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QUANTITATIVE ANALYSIS OF ANTIOXIDANT COMPOUND CLASSES IN VEGETATIVE ORGANS OF BITTER GOURD (*MOMORDICA CHARANTIA* L.)

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ABSTRACT

Bitter gourd (Brâncuși cultivar) stems, tendrils and leaves from local production were studied for their content in some classes of nutritive compounds. Chlorophyll, carotenoids (total, β-carotene and lycopene), phenolic compounds (total, flavonoids, anthocyanins, tannins) and ascorbic acid were determined.

Vegetative organs of M. charantia were found to contain high amounts of chlorophyll (1,643 mg/kg DW in leaves). The total carotenoid content was 176-386, with the highest amounts in leaves. While β-carotene was dominant in the stems (177 mg/kg), the other vegetative organs had significant amounts of lycopene (67 mg/kg in leaves).

The total phenolic inventory ranged from 6,283 in leaves to 9,657 in stems. Flavonoids were dominant, by 50-70%, with the highest amounts in stems. Of these, 501-997 mg/kg were anthocyanins. Tannins constituted 8.1-9.3% of the phenolic inventory.

Ascorbic acid concentration ranged between 1,7134 and 2,691 mg/kg, with the highest values in tendrils.

In conclusion, vegetative organs of M. charantia can be a valuable source of phytochemicals.

INTRODUCTION

Momordica charantia L. (bitter gourd, bitter melon, bitter cucumber, balsam pear) is a member of the Cucurbitaceae family, native of Africa, but widely grown in South Asia, and, in more recent times, in the Caibbean and Australia. Although a culture mostly specific to tropical and subtropical regions, its popularity is rising in other areas too. In Romania it was introduced in the 1990's and since then new varieties were developed. While dependent of greenhouse growing, it is now considered as the most profitable crop in our country.

It is a perennial, herbaceous plant, with climbing stems growing up to 5 m. Leaves are up to 5 cm long, with long, pubescent petioles. Spiral tendrils are present to ensure climbing. A monoecious plant, it has solitary yellow flowers, with long peduncles, 10-20 cm long petals. Male flowers outnumber female ones by 20:1. The fruit is a pepo, up to 20 cm long, cylindical, covered with proeminent ridges and warts. Ripe fruits are yellow to orange, with bright yellow pulp and multiple seeds covered by red arils.

It is a plant with known nutraceutical properties, being used as a hypoglycemic, anti-obesity, antibiotic, antimalaria, hepatoprotective, immuno-modulatory food (Șesan 2020).

Figure 1. *Momordica charantia* L.

Plants are known to contain several classes of valuable bioactive compounds.

Chlorophylls (of which chlorophylls a and b are found in land plants) are the main pigments used in photosynthesis. They also have anti-inflammatory activity for consumers, enhance wound healing and inhibit calcium oxalate dihydrate accumulation (kidney stones), while limiting dietary uptake of some known carcinogens. Chlorophylls are among the main antioxidant compounds, preventing oxidative stress-associated diseases (Inanç 2011).

Carotenoids (including carotenes, lutein, lycopene, zeaxanthin) are accessory photosynthetic pigments. Some of them (especially carotene) are precursors of retinol and key to melanin synthesis, thus important for eye and skin functioning. They are also antioxidant and antiproliferative and researches show that lycopene is among the most important dietary anticarcinogens (Eldahshan & Singab 2013).

Phenolic compounds are a variate group of bioactive compounds, functioning as plant pigments, but also as antimicrobials and antifungals. Flavonoids (among them being anthocyanins – plant pigments and strong antioxidants), phenolic acids and tannins belong to this group (Kivrak & Kivrak 2014). For consumers, phenolic compounds are a key class of antioxidant, reducing and radical scavenging agents (Zymonė et al. 2018).

Ascorbic acid (vitamin C) is a key antioxidant, countering lipid oxidation at cell level in all organisms (Riscahyani et al. 2019).

The objective of this paper was to determine the amount of several antioxidant compound classes in vegetative organs of bitter gourd.

MATERIAL AND METHODS

Bitter gourds (Brâncuși cultivar) were grown on the experimental farm of the "Ovidius" University of Constanța. Collected organs were stems (lateral branches), tendrils and leaves. Samples were dried and ground prior to analysis.

