International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 1721-1725 www.ijprajournal.com ISSN: 2249-7781

Anti tyrosinase Activity of Astaxanthin Cream Formulation for Hyper pigmentation

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Submitted: 15-04-2023 Accepted: 25-04-2023

ABSTRACT

The study aims to develop a cream formulation using astaxanthin. When an excess of melanin pigment forms deposits on the skin, it forms dark patches, thus causing a condition called hyperpigmentation. Astaxanthin is a xanthophyll carotenoid, present abundantly in Haematococcus pluvialis, a green microalga. Astaxanthin also plays a vital role in the food and cosmetic industry due to its antioxidant capacity. The xanthophyll pigment in astaxanthin can be characterized using Thin Layer Chromatography (TLC) followed by the HPTLC process. UV- Vis and FTIR analysis characterize the absorbance maxima and distinct functional groups in astaxanthin. Emulsifying wax, glycerine, stearic acid, and sodium benzoate are used as a base for developing a cream formulation. When a melanoma cell is exposed to UV light, increased oxidative stress is caused due to Reactive Oxygen Species (ROS). Tyrosinase enzyme contributes to the synthesis and accumulation of melanin in melanocytes. Astaxanthin and its esters are said to be able to downregulate tyrosinase and ROS [9].The antityrosinase activity of astaxanthin is evaluated using a tyrosinase inhibition assay. Astaxanthin showed potent inhibition of tyrosinase activity with IC50 values of 30.37 and 41.02 µg/ml at 60- and 120 min time intervals. Further studies include the evaluation of the physiochemical properties of the cream developed using astaxanthin.

Keywords: Astaxanthin, hyperpigmentation, cream, antioxidant, tyrosinase

I. INTRODUCTION

Astaxanthin is a xanthophyll carotenoid, present abundantly in Haematococcus pluvialis, a green microalga [2]. Products made using astaxanthin are used for commercial applications in dosage forms such as tablets, syrups, creams, and gels. Astaxanthin is an antioxidant that is used to treat Alzheimer's disease and the aging of the skin. [11]. The oxidative stress resulting from stimuli, such as UV exposure and skin dryness, triggers skin inflammation [10]. Tyrosinase (EC 1.14.18.1) is the critical enzyme in the first two steps of melanin biosynthesis, catalyzing the hydroxylation of L-tyrosine to the 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone. The increased levels of tyrosinase enzyme result in the enhanced synthesis and accumulation of melanin in melanocytes and causes various skin disorders. Astaxanthin possesses a protective capacity against the skin. It inhibits lipid peroxidation, thereby maintaining skin moisture, helping avoid early stages of wrinkle formation, and decreasing skin roughness. It efficiently inhibits atopic dermatitis and UVB-induced age spots [1]. Astaxanthin reduces Stem cell factor (SCF), decreasing melanin production by downregulating tyrosinase activity [7].

II. MATERIALS AND METHODS

2.1 Thin Layer Chromatography Analysis

Astaxanthin was subjected to thin-layer chromatography to separate the bioactive compounds [3]. TLC plates were prepared using Silica gel G and distilled water. The dried plate was then loaded with astaxanthin using a microcapillary tube and subjected to the TLC chamber with the mobile phase solvent system. As the solvent rises to 2cm below the top end of the plate, it was taken out of the chamber and examined under UV light.

2.2 HPTLC

The HPTLC process is performed for astaxanthin to separate and purify the bioactive compounds from natural materials. For the process, the HPTLC plates of width 5x10 cm2 were

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 1706-1711 www.ijprajournal.com ISSN: 2249-7781

precoated with silica gel with a thickness of 200 µm. For placing spots on the plates, a 10 µL micro-syringe was used. The standard solution was prepared with 0.93 g of AST dissolved in 10 mL of DMSO reagent in a 3:1 ratio with a 0.093 mg/ml concentration. In the mobile phase, n-hexane and acetone were used in a ratio of 7:2.The sample and the standard solutions were applied as dots on the plate with diameters less than 3 mm and a minimum distance of 8 mm. The plates were observed under UV and visible light at different wavelengths [14].

