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EVALUATION OF TOTAL ANTIOXIDANT POTENTIAL OF PERIPHYTIC CAROTENOIDS

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ABSTRACT

Isolation of bioactive compounds relies greatly upon proper extraction strategy. Sustainable aquatic resource periphyton was exploited to extract carotenoids by means of acetone-petroleum ether and ethanol and identified through paper chromatographic approach. Carotenoid extracts were studied for their antioxidant potential. Result showed that ethanol extraction method performed well over acetone-petroleum ether extraction with respect to total carotenoid yield. Whereas acetone-petroleum ether extracted carotenoids showed the highest antioxidant activity compared to ethanolic carotenoid extract in a dose dependent manner. Periphytic carotenoid can be beneficial as a natural antioxidant for the periphyton grazing fish in aquaculture.

Keywords: Periphyton, carotenoid, chromatography, antioxidant.

I. INTRODUCTION

In a freshwater ecosystem, the complex association of microfloral communities found on the surfaces of submerged objects is called periphyton. It harbors variety of organisms from zooplankton to microbes, with phytoplankton contributing a major share [1].These are quintessential to maintain and manage a healthy aquatic system. Microbes, algae and plants are some of the important sources of bioactive compounds. Antioxidants are the bioactive molecules that prevent oxidation of other molecules broadly divided as enzymatic (SODs, catalase, GSH-Px) and non enzymatic antioxidants (β-carotene, ascorbic acid, α-Tocopherol) [2] and are well known for their ability to scavenge free radicals produced in the living cell. In recent time, attention towards the antioxidants has increased a manifold due to their huge health benefits. Carotenoids are one of the major photochemical found in algae and higher plants. These are basically organic pigments broadly divided into xanthophylls and carotene. Their appearance of color varies from yellow to orange. Carotenoid is of great interest in food and nutrition due to the tremendous health benefits. It was supported by many studies that these exhibit novel properties like anti-oxidation [3][4][5], anti-inflammatory [6][7], immune-modulation [8] and anticancer [9][10][11]. Due to the carcinogenic effect of synthetic antioxidants [12] interest towards natural antioxidants is growing. In this perspective, carotenoids were extracted from periphyton by means of acetone-petroleum ether biphasic and ethanol solvent system and checked for their anti-oxidant potential.

II. MATERIALS AND METHODS

2.1 MATERIALS

The following chemicals and equipment were used for the study. Ethanol (99.9%), petroleum ether (Sigma), calcium carbonate (Himedia), sodium chloride (SRL), sulphuric acid(Merck), ammonium molybdate (SRL), sodium phosphate(SRL) were procured locally. The absorbance was read using UV- Visible Spectrophotometer (Thermo).

2.2METHOD

Periphyton was grown on vertically hanged PVC pipes in a water filled cement tank with fortnightly application of urea, single super phosphate and cow dung [13]. After a month, the dense green mucilaginous periphyton coating on the pipes was scraped out and used for the study. In the first method, 2 gram of collected periphyton was nicely macerated with 50 ml of acetone along with a pinch of calcium carbonate and transferred to separating funnel. Then 10 ml of petroleum ether was added along with a pinch of sodium chloride and partitioned with distilled water. Later, lower layer of acetone was discarded and the upper layer of petroleum ether was filter collected in a volumetric flask of 25ml. The process was repeated twice to ensure maximum extraction and the volume made up to 25 ml with petroleum ether [14]. In the second method, two gram of periphyton was macerated with 10 ml of absolute ethanol and filter collected in a volumetric flask of 10ml and the volume made up with ethanol [15]. Finally, extracts were read at an absorbance of 450nm using UV-Visible spectrophotometer. Total carotenoid content was calculated using the following formula [16].

$$\text{Carotenoids content } (\mu g/g) = \frac{A \times V\ (ml) \times 10^4}{A_{1cm}^{1\%} \times P\ (g)} \qquad \text{Formula (1)}$$

Where A = Absorbance; V = Total extract volume; P = sample weight; $A_{1cm}^{1\%}$= 2592 (β-carotene Extinction Coefficient in petroleum ether); $A_{1cm}^{1\%}$=2620 (β-carotene Extinction Coefficient in ethanol).

