REVIEW

COMPOSITION OF CAROTENOIDS IN CALENDULA (*CALENDULA OFFICINALIS* L.) FLOWERS

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Abstract

Calendula (*Calendula officinalis* L.) belongs to the *Asteraceae* family. It is a medicinal plant and its flowers are used as important ingredient of pharmaceutical and food production. Most calendula research has been focused on extraction and pharmaceutical properties of bioactive compounds from flowers. Fresh calendula flowers are suggested to use as edible flowers but dried flowers – as an herbal tea and as condiment of food. Calendula accumulates large amounts of carotenoids in its flowers. The yellow and orange colour of petals is mostly due to the carotenoids and the shade depends on the quantity and composition of pigments. Carotenoids are known as biologically active compounds with multiple applications in therapy. It is important to humans as precursors of vitamin A and retinoids. Major factors that impact differences in the amount of total carotenoids in calendula flowers is reported to be the plant variety, colour of the ligulate and tubular florets, the place of cultivation and time of harvesting. Nowadays many important carotenoids are used as pigments – food colorants in the food industry. Carotenoids composition in calendula flowers has been recently begun to explore and in future the scientific research on calendula will be increased. In this review, existing information of carotenoids composition in calendula flowers depending of variety are explored, as well as carotenoids multi-activity and usage of calendula flowers are highlight.

***Keywords**:* pot marigold, edible flowers, calendula, variety, carotenoids.

Introduction

Calendula (*Calendula officinalis* L.) belongs to the *Asteraceae* family. Depending on variety and culture, the plants grow up to 60 cm. Flowers color varies from yellow to orange and they bloom from June until late autumn (Kemper, 1999; Mills, 1999). Calendula is a native of southern Europe but on commercial scale it is cultivated in all around the world. Calendula is a medicinal plant and its flowers are used as important ingredient of pharmaceutical, cosmetic and food production (Cromack, Smith, 1998; Rubine, Enina, 2004). Most calendula research has been focused on extraction and pharmaceutical properties of bioactive compounds from flowers. The name carotenoids, is derived from the fact that they constitute the major pigment in the carrot root, *Daucus carota*, are undoubtedly among the most widespread and important pigments in living organism. Carotenoids are the pigments responsible for the colors of many plants (Eldahshan, Singab, 2013). The yellow and orange color of flower petals is mostly due to the carotenoids and the shade depends on the quantity and composition of pigments (Faulks et al., 2005; Fernandez-Garcia et al., 2012; Tanaka et al., 2008; Zhu et al., 2010).

In this review, there are explored the composition of carotenoids in calendula flowers in order to compare existing information on this plant and its different varieties as well as highlight its multi-activity.

Characterization and health applications of carotenoids

Carotenoids are tetraterpenoids synthesized in plants and microorganisms. They can also be found in many animal species and are important colorants in birds, insects, fish and crustaceans. However, animals and humans cannot synthesize carotenoids and their level in body depends on dietary supply (Britton, 1995; Olson, Krinsky, 1995). Up to now, more than 750 different carotenoids have been isolated from natural sources and new carotenoids continue to be identified (Ramawat, Merillon, 2013). However, only about 40 carotenoids can be absorbed, metabolized, and used in human body. That number is reduced to 6 if the carotenoid profile that is usually detected in human blood plasma is considered. This group includes α- and β-carotene, lycopene, β-cryptoxanthin, zeaxantin and lutein, which are regularly presented in food list of human diet (Britton, Khachik, 2009).

From the chemical point of view, structurally carotenoids are divided into two major groups: carotenes and xanthophylls. Firstly, carotenes or exclusively hydrocarbon carotenoids only composed of carbon and hydrogen atoms, examples of these compounds are α-carotene, β-carotene, lycopene, etc. Secondly, xanthophylls are known as oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy or carboxylic acid groups. Examples of these compounds are a zeaxanthin and lutein (hydroxy), spirilloxanthin (methoxy) and antheraxanthin (epoxy), etc. (Ramawat, Merillon, 2013).

