

# Isolation of $\beta$ -Carotenoids for Yellow Pigment as Food Colorant with Antioxidant Compound from *Cucurbita moschata* Flower

Yan YW, Simon SE\* and Jayakumar FA

Department of Biotechnology, Malaysia University of Science and Technology, Kota Damasara, Selangor, Malaysia

## Research Article

Received Date: 08/03/2017

Accepted Date: 20/03/2017

Published Date: 27/03/2017

### \*For Correspondence

Samson Eugin Simon, Department of Biotechnology, Malaysia University of Science and Technology, Kota Damasara, Selangor, Malaysia, Tel: +60174238264.

**E-mail:** samson.eugin@pg.must.edu.my

**Keywords:** Carotenoids, Antioxidants, *Cucurbita moschata*, Food colourant, DPPH, Thin layer chromatography

### ABSTRACT

In the current study, the carotenoid pigment was extracted from the corolla of *Cucurbita moschata* as food colourant with antioxidant property using acetone and hexane. The pure carotenoid was separated by giving water, brine and solvent wash. These carotenoid extract were further used for the TLC analysis. The carotenoid extract was compared with the  $\beta$ -carotene standard and resulted with the two spots. The R<sub>f</sub> value was 0.786 (dark orange) and 0.12 (light yellow). Antioxidant assay using TLC was done using DPPH method. Yellow and white band formation confirms that the carotenoid extract having antioxidant. The absorbance was scanned for the carotenoid extract using UV/VIS spectrophotometer and was found to have four compounds at 662.5, 613.0, 497.0, and 436.5 nm. Carotenoid test was carried out for the respective sample. Acetone disappearance confirms that the sample contain carotenoids. Two antioxidant activities were determined for the carotenoid extract. The Phosphomolybdenum method results with the 75.549% of antioxidant concentration and ferric ion reducing antioxidant power assay (FRAP) reveals with the 2.916% of antioxidant concentration. The carotenoid pigment not only plays a role in pharmaceutical, but also in nutraceutical applications. Thus the carotenoid content with antioxidant was detected by UV/VIS spectrophotometer and the compound was separated by column chromatography as food colourant.

## INTRODUCTION

Carotenoids are red, yellow and orange pigments that are widely distributed in nature. They are especially abundant in yellow-orange fruits and vegetables and dark green, leafy vegetables<sup>[1]</sup>. Carotenoids have an immense value for human health, and many people are seeking ways to incorporate these health-brimming compounds into their diet<sup>[2]</sup>. Nutritionists, physicians, and health organizations all recommend a diet high in antioxidants, including those that are derived from carotenoids. The most pronounced benefits of carotenoids are supports eye health, cardiovascular health, possible anti-tumor properties, male fertility, and skin health<sup>[3]</sup>. *Cucurbita moschata* is a species originating in either Central America or northern South America. But, carotenoid content was identified different in pumpkin from localities in Malaysia. Carotenoid content in fruits and vegetables varies due to certain factors such as variety, level of maturity, climate or geographic site of production, part of the plant utilized, environment conditions during agricultural production, post-harvest handling, processing, and storage conditions<sup>[4]</sup>. The pumpkins showed a large variability in the contents of total carotenoids varying, in fresh pumpkins from samples cooked in boiling water and steamed cooked<sup>[5]</sup>. Pumpkins were grown in the experimental field of Warsaw Agricultural University and determined the dry matter, soluble solids content, total carotenoids content, nitrates content, colour of flesh, antioxidant activity with DPPH method. Significant relationship between carotenoids content and antioxidant activity of pumpkin fruits was found<sup>[6]</sup>. Carotenoid pigments provide orange colors and antioxidant protection to a wide variety of plants, animals, bacteria, and fungi. They also serve as precursors to other biologically important compounds<sup>[7]</sup>. Oxidative stress is an important contributor to the risk of chronic diseases. Dietary guidelines recommend increased consumption of fruits and vegetables to combat the incidence of human diseases<sup>[8]</sup>.

## MATERIALS AND METHODS

### Sample Collection

Crookneck pumpkin known as *Cucurbita moschata* is known as Parangi in Tamil, where its flower was used for the project. The flowers were purchased from the local markets of Malaysia. The corolla was removed from the flowers and shadow dried. The dried corolla was powdered by the blender. The powdered sample was weighed using weighing balance and used for further studies.

