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# Determination of carotenoids in extracts from species of *Tagetes* and *Calendula*

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## Abstract

**Background**: Carotenoids have played a significant role in reducing the risk of chronic diseases. The most studied in this regard is β-carotene, present in species of *Tagetes* and *Calendula* genus. Objective of the study: Comparative analysis of β-carotene content in liquid and dry flowers extracts of *Tagetes* and *Calendula* species, cultivated in the collection of the Scientific Center for the Cultivation of Medicinal Plants of *Nicolae Testemitanu* SUMPh.
**Material and methods**: Dry extracts of flowers harvested in the budding-flowering phase, were obtained by repeated maceration and rotary evaporation, subjected to phytochemical evaluation by thin-layer chromatography (TLC) and UV-VIS spectrophotometry, equivalent to β-carotene.
**Results:** Beta-carotene was identified by TLC in hexan-ethyl acetate (50:50, v/v), retention factors were established. Caratenoid content (mg%) varied as follows: in *T. patula* L. (75.34±2.15), *T. erecta* L. (21.97±0.84), *C. officinalis* L. variety Natali (13.09±3.23), *C. officinalis* L. variety Diana (12.39±1.98), *C. officinalis* L. local population (10.99±0.06). The carotenoids content ranged in the dry extracts as well, determined in the highest amount in *T. patula* L. flowers (137.87±2.18 mg%).
**Conclusions:** This study demonstrated the opportunity for further research of *Tagetes* and *Calendula* varieties that could serve as sources of carotenoids for obtaining antioxidant phyto-pharmaceuticals.
**Key words**: carotenoids, vegetal products, dry extracts, spectrophotometry.

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## Introduction

Carotenoids are natural pigments responsible for many of the red, orange and yellow hues of plant leaves, fruits and flowers, as well as the colors of some birds, insects, fish and crustaceans [1]. Carotenoids are synthesized in plants but not in animals. They are localized in subcellular organelles (plastids), *i.e.* chloroplasts and chromoplasts. In chloroplasts, the carotenoids are chiefly associated with proteins and serve as accessory pigments in photosynthesis, whereas in chromoplasts they are deposited in crystalline form or as oily droplets [2]. Carotenoids play crucial roles in photosynthesis, photoprotection, development, as stress hormones and signaling molecules in plants. In addition, these colors serve to attract pollinating and seed dispersal agents. More than 600 carotenoids have been identified so far in nature. About 40 carotenoids are present in the typical human diet and only 20 of them have been found in human blood and tissues, close to 90% of the carotenoids in the diet and human body are represented by β -carotene, α-carotene, lycopene, lutein and zeaxanthin [3].

Several carotenoids act as precursors of vitamin A, which is an efficient antioxidant and is important for human nutrition. The provitamin A carotenoid, β-carotene, is a significant source of vitamin A [4]. In human body, β-carotene is broken down by β-carotene dioxygenase in the mucosa of small intestine into two retinyl molecules, which are later reduced to vitamin A (retinol). Beta-carotene is a colored red-orange pigment and widely found in plants and fruits, especially in orange fruits such as cantaloupe, mangoes, pumpkins and papayas, and orange root vegetables such as carrots and sweet potatoes [5].

Lycopene is an unsaturated acyclic carotenoid with open straight chain hydrocarbon consisting of 11 conjugated and two unconjugated double bonds. Lycopene has no provitamin A activity due to the lack of terminal β-ionic ring as the basic structure for vitamin A. The red color of lycopene is mainly due to many conjugated carbon double bonds, as it absorbs more visible spectrum compared to other carotenes [2]. Over the last decade, there has been increased recognition that lycopene plays an important role in preventing the development of coronary disease and retarding the progression of atherosclerosis. The antioxidant activity of lycopene is almost twice as high as that of β-carotene and has the greatest synergism with vitamin E. Aside from the popular

tomato, other sources of lycopene include red grapefruit, watermelon and apricots [5].

Lutein and zeaxanthin belong to the class of carotenoids called xanthophylls, they are the major constituents of macular pigment, a compound concentrated in the macula region of the retina that is responsible for fine-feature vision. Given their accumulation in the retina, has been investigated how consumption of these carotenoids may prevent and/or slow the progression of age-related macular degeneration, the leading cause of blindness in older adults [6].