Many research results shows, that the consumption of a diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases (Faulks et al., 2005). Carotenoids are considered as important to humans as precursors of vitamin A and retinoids. The major provitamin A carotenoids in Western diet is β-carotene, but also α-carotene, and β-cryptoxanthin contribute to vitamin A supply and may prevent vitamin A deficiency (Olson, Krinsky, 1995; Het Hof et al., 2000). The importance of carotenoids in food goes beyond as natural pigments and biological functions and actions have increasingly been attributed to these pigments (Faulks et al., 2005). Carotenoids in tissues reflect food choices too (Rock, 1997). Epidemiologic evidence links higher carotenoid intakes and tissue concentrations with reduced cancer and cardiovascular disease risk (Rock, 1997; Mayne, 1996). There is convincing evidence that carotenoids are important components of the antioxidant network (Rock, 1997). Photooxidative damage is suggested to be involved in the pathobiochemistry of several diseases affecting the skin and the eye, and carotenoids may protect light-exposed tissues.

Lutein and zeaxanthin are the predominant carotenoids of the retina and are considered to act as photoprotectants preventing retinal degeneration. The unique distribution, localization and high levels of both carotenoids within the macula lutea as well as their physicochemical properties make them suitable candidates for photoprotection (Laurence et al., 2000; Stahl, Sies, 2005; Richar, 2013). There are few reports on the toxicity studies of lutein and histopathological analysis of the organs supported the nontoxicity of lutein and its ester form (Harikumar et al., 2008).

β-carotene is one of the most abundant carotenoids in the human body. Comparing to carotenoids that can be metabolized into vitamin A, β-carotene has the highest provitamin A activity (Krinsky, Johnson, 2005). According to the researches, β-carotene is widely used as an oral sun protectant for the prevention of sunburn and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins (Sies, Stahl, 2004; Stahl, Sies, 2005; Berneburg, Krutmann, 2000; Stahl et al., 2000).

Protective effects are also achieved with a diet rich in lycopene. Many studies suggest that eating lycopene rich foods or having high lycopene levels in the body may be linked to reduced risk of cancer, heart disease, and age-related eye disorders. However, measures of lycopene intake have been based mainly on eating tomatoes and other products with high lycopene content, not on the use of lycopene supplements (Krinsky, Johnson, 2005; Rock, 1997; Sies, Stahl, 2004).

Carotenoids in calendula flowers

The several investigations of carotenoids compositions of calendula petals have been reported although the researches on it has been recently begun to investigate. Some report refers to qualitative aspects as separation and identification of carotenoids, and others to quantitative determination as total carotenoids. According to the researches, there are found out that calendula accumulates large amounts of different carotenoids in its flowers.

Bako et al. (2002) have studied the composition of carotenoids in calendula petals that were obtained from commercial garden in Pècs (southern Hungary) and found about 18 different types of carotenoids: neoxanthin, (9*´Z*)-neoxanthin, violaxanthin, luteoxanthin, auroxanthin, (9*´Z*)-violaxanthin, flavoxanthin, mutatoxanthin, (9*´Z*)-antheraxanthin, lutein, (9/9*´Z*)-lutein, (13/13*´Z*)-lutein, α-carotene, β-carotene. Kishimoto et al. (2005) were analyzed three orange flowered cultivars (‘Alice Orange’, ‘Orange Star’ and ‘Orange Zem’) and three yellow flowered cultivars (‘Alice Yellow’, Gold Star’ and ‘Golden Zem’) of calendula in their researches, grown in Tsukuba, Japan. They have identified 19 different carotenoids in their researches, such as, (8*´R*)-luteoxanthin, lutein-5,6-epoxide, flavoxanthin, (8R/8*´R*)-auroxanthin, (9*´Z*)-lutein-5,6-epoxide, lutein, antheraxanthin, (9*Z*)-lutein, (5*´Z*/9*´Z*)-rubixanthin, α-carotene, β-carotene, (5*´Z*)-rubixanthin, δ-carotene, (5*Z*/9*Z*/5*´Z*/9*´Z*)-lycopene, γ-carotene, (5*´Z*)-γ-carotene, (5*Z*/9*Z*/5*Z*)-lycopene, (5*Z*/9*Z*)-lycopene, all-E-lycopene. Two carotenoids of them ((5*´Z*)-γ-carotene and (5*´Z*/9*´Z*)-rubixanthin) have never been before identified. However, they have not identified numerous of carotenoids that have been found in by other researcher from other countries. From these results we can find out that the place of cultivation impact composition of different carotenoids in calendula flowers.