2.2.1 PAPER CHROMATOGRAPHY

Separation of carotenoid was performed using paper chromatography [17] with a slight modification. Instead of TLC plate, Whatman no 2 paper was used as a stationary phase. Chromatogram was developed in petroleum ether mobile phase for the first extract and ethanol for the second extract. Retention factor was calculated using the following formula.

$$R_f \text{ value} = \frac{\text{Distance from origin to solute front}}{\text{Distance from origin to solvent front}} \qquad \text{Formula (2)}$$

2.2.2 TOTAL ANTIOXIDANT ASSAY

The total antioxidant capacity was evaluated by phosphomolybdenum reduction assay [18] with little modofication. Phosphomolybdenum reagent was prepared by mixing 0.588ml of sulphuric acid, 0.049g of ammonium molybdate and 0.036g of sodium phosphate. 1ml of varying concentration (100, 200, 400, 600, 800 and1000 µg/ml)) of carotenoid extracts and standard antioxidant ascorbic acid were taken in a test tube and 1ml of phosphomolybdenum reagent was added to it and allowed to stand in a water bath at 95 °C for 90min. After

incubation, the blue colored complex formed was measured at 695nm using UV-Visible spectrophotometer. Results were expressed in ascorbic acid equivalents.

III. STATISTICAL ANALYSIS

All the extractions were carried out in triplicates and analyzed using one-way analysis of variance (ANOVA). Total carotenoid content of periphyton recorded in the ethanol extraction method and acetone-petroleum ether method differed significantly ($P<0.001$).

IV. RESULT AND DISCUSSION

Result showed a mean total carotenoid content of 125.89µg/g in acetone-petroleum ether extraction method and 173.79 µg/g in ethanol extraction method which is given in the table 1.Mean carotenoid contents ranging from 19.2 to 33.8 in methanol, 18.8 to 28.7 in diethyl ether and 20.1 to 29.5 µg/g in acetone were recorded for some algal species [19]. Periphyton harbors variety of different species of phytoplankton [1], hence variation in carotenoid contents are expected. Fig. 1 shows the paper chromatographic separation of carotenoid obtained from two different extraction methods. Orange yellow color band obtained during paper chromatographic separation along with retention factor confirmed the presence of carotenoid [20]. Acetone-petroleum ether extracted carotenoids showed the highest antioxidant activity compared to ethanolic carotenoid extract in a dose dependent manner (table. 2, Fig.2 and Fig.3).

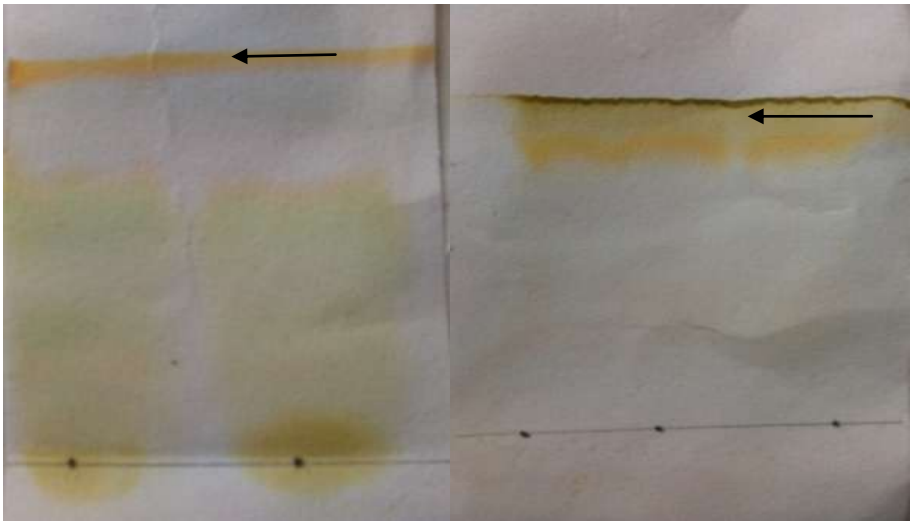
Antioxidant potential of carotenoid has long been studied and showed its efficacy in preventing inflammation, scavenging free radicals, and cardiovascular diseases [21] also in coloration of ornamental fish [22]. In case of phosphomolybdenum reduction assay Mo (VI) is reduced to Mo (V) by the antioxidants with production of a green phosphate/Mo (V) complex at acidic pH [23]. In our study acetone-petroleum ether extracted carotenoid exhibited highest antioxidant activity compared to ethanolic carotenoid extract in a dose dependant manner. Reduced oxidative stress was reported by dietary feeding of astaxanthin rich red yeast on rainbow trout [24] and carotenoid supplemented feed demonstrated good antioxidant potential in *Cyprinus carpio* [25]. Increased level of retinol and α-tocopherol level in the muscle and ascorbic acid in liver was observed on dietary astaxanthin fed Altantic salmon establishing a positive correlation between astaxanthin and antioxidant status in liver and muscle [26]. Enhanced antioxidant status by dietary astaxanthin was reported in penaeid postlarvae also increasing the dietary carotenoid concentration decreased the blood superoxide dismutase level in *Hyphessobrycon callistus* [27].

