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ORIGINAL PAPERS

Carotenoids Content in Plant Organs of *Taraxacum Officinale* (L.) Species from Two Romanian Regions

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Abstract. *Carotenoids are pigments that occur naturally in bacteria, algae, fungi and plants and give them their orange or red colour. The most important carotenoids that have beneficial effects on human health are beta-carotene, lutein, astaxanthin, lycopene and zeaxanthin. The main advantage of carotenoids lies in their antioxidant potential, the role of protecting the body's cells from damage by unstable oxygen molecules. The present paper presents the comparative content of carotenoid-type bioactive compounds present in the hydroalcoholic extracts obtained from different plant organs (roots, leaves and flowers) of the species Taraxacum officinale (L.), dandelion. The plant product was collected from the spontaneous flora, the southern area of Dobrogea, Constanța county and from the eastern area of Transylvania, Harghita county, in May 2022. The obtained hydroalcoholic extracts of 10% concentration in 50% ethanol and respectively 70% ethanol, using the cold maceration method, in the dark, for 14 days, were analysed by UV-Vis spectrophotometry to determine the total content of carotenoids, beta-carotene and lutein. Total carotenoids content was higher in ethanolic extracts of roots and flowers of plants collected from the southern area of Dobrogea, and for the leaves the content of total carotenoids was higher in the ethanolic extracts of the plants collected from the eastern area of Transylvania. The differential distribution observed in terms of the carotenoids content present in the various plant organs of the species Taraxacum officinale (L.), may be due to the influence of the climate and the type of soil characteristic of the two studied areas. Enriching the database regarding the concentration of analysed carotenoid-type bioactive principles can contribute to the development and testing of* *hypotheses about the therapeutic potential of this valuable component of the Romanian spontaneous flora.*

Key words: *Taraxacum officinale* (L.), total carotenoids, beta-carotene, lutein.

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1.Introduction

Carotenoid pigments are among the most important pigments and have many applications in various cosmetics, hygiene industries and biotechnology, pharma and agro food industries; the leading interest for humans is their nutritional value. These pigments are produced by plants, animals, algae, fungi and microorganisms. Until 2004 more than 700 carotenoids have been reported (Britton et al., 2004). Chemically carotenoid pigments are C40 tetraterpenoids with a long chromophore of conjugated double bonds, which is responsible for their red to yellow coloration (Weedon and Moss, 1995; Britton, 1995). Aside from being responsible for the colour of a large variety of structures, carotenoids are required for photosynthesis and photoprotection (Domonkos et al., 2013). Because carotenoids are fundamental for these processes, the variations of their levels in photo-synthetic tissues is changed by the need to maintain the functionality of chloroplasts.

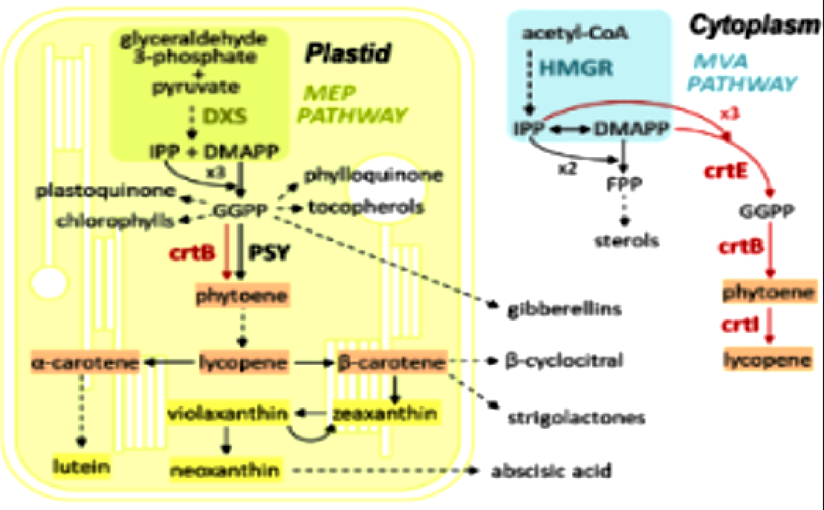

Fig. 1. Endogenous and engineered pathways for carotenoid biosynthesis in plant cells. (after Rodriguez-Concepción and Daros, 2022, in *Transient expression systems to rewire plant carotenoid metabolism*).

