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Research Paper

Antibacterial Activity of *Haematococcus pluvialis* Crude Astaxanthin Extract

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Abstract

H. pluvialis is the potential source of natural astaxanthin, which is considered as super antioxidant. In the present investigation, astaxanthin was extracted from the encysted cells of *H. pluvialis* with acetone, methanol, DMSO and hexane, the crude extracts were tested for four strain (Escherichia *coli, Salmonella typhi, Vibrio cholera and Staphylococcus aureus*)of bacteria for the antibacterial activity. Highest antibacterial activity was observed as 10.2 ±0.20 mm extracted with acetone on *Escherichia coli* while as least antibacterial activity was found as6.1±0.0 mm extracted with hexane on *Vibrio cholera*.

Keywords: *Haematoccoccus pluvialis*; Astaxanthin; Antibacterial.

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1. INTRODUCTION

The growing demand in the cosmetic formulation with natural ingredients continues to raise and now-a-days this has been a trend to replace the synthetic preservatives with natural ones. The natural preservatives are preferred from plants, bacteria, fungi, and algal sources. Astaxanthin is a xanthophyll carotenoid[1]. The main biological sources of astaxanthin include *crustacean crustacean* extracts, *Haematococcus pluvialis*, yeast *Rhodotorularubra*, and *Phaffia rhodozyma*. The aforementioned natural sources cannot compete with the synthetic products available [2]. *Haematococcus pluvialis*, freshwater green microalga is considered a potent producer of pigment astaxanthin [3]. In our previous study [4, 5] *H. pluvialis* was cultivated and astaxanthin was extracted from the green alga *H. pluvialis*. In present study, efforts have been made to study antibacterial activity of *Haematococcus pluvialis* crude astaxanthin extract.

2. MATERIALS AND METHODS

2.1. Cultivation

Haematococcus pluvialis culture was obtained from culture collection of algae at the University of Texas, Austin, USA. *H. pluvialis* culture was grown in the bold basal medium (BBM) [6]. The astaxanthin accumulated cells, were harvested, dried in the oven at 70 ^{o}C [7].

2.2. Preparation of Samples

10 mg biomass of *H. pluvialis* was mixed in mortar and pestleand extracted with acetone, methanol, DMSO and hexane, centrifuged at 2800x g for 10 min at 4 ^{o}C, the pellet was discarded and the supernatant was taken for the estimation of astaxanthin. The absorbances of the extracts were determined at 492 nm and the amount of the pigment was quantitatively measured according to Davies [8]. Acetone was evaporated in the rotary evaporator. The crude astaxanthin extracts were individually tested for *Escherichia coli, Salmonella typhi, Vibrio cholera and Staphylococcus aureus*. The antibacterial activity was tested using disc diffusion method. 1 ml of test organism at concentration of 10^{-5} cells mL^{-1} was spread on Mueller-Hinton agar plate. 10 µl astaxanthin, extracted with acetone, methanol, DMSO and hexane was poured in the wells. The plates of bacterial strain were incubated at 37 °C for 24. The inhibition zone discs were observed and the diameters of clear zones were measured. The bacteria strains were sub cultured and maintained on nutrient agar (Beef extract 3.0 g, Peptone 5.0 g, NaCl 5.0 g, Agar 15.0 g, pH 7.4, dissolved in one liter distilled water and sterilized at 121 ^{o}C for 15 minutes).

3. RESULTS AND DISCUSSION

3.1. Cultivation

Haematococcus pluvialis was grown in the bold basal medium for 30 days and the cells were harvested and astaxanthin was extracted with different solvents (**Figure 1**)

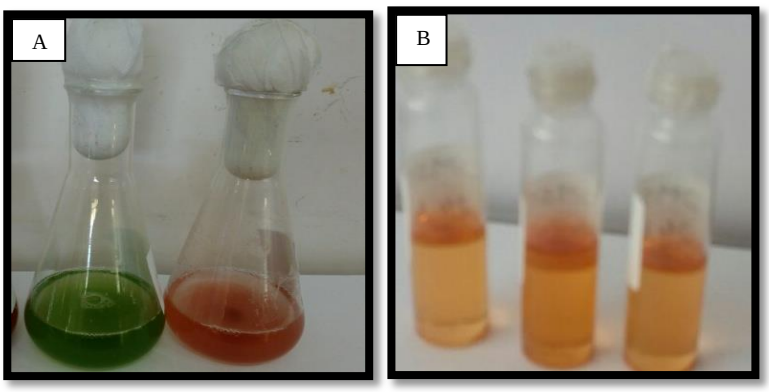

Figure 1: *Haematococcus pluvialis* (A) green stage and red stage (B) astaxanthin extract

Astaxanthin has been extracted with acetone, methanol, DMSO and hexane and the crude astaxanthin was tested for the *E.coli*, *Salmonella typhi*, *Vibrio cholera*, *Staphylococcus aureus*. The results showed that the green algae *H. pluvialis* crude extract (10µl) possess antibacterial activity by showing the inhibition zone around the discs. Highest antibacterial activity was found as 10.2 ±0.20 mm extracted with acetone on *Escherichia coli.* In methanol, DMSO and hexane extracts, highest antibacterial activity was found as 8.8±0.12, 9.4 ±0.15 and 8.7 ±0.12 mm on *Salmonella typhi*, *Salmonella typhi*, *Staphylococcus aureus* respectively as seen in **Table 1**.

Table 1: Antibacterial activity of crude *H. pluvialis* astaxanthin

	Inhibition zones of crude astaxanthin (mm) in different bacteria			
Solvents	*E.coli*	*Salmonella typhi*	*Vibrio cholera*	*S. aureus*
Acetone	10.2 ±0.20	9.8 ±0.15	10.1 ±0.15	9.1 ±0.12
Methanol	8.8±0.12	7.5 ±0.12	7.4 ±0.12	6.1 ±0.10
DMSO	8.2 ±0.15	9.4 ±0.15	7.3 ±0.20	7.6 ±0.20
Hexane	6.6 ±0.10	7.2 ±0.20	6.1 ±0.10	8.7 ±0.12

The antioxidant activities of astaxanthin are better than β-carotene and vitamin-E. This is the reason due to which makes it protects against inflammation, UV radiation, aging and skin cancer. Astaxanthin is used in cosmetic, food and feed industries. *H. pluvialis* astaxanthin is found effective agent for the regulation and maintenance of uric and hepatic enzymes in rat model animals [9]. The pharmacokinetic data has revealed that a single dose of 10 mg astaxanthin can remain in the human blood for a day and 100 mg for approximately three days [10]. A dose of 1 mg when given once daily for a month can increase the blood levels [11]. The animal study showed that astaxanthin higher than 120 mg a day in human [12], revealed no harmful effects. Gonzalez *et. al* [13] observed that extract of green algae have umpteen antimicrobial activities against *B. subtilis* and *S. aureus*. The green algae extract showed potential effects against Gram-positive bacteria [14].

4. CONCLUSION

The current results revealed that crude astaxanthin extracts prevented the bacterial activity hence *H. pluvialis* astaxanthin may be consider as a preservative in different food formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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