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Spectral Analysis Used in the Determination of β-Carotene from C*alendula Officinalis*

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Abstract

β-Carotene is part of the class of the carotenoidic pigments that have a special function in the pigmentation of flowers and fruits. These pigments are essential in photosynthesis where they are retaining energy and photoprotectors by forming redox system etc.

In the present paper the methods of extraction, separation and identification of β-carotene from leaves and stalk of Calendula officinalis are presented, there will also be determined the quantity of β-carotene through spectral analysis.

Keywords: β- carotene, Calendula officinalis, spectral determination

Introduction

β-Carotene is part of the class of the carotenoidic pigments that have an important role in the formation of photosensitive pigments involved in the process of seeing, in the growth and development of organisms, in the protection and recovery of epithelial tissue etc. In plants, carotenoids are essential in photosynthesis where they are retaining energy and photoprotectors by forming redox system etc. Carotenoids have a role in the pigmentation of flowers and fruits. Next to chlorophyll, carotenoids are the major components of the complex pigment-protein from the thylacoide membrane, and some of them, such as β-carotene, were for a long time considered to be protecting the membrane against destructive events caused by the over excitement of the chlorophyll.[1]

The impetuous development of the techniques of isolation and identification of natural components resulted in a serious growth of the number of carotenoid pigments. For their isolation from the mixtures of natural products, their physical and chemical features are to be considered.[2]

In the present paper the methods of extraction, separation and identification of β-carotene from leaves and stalk of Calendula officinalis are presented, there will also be determined the quantity of β-carotene through spectral analysis.

Materials and Methods

The following raw materials and instrument were used:

- fresh leaves and stalk of *Calendula officinalis* (marigold);
- ethylic ether (*Sigma*);
- KOH (*Aldrich*);
- silicagel tip G;
- benzene (*Sigma*);
- ethylic alcohol (*Chimopar*);
- Spectrophotometer type *Varian*.

1 g fresh leaves were mixed with quartz sand, after which the mixture was put in a plastic tube of a 100 ml and 10 ml of cold ethylic ether (5^0C) were added. The heterogeneous mixture was put under ultrasounds for 10 minutes, then was put under centrifugal motion at 2000 rot/min. The supernatant was separated and put it into a phial of 20 ml over being put 2 ml KOH 15% for the saponification of and removal of the lipids and chlorophyll's. The precipitate was subjected to extraction with ethylic ether, the operation being repeated twice, using the same procedure as the one of saponification. The three phials containing extracts were left at -20^0C for 24 h.

The same procedure was applied to the separation of β-carotene from stalk.

Following the saponification with KOH 15%, 2 phases was separated, the organic phase containing β-carotene.

The identification of β-carotene from the extract obtained was realized through thin layer chromatography (TLC) using as stationary phase silicagel G and as mobile phase the solvents system benzene: ethylic alcohol (1:2, v/v), and as martor pure β-carotene from Biochemika. All chromatographic operations were performed very quickly and in the dark because β-carotene can be distructed upon exposion to light. The identification of spots was achieved in 2 ways:

- visual, β-carotene being as 2 yellow spots, this way being used for further prelucration, it means taking off a colored spots from support and the elution with ethylic ether, and after that filtration. [3] An absorption spectrum for β-carotene on Varian spectrophotometer was achieved;
- the iodine vaporous, this procedure being used in the detection of some impurities from extract. This procedure can't be used further because iodine reacts to double bonds of β-carotene forming poliiodine compounds of β-carotene.

It was spotted extract of β-carotene from *Calendula officinalis* and solution of pure β-carotene on 2 chromatographic plates. The identification was carried out through the two methods described above.

Finally one spot for our extract was observed, corresponding to pure β-carotene, the conclusion being that our extracts haven't got impurities. The elution was achieved with ethanol 5-10%.

For the determination of chemical concentration [5] of extract of *Calendula officinalis*, was used as well as solutions with known concentrations: 50 μg/mL, 25 μg/mL, 12,5 μg/mL, 6,25 μg/mL, 3,125 μg/mL si 1,5625 μg/mL a standard solution of β-carotene in ethylic ether (100 μg/mL),. The concentrations of β-carotene from leaves and stalk of marigold were determined with the aid of the Varian spectrophotometer. [6]

Results and Discutions

The absorption spectra for pure β-carotene and the three extracts was determined. The spectra of extracts have 3 peaks in visible domain. In the following figures absorption spectra are presented. [7]

The extinction values of β-carotene solutions, as well as the extinction values of extracts of *Calendula officinalis* are presented in **Table 1**. The β-carotene concentrations from leaves and stalk of marigold were determined by extrapolation.