The carotenoid composition of chloroplasts is similar in most plant species, with lutein and β-carotene being the most abundant followed by xanthophylls (Domonkos et al., 2013; Rodriguez-Concepcion et al., 2018; Negreanu-Pirjol et al., 2021). Plants synthesise carotenoids in plastids using isoprenoid precursors (vitamin K), including chlorophylls and plastoquinone (Fig. 1, after Rodriguez-Concepcion and Daros, 2022), and also hormones such as gibberellins, abscisic acid and strigolactones, and retrograde signals such as beta-cyclocitral (Barja and Rodriguez-Concepción, 2021).

The scientific research interest is being focused on food carotenoids due to their health benefits - health-related properties associated with carotenoid-rich diets include a reduced risk of diseases such as age-related macular degeneration, cognitive malfunctioning, type-2 diabetes, obesity, cardiovascular diseases, and some types of cancer (Rodriguez-Concepcion et al., 2018; Amengual, 2019).

In vivo, carotenoids are found in precise locations and orientations in subcellular structures, and their chemical and physical properties are strongly influenced by other molecules in their vicinity, especially membrane proteins and lipids. In turn, carotenoids influence the properties of these subcellular structures. Structural features such as size, shape and polarity are critical determinants of a carotenoid's ability to fit properly into its molecular environment to allow it to function. The role of carotenoids in modifying the structure, properties and stability of cell membranes, and thus affecting the molecular processes associated with these membranes, may be an important aspect of their possible beneficial effects on human health (Britton, 1995a).

Extraction of carotenoids is commonly performed in organic solvents such as hexane, acetone, ethanol or methanol or in combination of these solvents (Inbaraj et al., 2008; Kao et al, 2011); it appears that a higher yield of carotenoid extraction can often be obtained by using a mixture of solvents. Among the carotenoids, zeaxanthin, lutein and ß-carotene from leafy vegetables have been intensively studied for their effects on human health (Landrum and Bone, 2001, Lakshminarayana et al., 2008, Martínez-García et al., 2023).

Analyses by Žnidarčič et al., 2011 revealed a value of 6.34 ± 0.94 mg/100 g D.W. of total carotenoids in dandelion leaves grown in the experimental field of the Biotechnical Faculty of Ljubljana, Slovenia, and lutein - 5.25 ± 0.62 mg/100 g D.W. Another study of Gomez, in 2017, analysed dandelion leaves from two different areas: Texas and New Jersey, in two different periods: autumn and summer, finding lower concentrations of β-carotene (2.69±0.39 mg/100 g D.W. and 4.27±0.58 mg/100 g D.W.) in Texas, but in New Jersey higher compared with Žnidarčič's study (14.13±1.01 mg/100 g D.W. and 23.18±0.54mg/100 g D.W.). The lutein concentration found was 25.48±2.45 mg/100 g D.W./25.99±3.37

mg/100 g D.W. for plants from Texas, for the two periods and respectively - 49.53±4.25 mg/100 g D.W. /53.65±4.74 mg/100 g D.W. for New Jersey plants.

In the 2022 study on the dandelion from the Dobrogea area (Tanasa et al., 2022b), in cold macerated hydroalcoholic extracts it was found a total carotenoids content of 134.44 mg/kg DW for the whole plant extract in ethanol 70% (coded Mix), respectively 104.01 mg//kg DW in *Taraxacum* leaves (coded Herba). For β-carotene the higher concentration was revealed in Herba hydroalcoholic extract in 70% ethanol (35.05 mg/kg DW) and a similar concentration between Herba hydroalcoholic extract in 50% ethanol (21.30 mg/kg DW) and Mix 70% ethanol (21.93 mg/kg DW). Another study in which the Soxhlet extraction of the bioactive principles from *Taraxacum* organs was applied (Tanasa et al., 2022a), it was obtained in hydroalcoholic extracts a total carotenoids value of 78.79 mg/kg DW in Herba and 4.77 mg/kg DW in Radix.

