

Obtaining and comparative analysis of some carotenoidic extracts from marigold (*Calendula officinalis* L.) flowers and celandine (*Chelidonium majus* L.) flowers

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Abstract

This paper presents obtaining and comparative characterization of two carotenoidic extracts: one from marigold (*Calendula officinalis* L.) flowers and the second, from celandine (*Chelidonium majus* L.) flowers. The extracts were obtained from plant material using a solvent mixture of petroleum ether: ethanol 96% (8:2, v:v) and then purified by removing sterols and saponification. Total carotenoids and β -carotene concentrations were determined by RP-HPLC. Noted a very high content of total carotenoids in marigold flowers (1667.42 $\mu\text{g/g}$), the amount of β -carotene being of 145.40 $\mu\text{g/g}$. In celandine flowers found an amount of total carotenoids of 1377.70 $\mu\text{g/g}$ and 369.50 $\mu\text{g/g}$ of β -carotene. It also has been determined the concentration of various minerals (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni), both in raw materials and in carotenoidic extracts by atomic absorption spectrometry. Potassium and calcium were found in greater quantity in celandine flowers (4420 ppm, 4402 ppm) than in marigold (4025 ppm, 2601.3 ppm), while the marigolds are richer in sodium, magnesium and iron. Macroelements from the carotenoidic extracts were found in much lower concentration than in the raw material, due to their elimination, in large quantities, during the extraction process.

Keywords: carotenoidic extract, β - carotene, RP-HPLC, minerals, marigold flowers, celandine, AAS.

1. Introduction

Today it attaches an increasingly greater importance to the medicinal plants, appreciating that they are inexhaustible sources of raw materials for the preparation of medicines and industrial extraction of active principles used in food, cosmetics, pharmaceutical.

Calendula (*Calendula officinalis* L.), family Compositae, annual herb, grows wild from the plains to the mountains, but it is often grown in gardens, parks, the systematic culture for industrial and medical uses. In the chemical composition of the marigold flowers stand în compoziția chimică a florilor de gălbenele se disting saponosides triterpenoid based on glucuronyl oleanolic acid derivatives, carotenoids (482- 2760 $\mu\text{g/g}$) [1,2] (especially lycopene, α and β -carotene, luteine),

flavonoids and flavonoid glycosides: izoramnetin 3 ramno glycosides, retinoids and quercetol derivatives; essential oil (about 0,02%), bitter substances with undefined structure, mucilage, cholesterol esters of lauric, myristic, palmitic and margaric acids; vitamin C, malic acid, protein substances etc [2,3]. Marigold is chiefly used as a local remedy. Its action is stimulant and diaphoretic. Given internally, it assists local action and prevents suppuration. The infusion of 1 ounce to a pint of boiling water is given internally, in doses of a tablespoonful, and externally as a local application. It is useful in chronic ulcer, varicose veins, etc.

Was considered formerly to have much value as an aperient and detergent in visceral obstructions and jaundice [2-7].

Celandine (*Chelidonium majus* L.), *Papaveraceae* family, is a herbaceous plant, perennial, hemicriptophytes, toxic, spontaneous, encountered by shady, by woods, bushes, gardens, in addition to fences, walls, ruins, around settlements, from the plains to the mountains at 800-1000 m. Is recently introduced into culture [3]. All parts of the plant contain a milky juice (latex) yellow, which, in contact with air, darkens. Chemical composition: alkaloids (chelidonine, homeochelidonine, oxychelidonine, mezoxy-chelidonine, cheleritrine, sanguinarine, coptisine tetrahydro coptisine, protopine, alocriptopine, berberine, sparteine), vitamin C, carotenoids (in the flowers between 1100-1400 µg/g)- especially lutein, lutein-5,6-epoxide, violaxanthin, β-carotene, resins, essential oil, flavonoids, minerals, saponozids. The maximum amount of alkaloids is in rhizomes and roots [3,4,8].

The whole plant is toxic as it contains a range of isoquinoline alkaloids, but there are numerous therapeutic uses when applied at the correct dosage. The effect of the fresh herb is of a mild analgesic, chola-gogic, antimicrobial and central nervous system sedative. Externally, the otherwise purgative, caustic, orange sap is an ancient remedy for warts, corns and ringworm [9].

