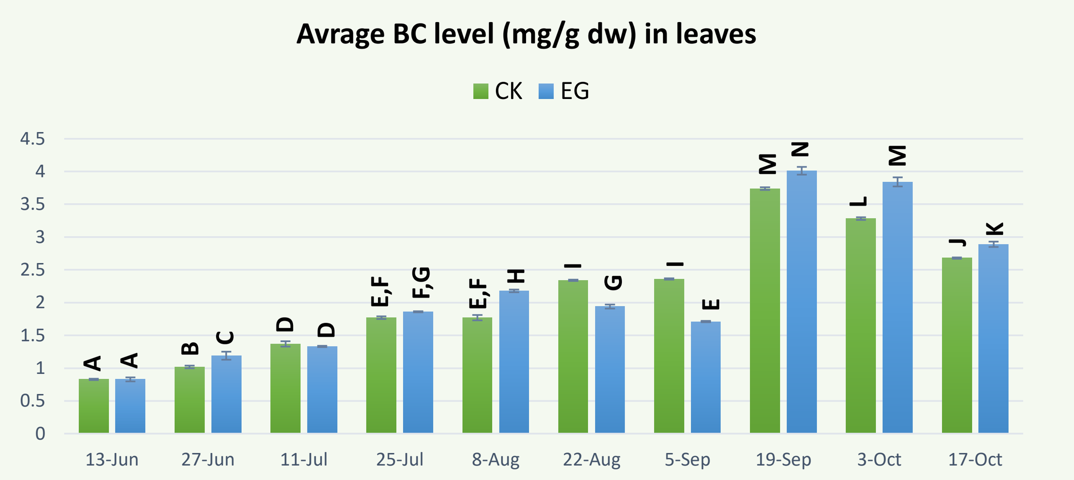

Fig 2. Total content of betalains (BC) in beetroot leaves, values marked with various letters indicate significant differences (p<0.05).

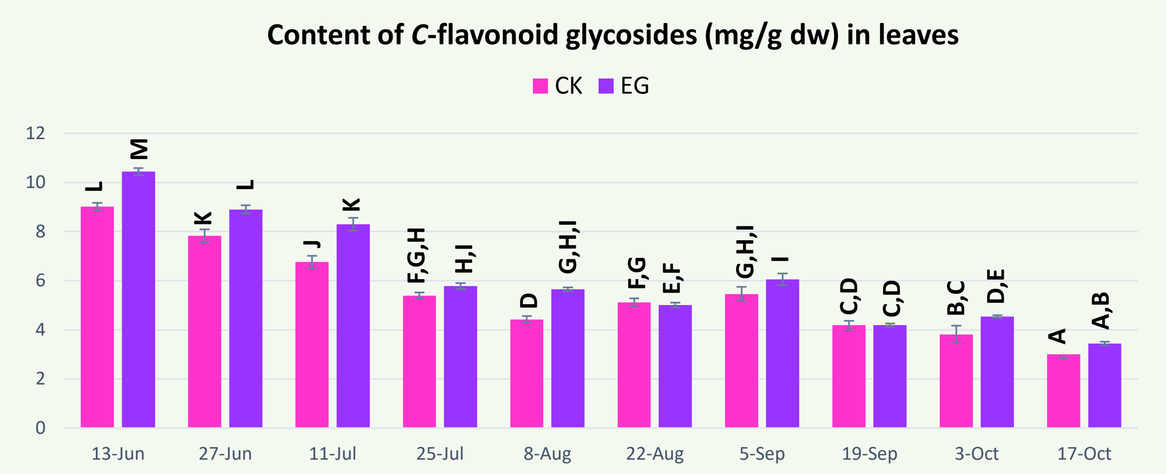

Fig 3. Total content of flavonoids (expressed as vitexin) in beetroot leaves, values marked with various letters indicate significant differences (p<0.05).

1-4, 6, 7, 9, 11, 13-15 – betacyanins (i.a. 1 – betanin, 2 - isobetanin)

5, 8, 10, 12 – betaxanthins (i.a. 5 – dopamine-betaxanthin)

1-12, 14, 16 – flavone *C*-glycosides (i.a. vitexin and methylvitexin derivatives)

13, 15 – phenolic amides (i.a. 13 – *N-trans*-feruloyltyramine)