Some studies revealed that green leaves are used for the isolation of neoxanthin, violaxanthin and lutein, palm oil for the isolation of ⅄-carotene and β-carotene or red peppers for the isolation of capsanthin, capsorubin and other carotenoids (Mınguez-Mosquera and Hornero-Ménde, 1993; Olatunde Farombi and Britton, 1999). Therefore, determination of carotenoid pigments from natural sources must be encouraged for accurate identifications.

2. Material and method

Mature vegetal product (whole plant) of *Taraxacum* was collected in the period of May 2022, from spontaneous flora of the southern area of Dobrogea, Mangalia locality (43° 49′ 1″ North, 28° 34′ 59″ East), Constanța county and from the eastern area of Transylvania, Dănești locality (46° 31′16″N 25°39′24″E), Harghita county. Mangalia has typical chernozemic and calcareous soils and average annual temperature of 11-13 °C because the climate is temperate-continental, closer to subtropical-humid, with certain peculiarities related to the physical-geographical components of the territory - the Black Sea shore, with a permanent evaporation of water, ensures the humidity of the air and at the same time causes the regulation of its heating. Dănești has average annual temperature of 4-6 °C because of the climate, it is temperate-continental, with certain particularities determined by the alternation of the mountain massifs with the series of intramontane depressions and the approximately perpendicular orientation of the relief units to the atmospheric circulation of air masses, and the soils are a combination of acidic podzolic clay-alluvial soils, brown and podzolic clay-alluvial soils, lithomorphic soils.

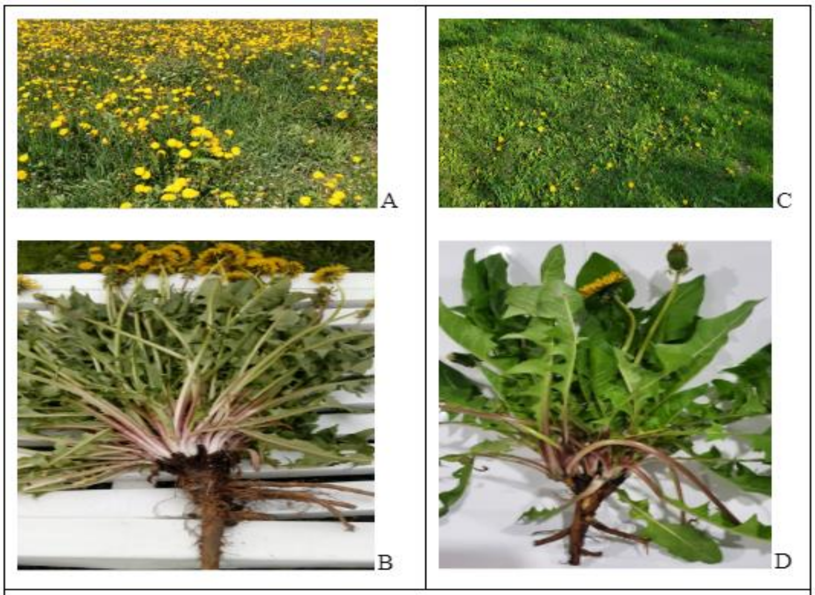

Fig. 2. ***Taraxacum officinale*** **- from Harghita county - A- dandelion meadows, B - whole plant; Constanța county - C - dandelion meadows, D - whole plant** (original photos, Tănasă (Acreței) M.V.)

The biological material was represented by roots (coded Radix) (Fig. 3. A), scapes and leaves (coded Herba) (Fig. 3. B) and flowers (Fig. 3. C), collected from plants at full maturity and all parts of the plant were separated, washed thoroughly with tap water. Fresh plant was dried at room temperature on metal sieves and grind to a fine powder.

