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EXTRACTION OF ASTAXANTHIN FROM THE ENCYSTED CELLS OF *HAEMATOCOCCUS PLUVIALIS* WITH DIFFERENT SOLVENTS

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ABSTRACT: The aim of the present investigation was to extract the astaxanthin, a pharmaceutical valuable secondary carotenoid from the *Haematococcus pluvialis* with different solvents. Extraction of astaxanthin from the encysted cells of *H. pluvialis* was done with Acetone, DMSO, Hexane, Methanol and pretreating the cells of *H. pluvialis* with HCL at 70 °C followed by Acetone extraction. The HCL pretreatment was done only in Acetone. 55%, 18.4%, 17.5% and 10% of astaxanthin extractability was found with Acetone, DMSO, Hexane and Methanol respectively without the HCL pretreatment. However, HCL pretreatment followed by Acetone facilitated 90% extractability of astaxanthin. HPTLC analysis of astaxanthin extracted with Acetone and Methanol was compared with standard astaxanthin. Results clearly shows the presence of astaxanthin in Acetone extract. From our present investigation, it is apparent that pretreatment of *H. pluvialis* cells with HCL followed with Acetone facilitates the astaxanthin extraction. Astaxanthin from *Haematococcus* will expand not only the consumer space but also medical institution worldwide, therefore optimal extraction method of astaxanthin is important for human welfare.

KEYWORDS: *Haematococcus pluvialis*, Astaxanthin, Carotenoid, Pretreatment, Extractability.

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1.INTRODUCTION

The antioxidant properties of astaxanthin are 500 and 38 times better than β-carotene and vitamin E respectively. This makes it capable of protecting against inflammation, UV radiation photooxidation, aging and age-related mucular degeneration, cancer and used in cosmetic, food and feed industries, in addition to maintaining normal liver and heart functions [1]. An extensive study has been done previously on β-carotene (pro vitamin-A) and vitamin E. However, in recent times there has been a shift towards the carotenoids like astaxanthin extracted from *H. pluvialis* which has additional positive features like potent quenching and anti-lipid peroxidation lacking both in β-carotene and vitamin E [2]. The skin cells irradiated with UVA significantly suffered less DNA damage when pretreated with astaxanthin (10μM). The skin endogenous antioxidants SOD and glutathione (GSH) were protected by astaxanthin from oxygen radical attack [3]. The pharmacokinetic data has revealed that a single dose of 10 mg astaxanthin can persist in the human blood for 24 hours and 100 mg 76 hours [4]. A dose of 1 mg when taken once daily for four weeks can increase the blood levels [5]. The animal experiments have revealed that astaxanthin at levels well above 120 mg a day in human equivalents [6], apparently causing no harm, confirmed by various experiments including acute toxicity, mutagenicity, teratogenicity, embryotoxicity and reproductive toxicity [7]. The various extraction techniques viz maceration (soaking), percolation, counter- current extraction and soxlet are reproducible, allowing the rapid extraction of chemicals but usually involve a use of large amounts of solvents and thus there is a risk of thermal denaturation or transformation of chemicals of interest [8]. Thus to enhance pigment extractability rates, enzymes such as xylanases, pectinases or cellulases were used for superior plant tissues [9-10], macroalgae [11] and could be of interest for unfrastulated microalgae. An extraction of 70% astaxanthin from *H. pluvialis* was achieved by use of different enzymes with 40% (v/v) acetone for 2 minutes at 80^0C [12]. Astaxanthin was also extracted from *H. pluvialis* by treating with several enzymes [13]. 88% of astaxanthin was extracted from *H. pluvialis* with common vegetable oils [14]. With DMSO along with acetic acid drops and heating at 70^0C for 10 minutes [15]. Similarily at low temperature with butylated hydroxyl toluene [16]. Therefore, in present investigation, efforts have been made to extract astaxanthin from *H. pluvialis* by using different solvents.

2. MATERIALSAND METHODS

The procurement of *Haematococcus pluvialis* culture

The *Haematococcus pluvialis* used in the present investigation, was procured from the Culture Collection of Algae at the University of Texas, Austin, USA.

Maintenance of culture

The culture was maintained in the both liquid and solid BBM [17]. The axenic cultures were incubated in an air-conditioned culture room maintained at 25 ± 2 ^{0}C under 16:8 h (L/D), at light intensity 35 μmol m^{-2} s^{-1}.The encysted *Haematococcus pluvialis* cells which appeared red due to the accumulation

of astaxanthin were harvested by centrifugation at 2800x g for 5 min at 4 °C. Biomass was dried in hot air oven at 70 °C for 8h and used for extraction process.