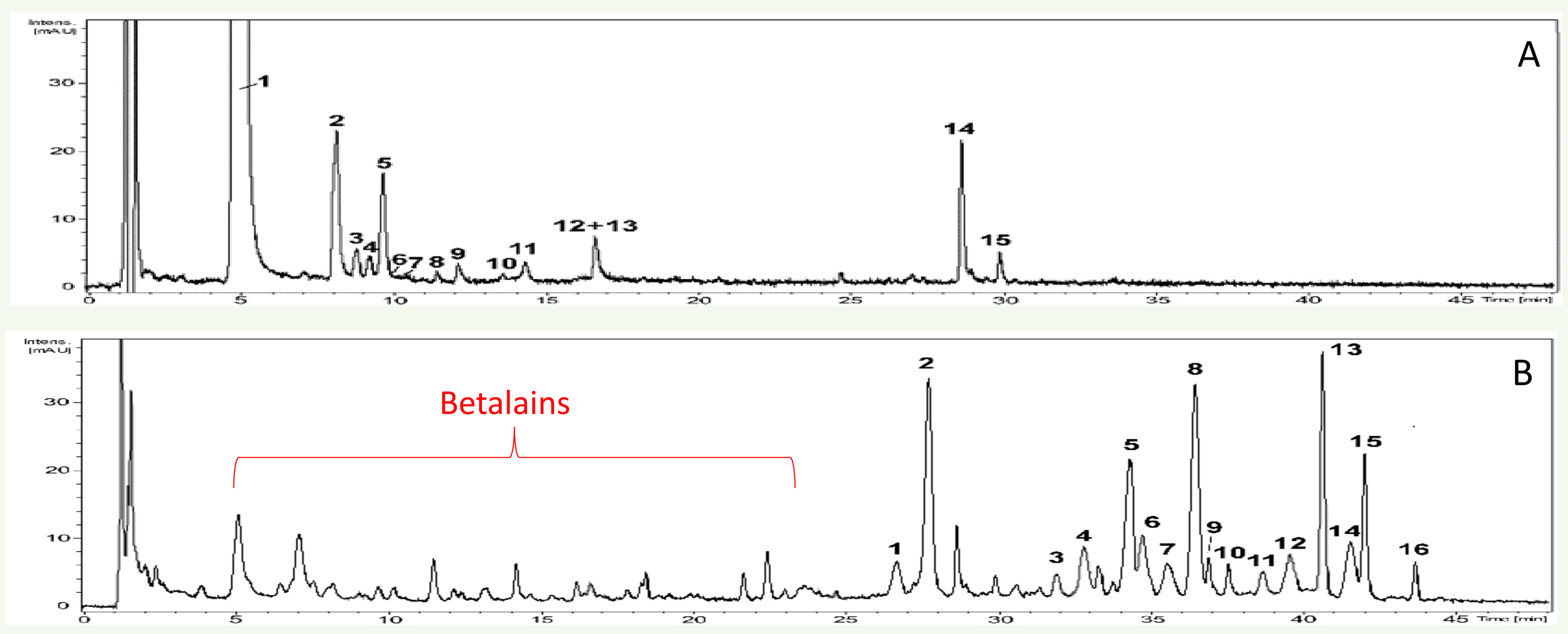

Fig 5. representative UHPLC chromatogram of the extract from 12-day-old sprouts; A (520 nm) and B (350 nm). Peak numbers refer to those implemented in Table 1 (A) and 2 (B).

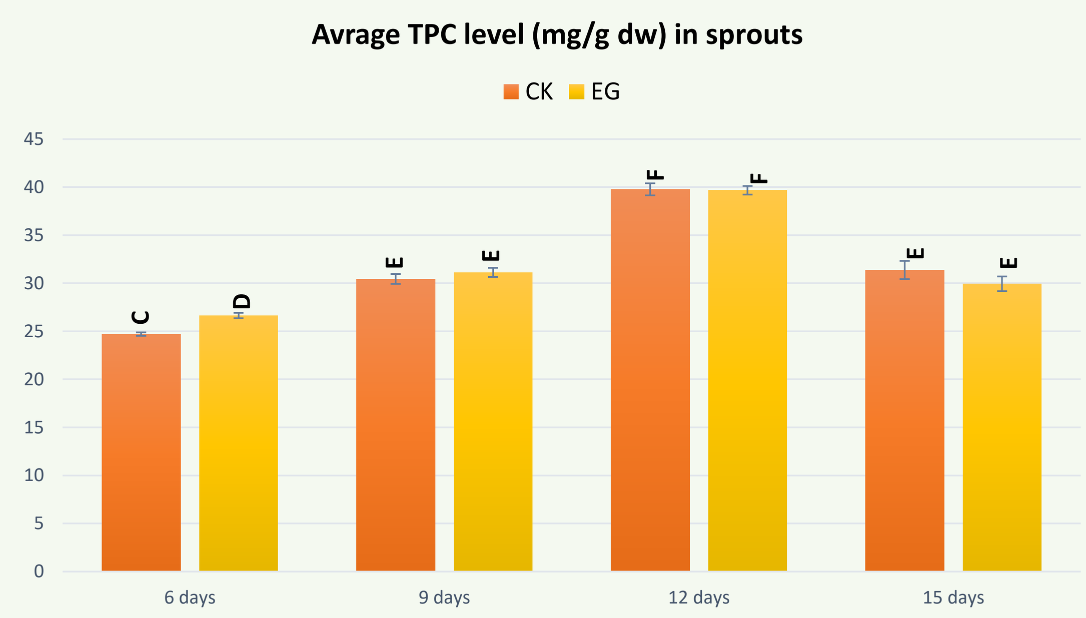

Fig 4. Total polyphenol content (TPC) in beetroot sprouts; values marked with various letters indicate significant differences (p<0.05).