Chlorophyll and carotenoid content was determined by 80% acetone extraction and spectrophotometric absorption reading (S106 WPA spectrophotometer) at 470, 647, 663 nm (Popoviciu et al. 2020). Concentrations were calculated according to Lichtenthaler & Buschmann 2001.

Among individual carotenoid compounds, β-carotene and lycopene were determined by extraction in acetone:hexane:petroleum ether and using the spectrophotometric method of Braniša et al. 2014.

The total concentration of phenolic compounds was determined by extracting plant tissue in methanol and reaction with Folin-Ciocâlteu reagent (10%) and sodium bicarbonate (7.5%) for 30 minutes. Absorbance was read at 765 nm against gallic acid calibration curve (Popoviciu et al. 2020). Flavonoids were determined by precipitation with hydrochloric acid and formaldehyde, followed by quantification of non-flavonoid phenolic content by Folin-Ciocâlteu reaction. Tannins were similarly determined after precipitation in gelatin and NaCl solution (de Lima et al. 2011). Anthocyanins were determined by 70% ethanol extraction and spectrophotometry at 520 and 700 nm (Braniša et al. 2014).

For total ascorbic and dehydroascorbic acid, ethanol extraction, reaction with ammonium molybdate and sulfuric acid, followed by spectrophotometric reading at 494 nm were employed (Riscahyani et al. 2019).

RESULTS AND DISCUSSIONS

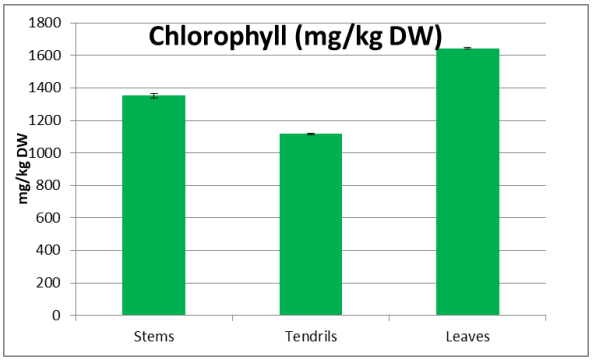

Figure 2. Chlorophyll concentration in bitter gourd vegetative organs (mg/kg DW).

The concentrations of various classes of compounds with bioactive potential are shown in Figures 2-5.

Total chlorophyll concentration was highest in leaves (1,643 mg/kg), followed by stems (1,353 mg/kg) and tendrils (1,117 mg/kg).

Average total carotenoid content was 386 mg/kg in leaves, 275 mg/kg in stems and 176 mg/kg in tendrils. While β-carotene was dominant in the stems (177 mg/kg and 64% of the total amount), the other organs had concentrations below detection limit, while possessing amounts of lycopene (67 mg/kg in leaves; 26 mg/kg in tendrils). While carotenoids in vegetative organs of Cucurbitaceae are

less studied, related *Momordica balsamina* leaves were found to contain over 344 mg/kg DW (Grassino et al 2023), similar to our findings, but with a dominance of β-carotene.

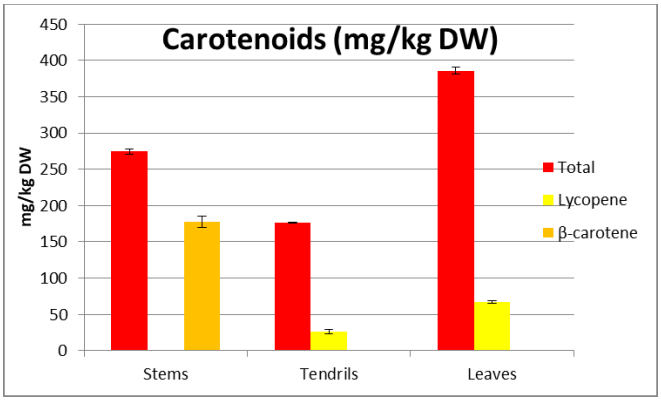

Figure 3. Concentrations of total and some individual carotenoids in bitter gourd vegetative organs (mg/kg DW).

