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Editorial
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Carotenoids of Therapeutic Significance from Marigold

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Use of flowers and plants for worshipping, decoration and personal adornment is an integral part of Indian cultural heritage since pre-historic times. Marigold is a potential ornamental plant grown commercially in different parts of the world and obtained from different species of *Tagetes* of family Asteraceae. The essential oil from *Tagetes erecta* flowers has been used in high class perfumery and also acts as antihaemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, diaphoretic and emmenagogue. The oil is very valuable in aromatherapy for its powerful skin healing effects and also possesses fly repellant properties. Marigold varieties have pesticidal value as they destroy ground pests particularly nematodes. The genus is also recognized as a potential source of very interesting biologically active products *viz.* carotenoids that are currently being used as food colorants, nutritional supplements and poultry feed additives and in ophthalmology for the treatment of age related ocular diseases *viz.* cataract and dry age related macular degeneration (ARMD). Several species of the genus *Tagetes* find tremendous application as per the traditional database for the treatment of various ailments and diseases.

Carotenoids from *Tagetes* species

The extracts of *Tagetes erecta* were earlier reported to contain epoxides such as lutein 5, 6-epoxide and other oxidation products of lutein. Some preliminary studies during the same phase demonstrated the predominance of lutein and zeaxanthin (88-92%) out of 17 different separated pigments, while less than 3% were reported to be epoxy pigments [1]. The carotenoid composition of marigold extract product was analyzed later via HPLC whereby epoxides namely violaxanthin and neoxanthin were it should be reported [2]. The saponified *Tagetes erecta* flower extract was reported for 93% utilizable pigments (detected at 450 nm), consisting of all *trans* and *cis*-isomers of zeaxanthin (5%), all *trans* and *cis*-isomers of lutein, and lutein esters (88%). Among the lutein isomers, *trans*-lutein was reported as the major component with several *cis*-lutein isomers as minor components from the commercial marigold flower extract. The yield of xanthophyll content in well preserved flowers of *Tagetes erecta* was reported to be 105.19 g/kg in contrast to the unpreserved flower sample (58.87 g/kg), thereby emphasizing the significance of flower preservation during the extraction of xanthophylls (Figures 1 and 2) [3].

Purification of carotenoids

On a commercial scale, marigold extract saponification is accomplished by dissolving the extract in ethyl ether and mixing it with 15% potassium hydroxide in methanol for 1h in the absence of light. This mixture is then washed with deionized water until a neutral pH is reached. The lower aqueous phase is washed with ethyl ether for re-extraction until the aqueous phase is colorless in white light (i.e. no carotenoids present). The re-extracted ethyl ether is then combined with the original upper solvent phase, which contains the lipid and carotenoid compounds, to be passed through sodium sulfate to dry. The sample is finally evaporated to dryness under nitrogen and stored frozen with nitrogen headspace at -20°C until further use [4]. For chromatographic and spectral analysis, dried samples are brought to the desired volume with the appropriate mobile phase. Carotenoids in the petal extracts are identified by their retention times in HPLC and by their UV/Visible absorption spectra compared to reference standards. For measurements and spectral determination, the diode array measurements of spectral properties for the individual peaks (from 300 to 600 nm) are determined at the up slope, apex and down slope for checking of peak purity [5].

Briefly, HPLC determination is usually carried out on normal phase silica columns using photodiode array detector in a range from 250 to 550 nm using hexane/ethyl acetate (75:25) mobile phase at a flow rate of 2 ml/min while the reverse phase HPLC is performed using silica C_{30} column using an isocratic mobile phase consisting of 3% methyl *tert*-butyl ether in methanol with a flow rate of 1 ml/min. For the mass spectrometry, positive ion electrospray ion source is generally used with samples redissolved in methanol/methyl *tert*-butyl ether (50:50 v/v) and infused at a rate of 10 µl/min. In addition, measures are also in progress to enhance carotenoids extraction at industrial level by using enzymes such as econase-CEP that have been reported to increase the extraction yield from 1.7 to 7.4 g/kg of marigold flower (*Tageteserecta*) in dry weight [6].