Aquatic animals undergo series of oxidative stress contributed by changes in temperature, salinity, oxygen level, and human born pollutants [28].The antioxidant activity of carotenoid from sustainable sources like periphyton would be beneficial to the aquatic organisms grazing on periphyton and hence additionally could be used as a functional food in aquaculture and their by facilitating a unique solution to aquatic health management.

V. FIGURES AND TABLES

Extraction method	Total carotenoid content (µg/ml)	Mobile phase	Retention factor (R_f)
Acetone -petroleum ether extraction	125.89±0.57	Petroleum ether	0.96
Ethanol extraction	173.79±0.91	Ethanol	0.92

Table 1. Total carotenoid yield (± SD; n=3) and retention factor.



Acetone-petroleum ether extract **Ethanol extract**

Figure.1 Paper chromatographic separation pattern of carotenoid.

Concentration of extract (µg/ml)	Total antioxidant activity in ascorbic acid equivalent (Acetone-Petroleum ether extract)	Total antioxidant activity in ascorbic acid equivalent (Ethanol extract)
100	48.33±0.10	16.67±0.24
200	68.00±0.13	20±0.42
400	137.33±0.09	32.67±0.20
600	202.66±0.03	53.33±0.39
800	252.33±0.04	76.33±0.29
1000	340.00±0.28	153±0.49

Table 2.Comparison of total antioxidant activity expressed in ascorbic acid equivalent.

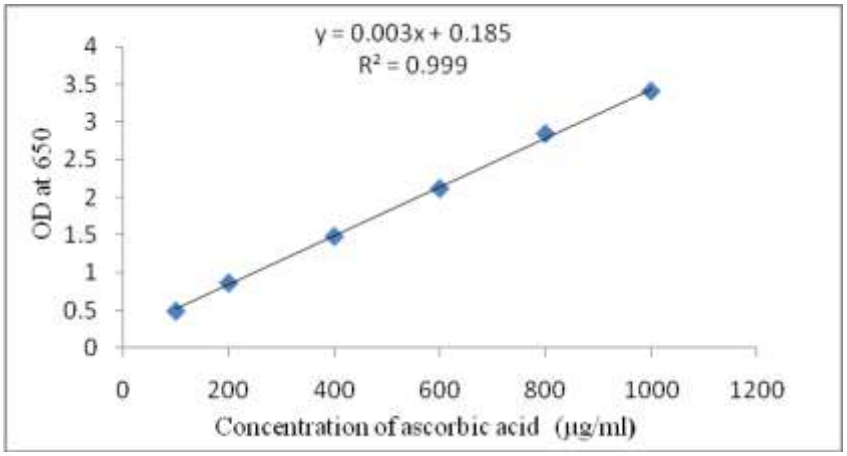


Figure 2.Ascorbic acid standard curve.

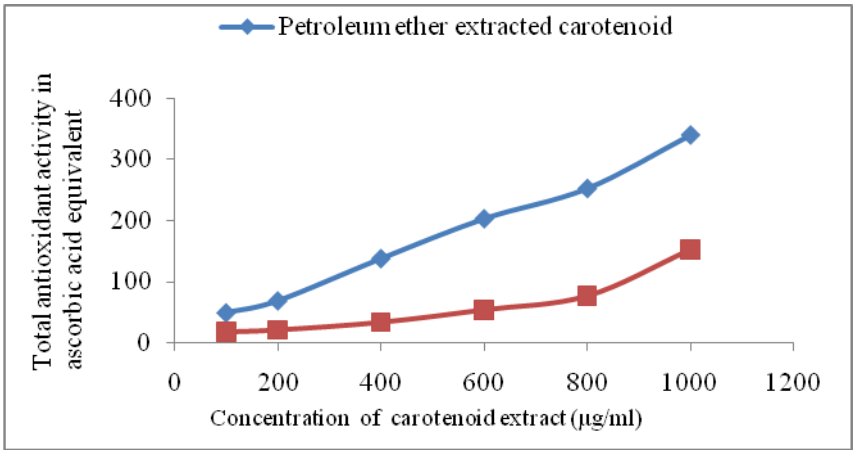


Figure3. Comparison of antioxidant activity of carotenoid extracts.

VI. CONCLUSION

Result showed that ethanol extraction method performed well over acetone-petroleum ether extraction with respect to total carotenoid yield. Petroleum ether extracted carotenoids showed the highest antioxidant activity compared to ethanolic carotenoid extract in a dose dependent manner. Periphytic carotenoid can be beneficial as an antioxidant for the periphyton grazing fish in aquaculture.

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