Carotenoids are generally responsible for petal colors in the yellow to red range and the wide range of petal colors in various varieties of calendula originates mainly from combinations of these pigments. Verghese (1998) has reported that marigold (*Tageteserecta*) flower petals are a significant source of the xanthophyll and have a much higher concentration of this pigment compared to other plant materials. Moehs et al. (2001) have reported that in marigold (*Tageteserecta*), the differences in petal color from pale-yellow to orange-red are caused by different levels of accumulation of yellow carotenoid lutein.

Kishimoto et al. (2005) have shown that distinctive differences in carotenoids content (characterized by HPLC chromatograms) were found between orange flowered varieties and yellow flowered varieties. In all, 19 carotenoids where observed in orange flowered varieties, and of these, 10 carotenoids were detected only in orange flowered varieties. They have found out that yellow colored petals don’t contain (5*´Z*/9*´Z*)-rubixanthin, α-carotene, (5*´Z*)-rubixanthin, δ-carotene, (5*Z*/9*Z*/5*´Z*/9*´Z*)-lycopene, γ-carotene, (5*´Z*)-γ-carotene, (5*Z*/9*Z*/5*Z*)-lycopene, (5*Z*/9*Z*)-lycopene, all-E-lycopene. Therefore we can consider that these carotenoids are responsible for the orange color formation of the petals. The similar results have shown Pintea et al. (2003) in their researches of four calendula varieties – two orange flowered (‘Double Esterel Orange’ and ‘Radio Extra Selected’) and two yellow flowered (‘Bonbon Abricot’ and ‘Double EsterelJaune’). They have found out that the composition of different carotenoids increases with color intensity, the dark orange variety being the richest one. The dark flowered variety (‘Double Esterel Orange’) is the richest in rubixanthin, lycopene and γ-carotene, but yellow flowered variety (‘Bonbon Abricot’) contains important amount of β-carotene, but very low amountof γ-carotene and it is especially low in lycopene. Kishimoto et al. (2007) have reported, that orange and yellow flowered cultivars of *O. ecklonis* (*Compositae* family) showed the same composition, the proportions of three reddish carotenoids – 5´Z-γ-carotene, 5Z/9Z/5´Z-lycopene, and lycopene – were higher in the orange flowered cultivar. In general, there is found out that orange flowered cultivars of calendula accumulate more reddish carotenoids than yellow flowered varieties (Kishimoto, Ohmiya, 2009). The color intensity of orange flowered varieties is primarily due to lycopene, which is absent from yellow flowered varieties. Lutein and β-carotene were the most abundant carotenoids in the Italian type of calendula, grown in Ravenna, northern Italy (Piccaglia et al., 1999).

More reports refer only to quantitative determination as total carotenoids. The major factors that impact differences in the amount of total carotenoids in calendula flowers is reported to be the plant variety, color of the ligulate and tubular florets, the place of cultivation and time of harvesting (Bako et al. 2002; Kishimoto et al. 2005; Kishimoto et al. 2007; Kishimoto, Ohmiya, 2009; Pintea et al., 2003; Raal et al., 2009), as well as, environmental factors, such as soil mineral composition, temperature, oxygen and light (Piccaglia et al., 1999).

A wide range of calendula varieties were analyzed in Estonia. Raal et al. (2009) and Toom et al. (2007) have found out that the total carotenoids content varied in the investigated 42 samples from 200 to 3510 mg 100 g^{-1} dried flowers. The highest total carotenoids content (3510 mg 100 g^{-1}) was found in brownish-yellow flowered variety 'Golden Emperor' (Table 1.). The lowest sum of pigments was found in the variety 'Touch of Buff' (200 mg 100 g^{-1}), with a very unconventional combination of flowers color – its tubular florets were sorrel, but the ligulate ones were maroon in the top and cream colored in the bottom. In their studies, they have observed that usually canary yellow, carnation-yellow and yellow flowered cultivars contain total carotenoids of less than 1000 mg 100 g^{-1} while cultivars with orange flowers contain pigments between 1000–2000 mg 100 g^{-1} or even more. Pintea et al. (2003) have found the highest total carotenoids content in fresh flowers of orange flowered cultivar 'Double Esterel Orange' with a total content 276 mg 100 g^{-1}, but lowest in yellow-orange flowered cultivar 'Bonbon Abricot' – 48.2 mg 100 g^{-1}. Also Kishimoto et al. (2007) have found differences in total carotenoids content between orange and yellow flowered cultivars.

