CHEMICAL RESEARCH OF CAROTENOIDS FROM *CHENOPODIUM BONUS HENRICUS* L. (*CHENOPODIACEAE*)

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Abstract

Chenopodium bonus henricus L. belongs to the *Chenopodiaceae* family and grows spontaneously in Romania. It is used for its wound healing, anti-arthritis and anti-cough properties. The plant also has nutritional value due to its high protein and iron content.

The studies of the carotenoids in *Chenopodium bonus henricus* L., performed by spectral and chromatographic (high pressure liquid chromatography - photodiode array: HPLC-PDA) methods, are mentioned for the first time in this study. The analysis were performed on both saponified and non-saponified extracts. The aerial part of the plant (herba) contains 344.1mg total carotenoids/100g dry weight (51.3mg total carotenoids/100g fresh weight). Five xanthophylls were identified and quantified: neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxantin, and two carotenes: α-carotene and β-carotene. In the non-saponified extract lutein was the major carotenoid (176.54mg/100g dry weight), followed by β-carotene (87.31mg/100g dry weight), then by violaxanthin and neoxanthin. The value of retinol equivalents (RE) calculated was 15320 by taking into account the α-carotene, β-carotene and β-criptoxanthin content. The results demonstrate that *Chenopodium bonus henricus* L. represents a rich source of provitamin A carotenoids and lutein, with high nutritional value. Raw plant or standardized extracts of *Chenopodium bonus henricus* L. can be used as dietary supplements for improving the vitamin A status and for prevention of Age-related Macular Degeneration.

Rezumat

Chenopodium bonus henricus L. (*Chenopodiaceae)*, denumită popular lobodă albă, este o plantă din flora spontană a României, utilizată pentru proprietăţile medicinale (cicatrizante, antiartritice, antitusive) şi alimentare (datorate conţinutului ridicat de proteine, fier). Cercetarea carotenoidelor prezentat pentru prima dată în această lucrare, a fost

efectuată prin metode spectrofotometrice şi cromatografice (*high pressure liquid chromatography - photodiode array:* HPLC-PDA). Analizele au fost efectuate atât pe extracte saponificate, cât şi pe extracte nesaponificate. Părţile aeriene ale plantei (herba) conţin 344,1 mg carotenoide totale/100 g produs vegetal uscat (51,3mg carotenoide totale/100 g produs vegetal proaspăt). Au fost identificate şi cuantificate cinci xantofile: neoxantina, violaxantina, luteina, zeaxantina, β-criptoxantina, şi două hidrocarburi carotenoidice : α- şi β-caroten. În extractul nesaponificat, luteina este principala carotenoidă (176,54mg/100g), urmată de β-caroten (87,31mg/100g), violaxantină şi neoxantină. Valoarea de echivalenţi de retinol (RE) a fost estimată la 15320, ţinându-se cont de conţinutul în α-caroten, β-caroten şi β-criptoxantină. Rezultatele obţinute demonstrează faptul că *Chenopodium bonus henricus* L. reprezintă o sursă bogată de carotenoide cu rol de provitamine A, având o valoare nutriţională ridicată. Produsul vegetal sau extractele standardizate pot fi utilizate ca suplimente alimentare pentru îmbunătăţirea nivelului vitaminei A şi în prevenţia degenerescenţei maculare senile.

Keywords: *Chenopodium*, carotenoids, HPLC-PDA, retinol equivalent, lutein

Introduction

Carotenoids are natural pigments produced by plants and microorganisms. In plants, these are involved in photosynthesis and photoprotection. In animals, besides the role of provitamin A, they act as antioxidants, tumor-preventing agents, photoprotection agents for skin and retina [1,2,10,13]. In consequence, carotenoids are largely used in food industry, cosmetics and medicine. Xanthophylls, mainly lutein and zeaxanthin, are of great importance for the well-functioning of the eye structures. Lutein and zeaxanthin are the only carotenoids present in human plasma, accumulating in human retina. It was demonstrated that a high concentration of these two pigments in plasma is correlated with an increase of macular pigment density and a reduced risk of Age-related Macular Degeneration (AMD). An important property of carotenoids in the retina is the absorption of blue-light by acting as filter pigments and reducing the photo-oxidation processes. Epidemiological and intervention trials demonstrated that common antioxidants (dietary carotenoids, vitamin C, vitamin E and zinc) are associated with a decreased risk of AMD [12,16,17].

Taking into account their beneficial effects on human health, there is an increasing interest for the screening of plant materials, such as leafy green vegetables, regarding their content in carotenes and xanthophylls (mainly lutein and zeaxanthin).

Chenopodium bonus henricus L. belongs to the *Chenopodiaceae* family and its aerial part (*Chenopodii boni henrici herba*) is used for a long time in Romanian folk medicine for its nutritional and medicinal properties [3,5]. The information about the chemical composition of the plant is poor.