Nowadays, many of ongoing research has focused on the identification of foremost sources of carotenoids for the use in ophthalmology for the treatment of age-related ocular diseases**.** Genus *Tagetes* (Asteraceae) is considered an important source of carotenoid pigments, especially of the yellow carotenoids (α-, β-carotenes) and xanthophylls (lutein, zeaxanthin, violaxanthin) [7]. Genus *Tagetes* contain about 50 species of annual or perennial herbaceous plant, native to Central and South America and naturalised elsewhere in the tropics and subtropics [8]. Some species such as *Tagetes erecta* L., *Tagetes patula* L. and *Tagetes tenuifolia* Cav., are cultivated as ornamental plants, while *Tagetes minuta* L. has become a noxious plant [9]. *T. erecta* L. has been used as coloring agent and nutritional supplement in a wide range of foods and beverages in levels ranging from 2 to 330 mg/kg for lutein and 0.5 to 70 mg/kg for zeaxanthin [10].

Genus *Calendula* (Asteraceae), native to the Mediterranean Basin, includes approximately 25 herbaceous annual or perennial species, most common being *Calendula officinalis* L., *Calendula arvensis* L., *Calendula suffruticosa* Vahl., *Calendula stellata* Cav., *Calendula alata* Rech. and *Calendula tripterocarpa* Rupr. [11]. Among the various species of the genus *Calendula*, *C. officinalis* L. is the only one which is extensively used clinically throughout the world. The inflorescence of *C. officinalis* L. has abundant amount of carotenoids that give flowers their yellow-orange color and the color shade depends on pigment content and pigment profile. Its yellow flower petals contain 19 carotenoids and orange flower contains 10 unique carotenoids. The main carotenoids present in the petals and pollens are flavoxanthin, luteoxanthin, auroxanthin, 9Z-antheraxanthin, neoxanthin, lutein and its Z-isomers, mutatoxanthin, violaxanthin, 9Z-neoxanthin, 9Z-violaxanthin, α- and β-carotene, and α- and β-cryptoxanthin with higher quantity of lycopene in petals [12]. *C. officinalis* L. is considered to offer protection against some cancers, UV-induced skin damage, coronary heart disease, cataracts and molecular degeneration [13].

The aim of the present study is to investigate and compare the β-carotene content in liquid and dry flowers extracts of *Tagetes* and *Calendula* species, cultivated in the collection of the Scientific Center for the Cultivation of Medicinal Plants of *Nicolae Testemitanu* State University of Medicine and Pharmacy (SUMPh) by thin-layer chromatography (TLC) and UV-VIS spectrophotometry.

## Material and methods

**Plant material.** Flowers of the species *Tagetes patula* L., *Tagetes erecta* L., *Calendula officinalis* L. and the varieties of *Calendula officinalis* L. Diana and Natali were collected, in the complete flowering phase, from the collection of the Scientific Center for Cultivation of Medicinal Plants of Nicolae Testemitanu SUMPh. The vegetal products were dried in natural conditions in the shade, in a well-ventilated place. The Natali and Diana varieties of *C. officinalis* L. were obtained by scientists from the Institute of Genetics, Physiology and Plant Protection.

**Extraction procedure**. 5.0 g of crushed vegetal product was placed in a 100 ml flask, added 70 ml of hexane and heated in a water bath at 60°C for 5 minutes. The extracts were filtered into a 100 ml flask. The extraction was carried out twice with hexane for 30 ml for 5 minutes in a water bath at a temperature of 50-60°C. The extracts after cooling were filtered and their volume was made up to 100ml with hexane.

**Thin layer chromatography (TLC) assay.** The identification of β-carotene in the studied vegetal products was performed by thin layer chromatography on chromatographic plates "Sorbphil" (Krasnodar). For the chromatographic separation of β-carotene three mobile phases: hexane-ethyl acetate (50:50 v/v), hexane-ethyl acetate (80:20 v/v), hexane-ethyl acetate-propanol-2 (75:18:7 v/v/v) were used. As a control served β-carotene (Sigma-Aldrich). The analyzed solutions were obtained by mixing 0.5 g of vegetal product (*flores*) with 15 ml of hexane. The extractive solutions and the solution of the reference substance (β-carotene) were applied on the start line of the chromatographic plates [14].

