



Extraction of Major Carotenoids from Flower Petals

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ABSTRACT

Carotenoids extraction and quantification was carried out from flowers of Yellow bloom, flame tree, Gerbera, yellow rose, solid aster and Golden aster. Six solvents viz., Hexane, acetone, ethanol, Chloroform, Petroleum ether and diethyl ether were used for extraction of carotenoids. The extracted carotenoids were quantified using UV-visible spectrophotometer by comparison with standard carotenoids. There are significant differences in the distribution of carotenoid components depending on the color, nature, and variety of selected flowers. Among the various carotenoids analyzed xanthophyll violaxanthin was found to have maximum concentration 12 µg/g, in Yellow bloom flowers. In Gerbera, the carotenoid antheraxanthin was found to possess the higher concentration of 11.91µg/g. The carotenoid auroxanthin obtained from ethanolic extracts has the higher concentration of 48.64 µg/g, 10.2µg/g, 22.1 µg/g, 29.1 µg/g from flowers of flame tree, yellow rose, solid aster and Golden aster respectively.

Keywords: Carotenoids, Ethanolic extracts, Solvent extraction.

INTRODUCTION

Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae, and photosynthetic bacteria. They comprise a class of natural lipid-soluble compounds which are found in numerous vegetables and fruits.¹ These are richly colored molecules which are the sources of yellow, orange, and red colors of many plants.² Carotenoids are singlet excellent oxygen scavenger and are used as food colorant, food additive, cosmetics, nutraceuticals etc.^{3,4} Animals are incapable of carotenoid biosynthesis, thus their carotenoids are diet derived, selectively or unselectively absorbed, and accumulated unchanged or modified slightly into typical animal carotenoids. Fruit and vegetables provide most of the carotenoids in the human diet. Carotenoids can be broadly classified into two classes, carotenes (α -carotene, β -carotene, and lycopene) and xanthophylls (β -cryptoxanthin, lutein, and zeaxanthin). Carotenes are found to serve as a source of vitamin A.⁵ In plants, carotenoids have the important antioxidant function of quenching (deactivating) singlet oxygen, an oxidant formed during photosynthesis.⁶ The role of antioxidant compounds like carotenoids in the prevention of cardiovascular disease has been studied.⁷ The majority methods of extraction of carotenoids from plant sources make use of organic solvents such as hexane, ethanol, acetone, methanol, tetrahydrofuran, benzene, and petroleum ether.⁸⁻¹⁰ It has been observed that the stability of carotenoid extracts obtained with hexane/acetone or hexane/ethanol was higher than that of extracts obtained with other organic solvents, such as chloroform, methanol or dichloromethane.¹¹ Solvent extraction method has been always the primary option as far as industrial point is concerned due to its simplicity and low costs.

MATERIALS AND METHODS

Sample Preparation

The flowers used for the study were Solidaster, Golden aster, Rose, Gerbera, Flame tree and Yellow bloom. The flowers used were bought fresh from the market and washed under tap water, cleaned with sterile water and air dried at room temperature. The raw materials were chosen based on their availability and also on the basis of information gathered during literature studies. The scientific and local names of the selected flowers that were used in this study were mentioned in Table 1.

Table 1: Scientific, family, common and local names of the plants investigated

Scientific name	Family name	Common name
<i>Solidasterlutens</i>	Asteraceae	Solidaster
<i>Chrysopsis</i>	Asteraceae	Golden aster
<i>Rosa</i>	Rosaceae	Rose
<i>Gerbera daisy</i>	Asteraceae	Gerbera
<i>Delonix regia</i>	Fabaceae	Flame tree
<i>Delonix regia var. flavida</i>	Fabaceae	Yellow Bloom

Extraction

Six different solvents such as Petroleum ether, diethyl ether, acetone, ethanol, hexane and Chloroform were used. Carotenes are readily soluble in petroleum ether, hexane, and toluene; xanthophylls dissolve better in methanol and ethanol. 2 g of flower petals were weighed and grounded in pestle and mortar with 5 ml of solvent. The volume was then made upto 20 ml with the respective solvent and the solution was filtered using Whatman No. 1 filter paper. The filtrate was taken in the separating funnel and 20 ml of Distilled water was added



along with 10ml of 10% KOH. The mixture was shaken vigorously and kept undisturbed for separation. The upper phase containing carotenoids was collected and its concentration was determined.