Table 1. Chromatographic characteristics of betalains in sprouts

No	Analyte	Retention time (min)	UV λmax (nm)	[M+H]+ (m/z)	Fragmentary ions
1	Betanin	5.2	529	551	389
2	Iso betanin	8.3	531	551	389
3	17-decarboxybetanine	9.1	505	507	345
4	17-decarboxyisobetanine	9.5	510	507	345
5	Dopamine betaxanthin	9.8	461	347	137;241;303
6	Iso betanidine	10.2	443	345	283; 301
7	Unidentified	10.4	460	457	442
8	Valine-betaxanthin	10.6	470	311	267
9	2-decarboxy neobetanine	12.3	445	505	343
10	Tyramine betaxanthin	13.8	463	331	287
11	Neo betanin	14.5	471	549	387
12	Methionine betaxanthin	16.8	400	343	299
13	Decarboxy betanidine	16.8	433	345	301
14	6'-O-feruloylbetaine	28.8	530	727	389
15	6'-O-feruloylisobetaine	30,0	530	727	389

Table 2. Chromatographic characteristics of polyphenols in sprouts

No	Analyte	Retention time (min)	UV λmax (nm)	[M-H]- (m/z)	Fragmentary ions
1	Vitexin 2-hexoside	26.8	332	593	413;293
2	Vitexin 2-pentoside	28.0	338	563	413;293
3	Vitexin derivative I	32,1	332	605	545;455;413;293
4	Vitexin derivative II	33.0	330	635	455;293
5	Vitexin derivative III	34.6	333	605	545; 455; 293
6	Methylvitexin 2-hexoside	35.0	333	607	427; 307
7	Methylvitexin 2-rhamnoside	35.8	333	591	427;307
8	Methylvitexin 2-pentoside	36.6	333	577	427;307
9	C-glycoside I	37,1	330	445	325
10	C-glycoside II	37,7	325	593	417
11	methylvitexin derivative |	39,4	335	695	649;487;307
12	methylvitexin derivative II	39.8	333	649	589;427;307
13	N-trans-feruloyltyramine	41.0	318	312	178
14	Methylvitexin derivative III	41,8	333	619	589; 469; 427; 307
15	N-trans-feruloylhomovanilylamine	42.1	325	342	178
16	C-glycoside III	43.8	325	487	337

Superoxide anion ($O_2^{\bullet-}$) scavenging capacity of leaves:

The biological activity test showed the high $O_2^{\bullet-}$ scavenging potential of dry leaf extracts, based on the xanthine oxidase/xanthine reaction system. In both cultivars the activity increased from spring to autumn, showing the same seasonal trend observed in determinations of TPC and BC levels. Consequently, the scavenging capacity peaked in September with the IC_{50} value of 44.67 µg extract dw/ml.

- Based on results showed at Fig. 1. TPC levels were 11.08-30.49 mg GAE/g dw and 13.48- 36.76 mg GAE/g dw of plant material in CK and EG leaves, respectively. The highest level was observed in 19-Sep and 3-Oct.
- As Fig. 2. illustrates, the peak levels of BC were observed for autumn leaves, with 3.74 mg/g dw and 4.01 mg/g dw in CK and EG plant material, respectively.
- As per Fig. 3., it can be observed that the concentration of flavone *C-glycosides* was the highest in spring and early summer, reaching its peak in June. This trend contrasts with the patterns observed for TPC and BC levels.
- In accordance with Fig. 4., the TPC level was 24.71-39.77 and 26.64-39.68 mg GAE/g dw for CK and EG sprouts, respectively. The amount of phenolic compounds increased during cultivation, and the highest TPC value was observed for 12-day-old sprouts. The TPC level for 15-day-old sprouts decreased slightly compared to the 12-day-old ones.
- According to the Fig. 5 and Table 1 and 2, over 30 compounds belonging to the groups of polyphenols and betalains were found in the tested sprouts. Among polyphenols, vitexin derivatives, methylvitexin derivatives, other *C*-glycosides and phenolic amides were recognized. In the betalains group, betacyanins and betaxanthins were identified.
- The LC-MS/MS analysis of leaves led to the complete or tentative identification of more than 40 components, primarily categorized as betalains and polyphenols, particularly flavone *C*-glycosides including vitexin and methylvitexin with their derivatives such as vitexin 2-hexoside, vitexin 2-pentoside, and methylvitexin pentoside, and other vitexin and methylvitexin derivatives.

6. Conclusion

In conclusion, 12 days old sprouts deserve further investigation into their biological activities. As to the leaves, the autumn material demonstrates significant phytochemical and biological potential due to the high total phenolic content and betalains. Apart from betalains, it contains other beneficial compounds, primarily flavone *C*-glycosides. These findings support the idea that autumn leaves, often considered waste, have significant potential for functional food applications. Therefore, the next phase of the PhD project will involve preparing extracts from autumn leaves and 12-day-old sprouts for biological activity testing in vitro and ex vivo to verify the research hypothesis eventually.

References:
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2. Burri, S.C., et al., Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. Journal of Functional Foods, 2017. 38: p. 119-127.