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Research Article

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Extraction, partially purification and study on antioxidant property of fucoxanthin from *Sargassum cinereum* J. Agardh

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ABSTRACT

Fucoxanthin is a Xanthophyll. It is found as an accessory pigment in the chloroplasts of brown algae, Sargassum cinereum. The crude pigment was initially screened through TLC and then fucoxanthin was separated and partially purified using silica column chromatography (230 – 400 mesh). The yield of fucoxanthin from Sargassum cinereum was 2.0225mg/g of sample. Fucoxanthin was found to be present in Sargassum cinereum extract, which was detected using UV vis spectrophotometer at 422.90nm and identified using FT-IR, HPLC and GC-MS. The fucoxanthin was later checked with free radicals for its antioxidant property. 60µg/ml of pigment showed 50% of scavenging activity of free radicals.

Keywords: Fucoxanthin, Brown seaweed, Antioxidant property, Silica gel Chromatography

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INTRODUCTION

Seaweeds are excellent sources of bioactive compounds such as polyphenols, carotenoids and polysaccharides [6, 10, 17, 21]. These bioactive compounds can be applied in functional food, pharmaceuticals and cosmetic products as they bring health benefits to consumers [12, 14, 21].

Sargassum sp is brown seaweed. The major pigment of brown seaweeds is fucoxanthin, which is one of the most abundant carotenoids in nature (10% estimated total production of carotenoids) [12]. It is an orange-coloured pigment, found in brown seaweeds along with chlorophyll, to give a brown or olive-green colour [5,8]. Fucoxanthin has an unique structure, where it contains an unusual allenic bond and a 5,6-monoepoxide in its molecule. However, different brown seaweed strains produce different compositions and profile of fucoxanthin [12]. This natural pigment exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities. Therefore, various natural pigments isolated from marine algae have attracted much attention in the fields of food, cosmetic and pharmacology [12].

EXPERIMENTAL SECTION

Sample collection

Marine algae *Sargassum cinereum* was collected from Tuticorin coast, Tamilnadu and identified by *Sargassum cinereum* was identified by Dr. P. Anantharaman Associate Professor, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamilnadu. The seaweed was directly handpicked from the 5cm depth of the sea. Collected seaweed was washed thoroughly with sea water followed by washed with distilled water to removed sand and salt particles adhere to tat. It was allowed for shadow drying for 5 days. Then it was powdered using mixer grinder and stored at room temperature.

Extraction of Fucoxanthin

1 g of seaweed powder was weighed and 10ml of ethanol was added. Incubated at 4°C in dark place for overnight. Centrifuged at 8000rpm for 15minutes. Supernatant was collected. Repeat that process till it become colourless. Stored in amber colour bottles to avoid degradation by light. The amount of fucoxanthin present in the sample calculated by the formula [15].

Fucoxanthin mg/g = A_{470} - 1.239 (A_{631}+A_{581} – 0.3× A_{664}) – 0.0275×A_{664}/141

Where,
A= Absorbance of particular wavelength

SCREENING AND PURIFICATION:

Thin layer Chromatography:

Thin layer chromatography was used to calculate the R_f value of the photosynthetic pigments such as Chlorophyll, carotenoids and fucoxanthin present in the extracted sample. In TLC pigments in the mixture are separated on the basis of their differences in solubility and partition co- efficient in a binary solvent system. Silica gel coated plates (Merck -10×6 cm) and the developing solvent (chloroform: ethanol) in the ratio 7: 3 were used for TLC. 5µl of sample applied on the plates and samples allowed to dry. Sample does not dip in the solvent system and kept undisturbed. Carotenes, chlorophyll a, fucoxanthin and Chlorophyll C. [7].

R_f = Distance moved by the pigment / distance moved by the solvent

Where,
$R_{f\,=}$ Retention factor

Purification of the pigment:

Column was rinsed with chloroform and was packed with wool or cotton at the bottom. Silica gel (100 – 200 mesh) mixed with the solvent chloroform. Poured into the column and tap it continuously without any breakage or bubbles. Then it is kept undisturbed for 1 day for proper binding of silica or else use within half an hour. Dried oily like pigment mixed with silica gel and loaded into the column. For the purification and separation of our target compound, eluting solvent chloroform, chloroform: ethanol was poured in different ratio. Compound was collected and stored in a brown bottle under 4-10°C.