2.3 Characterisation of Astaxanthin

2.3.1 Characterisation of Astaxanthin using UV-Vis Spectroscopy

Astaxanthin was the primary pigment isolated from the coral samples, followed by a canthaxanthin-like carotenoid. These carotenoids are characterized by specific absorption maxima situated between **409–476 nm** and **429–477 nm** in the in vivo and in vitro absorbance spectra, respectively [5].

2.3.2 Characterisation of Astaxanthin using FTIR Spectroscopy

Astaxanthin was analyzed using ATR-Fourier Transform infrared spectroscopy (Shimadzu, Japan), and the wavenumber was fixed in the range of **4000-500 cm^{-1}**. The results were plotted, and the functional groups were named using OriginPro.

2.4 Tyrosinase Inhibitory Activity Assay

Tyrosinase is a crucial enzyme of the melanogenesis pathway in the rate-limiting steps for melanin synthesis. Inhibiting tyrosinase activity helps in skin whitening and regulating hyperpigmentation [4]. Forty microliters of L-DOPA (10 mM, for the diphenolase activity assay) or L-tyrosine (5.0 mM, for the monophenolase activity assay) was mixed with 80 μL of phosphate buffer (0.1 M, pH 6.8) in a 96-well microtiter plate, and the resulting mixture was incubated for 10 min at 37∘C. Forty microliters of seed oil (50, 100, 200, 400, and 800 μg/mL in 50% DMSO) and 40 μL of mushroom tyrosinase (250 U/mL, in PBS) were then added to each well on the plate. The absorbance characteristics of the resulting mixtures were measured at 475 nm using an ELISA reader (Multiskan Sky High, Thermo Fischer Scientific) at 60-sec intervals for 120 min [12]. PBS was added instead of the test sample as a blank control, and ascorbic acid (50 μg/mL) was used as the positive control.

The inhibition for each enzyme assay was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

2. 5 Cream Formulation using astaxanthin

The cream formulation was developed as an oil-water emulsion [8]. Emulsifying waxand stearic acid were dissolved in the oil phase at about 75℃ in the water bath. Subsequently, in the water phase, glycerine and sodium benzoate were dissolved at 75℃ in the water bath. The water phase components were added to the oil phase, and both were dissolved in a water bath at 75℃. The components were further blended by placing them in a magnetic stirrer for about an hour. Continuous stirring resulted in the components being appropriately blended to form a lotion. To the lotion, astaxanthin was added at the concentration of 20 mg/ml and blended using a magnetic stirrer. The lotion was stored at room temperature for 24 h, and thus the lotion got solidified into a cream.

Table 1 Cream Formulation

COMPONENT	QUANTITY
Emulsifying Wax	3.75 g
Stearic acid	3.75 g
Glycerine	45 ml
Sodium benzoate	0.3 g
Astaxanthin	2 ml

III. RESULTS AND DISCUSSION

3.1 Characterisation of Astaxanthin

3.1.1 Characterisation of Astaxanthin using UV-Vis Spectroscopy

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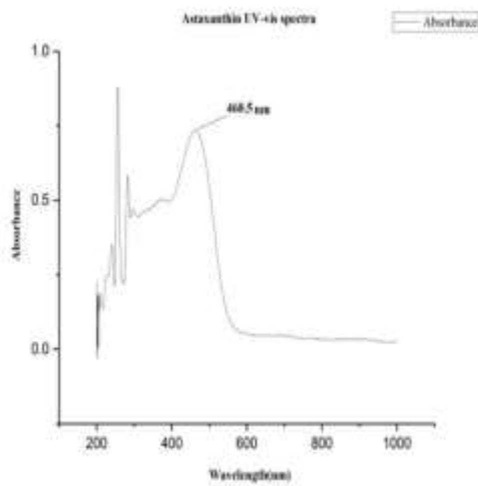