The presence in the plant of polyphenolic acids, flavonoids, ecdysteroids, polysaccharides, saponins was mentioned [6,7].

There are no data about the content in carotenoids of *Chenopodium bonus henricus L.* The aim of this study was the separation, identification and quantification of the main carotenoids from *Chenopodii bonii henrici herba*, in order to establish the nutritional and therapeutical value of the plant.

Material and Methods

a. Extraction and saponification

The fresh material (5g) was ground with acetone (50mL) using an Ultraturax homogenizer and extracted, under protection from light until the material became colorless. BHT (butyl hydroxy-toluene), as antioxidant, and $NaHCO_3$ were added in order to avoid the isomerization due to acid pH conditions. The extract was filtered and partially evaporated at 35ºC in a rotary evaporator (Heidolph). The extract was transferred into a separating funnel, diluted with equal volumes of diethyl ether and then a saturated salt (NaCl) solution was added to it. The upper phase was washed for several times and the obtained salty aqueous solutions were re-extracted with diethyl ether in order to avoid losses of pigments. Combined diethyl ether extracts were partially evaporated.

Saponification was carried out in diethyl-ether overnight and under permanent stirr by adding 30% w/v KOH in methanol to a final concentration of 15% w/v KOH. The carotenoids were extracted with diethyl-ether from a saline solution to neutral pH. The total carotenoid extract was evaporated, brought to a fixed volume and used in the quantitative evaluation of carotenoids and for separation by chromatographic methods [1,2,8].

b. Spectrophotometric determination of total carotenoids

The concentration of total carotenoids was calculated using the following relation: $X= (A\times Y\times 1000)/(2500\times 100)$, were: A= absorbance at λmax = 450nm, $A^{1\%}_{1cm}$ = 2500, specific absorbance coefficient of colored carotenoids), X = weight of carotenoids in the sample (mg), Y = volume of the sample, mL [1,2].

c. Separation on open alumina column

The total carotenoid extract was fractionated on open alumina column – Al_2O_3 grade III with 6% water. Three fractions were collected:

Fraction I – 100% petroleum ether; Fraction II – 50% petroleum ether /50% diethyl-ether; Fraction III – 95% diethyl ether/5 % ethanol. Each fraction was collected, evaporated and submitted to HPLC analysis and spectrophotometric quantification.

d. HPLC analysis

The separation was performed in a HPLC system including a Kontron System 322 pump and controller, a Waters 990 Photodiode Array Detector (PDA) and a reversed phase Nucleosil 120-5 C-18 250x4.6 mm column. The mobile phase was a gradient of acetonitrile: water (9:1, v/v) with 0.25 % triethylamine (A) and ethyl acetate with 0.25 % triethylamine (B). The gradient started with 10 % B and increased to 50 % B after 10min. The separation was continued isocratically for up to 25 minutes, and decreased to 10 % B after 30 minutes. The flow rate was 1mL/min and the detection was made at 450nm.

The identification of carotenoids was performed by comparing the retention times of sample compounds with the retention times of available standard compounds and according to the UV-VIS absorption spectra recorded with the PDA detector for not available carotenoids (cis isomers of β,ε-carotene and β,β-carotene). HPLC analysis was performed on both saponified (SE) and non-saponified (NE) extracts (Figure 1). Individual carotenoids were identified according to their retention time and spectral properties. Carotenoid standards (β-carotene, β-cryptoxanthin, lutein and zeaxanthin) were kindly provided by Dr. George Britton. The purity of these standards was estimated by HPLC and was: 95 % - β-carotene, 97.7 % - β-cryptoxanthin, 98.5 % - lutein and 98.8 % - zeaxanthin. Neoxanthin, violaxanthin and β,ε-carotene were from LGC Standards, UK.

The quantitative analysis was performed using calibration curves. The calibration curves for lutein, zeaxanthin, β-cryptoxanthin, β,β-carotene and β,ε-carotene were prepared at 5 concentrations (0-200μg/mL), by plotting the peak area recorded on PDA *versus* the concentration. The linear regression factor of the calibration curves was higher than 0.98. All chemicals were of analytical grade or HPLC grade and provided by Merck KGaA, Darmstadt, Germany.

Results and Discussion

The total content of carotenoids estimated by the spectrophotometric method in saponified extract was 51.3mg/100g fresh material or 344.1mg/100g dry weight (Table I).

Table I
The carotenoid content in fractions, separated on alumina column

Fraction	Carotenoids (mg/100g dry weight)
Fraction I	90.4
Fraction II	10.6
Fraction III	243.1
Total carotenoids	344.1

Fraction I contained carotenes (α-carotene and β-carotene), Fraction II contained β-cryptoxanthin and Fraction III was quantitatively the most important one, composed by lutein, zeaxanthin, neoxanthin and violaxanthin.