CHARACTERIZATION OF FUCOXANTHIN

Uv –Vis Spectrophotometric Analysis:

Sargassum cinereum extract was dissolved in Chloroform and the pigment profile was determined using a double – beam UV – Visible spectrophotometer at the wavelength range of 200 -700nm at room temperature.

Fourier Transform Infrared Spectroscopy analysis of Pigment:

The pigment was characterized using Fourier transform infrared Spectrophotometer, Perkin – Elmer FT-IR instrument, which helped to analyse different sulphate, carboxyl and hydroxyl groups of these sample molecules [20]. One part of extract was mixed with ninety parts of dried potassium bromide (KBr) separately and then compressed to prepare a salt disc of 3mm diameter. These discs were subjected to IR – spectrophotometer. The absorption was read between 400 and 4000 cm^{-1}.

HPLC:

The purified fucoxanthin was dissolved in adequate amount of methanol. The presence of fucoxanthin in the Sargassum sp was identified by comparing in the retention time of fucoxanthin standard and purified fucoxanthin from Sargassum sp using HPLC. The parameters of HPLC were obtained from Noviendri *et al*. [11]. With some modifications on the column brand and detector used. The HPLC shimadzu system (Shimadzu LC -20AT) with auto injector (Shimadzu prominence HPLC autosamplers, SIL, 20A HT) was equipped with a C18 column (Hypersil Gold, 5.0µm particle size, 250 mm × 4.6 mm i.d.). The fucoxanthin was eluted using methanol: acetonitile (7:3, v/v) mobile phase, with a flow rate of 1.0ml/min at 28°C. The eluent was detected using a UV – Vis detector (Shimadzu Prominence HPLC UV – Vis detectors) at 450nm [11].

GC –MS analysis:

A Agilent (model7820 A GC system and 5977E MSD) plus series equipped with a FID detector and DB5 column (105 meter, 0.32mm ID, 0.20 µm film thickness) was used. The sample was prepared with Chloroform solvent mixture. The samples were injected into the gas chromatograph where high pure helium was used as the carrier gas.

Injector and detector temperatures were maintained at 280°C. The column temperature was programmed from 70° to 300°C. Peak areas and retention times were measured by electronic integration with computer.

Antioxidant property

DPPH (1,1 –diphenyl- 2- picrylhydrazyl) stock (1M) was prepared using 0.394grams of DPPH in 1ml each of 90% methanol. DPPH working solutions (0.15mM) were prepared from 15µl stock mixed with 100ml of solvent. 2ml of 0.15mM/L DPPH radical was taken in a test tube, 1ml of sample extract was added and vortexed for 30 seconds. Reaction mixture was kept in dark at room temperature for 20 minutes. Control was prepared without adding the sample extract. Absorption was read at 517nm and the antioxidant activity was calculated using the standard formula [4].

DPPH scavenging activity = 1- (Absorbance of sample/Absorbance of control) × 100

RESULTS AND DISCUSSION

Pigment profile and yield of fucoxanthin

A broad series of polysaccharides from edible seaweeds have emerged as an important class of bioactive natural products, possessing many important properties of pharmacological relevance. [16]. The antioxidant activity of several naturally occurring compounds have been known for decades. Seaweeds can be used as food additives and can also provide protection [2].