**Determination of total carotenoid content.** The determination of the total carotenoids in flowers of *Tagetes* and *Calendula* species was performed spectrophotometrically on a Metertech UV/VIS SP 8001 spectrophotometer. 10 ml of the obtained solution was passed into a 25 ml volumetric flask, brought to the level with hexane. The optical density of the solution was determined at λ= 450 nm in a 10 mm thick cuvette. Reference solution – hexane. The carotenoid content (mg%) in the β-carotene equivalent was calculated according to the formula:

$$X = \frac{A * 0.00208 * 100 * 100 * 25 * 100}{A_0 * a * 10 * (100 - W)}$$

$$X = \frac{A * 0.00208 * 100 * 100 * 25 * 100}{A_0 * a * 10 * (100 - W)}$$, where:

A – the absorbance of the analyzed solution; $A_0$ – the absorbance of the standard solution; m – the mass of vegetal product (g); W – the weight loss on drying (%); 0.00208 – the amount of β-carotene, which corresponds to 1 ml of standard potassium dichromate solution (mg).

Preparation of the potassium dichromate solution: 0.0900 g of $K_2CrO4$ pass into a 250 ml volumetric flask, dissolve in water, make up to the mark with the same solvent. The solution obtained by color corresponds to 0.00208 mg of β-carotene in 1 mg.

**Obtaining of dry extracts**. The dry extracts were ob-

tained by the fractional maceration method with stirring from 5 g of dry vegetal products, treated 4 times with 100 ml of hexane, with an extraction cycle of 30 min. The extractive solutions of 4 fractions were combined and kept cold for 24 hours to sediment the resins, then filtered through the Buchner funnel. The concentration of the extractive solutions was performed on the rotating system Laborota 4011 – digital at 60°C.

**Sample preparation for spectrophotometric analysis.** 0.05 g of dry extract was placed in a 50 ml flask and diluted with 30 ml of hexane, then the solution was made up to the level with the same solvent. The optical density was determined at λ= 450 nm, in a 10 mm thick tank. The carotenoid content (mg%) in the dry extracts was calculated according to the formula [15]:

$$X = \frac{A*V*50*100*1000}{2592*m*(100-W)} \quad X = \frac{A*V*50*100*1000}{2592*m*(100-W)}$$, where:

A – the absorbance of the analyzed solution; V – the volume (ml) ; m – the mass of dry extract (g); W – weight loss on drying (%); 2592 – the absorbance of β-carotene at λ= 450 nm.

**Statistical analysis.** The average of multiple measurements (triplicates) are listed and expressed with the standard deviations. Statistical analysis was performed using Excel 2017 software package.

## Results and discussion

The TLC assay revealed the presence of β-carotene under the described chromatographic conditions through the determination of retention factors. The results of the qualitative study of the analyzed vegetal products are presented in *table 1*. Following the analysis of visible light chromatograms, were observed in all studied products yellow spots, where the retention factors (Rf) corresponded to the Rf of the reference substance β-carotene. It was shown that the clearest separation of β-carotene in hexane solutions from *Tagetes* and *Calendula* flowers occurred in the mobile phase hexane:ethyl acetate (50:50 v/v). The migration of the chromatographic systems was 10 cm.

Total carotenoid content of the extraction samples, obtained from the under consideration vegetal products was evaluated spectrophotometrically. The highest level of carotenoid content was identified in *T. patula* L. flowers (75.34±2.15 mg%) and with a slighter quantity in *T. erecta* L. (21.97±0.84 mg%). Scientific studies show that the carotenoid content differs a lot, depending on the geographical area, climatic conditions, as well as species, genetics and variety. In some studies, done by Akshaya et al. (2017) *T. patula* L. has the highest content of carotenoids compared to yellow colored flowers of *T. erecta* L., but lesser compared to those of dark orange hue. Among the *Tagetes* genotypes studied, the total carotenoid content ranges from 19.61 mg/100g fresh weight to 525.68 mg/100g fresh weight of petals. Maliugina et al. (2013) determined, that the most contents of biologically active carotenoids exist in the inflorescences of the low-growing cultivars of the genus *Tagetes*, such as Gold kopfen (159.25±15.93 mg%), Orange flamme (56.0±15.61 mg%), Carmen (144.4±14,5 mg%) and Fiesta (143.5±14.33 mg%).

The study of total carotenoid content for the *Calendula* species, revealed a top content in *C. officinalis* L. variety Natali (13.09±3.23 mg%), followed by *C. officinalis* L. variety Diana (12.39±1.98 mg%) and *C. officinalis* L. (10.99±0.06 mg%). In some studies Toiu et al. (2016) observed high variations in carotenoid concentrations in some analyzed varieties from Romania: *Calendulae flores* contains 0.99-1.32 mg carotenoids/g dried flowers and *Tagetes flores* contains 0.52-3.72 mg/g.