### Extraction of Carotenoids from the Corolla of *Cucurbita Moschata*

The powdered sample of *Cucurbita moschata* corolla (2 g) was taken and grinded well using mortar and pestle. Solvent namely acetone and hexane of equal proportion was added to extract the pigment from the sample. Then magnesium sulphate was added to remove the moisture content. The extract was filtered using anhydrous sodium sulphate (to remove the water content from the extract). To extract of carotenoid, the sample extract was given a water wash in the separating funnel. Two phases namely the upper phase (carotenoid) and the lower phase (water) were formed. The water phase was discarded. The brine wash was given. The lower phase was discarded. Solvent of acetone and hexane was washed to the sample to get a pure carotenoid extract, which was stored in dark place. Using rotary evaporator the pure carotenoids was dehydrated and powdered.

### Screening of Carotenoid using TLC

TLC silica gel plates of 60 F254 Aluminium sheets (20 x 20 cm) were used for screening the carotenoid. Petroleum ether and acetone (9:1) was used as mobile phase. A space of 1 cm between each spotted sample was retained to spot the sample. Beta-carotene as a standard and pure extract of carotenoid from the *Cucurbita moschata* corolla was allowed to run. Then TLC plate was viewed under UV chamber. R<sub>f</sub> value was calculated with the help of fluorescent spots, which was marked.

$$R_f = \text{Distance travel by the sample} / \text{Distance travel by the solvent}$$

### Antioxidant Assay by TLC

Carotenoids in TLC plate were determined by spraying spots with a DPPH (diphenylpicrylhydrazyl) ethanol solution for derivatization. Spots sprayed with the DPPH were observed for white to yellow bands on a purple background. Absorbance of carotenoid was scanned by UV-VIS Spectroscopy using acetone and hexane as a blank and sample were recorded over the wavelength range of 300 to 700 nm using quartz cuvettes with 1 cm diameters.

### Qualitative Analysis for Carotenoid

The colour of a solution of the sample in acetone disappears after addition of a 5% solution of sodium nitrite and 0.5 M of sulphuric acid.

### Total Antioxidant Capacity (TAC) by Phosphomolybdenum Method

To a series of fresh tubes respective volumes of ascorbic acid standard and sample was added. The volume was made up to 1 ml with distilled water. Reagent solution of 3 ml was added to the entire test tubes and vortexed well. The test tubes were incubated at 90 °C for 90 minutes. After cooling the tubes, the absorbance was read at 695 nm. A standard graph equivalent of ascorbic acid was determined to the antioxidant concentration of sample.

### FRAP-Ferric Ion Reducing Antioxidant Power Assay

To the test tubes, individual volume of standard and sample was added. Phosphate buffer (0.2 M) of 2.5 ml of volume was made to the respective test tubes. Potassium ferric cyanide (1%) of 2.5 ml was added to all the tubes and vortexed well. The test tubes were incubated at 50 °C in water bath for 20 minutes. The tubes were cooled and trichloroacetic acid (10%) of 2.5 ml was added. The tubes were vortexed well and 2.5 ml of distilled water was added. Ferric chloride (0.1%) of 0.5 ml was added to all the tubes and vortexed. The tubes were incubated in room temperature for 10 minutes. The absorbance was read at 700 nm.

## RESULTS AND DISCUSSION

The ornamental plants usually used to decorate the house as well as the environmental surroundings. Some people decorate flowers at the winter season in front of their house or at the auspicious day. These flowers, as soon as dried, were thrown as waste. Without the knowledge of ours, these types of flowers aid in health benefits as well as play a pertinent role in pharmaceutical applications. However, to the best of our knowledge, no single report is accessible in the global level, relating carotenoid extraction from the corolla of *Cucurbita moschata*. In the previous study, the carotenoids were extracted from the flowers, leaves, fruits, vegetables<sup>[8,9]</sup>.

In the present study, the carotenoids were extracted from the corolla of *Cucurbita moschata* using different solvents namely acetone and hexane in equal proportion (**Figures 1-4**). Finally the pure carotenoid extract was separated by giving water, brine and solvent wash. Thus the pure carotenoid extract was used for further experiments<sup>[10]</sup>.

Screening of TLC was done for the carotenoid extract using mobile phase of petroleum ether and acetone (7:3). The carotenoid extract was compared to the standard of  $\beta$ -carotene. Two spots were found (Dark orange and light yellow). Antioxidant assay was found by dipping TLC in DPPH with ethanol <sup>[11]</sup>. The carotenoid extract, results with yellow and white band formation in a purple colour background. The absorbance was scanned for the carotenoid extract, which was found to have four compounds at 662.5, 613.0, 497.0, and 436.5 nm.

