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ISOLATION OF ASTAXANTHIN FROM SHRIMP WASTE AND STUDY OF ITS PHARMACOLOGICAL ACTIVITY

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ABSTRACT

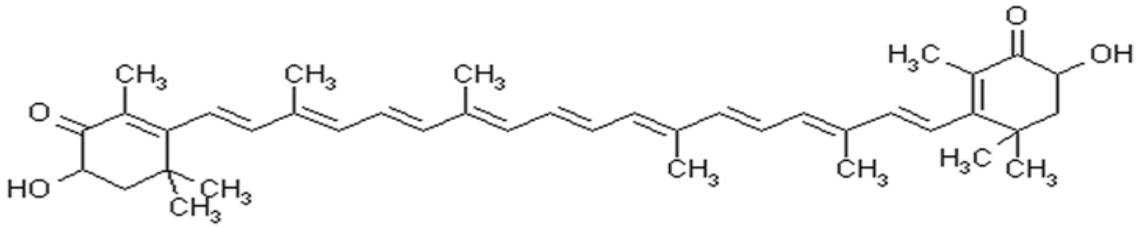
The aim of the present study was to isolate and study about the anti ulcer activity of astaxanthin from Shrimp of the species Aristeus alcocki. Astaxanthin a carotenoid present in marine yeast and crustaceans posses a wide range of pharmacological activity. Shrimp was collected from Cochin, Kerala during 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.The antibacterial activity was studied on several organisms like Bacillus Subtilis, Salmonella typhi, Staphylococcus aureus and Pseudomonas aeroginosa. The extract showed excellent antibacterial activity than the standard chloramphenicol. Among this pseudomonas aeroginasa showed maximum inhibition.

Key words Astaxanthin, , Metapenaeus dobsoni, chemical extraction Antibacterial activity, fermentation , well diffusion assay

INTRODUCTION

Astaxanthin (FIGURE NO 1), 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 is 10 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 sources.(Naguib YM. Antioxidant activities of astaxanthin and related carotenoids. J Agric Food Chem 2000 Apr;48(4):[1150-4].,)

FIGURE NO 1



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 research work is to study the anti ulcer activity of astaxanthin.(FISH PHYSIOLOGY AND BIOCHEMISTRY VOLUME 27, NUMBERS 1-2/SEPTEMBER 2002, PAGE NO: 71-80)

MATERIALS AND METHODS

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 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.

Chemical extraction of astaxanthin

Astaxanthin was extracted by mixing 5g shrimp waste powder homogenate, 50 mL of hexane and 5 mg 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 paper (Thompson, Alonzo C.; Hedin, P. A., Separation of organic acids by thin-layer chromatography of their 2,4-dinitrophenylhydrazide derivatives and their analytical determination 1966, Journal of Chromatography (1966), 21(1), 13-18.

STUDY OF ANTIULCER ACTIVITY

ANALYSIS OF ANTI ULCER ACTIVITY

Analysis of Proton Pump Activity

Preparation of H+, K+-ATPase.

Gastric membrane containing H+, K+-ATPase was prepared from mucosal stomach scrapings of sheep and was homogenized in 20 mM Tris–HCl buffer (pH 7.4). The homogenate was centrifuged for 20 min at 15,000 rpm and the resulting supernatant was used to determine the H+, K+-ATPase activity and its inhibition.

Estimation of Protein

The protein content of the supernatant was determined by Lowry's Method using bovine serum albumin as a standard.

Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1g/ ml) and water in the test tube. The final volume in each of the test tubes was 5 ml. The BSA range is 0.05 to 1 g/ ml. From these different dilutions, 100 µl protein solution was pipetted out to different test tubes and added 2 ml of alkaline copper sulphate reagent (analytical reagent). The solutions were mixed well and incubated at room temperature for 10 minutes. Then added 200 µl of reagent Folin Ciocalteau solution (reagent solutions) to each tube and incubated for 30 min. The colorimeter was set at zero with

blank and the optical density (measure the absorbance) was recorded at 660 nm. The absorbance was plotted against protein concentration to get a standard calibration curve. The absorbance of sample was measured and determined the concentration of the sample using the standard curve. The concentration of the protein was adjusted to a final concentration of 3 mg/ml.

H+, K+-ATPase Assay

The enzyme extract containing 100 µl (300 µg) proteins was taken for testing the activity of H+, K+ -ATPase. Reaction was carried out in 16mM Tris buffer (pH 6.5). The reaction was initiated by adding substrate (2mM ATP, 2mM MgCl2 and 10mM KCl), made up to 2 ml and incubated for 30 min at 37°C. The reaction was stopped by the addition (1 ml) of an assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid. Phosphomolybdate formed was measured spectrophotometrically at 400 nm.

Inhibition of H+, K+-ATPase in vitro.

The enzyme extract containing 100 µl of protein was taken for testing the activity of H+, K+-ATPase in the presence of different concentrations (10–200 µl) of astaxanthin extracts.

Astaxanthin extracts were incubated with H+, K+-ATPase for 30 min. Subsequently, reaction was carried out as described above. The results were expressed as percent inhibition of enzymatic activity at each concentration.

Lansoprazole (1mM), was employed as a standard anti-ulcer drug.

ANALYSIS OF ANTI ULCER ACTIVITY

Inhibition of H+, K+-ATPase in vitro.			
Amount of sample(µl)	OD @400 nm	% of inhibition	OD of Control
Lansoprazole (std)	0.37	44.78	0.67
10	0.61	8.96	
50	0.57	14.93	
100	0.49	26.87	
200	0.4	40.30	

CONCLUSION

The anti ulcer activity of astaxanthin was studied by using lansoprazole as standard anti ulcer drug. The results were obtained in percent inhibition of enzymatic activity at each concentration. It showed maximum inhibition at a concentration of 200µl.

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