Removal of Chlorophyll

The encysted cells of *Haematococcus* were treated with 5% methanolic KOH at 70 °C for 5 min. The cells were separated by centrifugation (Remi, CPR 24) at 2800x g for 10 min at 4 °C, washed with 2 mL of distilled water and processed for astaxanthin extraction [18].

Extraction of astaxanthin

10 mg of biomass was homogenized in mortar and pestle in the presence of neutral glass powder (Borosil Glass Works Ltd., India) extracted with acetone and centrifuged at 2800x g for 10 min at 4^0C. The supernatant was used for estimation of total astaxanthin. 10 milligram of biomass were individually extracted in acetone (1 h), (12 h), (24 h) and 48 h, Methanol (1 h), (12 h), (24 h) and 48 h (24 h), Dimethyl sulfoxide (DMSO) (1 h), (12 h), (24 h) and Hexane (1 h), (12 h), (24 h) and 48 h and with 5 drops of glacial acetic acid, centrifuged at 2800x g 10 min at 4 °C and supernatant were used for the estimation of extractable astaxanthin. Another set of 10 mg biomass of *Haematococcus pluvialis* was separately pretreated with different concentrations (1-10 N) of hydrochloric acid (HCl) at 70 °C, cooled, centrifuged, washed with 2 mL of distilled water, and extracted with acetone and the supernatants were used for the estimation of extractable astaxanthin. All the steps were carried out in dim light [18].

Determination of astaxanthin content in the extracts

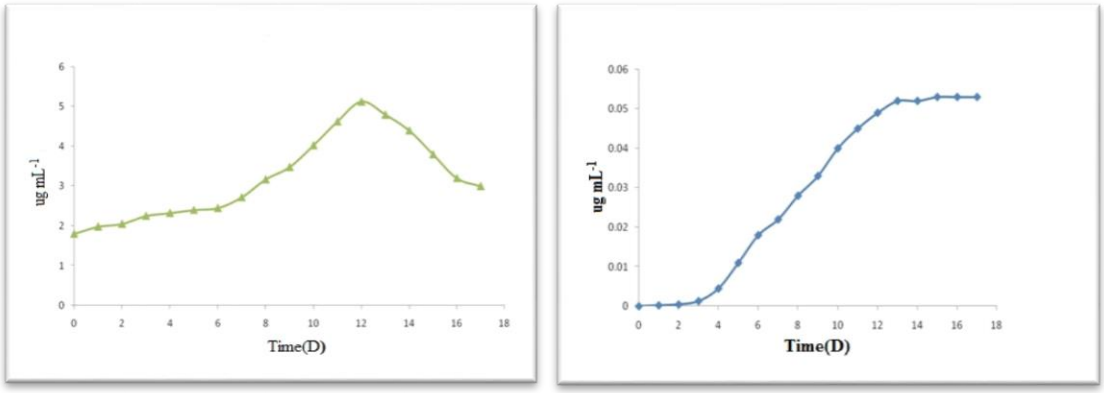
The concentration of astaxanthin in the extracts was analyzed using the Spectrophotometer (Systronics Visible Spectro, 105) [19] and the extractability of astaxanthin was calculated in different solvents [12].

Analysis of astaxanthin by HPTLC

Extracted astaxanthin from *Haematococcus* cells was separated by HPTLC (CAMAG Linomat5 200872" S/N 200872).

3. RESULTS AND DISCUSSION

Haematococcus pluvialis was grown in the Bold Basal medium for 30 days and growth was evaluated by different growth parameters.



A **B**

Fig.1.(A) Changes in *Chl b* content of *H.pluvialis* grown in Bold Basal medium. (B) Changes in astaxanthin content of *H.pluvialis* grown in Bold Basal medium.

A **B**

Fig. 2. *Haematococcus pluvialis* (A) Green Stage (B) Red Stage.