In general, that the richest in total carotenoids content are brownish-yellow and orange flowered calendula varieties. The total carotenoids content also depends on region of cultivation. The same variety grown in different regions has shown different content of total carotenoids. For example, variety 'Fiesta Gitana', grown in Central Estonia (Järvamaa) has shown 1060 mg 100 g^{-1},but towards to the south Estonia (Viljandimaa) total carotenoids content has increased to 1440.0 mg 100 g^{-1} (Raal et al., 2009). Pintea et al. (2003) have reported that the values of quantitative determinations are very different from one variety to another. Anyhow, the interconnection between calendula varieties and content of total carotenoids is not satisfactorily clear and therefore there more researches in future are needed.

Table 1

Amount of total carotenoids in flowers of calendula (*Calendula officinalis* L.) varieties

Variety (color of flowers)	Carotenoids, mg 100 g^{-1}	Place of cultivation (region, country)
Fresh flowers		
Alice Orange (orange)	170*	Tsukuba, Ibaraki, Japan
Alice Yellow (yellow)	125*	Tsukuba, Ibaraki, Japan
Bonbon Abricot (yellow-orange)	48**	Cluj-Napoca, Romania
Double EsterelJaune (lemon yellow)	97**	Cluj-Napoca, Romania
Double Esterel Orange (dark orange)	276**	Cluj-Napoca, Romania
Gold Star (yellow)	126*	Tsukuba, Ibaraki, Japan
Golden Gem (yellow)	118*	Tsukuba, Ibaraki, Japan
Orange Gem (orange)	107*	Tsukuba, Ibaraki, Japan
Orange Star (orange)	145*	Tsukuba, Ibaraki, Japan
Ponpon Orange (orange)	119*	Tsukuba, Ibaraki, Japan
Ponpon Yellow (yellow)	109*	Tsukuba, Ibaraki, Japan
Radio Extra Selected (orange)	112**	Cluj-Napoca, Romania
Dried flowers		
Apricot Beauty (dark orange-canary yellow)	530***	Järvamaa, Central Estonia
Apricot Beauty (yellow, top orange)	430***	Viljandimaa, Southern Estonia
Art Schades (canary yellow-light orange)	1780***	Järvamaa, Central Estonia
Art Schades (yellow-orange)	840***	Viljandimaa, Southern Estonia
Balls Orange (orange)	2000–2240***	Viljandimaa, Southern Estonia
Balls Orange (orange)	1150***	Järvamaa, Central Estonia
Balls Supreme (orange)	1750***	Viljandimaa, Southern Estonia
Cremeweiss (canary yellow)	370***	Viljandimaa, Southern Estonia
Cremeweiss (canary yellow)	270***	Pärnumaa, South-Western Estonia