Qualitative analysis was found for the carotenoid extract. Acetone disappearance in the sample was confirmed for its carotenoid test. Antioxidant activity was done for the carotenoid extract <sup>[12]</sup>. The corolla of *Cucurbita moschata* results with the 75.549% of antioxidant concentration by the phosphomolybdenum method and 2.916% of antioxidant concentration by ferric ion reducing antioxidant power assay (FRAP) (Tables 1-3).

**Table 1.** Rf values for  $\beta$ - carotene standard and carotenoid extract from the corolla of *Cucurbita moschata* (Mobile phase- Petroleum ether: acetone).

Sample	Spots (From Top)	Rf Value	Rf Value
$\beta$ -carotene Standard	Spot 1	Spot 1	Dark orange
<i>Cucurbita moschata</i>	Spot 1	0.786	Dark orange
	Spot 2	0.12	Light yellow

**Table 2.** OD values for the carotenoid extract from the corolla of *Cucurbita moschata*. Evaluation of carotenoid extract from the corolla of *Cucurbita moschata* by total antioxidant capacity (TAC) phosphomolybdenum method using UV/vis spectrophotometer.

Sample	OD (695 nm)
S1	0.031
S2	0.108
S3	0.166
S4	0.253
S5	0.286
C1	0.361
C2	0.509

**Table 3.** OD values for the standard and the sample. Estimation of antioxidants from the carotenoid extract of *Cucurbita moschata* corolla by FRAP-ferric ion reducing antioxidant power assay.

Sample	OD at 700 nm
S1	0.157
S2	0.188
S3	0.566
S4	0.642
S5	0.033



**Figure 1.** Carotenoid extract and powder from the corolla of *Cucurbita moschata*.

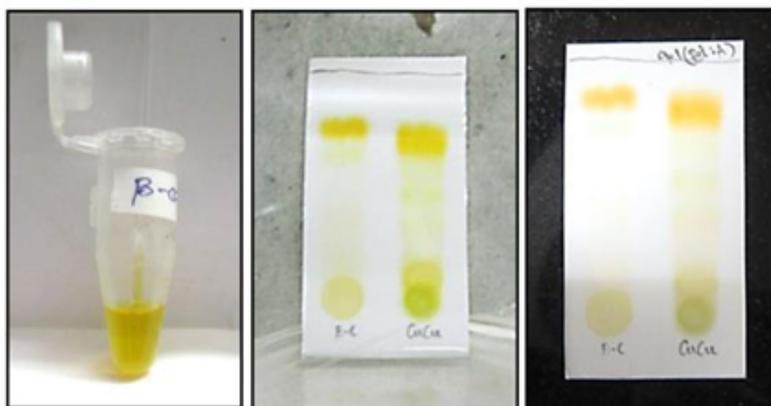


Figure 2. TLC analysis for carotenoid from the corolla of Cucurbita moschata.

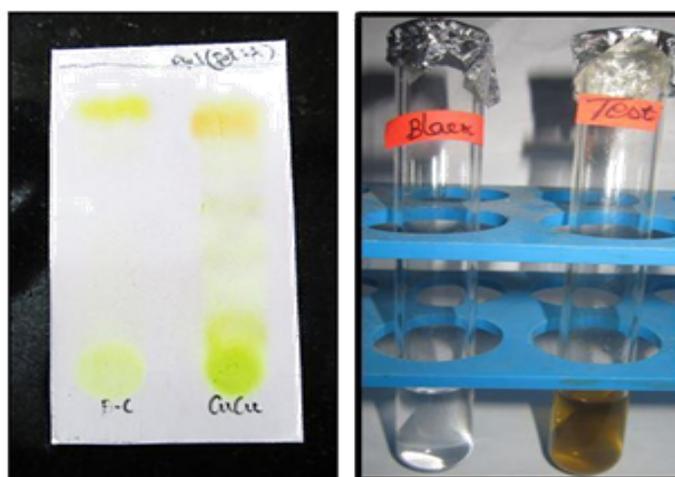


Figure 3. Antioxidant assay using TLC by DPPH method for carotenoid.