Figure 1UV-Visible spectra of peaks of Astaxanthin

3.1.2 Characterisation of FTIR using astaxanthin

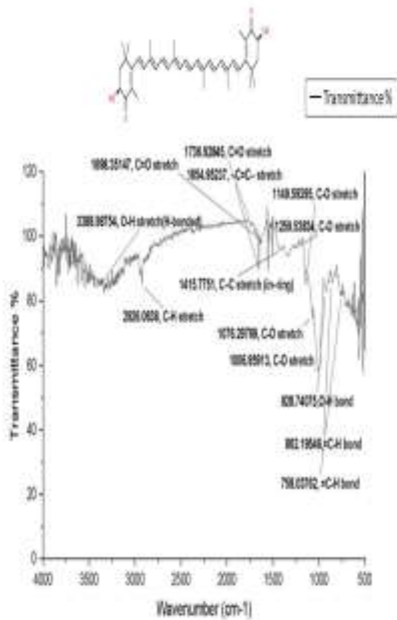

Figure 2 FTIR Spectroscopy of Astaxanthin

3.2 FTIR Spectra Analysis of Cream

FTIR of the cream sample incorporated with astaxanthin is done to analyze its organic and inorganic materials. The graph of FTIR was plotted by using Origin Pro software, 2022.

	Astaxanthin	Ascorbic acid
log (inhibitor) vs. response (three parameters)		
Best-fit values		
Bottom	69.70	124.4
Top	-3.865	4.736
Log IC50	1.482	1.532
IC50	30.37	34.05
Span	-73.57	-119.7

Table 2 IC50 value of astaxanthin at 60 min time interval

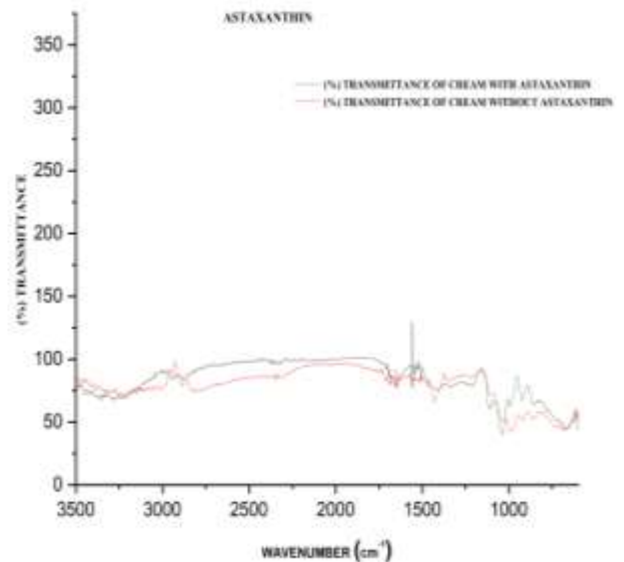

Figure 3FTIR spectra of cream sample incorporated with Astaxanthin

3.3 Tyrosinase Inhibition Assay

In the Tyrosinase inhibition assay, each experiment was carried out in triplicate (n=3). The IC50 value, i.e., the concentration of ascorbic acid or sample required to inhibit the enzyme's activity by 50%, was calculated from the dose-response curves using a Graph pad prism [13].