Measurement of Absorbance

Carotenoids in solution obey the Beer-Lambert law- their absorbance is directly proportional to the concentration. Thus, carotenoids were quantified spectrophotometrically. Absorbance was measured using UV-Vis spectrophotometer. The λ_{max} values of common carotenoids are obtained from Britton's 1995 compilation.¹²

Concentration of carotenoids was calculated using the following formula:

$$\text{Concentration of carotenoids } X (\mu\text{g}) = \frac{A \times Y (\text{ml}) \times 10^5}{A^{1\%} \text{ cm} \times 100}$$

$$X (\mu\text{g/g}) = \frac{X (\mu\text{g})}{\text{Weight of sample in (g)}}$$

Where A = Absorbance, Y = Volume (ml), $A^{1\%} \text{ cm}$ = Absorption coefficient. The absorption coefficient $A^{1\%} \text{ cm}$ of a carotenoid (absorbance at a given wavelength of a 1% solution in spectrophotometer cuvette with a 1-cm light path) used in the calculation of the concentration also varies pronouncedly in different solvents.

Test for carotenoids

The color of sample containing pigments in solvent disappears after the addition of 5% solution of sodium nitrite and 0.5M H_2SO_4 . This test was performed for samples in order to confirm the presence of carotenoids.

RESULTS AND DISCUSSION

The carotenoid extraction was carried out in all the sources mentioned above with different solvents such as Petroleum ether, hexane, diethyl ether, chloroform, acetone and ethanol, the most preferred solvents for extracting carotenoids. The concentration of carotenoids like antheraxanthin, α - carotene, β - carotene, γ - carotene, δ - carotene, Lycopene, Astaxanthin, auroxanthin, bixin, canthaxanthin, crocetin, lycoxanthin, mutatochome, rubixanthin and xanthophylls such as violaxanthin, neoxanthin and zeaxanthin were estimated. The color of the flower petals used in the study varies from red to yellow. The carotenoid content in the flowers of flame tree (Figure 1), Yellow bloom (Figure 2), Gerbera (Figure 3), Rose (Figure 4), solid aster (Figure 5) and Golden aster (Figure 6) were illustrated graphically. Extraction duration, solvent-solid ratio and extraction temperature were assumed to be the most important factors affecting solvent extraction for the determination of carotenoids.

In Flame tree among the various carotenoids quantified, auroxanthin was found to be in higher concentration of 48.64 $\mu\text{g/g}$ from the ethanolic extracts. The yield of carotenoid astaxanthin was lowest 2.9 $\mu\text{g/g}$ from the extracts of Hexane. In flame tree flowers the

concentration of xanthophyll such as Violaxanthin (38.72 $\mu\text{g/g}$), Neoxanthin (38.72 $\mu\text{g/g}$) and Zeaxanthin (36.69 $\mu\text{g/g}$) was found to be maximum among the xanthophylls quantified in other flowers employed for this study. Carotenoids are non polar in chemical nature, and therefore shows their higher affinity towards polar solvents which is well documented by several earlier findings.¹³⁻¹⁵

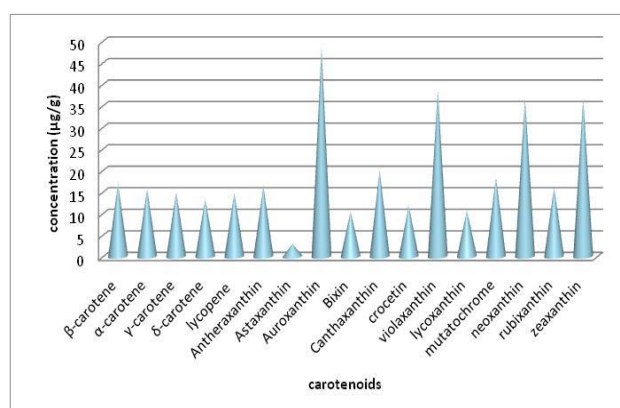


Figure 1: Carotenoids from flame tree flowers

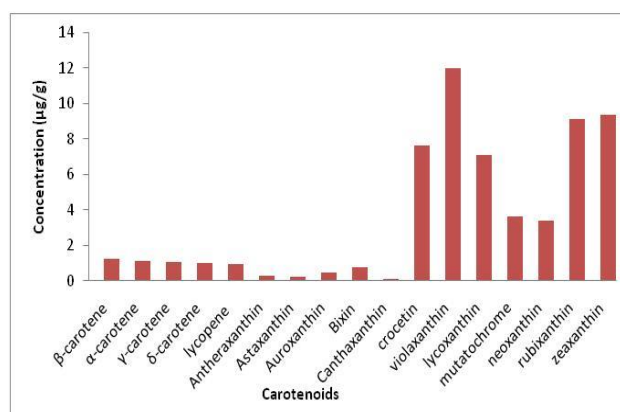


Figure 2: Carotenoids from Yellow bloom flowers

In yellow bloom, the Xanthophyll violaxanthin from extracts of ethanol was found to have higher yield 12 $\mu\text{g/g}$. The yield of carotenoid canthaxanthin 0.13 $\mu\text{g/g}$ was found to be lowest from the extracts of petroleum ether.