We achieved a good separation of carotenoids in herb using the above mentioned HPLC conditions, within 20 minutes. Typical green leaves carotenoids were identified in both extracts: neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, β-carotene and α-carotene [1]. Their retention time and spectral characteristics are presented in Table II and Figure 2. Absorption maxima of identified carotenoids were in good agreement with values reported in literature [1,2].

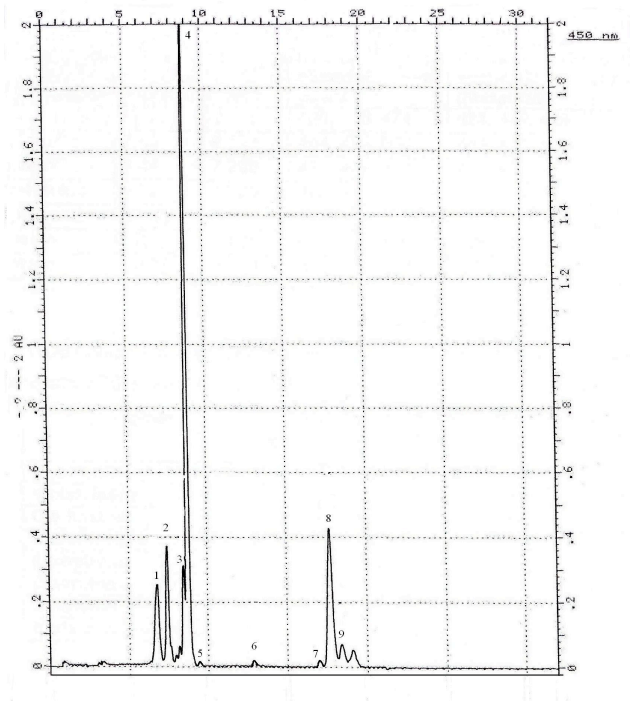

Figure 1
HPLC separation of saponified extract (SE) of *Chenopodium bonus henricus*

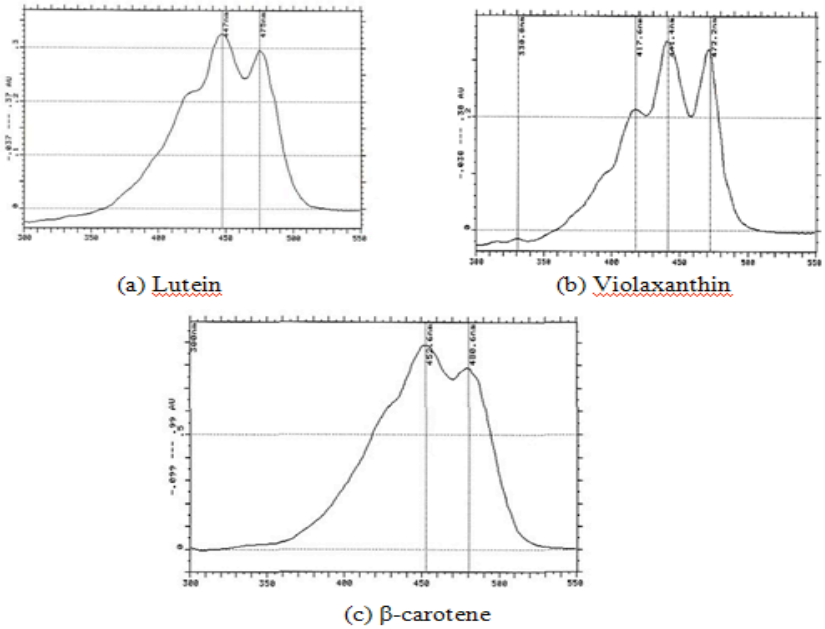

Figure 2
Absorption spectra of main carotenoids in *Chenopodium*

Table II
VIS spectroscopic data for identified carotenoids

Identified compounds	**Retention time (min)**	**Observed absorption maxima**	**Reported absorption maxima***	**Ratio III/II [1,2] ****
Neoxanthin	6.21	416, 438, 467	415,439,467	80 %
Violaxanthin	7.50	418, 441, 472	419, 440, 470	95 %
Lutein	8.97	422, 446, 475	422, 445, 474	60 %
Zeaxanthin	9.46	428, 452, 479	428, 450, 478	26 %
β-cryptoxanthin	13.75	428, 450, 474	428, 450, 478	27 %
β,ε-Carotene	17.03	422, 444, 473	423, 444, 473	55 %
β,β -Carotene	17.68	426, 452, 480	425, 450, 478	25 %

*Reported in references [1,2]
**Spectral fine structure of carotenoids is expressed as the ratio of peak heights III/II as percentage (%III/II).The peak height of the longest-wavelenght absorption band is designated as III and the peak height of the middle absorption band as II.