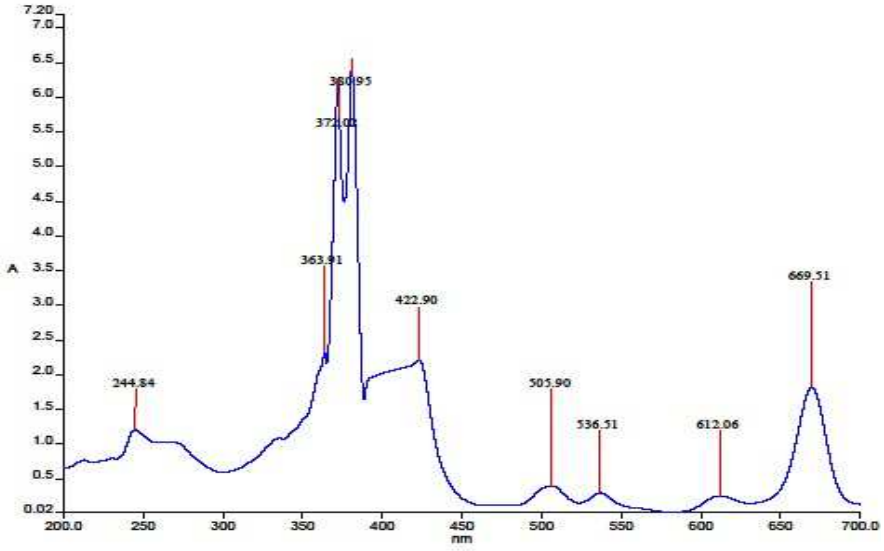

Fig 1: UV scan for Crude pigment

The yield of fucoxanthin from *Sargassum cinereum* 2.0225mg/g. In the present study the pigment from brown seaweed *Sargassum cinereum* was isolated and purified by silica gel chromatography. The employment of silica gel as a matrix has been widely reported for pigment separation and also to reveal the characteristics of different algae. In the present investigation pigment production 2.0225 mg/g. Zailanie and Sukoso [9] suggested that the fucoxanthin pigment from *Sargassum filipendula* is 0.1957 ± 0.0173mg/g. Sudhakar *et al.*,[18] experimented that the pigment extracted using 100% acetone showed values lower than that of 90% acetone and higher than that of 90% ethanol extracted samples. *Padina sp* and *Turbinaria sp* contain more amount of pigment 0.38 ± 0.046 mg/g.

UV – Visible spectrophotometer:

The absorption peak at 422.90nm. The crude extract purified using column chromatography and analysed by HPLC, FTIR to serve as further confirmation steps on the presence of fucoxanthin in the *Sargassum cinereum* extracts. (fig1). Fucoxanthin absorbs light primarily in the blue green to yellow green part of the visible spectrum peaking at around 510 – 525 nm by various estimates and absorbing significantly in the range of 450 – 540nm. From this UV vis spectrum, this pigment extract consisted of higher proportion of carotenoid compared with chlorophyll. Since carotenoids have beneficial functions and values in food and pharmaceutical industries, hence further analysis in this research was focused on the carotenoid. According to Chandini *et al.*, [5] and Hosokawa *et al.*, [8], fucoxanthin is a major carotenoid found in brown seaweeds. In order to determine whether the carotenoid present in pigment extract was fucoxanthin, was dissolved in methanol: water (1:9, V/V) and analysed by using UV Vis Spectrophotometer..

Sudhakar *et al.*, [18] showed that the fraction of yellow orange colour was subjected to spectral analysis and based on the absorbance maximum at 446 nm and it was confirmed to be fucoxanthin. According to [13], the absorption of fucoxanthin ranges from 420 – 470nm and pointed that standard fucoxanthin pigment showed peak at 423nm.