Fiesta Gitana (orange- yellow-canary yellow)	1440***	Viljandimaa, Southern Estonia
Fiesta Gitana (orange- yellow-canary yellow)	1140***	Pärnumaa, South-Western Estonia
Fiesta Gitana (orange-yellow-canary yellow)	1060***	Järvamaa, Central Estonia
Golden Emperor (brownish-yellow)	3510***	Viljandimaa, Southern Estonia
Golden Emperor (brownish-yellow)	3420***	Järvamaa, Central Estonia
Kablouna (yellow-orange)	1460***	Viljandimaa, Southern Estonia
Kablouna (yellowish)	770***	Järvamaa, Central Estonia
KablunaOranz (orange)	1840***	Viljandimaa, Southern Estonia
Medetkos (orange)	890***	Pärnumaa, South-Western Estonia
NizkyPlnokvety (orange-canary yellow)	650***	Pärnumaa, South-Western Estonia
Orange King (orange)	1630–2750***	Viljandimaa, Southern Estonia
Pacific Beauty (goldenrod)	2100***	Järvamaa, Central Estonia
Pacific Beauty (orange-yellow)	760–1060***	Viljandimaa, Southern Estonia
Pacific Deep Orange (dark orange)	1190***	Pärnumaa, South-Western Estonia
Pacific Lemon Yellow (lemon yellow)	1480***	Viljandimaa, Southern Estonia
Pink Surprise (carnation-yellow)	960***	Viljandimaa, Southern Estonia
Pomyk (orange)	1680***	Pärnumaa, South-Western Estonia
PrinzesGenischt (yellow-orange)	1330***	Viljandimaa, Southern Estonia
RozovoiSjurpris (carnation-orange)	630***	Viljandimaa, Southern Estonia
RusskiiRazmer XXL (orange)	2970***	Viljandimaa, Southern Estonia
Tokaj (brownish-yellow)	1600***	Järvamaa, Central Estonia
Touch of Buff (top maroon, bottom cream-colored)	200***	Pärnumaa, South-Western Estonia
Touch of Red (orange from upper, brown from underside)	2050***	Viljandimaa, Southern Estonia
Touch of Red (orange from upper, brown from underside)	1610***	Järvamaa, Central Estonia
Unknown, Hungarian type of calendula (orange)	770****	Pècs, Southern Hungary
VysokyKablouna (canary yellow-yellow)	1560***	Pärnumaa, South-Western Estonia
ZeljonoeSertse (orange)	1490***	Viljandimaa, Southern Estonia
Zjelt'IGigant (goldenrod)	2510***	Järvamaa, Central Estonia

* Kishimoto et al., 2007; ** Pintea et al., 2003; *** Raal et al., (2009); **** Bako et al., 2002.

Usage of calendula flowers

Calendula is with huge potential in medicine, food and cosmetic production (Rubine, Enina, 2004; Sausserde, Kampuss, 2014). It is used as therapeutically plant from ancient time. In human medicine calendula flowers are used in tea, infusions, tinctures etc. (Loranty et al., 2010; Rubine, Enina, 2004). It was efficient for liver illness, cramps, ulcers, jaundice, hemorrhoids, internal healing, skin cancer treatment (Khalid, Jaime, 2012; Muley et al., 2009; Preethi et al., 2009) and also with wide spectra of action, such as, choleretic, antispasmodic, bactericidal, anticancer, wounds, frostbite, burns, acne. Calendula is applied in a form of lotion for skin illness, ulcerations, frostbites, burns, fungus (Jorge et al., 1996; Preethi et al., 2009). Extracts derived from dried flowers inhibit the replication of HIV-1 (Kalvatchev et al., 1997). In veterinary medicine the raw material of calendula flowers is used for the preparation of different tinctures (Grela, Sembratowicz, 1998).

Dried and fresh flowers of calendula are widely used as food. In many parts of the world people continues to prepare food with old traditions. More and more the assortments of foodstuffs produced begin to be markedly extended with edible flowers. And these flowers could be potential rich resources of natural antioxidants and carotenoids and could be developed into functional foods, which increases and improves the appearance, taste and aesthetic value of these foodstuffs (Tanji, Nassif, 1995; Mlcek, Rop, 2011; Li et al., 2014). According to the researches, calendula flowers accumulate high amount of different carotenoids. Since the content of these compounds are not very high in the majority of food stuffs (Mlcek et al., 2011), calendula flowers as edible flowers could be one of the sources of valuable substances. Fresh flowers are served as garnish and trimming of various meals and cold buffet food, and petals are used to decorate salads, sweet meals, fruit and ice-cream sundae, drinks, etc. In addition to the esthetic appearance they also correspond to specific taste and smell of served food (Rubine, Enina, 2004; Scherf, 2004). It has long been shown in human studies that fat enhances the absorption of carotenoids (Castenmiller, West, 1998). In recent studies, greater absorption of carotenoids was observed when salad was consumed

with full-fat than with reduced-fat salad dressing (Brown et al., 2004; Rodriguez-Amaya, 2010).
Calendula flowers can be also dried, soaked in alcohol or sugar, frozen either directly or in form of ice cubes, added in to cocktails, etc. The freeze drying is not cost effective but preserves the original appearance, color, shape, and gloss of flowers (Mlcek et al., 2011; Mohammad, Kashani, 2012; Rubine, Eniņa, 2004).
At the beginning of the food industry, pigments – natural of synthetic, were used to give an attractive appearance, perception of freshness, taste, and quality of food. Today, natural colorants are emerging globally due to the perception of its safer and eco-friendly nature (Pratheesh et al., 2009; Abbey et al., 2014). Carotenoids pigments are of interest to the food scientists, nutritionists and food industries due to their positive impact on human health and their economic benefits. Carotenoids are responsible for the attractive color of many plant foods, which is perhaps the first attribute that consumers assess when determining the quality and appearance of a product, and therefore conditions of its acceptability (Fernandez-Garcia et al., 2012).