The purpose of this paper is to analyze and compare two carotenoidic extracts: from marigold and celandine flowers, in terms of the content of β-carotene, total carotenoids and minerals.

2. Materials and methods

It was used raw materials (marigold, respectively celandine flowers) from Didactic Station of the University of Agricultural Sciences and Veterinary Medicine of Banat. Extracts were obtained from dried raw materials.

2.1. Carotenoidic extracts obtaining. Carotenoid pigments were extracted from finely pulverized plant material with a solvent mixture consisting of petroleum ether:acetone (8:2, v/v), in an extraction flask. The extraction was repeated several times with a new solvent mixture until it remained colorless. The combined carotenoidic extracts were then subjected to concentration in vacuo, at 35 °C, in a rotary evaporator (model VR-05, basic 1-B, Shimadzu Japan) to a small volume (15-20 ml). The primary extract obtained was treated with 40 ml of petroleum ether and allowed to stand overnight (16 hours) at a temperature of -10 °C, for the removal of sterols [1]. The precipitated

sterols were removed by centrifugation for 10 minutes at 2000 rpm (centrifuge Universal 32 R model, Hettich, Germany). The supernatant was then concentrated in vacuo on the rotary evaporator at 35 °C to a small volume (10-15 ml). The extract remaining after the removal of sterols was further subjected to saponification in order to remove lipids and esters by treatment with 40 ml 20% alcoholic solution of potassium hydroxide and leaving overnight (16 hours) at room temperature under an atmosphere of nitrogen and in the dark [10-12]. Carotenoids were then back-extracted with petroleum ether in a 500 ml separatory funnel, washed several times with a saturated sodium chloride solution and then with distilled water until complete removal of the alkali and soap. The combined ether extracts were passed over anhydrous sodium sulfate to remove traces of water and then concentrated in vacuo at 35 °C in a rotary evaporator until complete removal of the solvent. Carotenoids obtained was re-dissolved in a volume of acetonitrile and stored in brown bottle at -20 °C under an atmosphere of nitrogen, to be then subjected to testing. For each type of raw material were made three parallel samples, starting from the same quantity of raw material and working under the same conditions

2.2. RP-HPLC analysis. In order to determine the concentration of β-carotene and total carotenoids from the samples, all carotenoidic extracts obtained were analyzed by reverse phase- high performance liquid chromatography (RP-HPLC). For this we used a standard Agilent 1100 HPLC chromatograph (Agilent, USA) with a Zorbax SB-C18 column with dimensions: 250 x 4.6 mm, particle diameter 5 µm. As eluent used a mixture of acetonitrile: methanol (20:80), both reagents from Merck & Co.. Inc., New Jersey. We worked at an eluent flow rate of 1 ml/minute, column temperature of 30° C and a wavelength of 450 nm. Samples were injected by 20 µl, and for determining the concentration of β-carotene of the samples was used a calibration curve (Figure 1.) obtained with standard β-carotene of purity > 97%, from Sigma Chemical Company.

2.3. Determination of mineral elements in raw materials and extracts by atomic absorption spectrometry

Samples mineralization. In porcelain capsules, previously brought at constant weight by drying at 105 °C, was weighing 1,000g raw material, respectively 0,200g carotenoidic extract both from marigold and celandine dried flowers. The capsules

with samples were introduced into drying stove at 50-60 °C for almost 8 hours. Then the temperature was increased at 105 °C for 5-6 hours. After this time, the sample capsules were took out from drying stove and introduced into calcinations oven, at cold. It was increased progressively the temperature at 200-250 °C and it was maintained to complete calcinations of the samples [13]. Then the temperature was increased to 500 °C and the samples were calcined for 6-8 hours to a white ash was obtained. The samples that were incompletely calcined were treated with 1ml concentrated nitric acid, drying on the sand bath and then calcined at 500°C for others 2 hours. After cooling, the ash was treated with 0,5 ml bidistilled water and 1 ml hydrochloric acid 6N and was evaporated to dry, on the sand bath; the operation was repeated for two times. The residue was dissolved in small portions of 5 ml HCl 0,5N, passed quantitatively into a 50 ml glass balloon and completed to exactly 50 ml with HCl 0,5N solution. The balloon content was finally filtered in a perfectly dry flask. For each sample set was achieved a control sample [13].