It is important to note that flowers from *Tagetes* species, grown in the Republic of Moldova have been determined to have the higher concentration of carotenoids, compared to varieties of *Calendula*. Among the varieties of *Calendula* the maximum concentration was demonstrated in flowers of *C. officinalis* L. variety Natali.

Since carotenoids are widely utilized in pharmaceutical industry as dry extracts we also aimed to obtain and evaluate the total carotenoid content in the dry extracts. The highest content (fig. 1), was identified in the extract obtained from flowers of *T. patula* L. (137.87±2.18 mg%), followed by *T. erecta* L. (57.88±7.14 mg%). In the extracts obtained from marigold flowers, the caratenoid content varies as follows: *C. officinalis* L. variety Natali – 39.98±0.93 mg%, *C. officinalis* L. variety Diana – 34.05±5.34 mg%, *C. officinalis* L. – 27.38±2.02 mg%. Furthermore, the results show that the total carotenoids content differs statistically significant both for liquid extracts obtained from the flowers of the marigold varieties and for dry extracts as well ($p<0.05$).

**Table 1**

**Values of Rf of β-carotene spots separated by TLC in the extractive solutions from *Tagetes* sp. and *C. officinalis* L. varieties**

| No | Extractive solutions obtained from vegetal product – flores / reference substance | Mobile phases/Rf of β-carotene | | |
|---|---|---|---|---|
| | | Hexane-ethyl acetate (50:50) | Hexane-ethyl acetate (80:20) | Hexane-ethyl acetate propanol-2 (75:18:7) |
| 1 | *T. patula* L. | 0.90 | 0.77 | 0.89 |
| 2 | *T. erecta* L. | 0.90 | 0.76 | 0.89 |
| 3 | *C. officinalis* L. | 0.90 | 0.76 | 0.87 |
| 4 | *C. officinalis* L. variety Diana | 0.89 | 0.77 | 0.86 |
| 5 | *C. officinalis* L. variety Natali | 0.90 | 0.77 | 0.87 |
| 9 | β-carotene – reference substance | 0.90 | 0.75 | 0.87 |

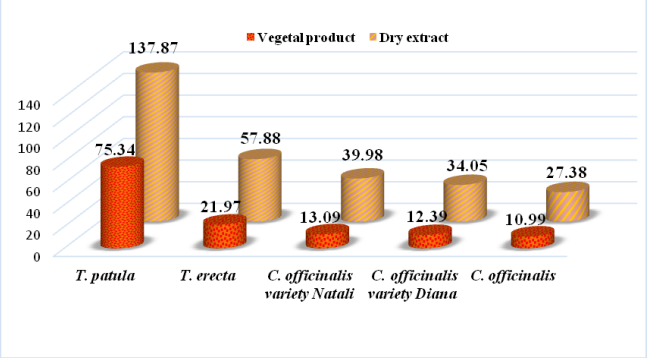


**Fig. 1. The total carotenoid content, equivalent to β-carotene, in vegetal products (*flores*) and in dry extracts**

The species of *Tagetes* and *Calendula* are cultivated as vegetable products with high carotenoids content. Concomitant with the pharmaceutical industry, where carotenoids are used as compounds with anti-inflammatory and antioxidant properties, they are processed in cosmetics and food production as natural dyes and preservatives. *Calendula officinalis* L. is listed in German Commission E, European Scientific Cooperative on Phytotherapy, British Herbal Pharmacopoeia, World Health Organization monographs for wound healing and anti-inflammatory actions [11], whereas species of the genus *Tagetes* are not found in the reference Pharmacopoeia, being used in folk medicine and often as ornamental plants. Different parts of these plants including flowers are used traditionally to cure various diseases, as the leaves are reported to be effective against piles, kidney troubles, muscular pain, ulcers, wounds and earaches [8]. The results of the present study showed, that carotenoid content, especially of β-carotene, from *Tagetes* genus is a source that needs further validation for correlation to biological activity and elaboration of normative documentation.

## Conclusions

In this work, during the phytochemical evaluation, we have determined that the richest in carotenoids are the dark orange inflorescences of the species *T. patula* L., followed by *C. officinalis* L. varieties Natali and Diana, which recommends them to be grown for medicinal use.

The results indicate that the flowers of *Tagetes* species and *C. officinalis* L. varieties, cultivated in the Republic of Moldova, can be used as vegetal products with high carotenoid content in the pharmaceutical, cosmetic and food industries.

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**Authors' contributions:** AB designed the study, performed the laboratory work and drafted the first manuscript; CC interpreted the data, revised the manuscript; MC-T revised the manuscript; NC conducted the laboratory work, revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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