Conclusions

Nowadays many important carotenoids are used as pigments – food colorants in the food industry Calendula accumulates large amount of different carotenoids in its flowers, therefore its promising plant for condiment of food production, as well as – food colorants in the food industry. Nowadays, a trend towards researches of calendula represents a challenge for food manufactures, because of its widely therapeutically applications, and diet rich in carotenoids represents lower risk for several diseases. The addition of many colors to healthy food and food products may become superfluous in the future. In collaboration of specialists of agriculture, bioengineering and food science it is possible to develop innovative techniques to produce foods that could be potential rich of natural carotenoids and to find out good colorants and to improve retention of total carotenoids pigments, as well as flavors and nutrients in cooked food.

References

1. Abbey J., Fields B., O'Mullane M. (2014) Food Additives: Colorants. *Encyclopedia of Food Safety*, Vol. 2, p. 459–465.
2. Bako E., Deli J., Toth G. (2002) HPLC study on the carotenoids composition of *Calendula* products. *Journal of Biochemical and Biophysical Methods*, Vol. 53, p. 241–250.
3. Berneburg M., Krutmann J. (2000) Photoimmunology, DNA repair and photocarcinogenesis. *Journal of Photochemistry and Pphotobiology, B: Biology*, Vol. 54, p. 87–93.
4. Britton G. (1995) Structure and properties of carotenoids in relation to functions, *The FASEB Journal*, Vol. 9, p. 1551–1558.
5. Britton G., Khachik F. (2009) Carotenoids in food. In: *Britton G., Liaaen-Jensen S., Pfander H.P. (Eds.) Carotenoids. Nutrition and Health*, Vol. 5, p. 45–66.
6. Brown M.J., Ferruzzi M.G., Nguyen M.L., Cooper D.A., Eldridge A.L., Schwartz S.J., White W.S. (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electro-chemical detection. *American Journal of Clinical Nutrition*, Vol. 80, p. 396–403.
7. Castenmiller J.J.M., West, C.E. (1998) Bioavailabilityandbioconversionof carotenoids. *Annual Review of Nutrition*, Vol. 18, p. 19–38.
8. Cromack H., Smith J. (1998) *Calendula officinalis* – production potential and crop agronomy in southern England. *Industrial Crops and Products*, Vol. 7, p. 223–229.
9. Eldahshan O.A., Singab A.N.B. (2013) Carotenoids. *Journal of Pharmacognesy and Phytochemistry*, Vol. 2, No. 1, p. 225–234.
10. Faulks R.M., Southon S. (2005) Chalanges to understanding and measuring carotenoids bioavailability. *Biochimica et Biophysica Acta*, Vol. 1740, p. 95–100.
11. Fernandez-Garcia E., Carvajal-Lerida I., Jaren-Galan M., Garrido-Fernanez J., Perez-Galvez A., Hornero-Mendez D. (2012). Carotenoids bioavailability from foods: From pigments to efficient biological activities. *Food Research International*, Vol. 46, p. 438–450.
12. Grela E.R., Sembratowicz I. (1998) Immunostimulatory activity of herbs. *Medycyna Weterynaryjna, Poland*, Vol. 54, No. 3, p. 152–158.
13. Harikumar K.B., Nimita C.V., Preethi K.C., Kuttan R., Shankaranarayana M.L., Deshpane J. (2008) Toxicity profile of lutein and lutein ester isolated from marigold flowers (*Tagetes erecta*). *International Journal of Toxicology*, Vol. 27, p. 1–9.
14. Het Hof K.H., West C.E., Weststrate J.A., Hautvast J.G. (2000) Dietary factors that affect the bioavailability of carotenoids. *The Journal of Nutrition*, Vol. 130, p. 503–506.
15. Jorge N.J., Fracasso J.F., Neves M.D.C.L.C., Santos L.E.D., Banuth V.L. (1996) Treatment of varicose ulcer and skin lesions with Calendula officinalis L. or Stryphnodendron barbadetiman Martius. *Revista De Ciencias Farmaceuticas*, Vol. 17, p. 181–186.
16. Kalvatchev Z., Walder R., Garzaro D. (1997) Anti-HIV activity of extracts from Calendula officinalis flowers. *Biomed Pharmacother*, Vol. 51, No. 4, p. 176–180.
17. Kemper K. (1999) Calendula (C*alendula officinalis*). The long wood Herbal Task Force and the Centre for Holistic *Pediatric Education and Research*. 767 p.
18. Khalid A.K., Jaime A.T.S. (2012) Biology of *Calendula officinalis* Linn.: Focus on pharmacology, biological activities and agronomic practices. *Medicinal and Aromatic Plant Science and Biotechnology*, Vol. 6, No. 1, p. 12–27.
19. Kishimoto S., Maoka T., Sumitomo K., Ohmiya A. (2005) Analysis of carotenoid composition in petals of calendula (*Calenula officinalis* L.). *Bioscience, Biotechnology, and Biochemistry*, Vol. 69, No. 11, p. 2122–2128.
20. Kishimoto S., Ohmiya A. (2009) Studies in carotenoids in the petals of composite plants. *Journal of the Japanese Society for Horticultural Science*, Vol. 78, No. 3, p. 263–272.
21. Kishimoto S., Sumitomo K., Yagi M., Nakayama M., Ohmiya A. (2007) Three routes to orange petal color via carotenoids components in 9 compositae species. *Journal*