Commercial Applications

African marigold (*Tageteserecta*) petals are commercially valuable as a natural source of lutein (yellow-orange) pigments and are primarily being used by the poultry industries as feed additives to color egg yolks orange and poultry skin yellow. The orange egg yolks are generally considered as healthy by the consumers in comparison to the colorless bland products and therefore there arises a considerable need for the inclusion of these pigments as feed additives in order to attain the desired color since birds lack the ability to synthesize them. Lutein is the primary xanthophyll pigment that produces the orange color in marigold flowers, roughly comprising of 90% of the total pigments from the petals. Additionally, marigold has been most commonly used by the poultry industries to augment the xanthophyll present in corn and alfalfa feed to standardize the feed's xanthophyll content. Poultry uses carotenoids for pigmentation as the color of poultry skin is provided by these pigments and they are also involved in growth metabolism and fertility. Some carotenoids even serve as precursors for the synthesis of vitamin A while some provide protection against damaging reactions in the body, acting as physiological antioxidants and thereby enhancing immune responses. Carotenoids are required by the immune system where they act as detoxifiers neutralizing free radicals before they damage DNA, lipids & proteins. Poultry cannot synthesize these compounds and must obtain carotenoids from their diets. The amount and availability of carotenoids in poultry feed ingredients fluctuate considerably and it has therefore been a common practice by the poultry industries to add carotenoids to the feed to assure the necessary amount of pigments in order to achieve the

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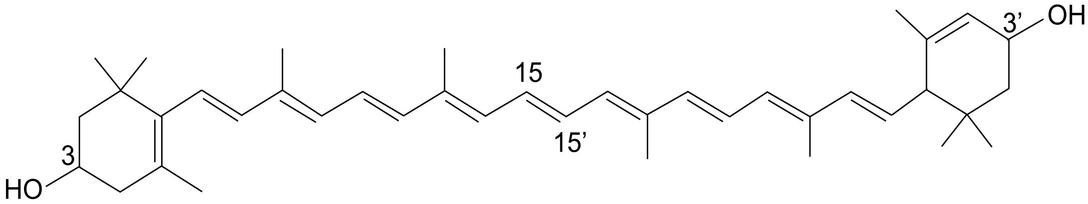

Figure 1: Lutein.

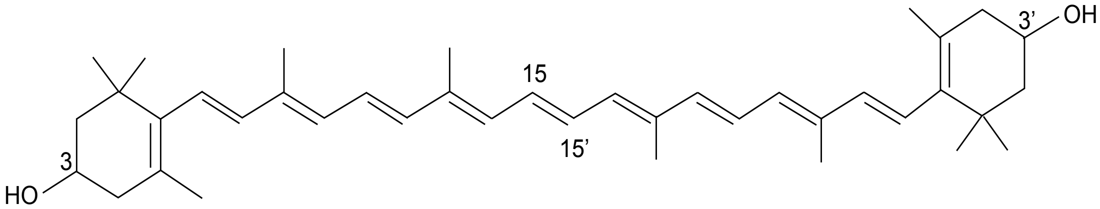

Figure 2: Zeaxanthin.

desired color.

Carotenoids furnish flowers and fruits with distinct colors ranging from yellow to red and are essential components for photosynthesis and they also play an important role in human nutrition and health, participating in pro-vitamin A and anticancer activities. Lutein and zeaxanthin are the only dietary carotenoids present in the macular region of the retina and in the lens of the eye and some epidemiologic studies have shown that the risks of ARMD and cataract are inversely correlated with dietary intake and the concentration of xanthophylls in the serum and macula. However, the consumption of lutein and zeaxanthin reduces around 40% of the dry age related macular degeneration and marigold flower petals offer the richest source of these xanthophylls particularly *Tagetes erecta* and *Tagetes patula*. Lutein and its isomer zeaxanthin are naturally existing in the flower in the ester form, normally as acetyl (palmitate) ester and are together present in the macular region of the eye where they make up the macular pigment and protect the eyes from oxidative damage by filtering out damaging near-UV blue light. As antioxidants, they inhibit the formation of damaging free radicals by quenching singlet oxygen.

Lutein and zeaxanthin from *Tagetes erecta* have been used as coloring agents and nutritional supplements (food additives) in a wide range of baked foods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes in levels ranging from 2 to 330 mg/kg for lutein and 0.5 to 70 mg/kg for zeaxanthin. Xanthophylls are one of two classes of carotenoid pigments which are also beneficial as a natural pigment source and have many commercial applications. Carotenoid pigments have shown positive benefits in slowing the growth of induced skin tumors, treating dermatological diseases and lowering overall risk of cancer in human beings. Lutein has a special pharmacological use as an ophthalmological ointment with the trade name Adaptionol. Thus, the potential for broad commercial use of carotenoids should generate further interest in marigold as an alternative crop throughout the world as of now it is mainly grown for pigment production in Mexico, Peru and India.

A large variety of phytoconstituents have been revealed in the genus *Tagetes*, of which carotenoids lutein and zeaxanthin have been proven to be of great therapeutic concern. These xanthophyll esters are currently under preclinical trials for the treatment of age related ocular diseases like cataract and dry ARMD as they have successfully slowed down the progression of these diseases due to their antioxidant properties and have also played an important role in improving visual acuity. Therefore, further exploration of the pharmacological potential of all the carotenoids from *Tagetes* species may lead to generation of new leads in future for the treatment of severe, life threatening and age related disease.

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