Extraction of Astaxanthin from Shrimp Shell (*Metapenaeus dobsoni*), Formulation and Evaluation of Sunscreen Lotion

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ABSTRACT

Aim: The present study focuses on the extraction of astaxanthin, a potential natural antioxidant and carotenoid from the shells of Metapenaeus dobsoni which is a species of shrimp, followed by incorporation into a tropical sunscreen lotion. Materials and Methods: Astaxanthin was extracted using an optimized solvent extraction method and its solvent assisted extraction. The presence of Astaxanthin is analyzed by using the FTIR spectrometer, Thin layer chromatography, UV-visible spectrophotometer were the various test which have been used for testing the presence of astaxanthin in the shrimp shells. The purified extract was incorporated into a tropical sunscreen formulation and the product was evaluated for the key physiochemical properties like pH, viscosity, spreadabilty and washability. The confirmatory test for astaxanthin was done using Sulphuric acid test, Iodine test, Bromine water test and antioxidant acticity with DPPH assay, UV production with UV-A and UV-B rays, antimicrobial activity, Vitamin C activity, Thin layer chromatography, FTIR analysis were determined, and the sunscreen was formulated. Results: It indicated that the sample was rich in antioxidant property and helps in exhibiting UV absorbing capacity and it is favorable in demonstrating its potential as a natural and ecofriendly alternative to synthetic UV filters. Conclusion: This study supports the potential of marine derived astaxanthin as a sustainable, natural UV protective agent in cosmetic formulation. It highlights the value-added utilization of shrimp shell waste in the development of sustainable skincare

Keywords: Astaxanthin, Shrimp shell, Sunscreen formulation, UV radiation, Antioxidant.

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INTRODUCTION

Marine ecosystems are rich sources of biologically active compound are byproduct that have significant environmental applications since they produce a variety of substances that can aid in pollution control, climate regulation and sustainable industrial practices. In this, the shrimp shell plays a vital role since it is a rich source of astaxanthin which offers a sustainable way to harvest the powerful antioxidants. In the cosmetic industry, astaxanthin is increasingly used in anti-aging and skin care products due to its ability to combat oxidative stress. [1]

Astaxanthin provides an additional layer of protection against UV radiation, specifically UV-A and UV-B rays, and hence used in the formulation of sunscreen. The primary role of astaxanthin as a sunscreen protectant is its antioxidant activity. UV exposure generates free radicals in the skin leading to oxidative stress and

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damages the skin cells, DNA and increases the risk of skin cancer. Astaxanthin has a unique structure which may neutralize free radicals efficiency and reduces the oxidative stress. [2]

The astaxanthin ensures the sunscreen protective properties for long lasting and eco-friendly effects compared to synthetic alternatives. The use of astaxanthin in sunscreen aligns with growing demands for natural, biodegradable ingredients that are safe for both humans and environment. The main aim of this project is to formulate a sunscreen lotion using natural derivatives and to reduce the use of chemical sunscreens which may harm marine life that leads to the ecological risks.^[3]

MATERIALS AND METHODS

Collection of Samples

The *Metapenaeus dobsoni* samples were collected from the district of Nagapattinam, Tamil Nadu which is located by the coastline.

Preparation and Extraction of Astaxanthin

The collected *Metapenaeus dobsoni* were sun dried and oven dried to remove the moisture content then finely powdered and stored.



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Using the solvent extraction method, to 100 mL of ethanol, 10 g of the substance was added and filtered and stored at 4°C for further use.

Confirmatory Tests

Confirmatory tests including the iodine, bromine, and sulfuric acid tests were done to identify the presence of astaxanthin.

Antioxidant Activity

The DPPH assay was used in an antioxidant test to determine the free radical scavenging activity of the extract.

Vitamin C Activity

Using the DNPH assay, vitamin C activity was carried out to determine the amount of vitamin C contained in the sample.

Antimicrobial Activity

To determine the sample's ability to eradicate bacteria, including both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) organisms, antimicrobial activity was performed.

Thin Layer Chromatography

Astaxanthin was measured via TLC by comparing the intensity of the sample spot to that of a reference astaxanthin sample.

FTIR

FTIR spectroscopy was used to determine the molecular structures and types of chemical bond present in astaxanthin.

Formulation of Sunscreen Lotion

The water phase is made by heating distilled water, Aloe vera gel, and glycerine at about 70°C. The oil phase is also prepared by heating the emulsifying wax. The water and oil phases are made separately and mixed together in a separate container to form an emulsion with other essential oils and preservatives.