of the Japanese Society for Horticultural Science, Vol. 76, No. 3, p. 250–257.

22. Krinsky N.I., Johnson E.J. (2005) Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, Vol. 26, No. 6, p. 459–516.
23. Laurence M.R., Seema S.M., Jung H.C. (2000) Lutein and zeaxanthin concentrations in Rod Outer segment membranes from perifoveal and peripheral human retina. *Investigative Ophthalmology and Visual Science*, Vol. 41, No. 5, p. 1200–1209.
24. Li A.N., Li H.B., Xu D.P., Xu X.R. (2014) Total phenolic content and antioxidant capacities of 51 edible and wild flowers. *Journal of Functional Foods*, Vol. 6, p. 319–330.
25. Loranty A., Rembialkowska E., Rosa E.A.S., Bennett R.N. (2010) Identification, quantification and availability of carotenoids and chlorophylls in fruit, herb and medicinal teas. *Journal of Food Composition and Analysis*, Vol. 23, p. 432–441.
26. Mayne S.T. (1996) Beta-carotene, carotenoids, and disease prevention in humans, *The FASEB Journal*, Vol. 10, p. 690–701.
27. Mills S. (1999) *The essential Book of Herbal Medicine*. Penguin Books Ltd, Harmondsworth, Middlesex, 765 p.
28. Mlcek J., Rop O. (2011) Fresh edible flowers of ornamental plants – A new source of nutraceutical foods. *Trends in Food Science and Technology*, Vol. 22, p. 561–569.
29. Moehs C.P., Tian L., Osteryoung K.W., DellaPenna D. (2001) Analysis of carotenoids biosynthetic gene expression during marigold petal development. *Plant Molecular Biology*, Vol. 45, p. 281–293.
30. Mohammad S.M., Kashani H.H. (2012) Pot marigold *(Calendula officinalis*) medicinal usage and cultivation. *Scientific Research and essays*, Vol. 7, No. 14, p. 1468–1472.
31. Muley B.P., Khadabadi S.S., Banarase N.B. (2009) Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): A review. *Tropical Journal of Pharmaceutical Research,* Vol. 8, No. 5, p. 455–465.
32. Olson J.A., Krinsky N.I. (1995) Introduction: the colourful fascinating world of the carotenoids: important physiologic modulators, *The FASEB Journal*, Vol. 9, p. 1547–1550.
33. Piccaglia R., Marotti M., Chiavari G., Gandini N. (1999) Effects of harvesting date and climate on the flavonoid and carotenoid content of marigold (*Calendula officinalis* L.). *Flavour and Fragrance Journal*, Vol. 12, p. 85–90.
34. Pintea A., Bele C., Andrei S., Socaciu C. (2003) HPLC analysis of carotenoids in four varieties of *Calendula officinalis* L. flowers. *Acta Biologica Szegediensis*, Vol. 47, No. 1–4, p. 37–40.
35. Pratheesh V.B., Benny N., Sujatha C.H. (2009) Isolation, stabilization and characterization of xanthophyll from marigold flower – Tageteserecta-L. *Modern Applied Science*, Vol. 3, No. 2, p. 19–28.