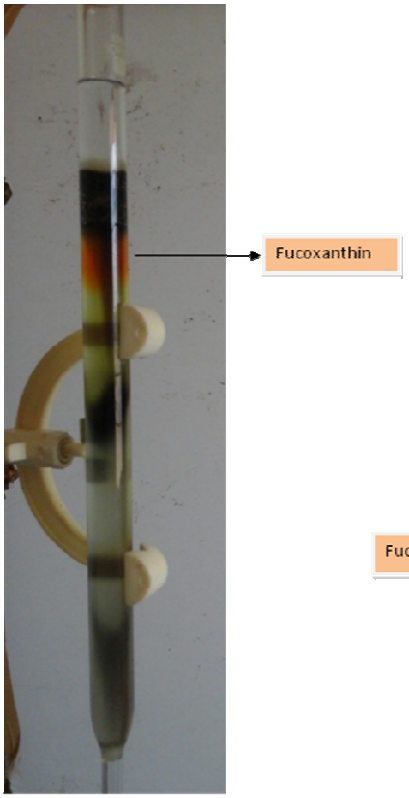

Fig 3: Silica gel column chromatography

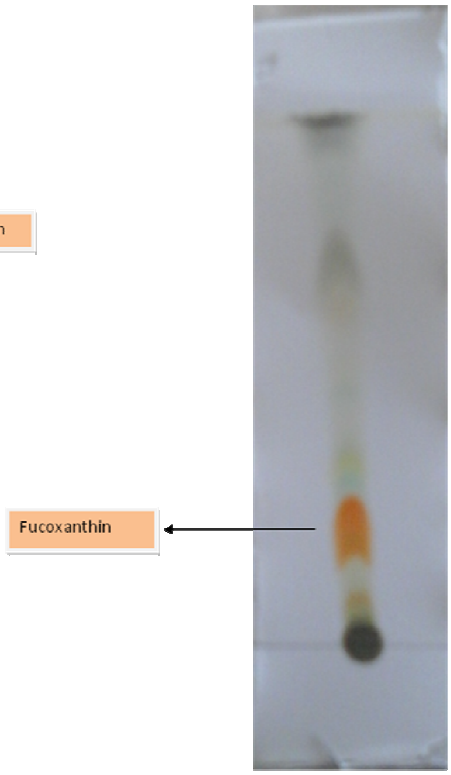

Fig 2: Thin layer Chromatography

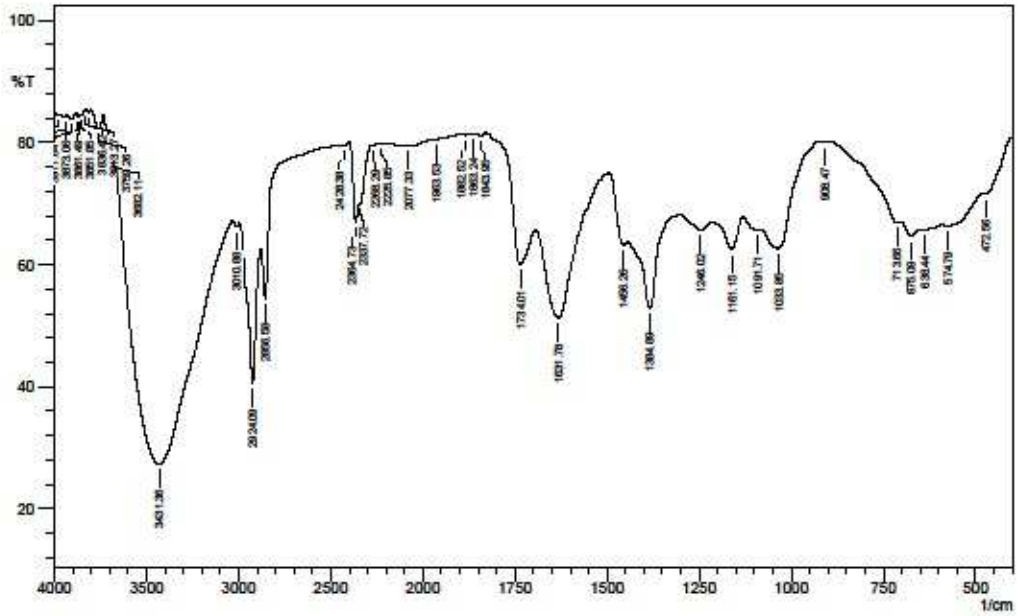

Fig 4: Fourier Transform Infrared Spectroscopy

Thin layer Chromatography:

From the crude pigment fucoxanthin was separated by using TLC. Orange coloured band showed the presence of fucoxanthin. R_f value – 0.26 (fig 2).