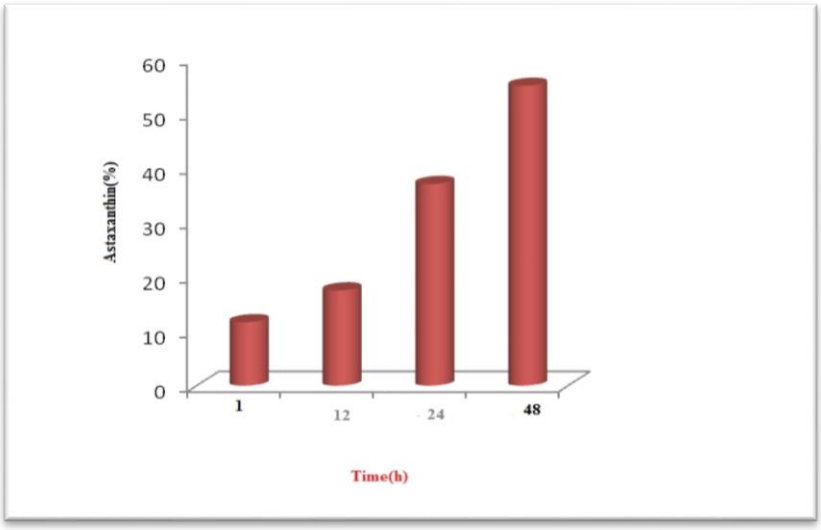


Fig.3.Percentage of astaxanthin extracted at various time intervals with Acetone without HCL pretreatment**.**

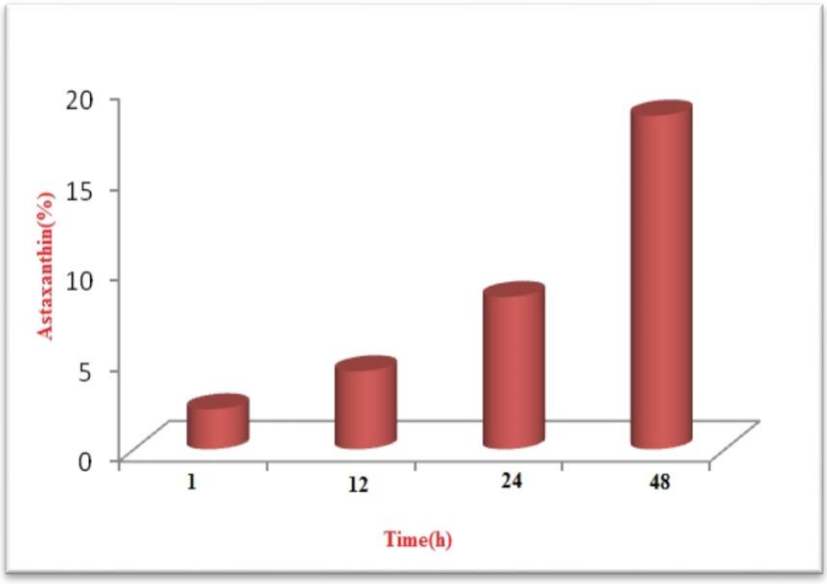


Fig.4. Percentage of astaxanthin extracted at various time intervals with DMSO without HCL pretreatment.

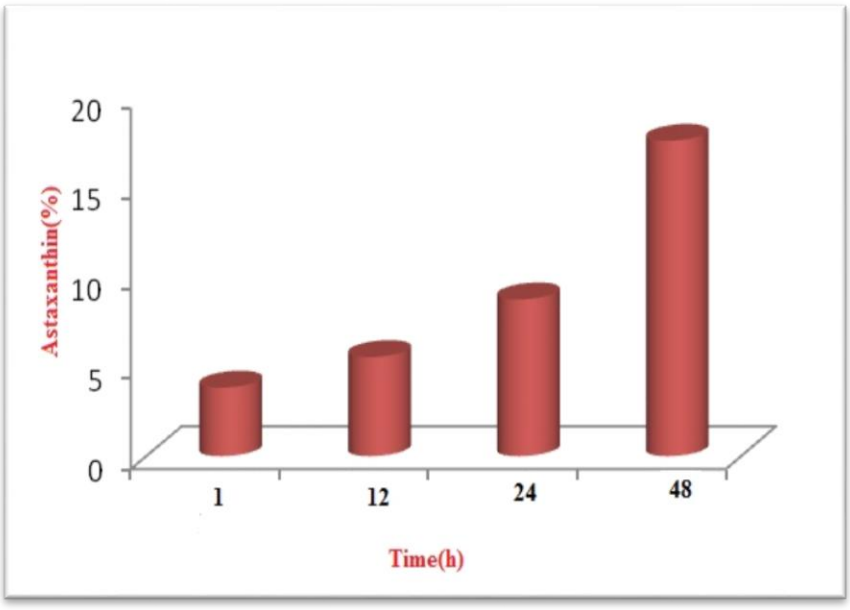


Fig.5. Percentage of astaxanthin extracted at various time intervals with Hexane without HCL pretreatment.