Table 1. The extinction values of β-carotene solutions from leaves and stalk of *Calendula officinalis*

Standard solution of β-carotene in ethylic ether		**Leaves and stalk extracts in ethylic ether**		
Concentration (μg/ml)	**Extinction (λ=450 nm)**	**Extract**	**Extinction (λ=450 nm)**	**Concentration chlorophyll + β-carotene (μg/ml)**
0.78125	0.17628	E_1 (leaf)	1.14113	5,07721
1.5625	0.34421	E_2 (leaf)	0.75067	3,33994
3.125	0.6793	E_3 (leaf)	0.2719	1,20976
6.25	1.37547	E_1 (stalk)	0.68489	3,04727
12.5	2.81013	E_2 (stalk)	0.21467	0,955528
25	3.93893	E_3 (stalk)	0.10027	0.446129
50	4.03482			

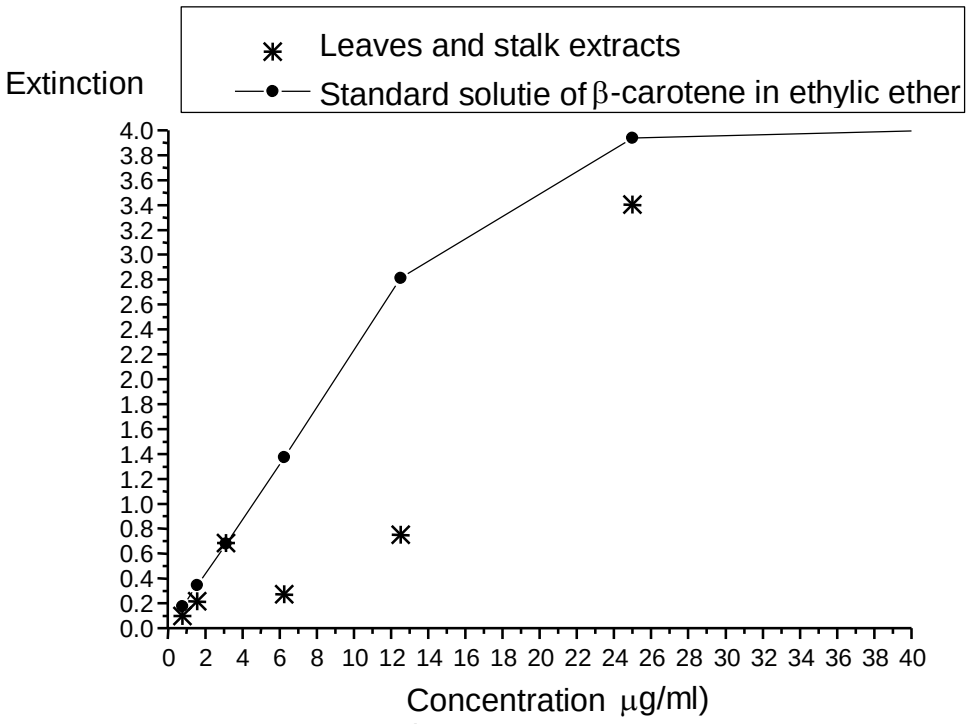

Figure 1. Etalonation curve of β-carotene in ethylic ether

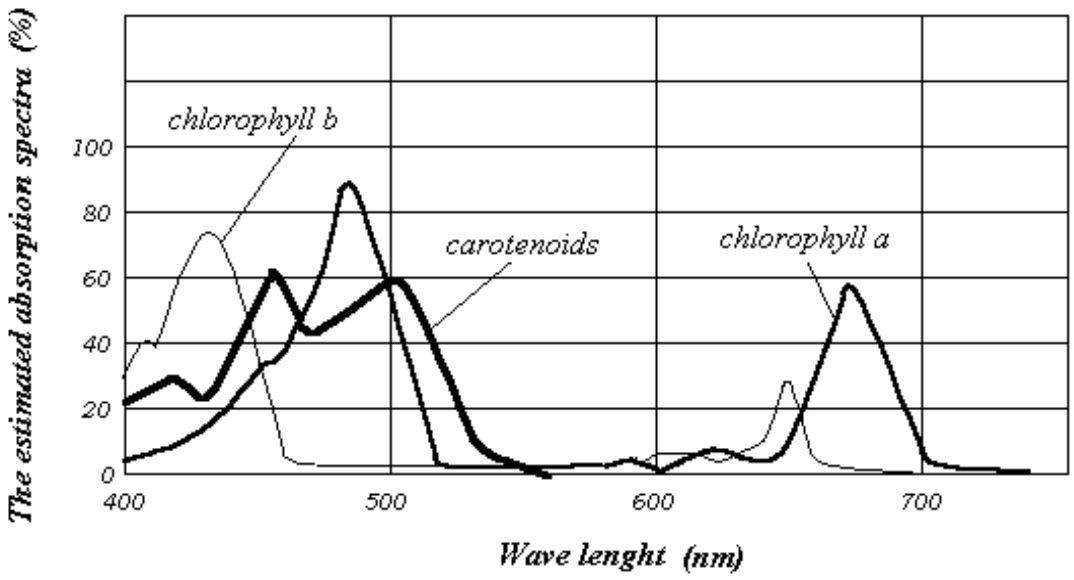

Figure 2. The absorption spectrum of mixture of carotenoids and chlorophyll

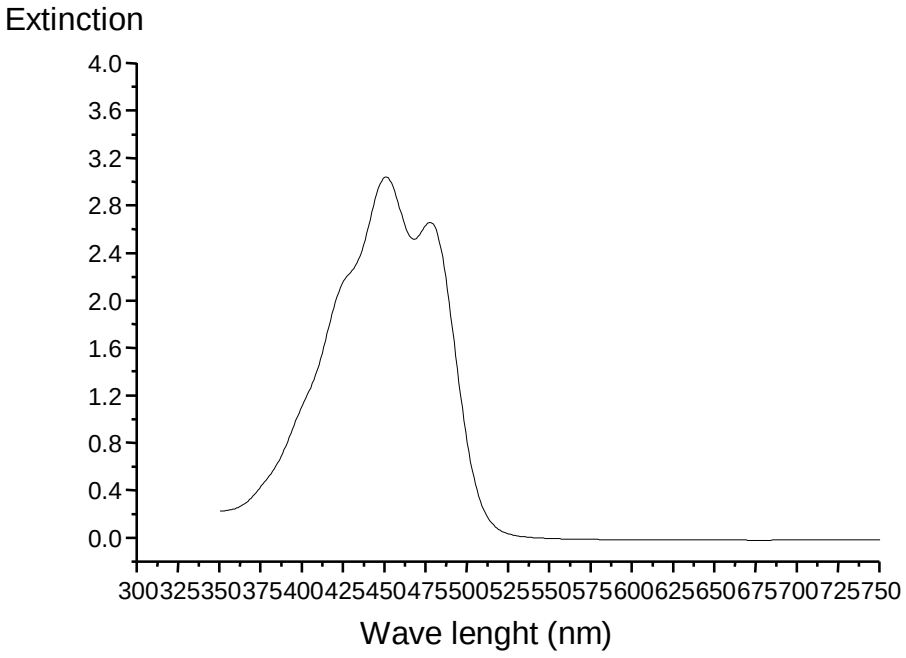

Figure 3. The absorption spectrum of β-carotene in ethylic ether (12,5 μg/ml)