Tests for Sunscreen Lotion

The sample was subjected to pH test, spread ability test, washability test and Sun protection factor test.

RESULTS

Preparation and Extraction

To remove the moisture, the gathered *Metapenaeus dobsoni* were dried in the sun and oven dried and used for confirmatory tests.

Confirmatory Tests

Sulfuric acid test

The sulphuric acid test is a simple chemical confirmatory test used to detect Sulfuric acid reacts with the conjugated polyene

system of Astaxanthin, producing a characteristic orange to reddish-brown coloration which indicated the presence of carotenoids like astaxanthin.

lodine test

This test turned positive indicating the presence of carotenoids due to its ability of iodine to form complexes with conjugated polythene systems, resulting in a characteristic of orange colouration.

Bromine test

The sample was mixed with a bromine solution, which causes bromine to react with unsaturated molecules. As a highly unsaturated molecule with numerous double bonds, astaxanthin interacts easily with bromine. The result of this reaction is a reddish-brown hue indicating the presence of carotenoids in astaxanthin. Both the sun dried and oven dried samples showed positive results.

Antioxidant Activity

DPPH Assay

Ethanolic extract of *Metapenaeus dobsoni* was used for the antioxidant assay (900 μ L, 920 μ L, 940 μ L, 960 μ L, 980 μ L) with standard as ascorbic acid and the absorbance was measured at 517 nm in UV-visible spectroscopy by taking methanol as control. Based on a study by^[4] the radical scavenging activity of various fruits (cucumber, muskmelon, orange) showed good scavenging activity in DPPH assay. The results were recorded and shown in Table 1.

Vitamin C activity

Ascorbic acid is used as a standard and different concentrations of sample were analyzed (50 μL , 100 μL , 150 μL , 200 μL , 250 μL) and found that with increasing concentration of the extract the Vitamin C activity also increased. [5] Conducted vitamin C activity in fruits such as Muskmelon, Mangoes, watermelon, waxapple and observed similar trend in their results. The results were recorded and shown in Table 2.

Thin layer chromatography

The result of TLC profiling is summarized in the Table. Ethanolic extract of *Metapenaeus dobsoni* showed the presence of Astaxanthin with R_f value of 0.45 (Table 3) which was indicated by the yellowish orange colored spot on the TLC plate. The bioactive compound was confirmed by the yellowish-orange spot after placing under UV light. In a study conducted by [6] it was proved that TLC is done for better identification of astaxanthin. *Metapenaeus dobsoni* extract possesses astaxanthin compounds.

The R_f value of the standard astaxanthin was 0.43. Test Sample 1 (Sun Dry) showed a lower R_f value of 0.33, while Test Sample 2 (Oven Dry) had a value of 0.4.

Antimicrobial Activity

The Metapenaeus Dobsoni extract prepared from ethanol solvents were tested on bacterial sample such as E. coli and Staphylococcus aureus compared with known antibiotics such as Streptomycin using agar well diffusion method. The extent of the zone of inhibition represents the antibacterial activity of ethanolic Shrimp shell extract. which displayed immense potential as that natural alternative, especially with its antimicrobial property. In a study by^[7] on the antibacterial effects of carotenoids, including astaxanthin extracted from various organisms such as shrimp, lobster, and algea showed promising antibacterial activity, inhibiting the growth of E. coli and S. aureus. The researched Highlighted ther of astaxanthin antioxidant properties in exerting its antimicrobial effects. In a study by[8] on the antimicrobial activity of carotenoid extracts, particularly astaxanthin, from shrimp (Metapenaeus dobsoni) showed significant antimicrobial effects against both S. aureus and E. coli. Antimicrobial activity

of *Metapenaeus dobsoni* extract shown significant effect against *Staphylococcus aureus* and *Escherichia coli*. The results were observed and recorded and it is shown in Table 3, Figure 1.