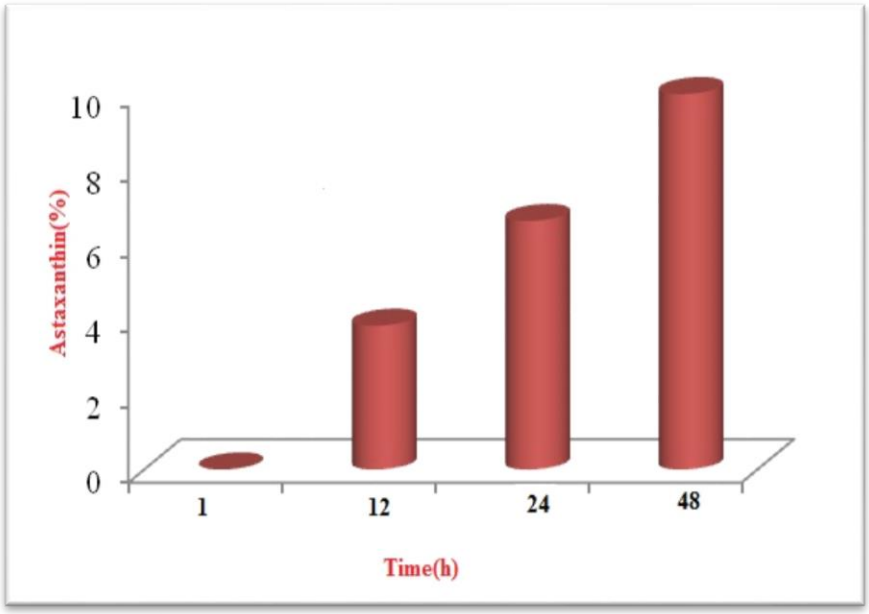
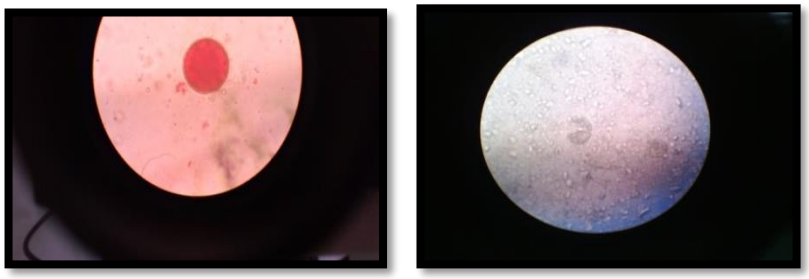


Fig.6. Percentage of astaxanthin extracted at various time intervals with Methanol without HCL pretreatment



(A) **(B)**

Fig.7. Encysted cells of *H. pluvialis* (A) before extraction (B) after extraction of astaxanthin.

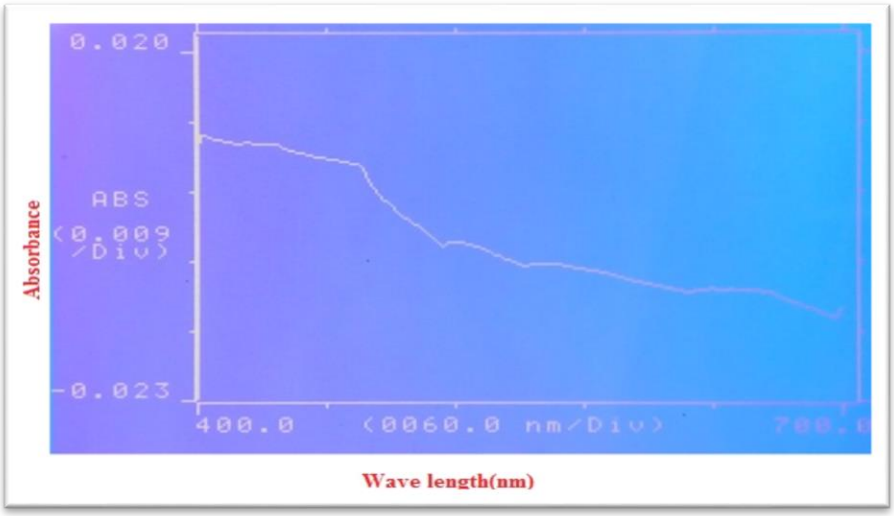


Fig. 8. Absorption spectra (400nm-700nm) of Astaxanthin extract from encysted cells of *H. pluvialis*.