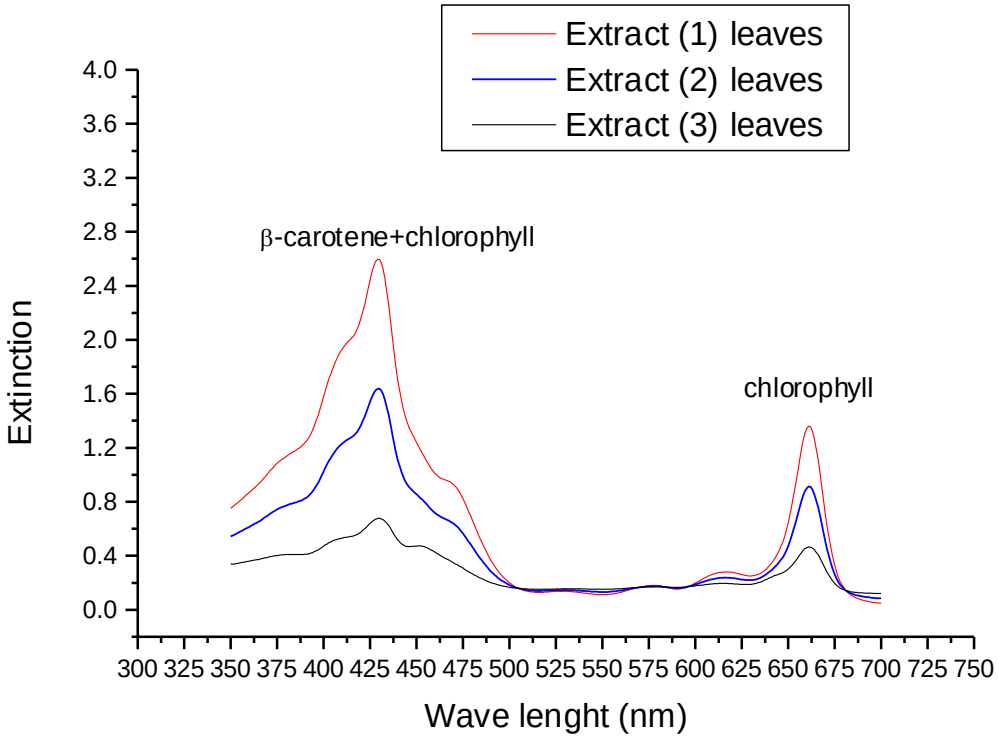

Figure 4. The absorption spectra of leaves extracts of *Calendula officinalis*

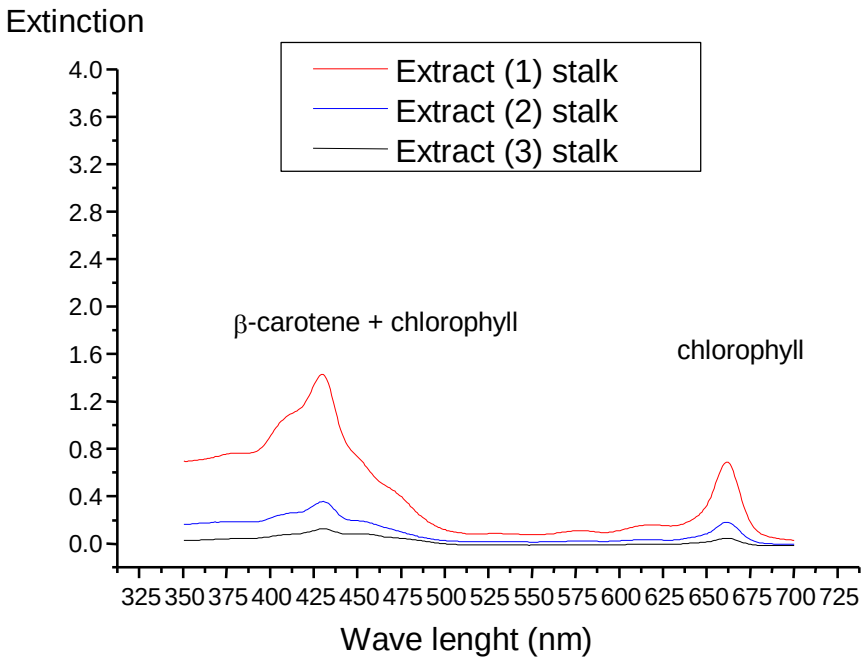

Figure 5. The absorption spectra of stalk extracts of *Calendula officinalis*

Quantitative determination of β-carotene through spectral analysis was done by analysis of mixture of carotenoids and chlorophyll spectrum [8]. In **Figure 2** this spectrum is shown. It was determined that the ratio between chlorophyll peaks on 6 different wave lengths, the ratio being the same for the 6 extracts. It was determined the unknown extinction for chlorophyll a, that absorbs in the same domain like β-carotene (350-500 nm).