$\beta$ -carotene is used as standard and carotenoid extract as sample. Spots sprayed with the DPPH were observed for white to yellow bands on a purple background shows antioxidant presence of carotenoids.

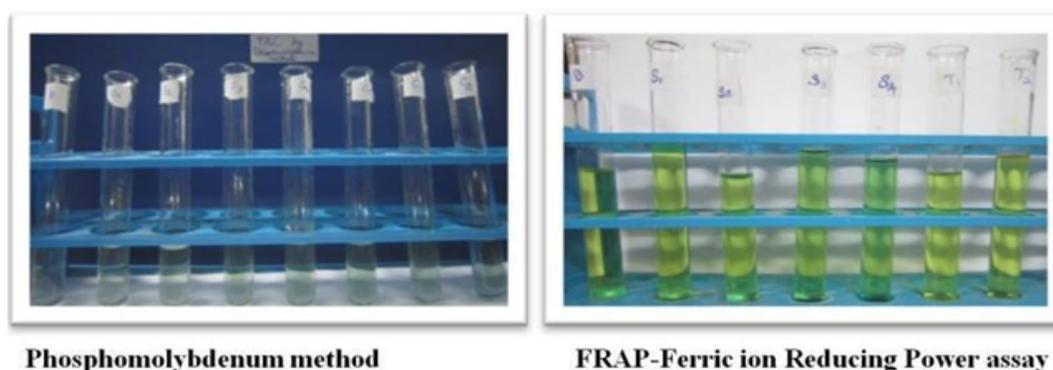


Figure 4. Antioxidant activity of carotenoid by phosphomolybdenum method and FRAP-ferric ion reducing power method.

The carotenoid extract from the corolla of *Cucurbita moschata* results with the 75.549% of antioxidant concentration by the Phosphomolybdenum method. The carotenoid extract from the corolla of *Cucurbita moschata* results with the 2.916% of antioxidant concentration by FRAP-Ferric ion Reducing Antioxidant Power assay.

## CONCLUSION

The *Cucurbita moschata* flower acts as antioxidants which has the capacity to scavenge the free radicals. The carotenoid extracted from the corolla of *Cucurbita moschata* can also be used as a dye in fabrics and cosmetics etc. The carotenoids are the bioactive compounds, which yields medicinal values like preventing eye diseases, skin health, cardiovascular health, male fertility as well as antioxidants. Thus the carotenoid pigment not only plays a role in pharmaceutical, but also in nutraceutical applications.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests regarding the publication of this paper.

## ACKNOWLEDGEMENT

It is a pleasant aspect that I now have the opportunity to express my gratitude for all who accompanied and supported during my dissertation work.

## REFERENCES

1. Bauernfeind JC. Natural food colors. In: Bauernfeind JC (ed.). Carotenoids as Colorants and Vitamin A Precursors. Academic Press, New York. 1981:1-45.
2. Antolovich M, et al. Methods for testing antioxidant activity. The Analyst. 2002;127:183-198.
3. Demmig-Adams B and Adams WW. Antioxidants in photosynthesis and human nutrition. Science. 2002;298:2149-2153.
4. Norshazila S, et al. Scheme of obtaining  $\beta$ -carotene standard from pumpkin (*Cucurbita moschata*) flesh. Int Food Res J. 2012;19:531-535.
5. Lucia MJC, et al. Total carotenoid content,  $\alpha$ -carotene and  $\beta$ -carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. Food Res Int. 2012;47:337-34.
6. Gajewski M, et al. Quality of pumpkin cultivars in relation to sensory characteristics. Not Bot Hort Agrobot Cluj. 2008;36:73-79.
7. Giuliano G, et al. Carotenoid oxygenases: Cleave it or leave it. Trends Plant Sci. 2003;8:145-149.
8. Rao AV and Rao LG. Carotenoids and human health. Pharmacol Res. 2007;55:207-216.
9. Omayma AE and Abdel Nasser BS. Carotenoids. J Pharmacogn Phytochem. 2013;2:225-233.
10. Sidney P, et al. Microscale extraction method for HPLC carotenoid analysis in vegetable matrices. Sci Agric. 2014;71:345-355.
11. Wilhelm S and Helmut S. Antioxidant activity of carotenoids. Mol Aspect Med. 2003;24:345-351.
12. Sadineni V, et al. Carotenoid composition of mango (*mangifera indica* L.) wine and its antioxidant activity. J Food Biochem. 2011;35:1538-1547.