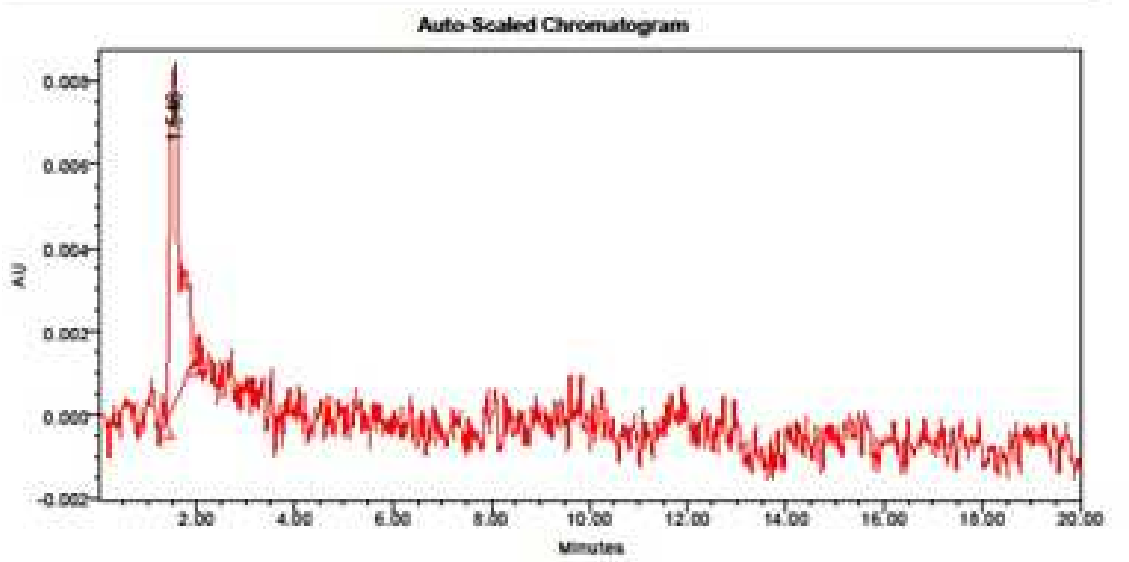

Fig 5: High performance liquid Chromatography

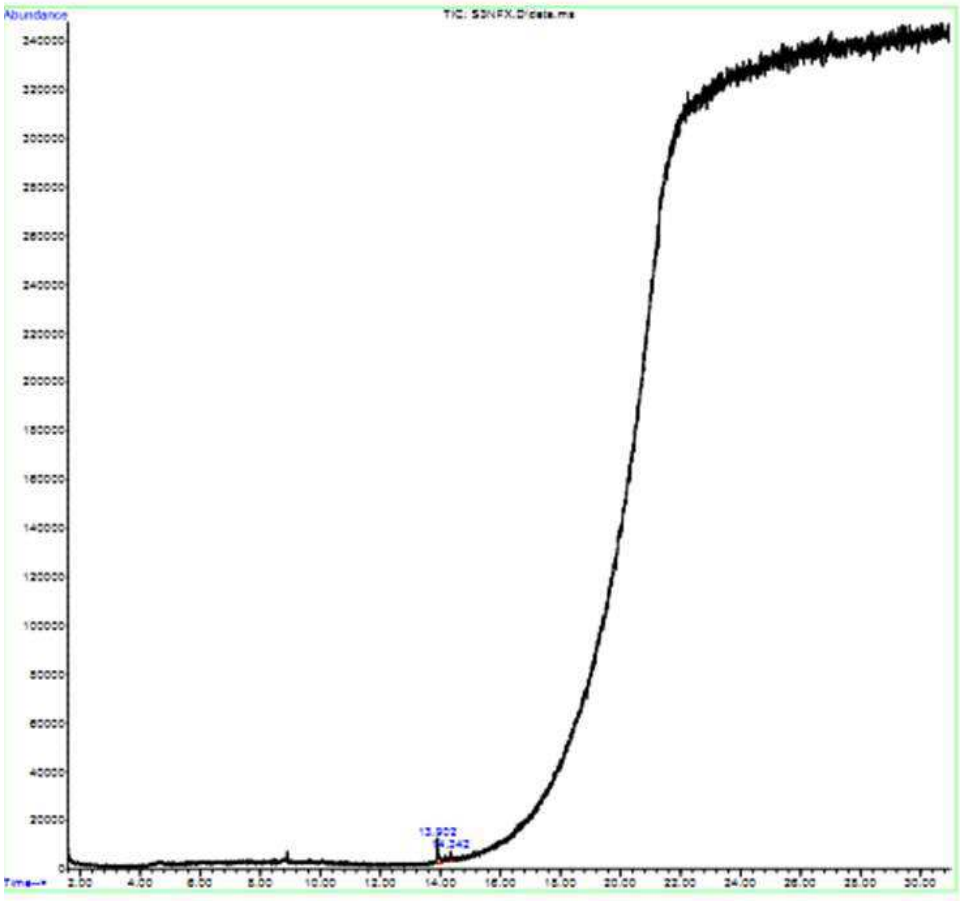

Fig 6: Gas Chromatography Mass spectrum of pigment

Silica gel Column chromatography : Purification

Crude pigment was separated using Silica gel column chromatography. Orange coloured band indicated fucoxanthin. (Fig 3)