Differences between total extinction of the 6 extracts and extinction of chlorophyll lead to extinction of β-carotene, that permits determination of concentrations of β-carotene. The medium error was determined with the following relation:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

where: x_i – variable;

$\bar{x}$ – medium value: $\frac{\sum_{i=1}^{6} x_i}{n}$;

n – number of determinations.

Table 2. The concentration values of β-carotene determined through spectral analysis (leaves extract)

Wave lenght (nm)	Total extinction	Extinction of chlorophyll	Extinction of β-carotene (E_{total}-$E_{chlorophyll}$)	Concentration of β-carotene (μg/ml)	Concentration of β-caroten medium (μg/ml)
Extract 1 (leaf)					
400	1.578	0.817	0.761	8.48	**5.26**±2.91
412	1.968	1.021	0.947	7.58	
424	2.382	1.089	1.293	7.53	
436	2.138	1.716	0.422	2.17	
448	1.292	0.953	0.339	2.17	
460	1.009	0.204	0.805	3.68	
Extract 2 (leaf)					
400	1.052	0.548	0.504	5.63	**3.36**±2.05
412	1.252	0.685	0.567	5.26	
424	1.504	0.730	0.774	4.50	
436	1.370	1.151	0.219	1.13	
448	0.881	0.639	0.242	1.00	
460	0.717	0.137	0.580	2.65	
Extract 3 (leaf)					
400	0.471	0.279	0.192	2.14	**1.23**±0.75
412	0.536	0.349	0.187	1.50	
424	0.626	0.372	0.254	1.48	
436	0.602	0.586	0.016	0.08	
448	0.470	0.325	0.145	0.59	
460	0.425	0.069	0.356	1.62	

Table 3. The concentration values of β-carotene determined through spectral analysis (stalk extract)

Wave lenght (nm)	Total extinction	Extinction of chlorophyll	Extinction of β-carotene (E_{total}-$E_{chlorophyll}$)	Concentration of β-carotene (μg/ml)	Concentration of β-caroten medium (μg/ml)
Extract 1 (stalk)					
400	0.917	0.415	0.502	5.6	**3.27**±1.85
412	1.103	0.519	0.584	4.67	
424	1.32	0.554	0.766	4.46	
436	1.193	0.872	0.321	1.65	
448	0.764	0.484	0.28	1.15	
460	0.561	0.103	0.458	2.09	
Extract 2 (stalk)					
400	0.222	0.11	0.112	1.25	**0.75**±0.39
412	0.263	0.138	0.125	1.002	
424	0.323	0.147	0.176	1.02	
436	0.301	0.232	0.069	0.35	
448	0.201	0.129	0.072	0.29	
460	0.165	0.02	0.145	0.63	
Extract 3 (stalk)					
400	0.066	0.03	0.036	0.4	**0.32**±0.06
412	0.085	0.038	0.047	0.37	
424	0.114	0.04	0.074	0.43	
436	0.111	0.064	0.047	0.24	
448	0.088	0.035	0.052	0.21	
460	0.076	0.007	0.069	0.31	

The average of β-carotene from tissue was:

$$\mu g\ \beta\text{-carotene/g tissue} = c_1 \times V_1 + c_2 \times V_2 + c_c \times V_3$$

where: c_1 – concentration of extract 1 (μg/mL)
V_1 – volume of extract 1 (ml)
c_2 – concentration of extract 2 (μg/mL)
V_2 – volume of extract 2 (ml)
c_3 – concentration of extract 3 (μg/mL)
V_3 – volume of extract 3 (ml)

μg β-carotene/g tissue (leaves) = 98.5
μg β-carotene/g tissue (stalk) = 43.4

Conclusions

The extraction, separation and physico-chemical characterization of β-carotene from leaves and stalk of *Calendula officinalis* were done, using ultrasonication and centrifugation operations for extraction and separation, and for identification – thin layer chromatography, using silicagel G and solvents system benzene: ethylic alcohol (2:1, v/v).

The average of β-carotene in different parts of the plant was determined through spectral analysis method, using absorption spectra of pure β-carotene in ethylic ether and of mixture of carotenoids and chlorophyll.

The concentrations of β-carotene were:

- c_m =98.5 μg β-carotene /g tissue (leaves);
- c_m = 43.4 μg β-carotene /g tissue (stalk).

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