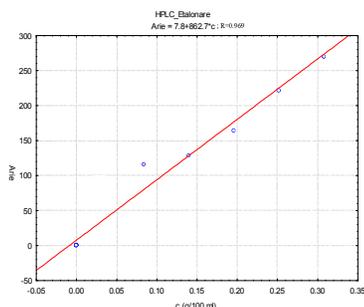


Figure 1. Calibration curve for standard β - carotene

Minerals determination by atomic absorption spectrometry. The minerals (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni) was measured from the obtained hydrochloric solution by pulverization in the air-acetylene flame and measurement of the absorbance (for Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni), respectively emission (for K and Na) at the characteristic wavelength for each analyzed element. It was used an atomic absorption spectrophotometer Varian Spectra AA 110, with four lamps, controlled by PC. For the spectrophotometer calibration were prepared sets of etalons of different concentrations in HCl 0,5N solution for each analyzed element, starting to the concentrated standard solutions.

The concentration (C) for each determined element was calculated with the following formula:

$$C \text{ (mg/kg or ppm)} = a \times f / m,$$

where:

f- dilution factor; a- element content indicated by apparatus (mg/l); m- sample initial weight.

3.Results and discussions

3.1. Carotenoids concentration.By the RP HPLC analysis of the carotenoid extract of dried marigold and celandine flowers were obtained the results shown in Figures 2. -3.

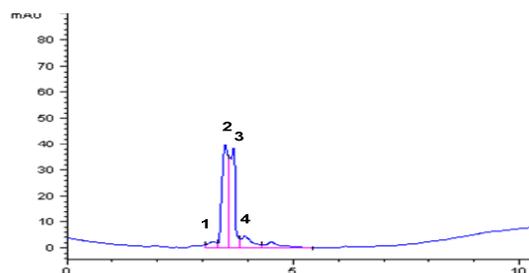


Figure 2. RP_HPLC chromatogram of carotenoid extract from marigold flowers

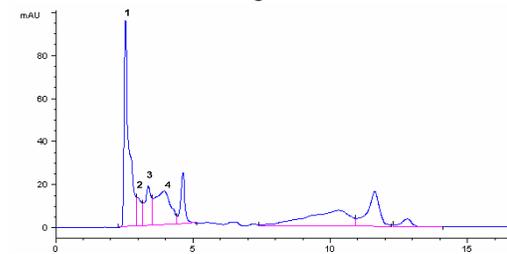


Figure 3. RP_HPLC chromatogram of carotenoid extract from celandine flowers

It is noted that marigold flowers have a total content of carotenoids (1667.42 $\mu\text{g/g}$) higher than celandine flowers (1377.70 $\mu\text{g/g}$), but β -carotene is in lower concentration (145.40 $\mu\text{g} / \text{g}$) than in celandine flowers (369.5 $\mu\text{g} / \text{g}$). The values are within the range found in the literature data [1,2,8].

3.2. Minerals content. The results concerning the minerals content of the two raw materials and of their carotenoidic extracts are showed in tables 1 and 2 (figure 5. and 6.)

Calendula flowers have a high content of macroelements essential for the body: K-4023.00 ppm, Mg-2992.00, 2795.00 ppm, Na, Ca-2613.30 ppm and iron -1050.40 ppm. The amounts of minerals in the carotenoidic extracts are much lower than in raw material, they largely eliminating in the process of extraction.