Quantitative determination of individual carotenoids by HPLC is presented in Table III. There is a good agreement between the quantitative data obtained by spectrophotometric determination of carotenoid fraction and the quantitative data obtained by HPLC using calibration curves for individual carotenoids. However, the presence of *cis*-isomers of lutein and β-carotene can be observed in the saponified extract, probably obtained during the samples preparation.

The literature presents researches on carotenoid composition in 30 green leafy vegetables with nutritional value that revealed a high content of carotenoids in *Chenopodium album* – 449.90mg/100g dry weight. In this study the authors found a very high level of lutein (187.59mg/100g dry weight), violaxanthin (142.59mg/100g dry weight) and β-carotene (114.61mg/100g dry weight). The ratio between xanthophylls and carotenes was 2.92 [14]. Our results revealed a total of 342.39mg carotenoids/100dry weight in non-saponified extract, and a ratio of 2.67 between xanthophylls and carotenes in *Chenopodium bonus henricus*. The amount of individual carotenoids and the ratio between xanthophylls and carotenes is highly variable in plant species and it depends on specific biosynthetic pathways [1,4 ,9].

It is remarkable that *Chenopodium bonus henricus* biosynthesizes high amounts of both carotenes, having provitamin A properties, and xanthophylls (mainly lutein), important for the prevention on Age-Related Macular Degeneration (Table IV). The retinol equivalents (RE) were calculated according to *in vivo* conversion factors established by the World Health Organisation (WHO) [18].

The best known sources of carotenoids among green leafy plants are spinach, kale, lettuce, parsley. A recent study revealed that raw spinach contains 6.60 mg lutein/100 g, cooked spinach – 12.64mg/100g and cooked kale – 8.85mg/100g [13]. If calculated to the wet material, *Chenopodium bonus henricus* contains more than 20mg lutein/100g, similar to values reported for *Chenopodium album* [11,14], and significantly higher than in commonly used vegetables like spinach.

Table III

The carotenoid composition of *Chenopodii boni henrici herba*

Nr	Compound	Retention time (min)	mg/100 g dry weight (SE)	% of total carotenoids (SE)	mg/100 g dry weight (NE)	% of total carotenoids (NE)
1	neoxanthin	6.21	30.41	9.03	29.80	8.64
2	violaxanthin	7.50	36.14	10.71	35.87	10.40
3	*cis*-lutein	8.53	23.62	7.01	-	-
4	*trans*-lutein	8.97	150.96	44.73	176.54	51.19
5	zeaxanthin	9.46	3.44	1.02	3.60	1.04
5	β-cryptoxanthin	13.75	3.07	0.91	3.24	0.94
6	α-carotene	17.03	4.52	1.34	6.03	1.74
6	*all trans* β-carotene	17.68	70.37	20.85	87.31	25.32
7	*cis* β-carotene	18.42	14.01	4.15	-	-
			336.54		**342.39**	

where: SE- saponified extracts; NE – non-saponified extracts

Table IV

Nutritional value of carotenoids in *Chenopodium* non-saponified extract

Parameter	
Total Provitamin A carotenoids (mg/100g dry weight)	96.58
RE - Retinol equivalents*	15320
Total anti-AMD xanthophylls (Lutein + Zeaxanthin) (mg/100g dry weight)	180.14

*1 RE = 6 μg β-carotene,12 μg α-carotene and 12 μg β-cryptoxanthin

Conclusions

Chenopodium bonus henricus L. is a rich source of carotenoids, containing 344.1mg total carotenoids/100g dry weight (51.3mg carotenoid/100g fresh weight). The HPLC analysis performed on saponified and non-saponified extracts revealed a typical green leaves composition in carotenoids, with an important fraction of xanthophylls (neoxanthin, violaxanthin, lutein, zeaxanthin and β-cryptoxantin) and two carotenes: α-carotene and β-carotene.

Lutein was found to be the major carotenoid, estimated at 176.54mg/100 g dry weight, followed by β-carotene with 87.31 mg/100 dry weight. The results demonstrate that *Chenopodium bonus henricus* L. has a

high nutritional and therapeutical value, as revealed by the high retinol equivalents (RE) value – 15320, and by the high lutein content. Our finding is important since *Chenopodium bonus henricus* is an edible, but also a medicinal plant, available in the spontaneous Romanian flora. Raw plant or standardized extracts of *Chenopodium bonus henricus* can be used as dietary supplements for improving of vitamin A and as a source of anti-AMD xanthophylls status.

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