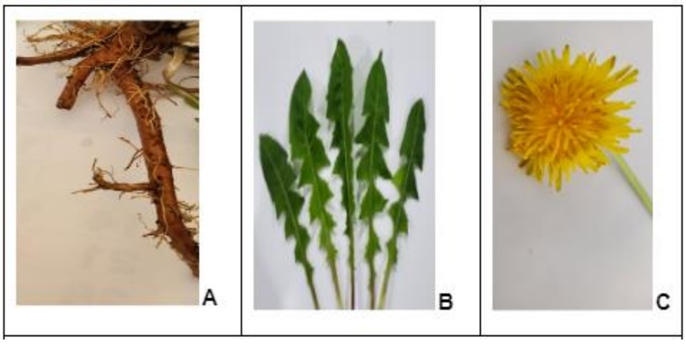

Fig. 3.*Taraxacum officinale*: (A) Radix; (B) Herba; (C) Flower

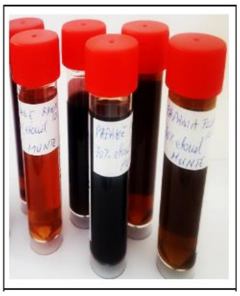

Fig. 4. Radix, Herba and Flower fluid extracts

(original photos, Tănasă (Acreței) M.V.)

Vegetal fluid extracts were obtained using the standard method of cold maceration in the dark for 14 days, followed by normal pressure filtration. For cold maceration, *Taraxacum* powders were mixed with 50% and 70% ethanol in a conical flask – 10 g/100 mL. The mixture was stirred thoroughly with a glass rod. The conical flask was kept with intermittent shaking for 14 days, in darkness. The mixture was filtered at normal pressure through quantitative Whatman filter paper; extractive solutions present various colours, from light-brown to green-brown (Fig.4).

Obtained hydroalcoholic extracts were analysed by UV-Vis spectrophotometry method for determining total carotenoids, lutein and ß-carotene content.

● For determining the concentration of total carotenoids, 1 mL vegetal extract was diluted in 9 mL 80% acetone (triplicate samples for each extract). The resulting extract was filtered at normal pressure through Whatman blue band filter paper and the spectrophotometric absorbance was read, using a S106 WPA UV-Vis spectrophotometer, against an 80% acetone as blank, at 470 nm, 647 nm and 663 nm of wavelengths (Lichtenthaler and Buschmann, 2001). Absorbance values were used to calculate carotenoids concentration, according to the specific trichromatic equations (Lichtenthaler and Buschmann, 2001; Negreanu-Pirjol et al, 2019; Popoviciu et al., 2019; Popoviciu et al., 2020; Popoviciu et al., 2021; Tanasa et al., 2022a).

● For determining the concentration of lutein, the extracts were diluted in petroleum ether and the absorbance reading was performed at 446 nm wavelength (Sujith et al., 2010).

● For determining the concentration of ß-carotene content, 1 mL extract in 9 mL acetone:petroleum ether (2:3), absorbance readings at λ = 453, 505, 645, 663 nm. (Branisa et al., 2014).

3. Results and Discussions

➢ *Total carotenoids content*

The total carotenoid content was higher in the ethanolic extracts of Radix and Flowers of the plants collected from Dobrogea area, especially the values of total carotenoids in Radix were higher than those for Radix of plants collected from the Transylvania area, but also compared to the levels in Flower extracts (Fig. 6). The highest value was recorded for the 50% ethanol extract of Radix - 242.98 mg/kg D.W., while the 70% concentration ethanol extracted only 157.18 mg/kg D.W. from the same plant organ (Fig. 5).

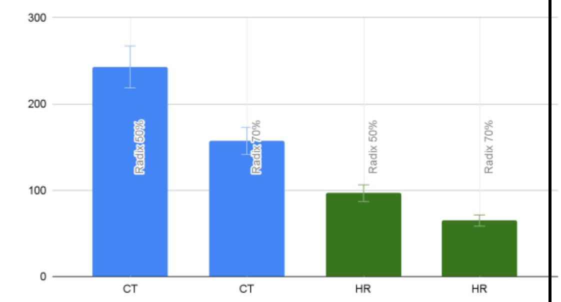

Fig. 5. Total carotenoids in root extracts (mg/kg D.W.)