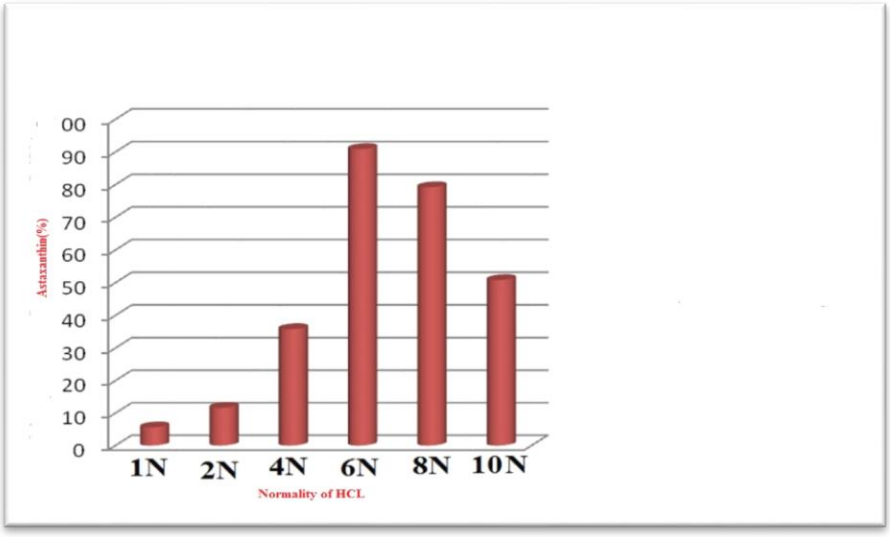


Fig.9. Extraction of astaxanthin with Acetone following pretreatment of cells with HCL (1N-10N)

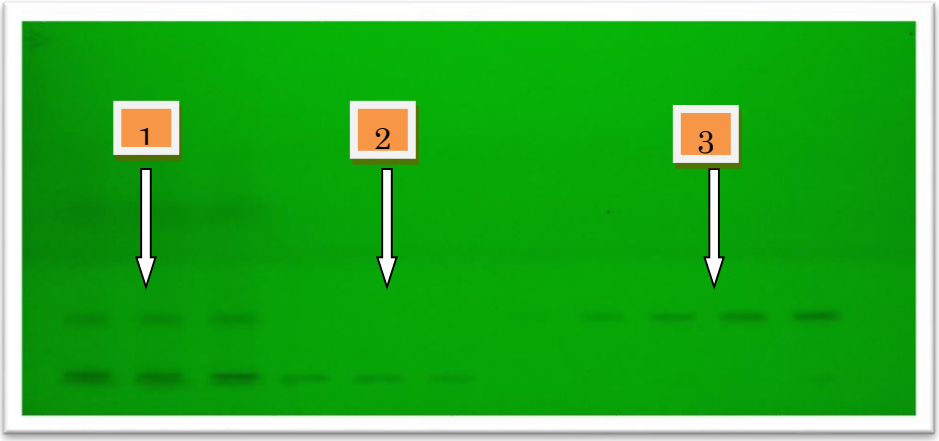
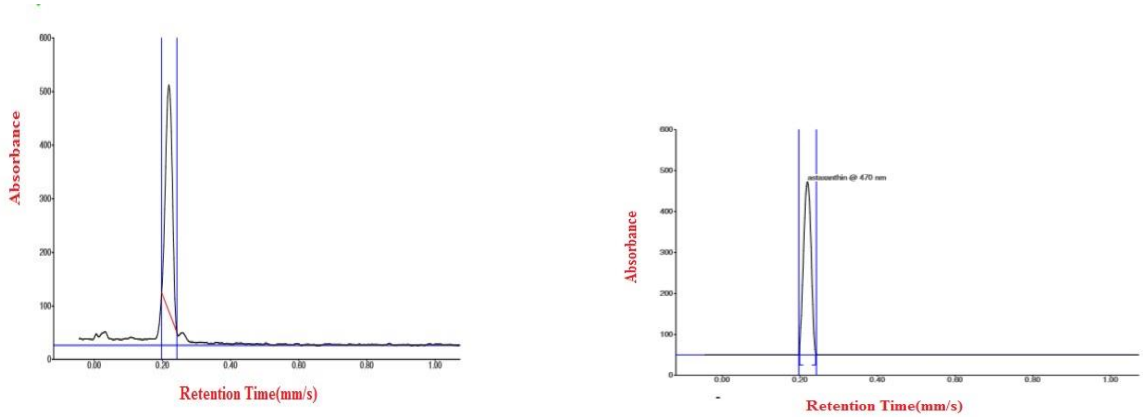
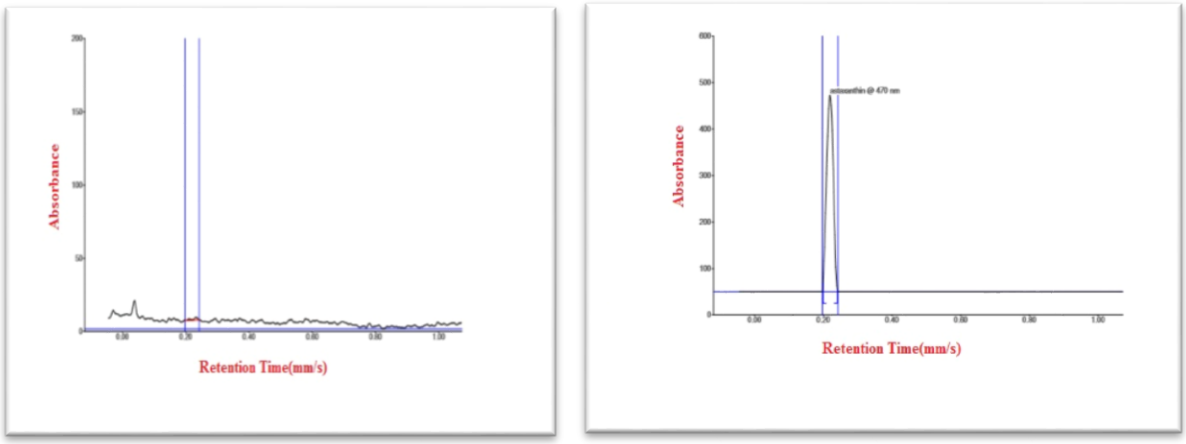