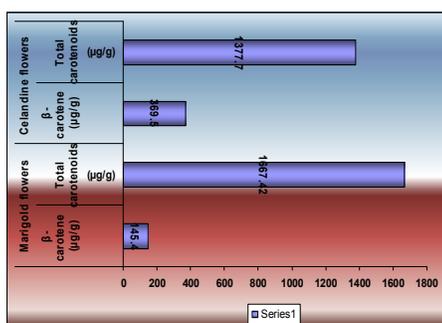


Figure 4. Carotenoids content in dried marigold and celandine flowers

Table 1. Minerals content (ppm) in marigold flowers and their carotenoidic extract

Sample Minerals	Marigold flowers	Marigold flowers extract
K	4023.00	1732.00
Na	2795.00	82,30
Ca	2601,30	211,40
Mg	2992.00	31,14
Fe	1050,40	87,50
Mn	0,339	0
Cu	0,282	0,068
Zn	2,61	1,715
Pb	0,08	0
Co	0,007	0
Cr	0	0
Ni	0,069	0,006

Table 2. Minerals content (ppm) in celandine flowers and their carotenoidic extract

Sample Minerals	Celandine flowers	Celandine flowers extract
K	4420.00	998.00
Na	342,50	25,20
Ca	4402.00	90,11
Mg	443,26	0
Fe	354.00	61,18
Mn	0,065	0
Cu	0,11	0,0136
Zn	2,089	0,413
Pb	0,20	0,047
Co	0	0
Cr	0,132	0,021
Ni	0,212	0,027

In the marigold flowers have been identified manganese (0.339 ppm) and cobalt (0.007 ppm), but in the carotenoidic extract these metals can not be found. Also, chromium is not found either in the raw material or in the extract.

The concentration of heavy metals in marigold flowers is below the statutory maximum.

And in the case of celandine flowers can be observed that the carotenoidic extract is much lower in minerals than the raw material.

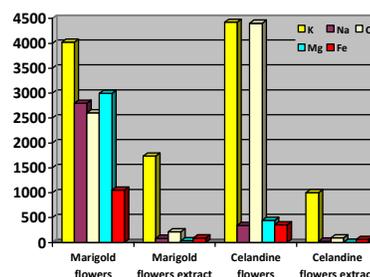


Figure 5. Macroelements (ppm) in dried marigold and celandine flowers and their carotenoidic extracts

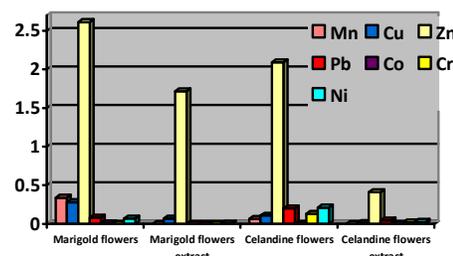


Figure 6. Heavy metals (ppm) in dried marigold and celandine flowers and their carotenoidic extracts

The most widespread both in celandine flowers and the extract is also potassium (4420 ppm in the raw material, ie 998 ppm in extract), followed by calcium (4402 ppm in the raw material, ie 90.11 ppm in extract), these elements being better represented in the celandine flowers than in marigold flowers. It appears that magnesium, although well represented in the raw material (443.26 ppm), does not appear at all in the carotenoidic extract; it is completely removed during the extraction process. The iron is found in appreciable quantities in celandine flowers (354 ppm), and the carotenoidic extract (61.18 ppm), but less than in marigold flowers. Regarding heavy metals in the raw material was not identified cobalt, and in the extract: magnesium, manganese and cobalt. All other heavy metals are below the maximum allowed by law [14], both in the raw material and the extract.

4. Conclusions

Results of this research lead to the following conclusions:

- Marigold flowers are somewhat high in total carotenoids than celandine flowers, while celandine flowers have a higher content of beta carotene.
- All macroelements of raw materials are in concentrations that fall within the limits of literature. The macroelements in the carotenoidic

- extracts are found in much lower concentrations than in the raw materials, they largely expunged during the extraction process.
- Potassium is the mineral element best represented in all samples, both in raw materials and in extracts.
 - The highest concentration of potassium in the analyzed raw materials was found in the flowers of celandine (4420.00 ppm), while sodium is in greater quantity in marigold flowers (2795 ppm). The highest concentration of calcium is celandine flowers (4402 ppm), magnesium is found more in marigold flowers (2992.00 ppm) and also iron (1050.40 ppm).
 - In all samples analyzed heavy metals are found below the maximum allowed by law.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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