FTIR Sun dried and Oven dried sample

The FTIR peak at 3332 cm⁻¹ is due to the presence of O-H stretching vibration. The sharp peak at 2978 and 2893 cm⁻¹ is due to C-H stretching vibration of CH3. The peak at 1651 cm⁻¹ is attributed to C=O stretching attributed to ketonic group present in the compound. The minor peaks from1381-1273 is attributed to the antisymmetric deformation of CH3 and CH2 groups. The sharp peak at 1041 cm⁻¹ is due to C-O stretching vibrations. The sharp peak at 879 indicates the presence of aromatic C=C bond. [9] The broadpeakat686 cm-1corresponds to C-H bending vibration due to aromatic alkene [10] employed FTIR spectroscopy to analyze the biochemical profile of various algae species and revealed key absorptionpeaksat2920 cm⁻¹ and 2855 cm⁻¹, corresponding to lipid content, while peaks at 1742 cm⁻¹ indicated the presence of

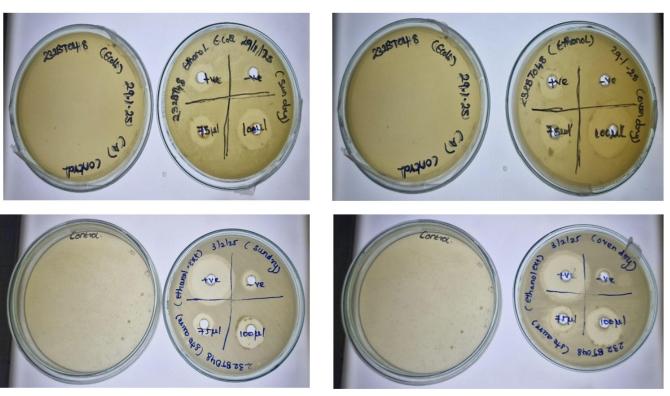


Figure 1: Antimicrobial activity of *Metapenaeus dobsoni*. (a) Anti-microbial activity of *Metapenaeus dobsoni* (*Escherichia coli*), (b) Anti-microbial activity of *Metapenaeus dobsoni* (*Staphylococcus aureus*).

Table 1: Antioxidant activity of Metapenaeus dobsoni Ethanol extract.

SI. No.	Sample	Absorbance 517nm		Scavenging Activity	
	Concentration	Sun dry	Oven dry	Sun dry	Oven dry
S1	900 μL	0.196	0.223	49.02%	41.12%
S2	920 μL	0.175	0.218	54.23%	43.26%
S3	940 μL	0.166	0.198	56.47%	48.29%
S4	960 μL	0.141	0162	63.21%	57.14%
S5	980 μL	0.060	0.054	84.11%	85.34%

ketonic groups, commonly found in triglycerides. The results of our study were performed and it's recorded as shown in Figure 2.

Formulation of Sunscreen Lotion

Formulation of sunscreen lotion both water and oil phase were prepared separately in a beaker. Slowly the oil phase was mixed together with water phase and was stirred gently to obtain the creamy texture and then added with certain preservatives, colour, fragrance and it is kept stored inside an air tight container. The ingredients for Water phase, Oil phase, Preservatives and the formulation of Sunscreen lotion.

The sunscreen formulation consists of two phases: a water phase and an oil phase. In the water phase, 60 mL of water is mixed with 0.3 g of xanthan gum, 2 g of aloe vera gel, and 3 mL of glycerine. The oil phase includes 5 g of beeswax, 5 g of cetearyl alcohol, and 2.5 g of titanium dioxide, which acts as the UV-blocking agent.

Tests for Sunscreen Lotion

pH test

The pH range of the sunscreen lotion formulation was determined in our study using a pH meter, and it was found to be approximately 7.8, which is considered to be skin-friendly. In particular^[11] study looked into the pH levels of different cosmetics, such as sunscreen lotions. The results were observed and recorded.

Spread ability test

2 g of formulated sunscreen lotion of about 20 mm is placed between two glass plates, and weight is applied to spread it. The diameter of the spread cream is measured and it is found to be about 86 and 60 mm. As such as in a study conducted by. [12] The results were observed and recorded and it is shown in Figure 3a.

Washability test

 $2\,\mathrm{g}$ of sunscreen lotion was placed on a microscopic slide, allowed to dry for around 30 min, and thoroughly washed to determine

the washability of the cream.^[13] Examined the significance of skin cleansing techniques for cosmetic washability. The results were performed and recorded it is shown in Figure 3b.

Control Sun Dry Oven Dry

Determination of sun protection factor test

SPF testing was conducted in our study using a variety of commercially available sunscreen lotions. Along with our test sample, three different commercially available sunscreen lotions were chosen as Controls 1 (Lakme), 2 (Dermaco), and 3 (Dr. Sheth's). Their absorbance was measured at specific wavelengths, including 320, 340, 360, 380, and 400 nm, respectively as shown in Figure 4 below. Accordingly, in a study by,^[14] the SPF test of various sunscreen lotion was measured by looking at them at about 400 nm. Our test sample's absorbance value is found to be close to Control 2's (Dermaco). The results were observed and recorded and it is shown in Figure 4.