36. Preethi K.C., Kuttan G., Kuttan R. (2009) Anti-inflammatory activity of flower extract of *Calendula officinalis* Linn. Andits possible mechanism of action. *Indian Journal of Experimental Biology*, Vol. 47, p. 113–120.
37. Raal A., Kirsipuu K., Must R., Tenno S. (2009) Content of total carotenoids in *Calendula officinalis* L. from different countries cultivated in Estonia. *Natural Products Communications*, Vol. 4, No. 1, p. 35–38.
38. Ramawat K.G., Merillon J.M. (2013) Tetraterpenes: Carotenoids. **In**: *Natural Products*. Vol. 1: Phytochemistry, Botany and Metabolism of alkaloids, phenolics and Terpenes. Ramawat K.G., Merillon J.M. (eds.). Germany: Springer Berlin Heidelberg, p. 3251–3283.
39. Richard L.R. (2013) Lutein, Zeaxanthin, and skin health. *American Journal of Lifestyle Medicine*, Vol. 7, No. 3, p. 182–185.
40. Rock C.L. (1997) Carotenoids: biology and treatment. *Pharmacology & Therapeutics*, Vol. 75, No. 3, p. 185–197.
41. Rodriguez-Amaya D.B. (2010) Quantitative analysis, *in vitro* assessment of bioavailability and antioxidant activity of food carotenoids – A review. *Journal of Food Composition and Analysis*, Vol. 23, p. 726–740.
42. Rubine H., Eniņa V. (2004) *Ārstniecības augi.*Rīga: Zvaigzne ABC, 344 lpp.
43. Sausserde R., Kampuss K. (2014) Calendula (*Calendula officinalis* L.) – promising medicinal plant. **In:** *Proceedings of the Scientific and Practical Conference "Harmonious Agriculture"*, Latvia, Jelgava, p. 161–165.
44. Scherf G. (2004) *Plane rostliny a jejichpouziti v kuchyni*. Praha: Beta-Dobrovsky, p. 8–15.
45. Sies H., Stahl W. (2004) Nutritional protection against skin damage from sunlight. *Annual Review of Nutrition*, Vol. 24, p. 173–200.
46. Stahl W., Heinrich U., Jungmann H., Sies H., Tronnier H. (2000) Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. *American Journal of Clinical Nutrition*, Vol. 71, p. 795–798.
47. Stahl W., Sies H., (2005) Bioactivity and protective effects of natural carotenoids. *Biochimica et BiophysicaActa (BBA) – Molecular Basis of Desease*, Vol. 1740, p. 101–107.
48. Tanaka Y., Sasaki N., Ohmiya A. (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, Vol. 54, 733–749.
49. Tanji A., Nassif F. (1995) Edible weeds in Marocco. *Weeds Technology*, Vol. 9, p. 617–620.
50. Toom M., Vares L., Kirsipuu K., Must R., Tenno S., Raal A. (2007) Content of the carotenoids in the pot marigold's sorts cultivated in Estonia. *European Journal of Pharmaceutical Sciences*, Vol. 32, No. 1, p. S. 25.
51. Verghese J. (1998) Focus on xanthophylls from TagetesErecta L. the giant natural complex-I. *Indian Spices*, Vol. 33, No. 4, p. 8–13.
52. Zhu C., Bai C., Sanahuja G., Yuan D., Farre G., Naqvi S., Shi L., Capell T., Christou P. (2010) The regulation of carotenoids pigmentation in flowers. *Archives of Biochemestry and Biophysics*, Vol. 504, p. 132–141.