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ANTIOXIDANT ACTIVITY OF ASTAXANTHIN

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ABSTRACT

Astaxanthin a carotenoid present in marine yeast and crustaceans posses a wide range of pharmacological activity. Shrimp of the species Aristeus alcocki was collected from Cochin, Kerala during the month of August 2012. The samples were collected and transported to the laboratory under iced conditions. The yield of dried shell was determined by weighing after dried at 50°C in oven for 24h. Samples were stored at two temperatures, of 25°C and -20°C until use. The material was thawed in running water before use and homogenized in a laboratory mixer.

KEYWORDS: Astaxanthin, Aristeus alcocki, carotenoid.

INTRODUCTION

Astaxanthin, unlike some carotenoids, does not convert to Vitamin-A (retinol) in the human body. Too much Vitamin A is toxic for a human, but astaxanthin is not. However, it is a powerful antioxidant. It is10 times more capable than other carotenoids. While astaxanthin is a natural nutritional component, it can be found as a food supplement. The supplement is intended for human, animal, and aquaculture consumption. The commercial production of astaxanthin comes from both natural and synthetic sources1.

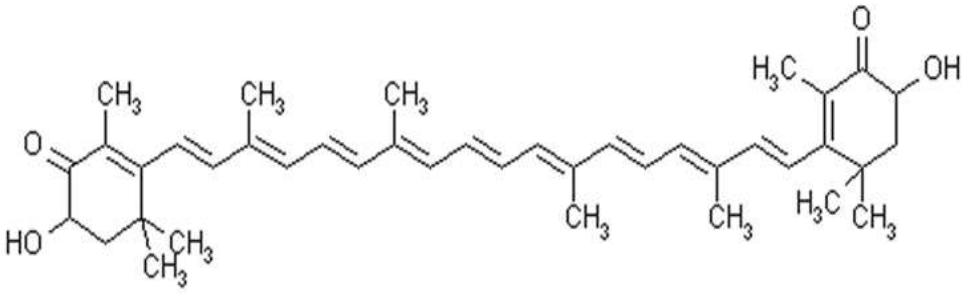

Fig. 1: Astaxanthin

The U. S. Food and Drug Administration (FDA) approved astaxanthin as a food colouring (or colour additive) for specific uses in animal and fish foods. The European Union (actually European Commission)considers it food dye within the E number system.

Astaxanthin pronounced as (as-tuh-zan'-thin) is a carotenoid. It belongs to a larger class of phytochemicals known as terpenes. It is classified as a xanthophyll, which means "yellow leaves".Like many carotenoids, it is a colorful, fat/oil-soluble pigment. Astaxanthin can be found in microalgae, yeast, salmon, trout, krill, shrimp, crayfish, crustaceans, and the feathers of some birds. Professor Basil Weedon was the first to map the structures of astaxanthin. The study of the pharmacological activity of astaxanthin is vast and wide. The aim and scope of the present work is to study the antioxidant activity of astaxanthin.

MATERIALS AND METHODS

Experimental

Sample Collection

Shrimp was collected from coastal areas of Cochin, Kerala during the month of August 2012. The samples were collected and transported to the laboratory under iced conditions. The yield of dried shell was determined by weighing after dried at 50°C in oven for 24 h. Samples were stored at two temperatures, of 25°C and -20°C until use. The material was thawed in running water before use and homogenized in a laboratory mixer.

Chemical Extraction of Astaxanthin

Astaxanthin was extracted by mixing 5g shrimp waste powder homogenate, 50 mL of hexane and 5mg of glass beads and vortexed for 30 seconds, place in the 50°C water bath for 10 minutes. Aqueous and organic layers were separated by 3000 rpm for 5 minutes. This step

repeat until the hexane is colorless. At the final step 6 mL of di- methyl sulfoxide (DMS0) was added to the tube and vortex vigorously and place in the water bath for 10 minutes and vortex again. Concentrated carotenoid was subjected to Thin Layer Chromatography (TLC) using silica gel 60 F MERCK TLC paper5.

Measurement of Superoxide Scavenging Activity

The superoxide scavenging ability of the extracts was assessed by the method of Winterbourn et al. (1975). Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the astaxanthin (20 ng/ml), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the astaxanthin. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer, (Model SL 160, UV-VIS Spectrophotometer, ELICO, India). The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

Quercetin (Sigma Chemicals, USA) was used as standard.

$$\% \text{ inhibition} = \frac{\text{OD of control - OD of test X 100}}{\text{OD of control}}$$

Inhibition of Lipid Peroxidation

Estimation of lipid peroxidation in terms of malonaldehyde (MA): Thiobarbituric acid reaction (Ohkawa et al., 1979).

The reaction mixture contained 0.1 ml of beef liver homogenate (25 % w/v) in Tris-HCl buffer (20mM, pH 7.0); 30 mM KCl; 0.16 mM $FeSO_4(NH_4)_2SO_4$. 6 H_2O; 0.06 mM ascorbate and various concentrations of astaxanthin (5 ng/ml to 50 ng/ml) in a final volume of 0.5 ml. The reaction mixture was incubated at 37^0C for 1h. After the incubation period, 0.4 ml was removed and treated with 0.2 ml of sodium dodecyl sulphate (SDS) (8.1 %); 1.5 ml of 0.8 % thiobarbituric acid and 1.5 ml of 20 % acetic acid (pH 3.5). The total volume was made up to 4 ml with distilled water and then kept in a water bath at 95-1000C for 1h. After cooling 1 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1, v/v) were added to the reaction mixture, shaken vigourously in an electric cyclomixer and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and absorbance at 532 nm was measured

using UV/VIS Spectrophotometer, (Model SL 160, UV-VIS Spectrophotometer, ELICO, India). Inhibition of lipid peroxidation was determined by comparing the OD of treatment with that of control. Catechin (Sigma Chemicals, USA) was used as the standard at a concentration ranging from 100µg/ml to 1000µg/ml.

Measurement of Hydroxyl Radical Scavenging Activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao (1990). The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of $FeCl_3$, 0.1ml of EDTA, 0.1ml of H_2O_2, 0.1ml of ascorbate, 0.1ml of KH_2PO_4-KOH buffer and and various concentrations of astaxanthin (10 ng/ml to 100 ng/ml) in a final volume of 1 ml. The mixture was incubated at 37°C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95°C for 20 minutes to develop the colour. After cooling, the TBARS formation was measured spectrophotometrically (Model SL 160, UV-VIS Spectrophotometer, ELICO, India) at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the samples. Catechin (Sigma Chemicals, USA) was used as standard at a concentration ranging from 100µg/ml to 1000µg/ml.

RESULTS

Activity	Antioxidant activity of Astaxanthin	Antioxidant activity of standard
Superoxide radical scavenging activity	26.87 µg/ml	40.11 µg/ml (Quercetin)
Inhibition of lipid peroxidation	27.15 µg/ml	451.3 µg/ml (Catechin)
Hydroxyl radical scavenging activity	57.3 µg/ml	798 µg/ml (Catechin)

CONCLUSION

Astaxanthin is a substance with wide range of pharmacological activities like antioxidant. Here the antioxidant activity is studied as it is free from the toxic effects of the available compounds with same activity.

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