Fig.10. HPTLC separation of Astaxanthin extracted from *Haematococcus* cells: with Acetone (1) Methanol (2) and Standard astaxanthin (3).



(A) **(B)**

Fig.11. HPTLC analysis of Astaxanthin (A) Acetone extract (B) Astaxanthin Standard.



(A) **(B)**

Fig.12. HPTLC analysis of (A) Methanol extract (B) Astaxanthin Standard.

The *H. pluvialis* cells, that used for the study were 30 days old, when the green stage of *H. pluvialis* was fully converted into astaxanthin accumulated red stage (fig. 2). The extractability of astaxanthin was evaluated using different solvents and HCL treatment followed by acetone. The extractability of astaxanthin from *H. pluvialis* with different solvents were shown in (fig. 3-6). The results indicate that highest amount of astaxanthin 55% was found in acetone without the HCL treatment while as lowest astaxanthin 10% was found in methanol. However, the extractability of astaxanthin was significantly increased by 40% when the *H. pluvialis* were pretreated with the 6N HCL followed by the acetone extraction as seen in (fig.9**)**. However, at higher concentration of HCL (≥8N) the cells were observed to be floating which lowers the extractability of astaxanthin. The cells after the treatment of HCL becomes almost colorless and were observed under microscope as seen in (fig.7) which indicated that the treatment facilitated the extraction of astaxanthin. The HPTLC astaxanthin profiles showed the presence of astaxanthin present in the acetone extract (fig. 10, 11). Astaxanthin being a powerful antioxidant [20], acetone extraction led to a higher concentration of astaxanthin [16]. Different types of enzymes were also used for the extraction of astaxanthin from cells of *H. pluvialis* cells with 40% (v/v) acetone for 2 min at 80^0 C, and the extractability of astaxanthin was achieved 70% [12-13]. In vegetable oils extractability was found 88% [14]. However, aforementioned procedures lead to the transformation and loss of valuable molecules [9]. Therefore, present study, have some advantages such as being simple, quick, reproducible, free of corrosible material and without presenting significant losses.

4. CONCLUSION

Haematococcus pluvialis is one of the richest source of natural astaxanthin which is considered as super anti-oxidant. Astaxanthin has umpteen applications in the nutraceuticals, cosmetics, food, and aquaculture industries, can significantly decimate free radicals, oxidative stress and help human body maintain a healthy state, therefore optimal extraction method of astaxanthin is important for human welfare

5. ACKNOWLEDGEMENT:

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6. CONFLICT OF INTEREST:

The authors have no conflict of interest.

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