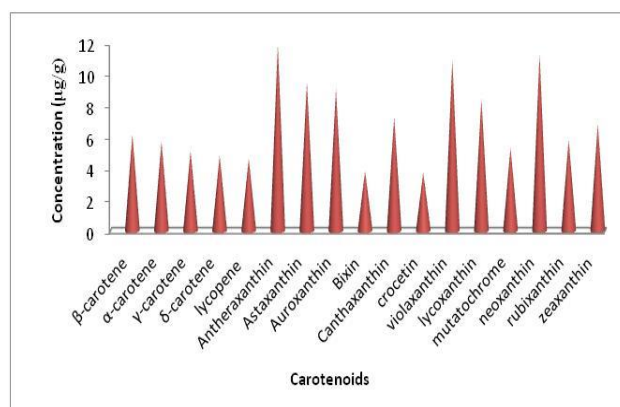


Figure 3: Carotenoids from Gerbera

In Gerbera the concentration of carotenoid, antheraxanthin obtained from the ethanolic extracts was

found to be higher 11.91 $\mu\text{g/g}$. The yield of carotenoid crocetin from petroleum ether extracts was lower 3.7 $\mu\text{g/g}$ among the various carotenoids analysed.

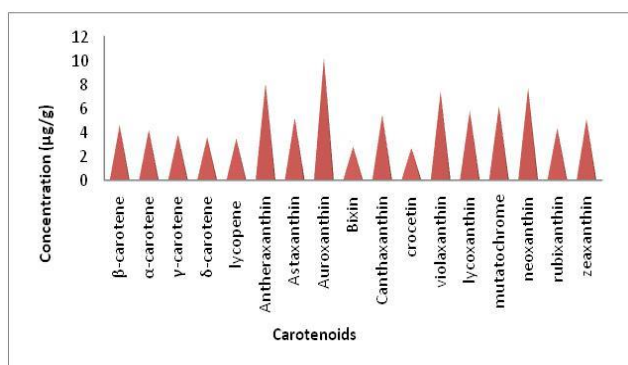


Figure 4: Carotenoids from Yellow Rose

The Yellow Rose petals were found to contain the auroxanthin carotenoid in higher concentration 10.2 $\mu\text{g/g}$ from ethanolic extracts. The yield of carotenoid crocetin was lower 2.7 $\mu\text{g/g}$ from the extracts of petroleum ether.

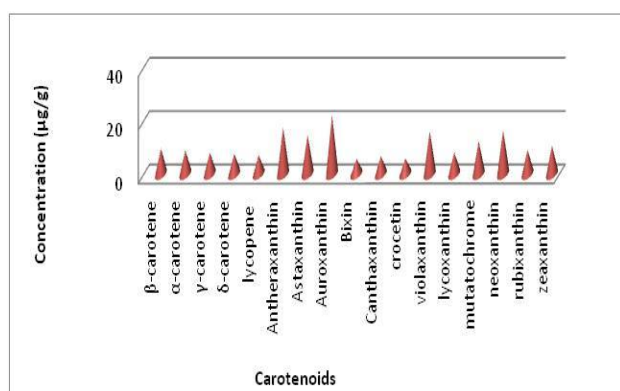


Figure 5: Carotenoids from solid aster

The petals of solid aster was found to contain the carotenoid, auroxanthin 22.1 $\mu\text{g/g}$ in higher concentration from ethanolic extracts and the carotenoid bixin had the least yield of 5.7 $\mu\text{g/g}$ from extracts of petroleum ether.

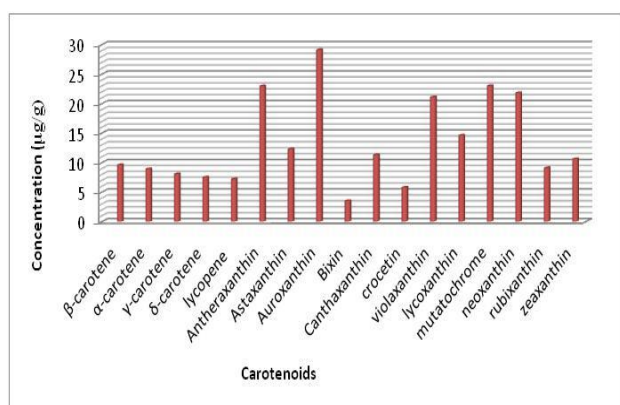


Figure 6: Carotenoids from Golden aster

In Golden aster the carotenoid auroxanthin predominates and the concentration was found to be higher in Ethanolic

extracts 29.1 $\mu\text{g/g}$. The yield of carotenoid bixin 3.5 $\mu\text{g/g}$ was found to be low in the extracts of petroleum ether.

CONCLUSION

For carotenoid extraction much work has been focused on vegetables, which was found to be the major source. As far as flowers are concerned, many publications are from marigold. From the present study, it can be concluded the flowers investigated can be exploited for carotenoid extraction and the yield depends on the chemical nature of solvents. However, purification, optimization and scale up studies are needed for commercialization.

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