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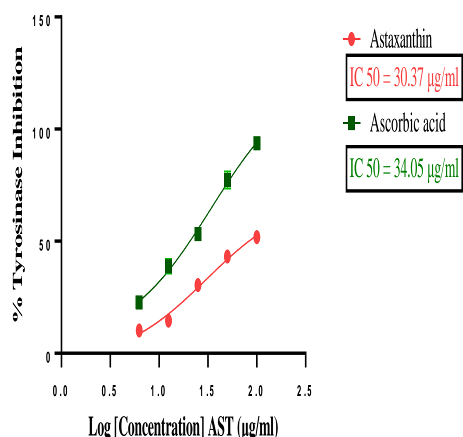

Figure 4Effect of Astaxanthin on Tyrosinase inhibition (60 min)

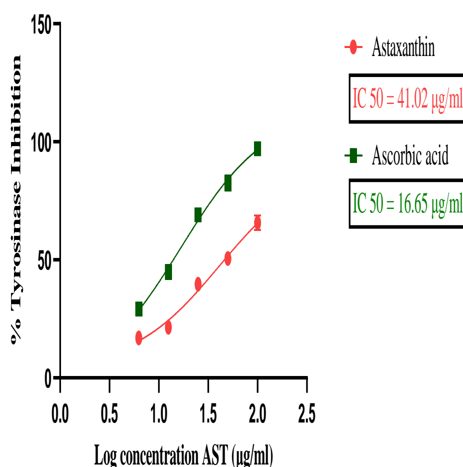

Figure 5Effect of Astaxanthin on Tyrosinase inhibition (120 min)

Table 2IC50 value of astaxanthin at 120 min time interval

	Astaxanthin	**Ascorbic acid**
log (inhibitor) vs. response (three parameters)		
Best-fit values		
Bottom	90.62	113.2
Top	4.238	-3.184
Log IC50	1.613	1.222
IC50	41.02	16.65
Span	-86.38	-116.4

3.4 Thin Layer Chromatography Analysis

The dried TLC plate loaded with astaxanthin is examined under UV and visible light.

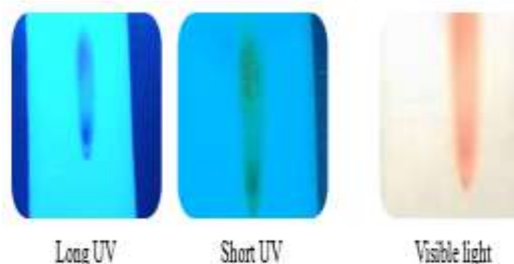

Figure 6Thin layer chromatography of Astaxanthin under UV and visible light

3.5 HPTLC Analysis

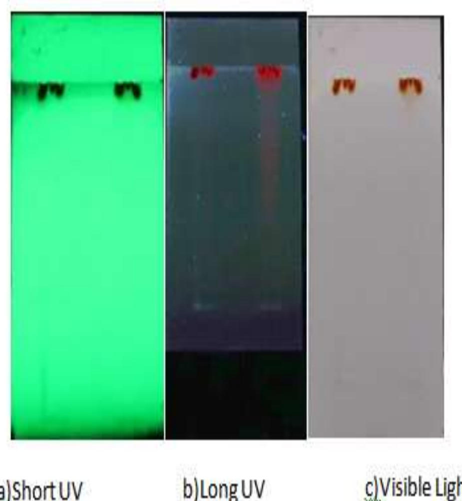

Figure 7 Astaxanthin spot observed under UV and Visible Light

3.6 Cream Formulation

Figure 8 Cream Formulation incorporated with Astaxanthin

IV. CONCLUSION

Astaxanthin is said to be a powerful antioxidant, which does not exert a prooxidant effect, unlike other carotenoids. Astaxanthin, abundantly found in the aquatic environment,

IJPRA Journal

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 1721-1725 www.ijprajournal.com ISSN: 2249-7781

enables various biological functions in marine animals. Natural astaxanthin is said to have singlet oxygen quenching activity and lipid peroxidation potential 40 times of beta carotene and 1000 times of vitamin E. Due to its potential properties, astaxanthin is used effectively in the food and pharmaceutical industry for various applications. In this study, the tyrosinase inhibition activity of astaxanthin was studied. As the astaxanthin showed antityrosinase activity, a cream was formulated using it.

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