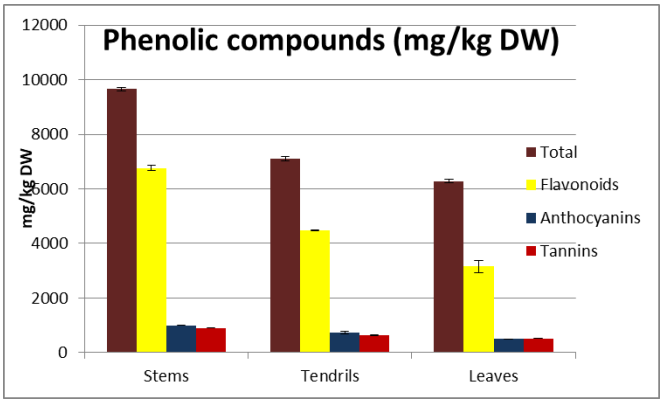

Figure 4. Concentrations of total phenolic compounds, flavonoids, tannins and anthocyanins in bitter gourd fruits (mg/kg DW).

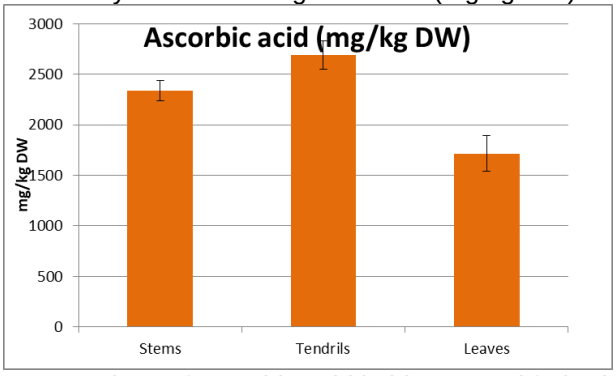

Figure 5. Concentrations of ascorbic acid in bitter gourd fruits (mg/kg DW).

Average total phenolic content was 9,657 mg/kg in stems, 7,107 mg/kg in tendrils and only 6,283 mg/kg in leaves. Of these, the flavonoid fraction was majoritary, ranging from over 50% in leaves to 70% in stems. Among flavonoids, 14-16% were anthocyanins, with the highest amount (997 mg/kg in stems) and the lowest (501 mg/kg) in leaves. Tannins reached also the highest concentration in stems (904 mg/kg) and the lowest (514 mg/kg) in leaves, making up 8.1-9.3% of the total phenolic inventory.

For comparison, amounts of phenolic compounds found in other Cucurbitaceae are below 5,000 mg/kg DW in *Citrullus colocynthis* leaves (Saleem et al. 2014), 5,500 mg/kg – of which 3,940 mg/kg flavonoids – in *Momordica foetida* leaves (Molehin & Adefegha 2014), 18,900-44,100 mg/kg DW (11,500-22,000 mg/kg flavonoids) in various aerial parts of *Echinocystis lobata* (Ielciu et al. 2017), up to 26,500 mg/kg DW in leaves and 5,000 mg/kg in other aerial organs of *Sechium edule* (Vieria et al. 2022).

Ascorbic acid concentration was higher in tendrils (2,691 mg/kg) than in stems (2,336 mg/kg) or leaves (1,713 mg/kg)/ values much higher than those found, for instance in *Cucurbita maxima* fruits (492-842 mg/kg DW; Kulczyński & Gramza-Michałowska 2019).

CONCLUSIONS

Vegetative organs of *M. charantia* were found to contain high amounts of chlorophyll (1,643 mg/kg DW in leaves). The total carotenoid content (176-386, with the highest amounts in leaves) was similar to that found in related species. While β-carotene was dominant in the stems (177 mg/kg), the other vegetative organs had significant amounts of lycopene (67 mg/kg in leaves).

The total phenolic inventory ranged from 6,283 in leaves to 9,657 in stems, an average amount when compared to known related species. Flavonoids were dominant, by 50-70%, with the highest amounts in stems. Of these, 501-997 mg/kg were anthocyanins. Tannins constituted 8.1-9.3% of the phenolic inventory (514-904 mg/kg).

Ascorbic acid concentration ranged between 1,713 and 2,691 mg/kg, with the highest values in tendrils.

In conclusion, vegetative organs of *M. charantia* can be a valuable source of phytochemicals. While leaves are the richest in chlorophylls and carotenoids, stems have the highest amounts of potential antioxidants, like phenolic compounds and ascorbic acid.

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