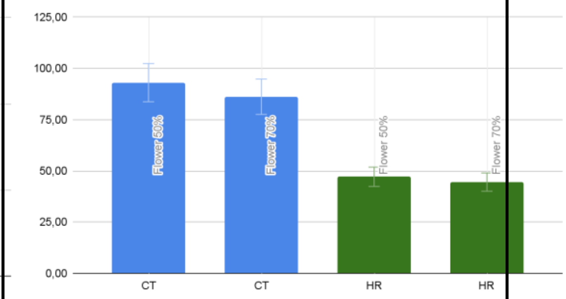

Fig. 6. Total carotenoids in flower extracts (mg/kg D.W.)

In the hydroalcoholic extract of Herba (Fig. 7), the content of total carotenoids was higher in the plant extracts collected from the eastern area of Transylvania - 186 mg/kg D.W. for the leaf extract in 50% ethanol compared to 93.94 mg/kg D.W. for the same plant extract from the Dobrogea area.

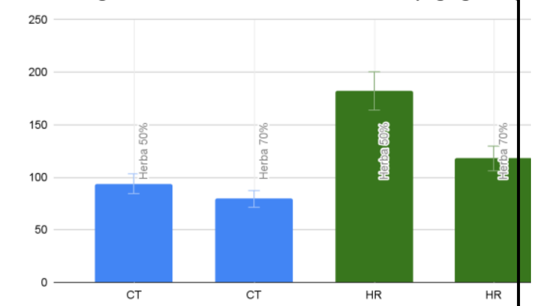

Fig. 7. Total carotenoids in leaf extracts (mg/kg D.W.)

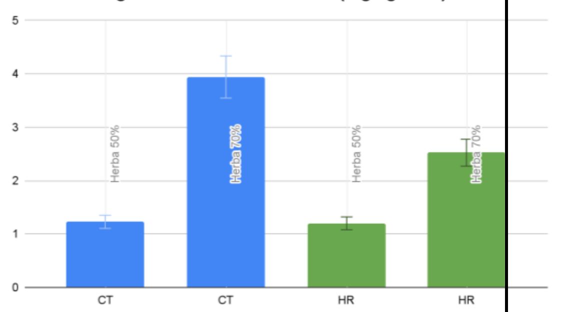

Fig. 8. Lutein in leaf extracts (mg/kg D.W.)

➢*Lutein content*

It was found higher values of the lutein content in the hydroalcoholic extracts of Radix and Flower in 50% and 70% ethanol of the plants collected from the Transylvania area, but for the ethanol extracts of the leaves we recorded slightly increased values in the Dobrogean plants. The highest value of the lutein concentration was recorded for the 70% Herba hydroalcoholic extract (Fig.8), from leaves collected from Dobrogea area - 3.94 mg/kg D.W. compared to 2.52 mg/kg D.W. for the same type of plant extract collected from the area of Transylvania; in the leaves we found a higher content of lutein compared to Flowers (Fig. 9) or Radix extract (Fig. 10).

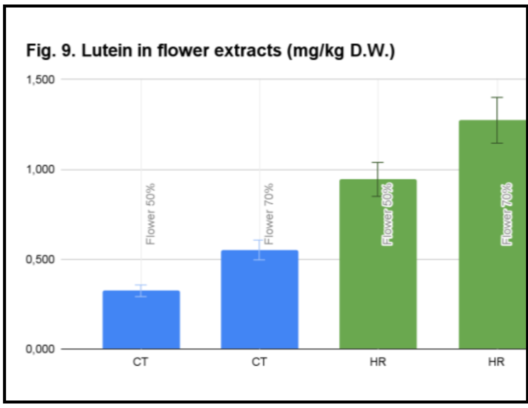

Fig. 9. Lutein in flower extracts (mg/kg D.W.)