DISCUSSION

Through TLC profiling, FTIR spectroscopy, and routine chemical testing (sulfuric acid, iodine, and bromine), the study verified the existence of astaxanthin in *Metapenaeus dobsoni*. According to earlier research, the DPPH and vitamin C assays demonstrated

Table 2: Vitamin C activity Metapenaeus dobsoni extract.

SI. No.	Concentration	Absorbance reading
S1	50 μL	0.270
S2	100 μL	0.630
S3	150 μL	0.922
S4	200 μL	1.250
S5	250 μL	2.496
Sample (Sun dry)	250 μL	0.843
Sample (Oven dry)	250 μL	0.583

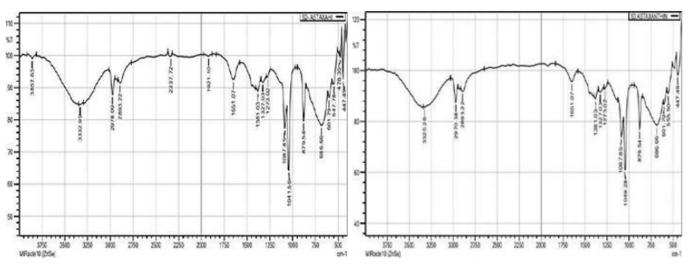


Figure 2: FTIR analysis of astaxanthin. (a) FTIR analysis of astaxanthin for sun dry method. (b) FTIR analysis of astaxanthin for oven dry method.

Table 3: Antibacterial activity of Metapenaeus dobsoni ethanolic extract.

Sl. No.	Microorganisms	Concentration of extract				
		75 μL		100 μL		
		Sun dry	Oven dry	Sun dry	Oven dry	
1	Positive (Streptomycin)	17 mm	25 mm	24 mm	28 mm	
2	Escherichia coli	15 mm	20 mm	22 mm	29 mm	
3	Staphylococcus aureus	14 mm	23 mm	20 mm	24 mm	

3 a



3 b







Figure 3: (A) Spread ability test, (B) Wash ability test.

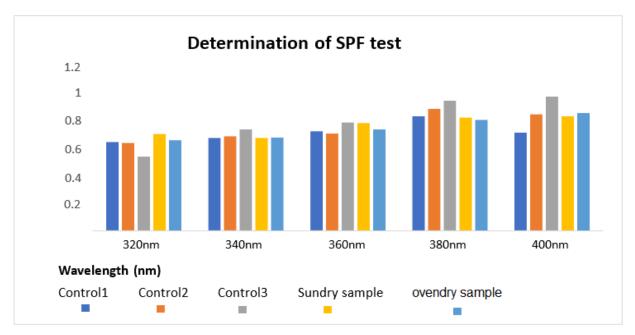


Figure 4: Determination of SPF test.

the antioxidant capability, exhibiting enhanced activity with greater extract concentrations.

The presence of astaxanthin was confirmed by TLC findings, with R_f values that were nearly normal. The antimicrobial assay showed excellent antibacterial potential by effectively inhibiting *S. aureus* and *E. coli*, particularly in oven-dried samples.

The functional groups that are characteristic of carotenoids were further confirmed by FTIR analysis. The sunscreen lotion's formulation demonstrated good pH, washability, and spreadability. Similar UV protection to store-bought sunscreens was found by SPF analysis, suggesting potential for cosmeceutical application.

As a natural sunscreen, antibacterial, and antioxidant, *M. dobsoni* extract exhibits great promise overall.

CONCLUSION

Metapenaeus dobsoni extract not only demonstrates strong antioxidant and antimicrobial properties but also shows promise as a bioactive ingredient in natural sunscreen formulations, thus paving the way for its application in pharmaceuticals and cosmetics. Further studies could focus on the formulation's long-term stability, *in vivo* efficacy, and potential synergistic effects with other natural compounds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; **FTIR:** Fourier Transform Infrared Spectroscopy; **TLC:** Thin Layer Chromatography.

SUMMARY

The present study provides an insight into the ability to produce an UV protection Sunscreen by using the astaxanthin extracted from the waste material which is shrimp shell *Metapenaeus* dobsoni. This study may helps in developing cosmetics along with the therapeutic applications keeping in mind the goals of Sustainable Development Goals postulated by WHO in order to create eco eco-friendly environment by creating wealth out of waste.

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