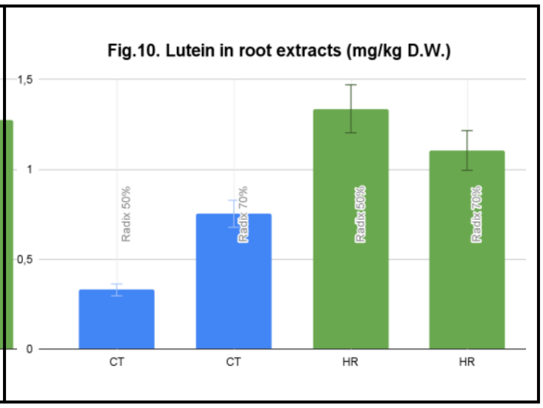

Fig.10. Lutein in root extracts (mg/kg D.W.)

➢*ß-carotene content*

Regarding the content of beta-carotene, higher values were recorded in the extracts of Radix and Herba in ethanol concentration 50% and 70% for the plants collected from the area of Dobrogea: 95.67 mg/kg D.W. for the leaf extract in 70% ethanol vs. 44.02 mg/kg D.W. for the extract from leaves collected from the area of Transylvania (Fig. 11). For the extract from Flowers in 70% ethanol we found a small increase in beta-carotene of the plants collected from the Transylvania area - 18.83 mg/kg s.u. compared to 15.89 mg/kg D.W. for the flowers from Dobrogea area, while for the extract in 50% ethanol the values are very close: 7.20 mg/kg D.W. compared to 7.33 mg/kg D.W. for flowers from the Dobrogea area (Fig. 12).

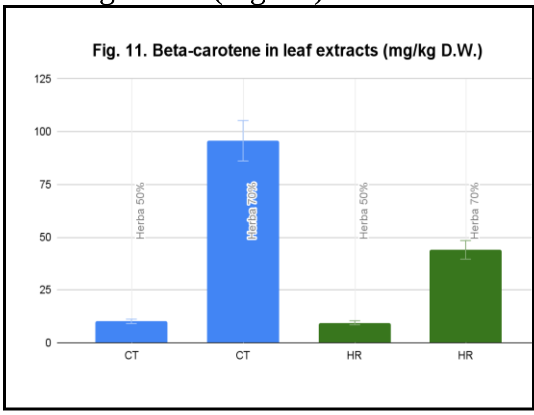

Fig. 11. Beta-carotene in leaf extracts (mg/kg D.W.)

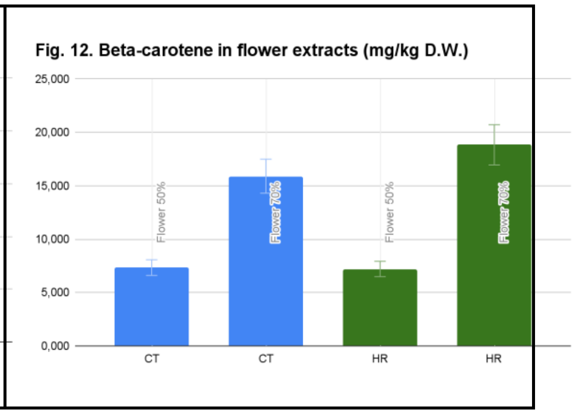

Fig. 12. Beta-carotene in flower extracts (mg/kg D.W.)

Conclusions

The *Taraxacum officinale* (L.) plants collected from Dobrogea area recorded higher values of the bioactive components in the hydroalcoholic extract of Radix: total carotenoids in 50% ethanol extract and ß-carotene in 70% ethanol extract, and in the Herba extracts: lutein and ß-carotene in ethanol 70%.

Taraxacum officinale (L.) plants collected from the Transylvania area recorded higher values in the hydroalcoholic extracts of Radix and Flower 70% ethyl alcohol for lutein.

The extraction of bioactive principles by the cold maceration method proved to be more effective for lutein and ß-carotene in 70% ethanol, and for total carotenoids in 50% ethanol.

The influence of climate and soil on *Taraxacum* plants from Dobrogea and Transylvania determined significantly different values of the concentration of bioactive principles analysed and, following these results, it can state that the potential benefits of natural compounds from *Taraxacum* and their therapeutic activity require further investigation.

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