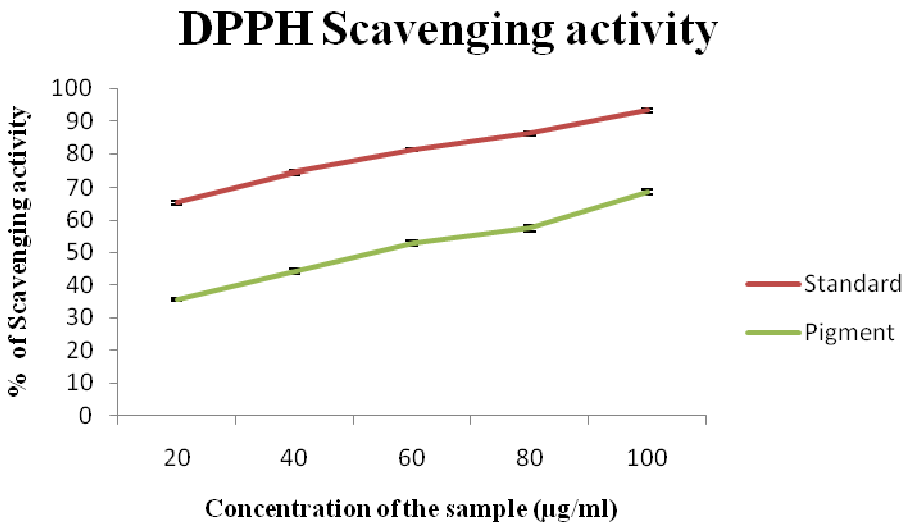

Fig 7: DPPH Scavenging activity

FTIR:

The signals at 3431.36 showed the presence of Heterocycline amine NH- Stretch. The signals at 2268.29 and 2225. 85 indicated the presence of aliphatic cyanide and aromatic groups. Alkynes groups presence was indicated by the signal 2077.33. The signals at 1863.24 indicated the presence of five membered ring anhydride. The stretch band at 1843.95 due to the presence of open chain acid anhydride. The signals at 1734.01 indicate the presence of aldehyde. Quinone or conjugated ketone indicated by signals at 1631.78. The signals at 1456.26 indicated nitrogen compounds. Alkane group presence was indicated by 1384.89. 1161.15 showed the presence of sulfonates. 1091.71 showed the presence of organic siloxane or silicone.(fig 4) Sudhakar *et al.*,[18] showed that the partially purified fucoxanthin showed that the R_f value is 0.21. In the present study orange band of fucoxanthin showed that the R_f value is 0.26. According to Yip *et al.*,[22] Fucoxanthin showed similar absorption bands with fucoxanthin standard the functional group that is descriptive of fucoxanthin in the presence of allenic bond (C=C=C), where it is found in the spectrum at 1929.21cm^{-1} and 1929.03 cm^{-1} for fucoxanthin standard and purified fucoxanthin from *Sargassum binderi* respectively [12]. The signals at 3031.74cm^{-1} showed the presence of –CH group. Therefore presence of all these functional groups in the spectrum validates the UV- Vis and HPLC results that the purified fucoxanthin is indeed fucoxanthin.

HPLC:

The peak showed the presence of pigment. (fig 5)

GC- MS analysis:

GC – MS analysis indicated the presence of Compound such as 2-4 – (Fluoro –phenyl) - 4 – (3 –methyl – benzylidene) 4-H –oxazol – 5 –one and 4H -1, 3 –Benzodioxin. (fig 6)

DPPH radical scavenging activity

The free- radical scavenging activity (DPPH) assay examines the primary antioxidant potential. Primary antioxidants, also referred to as chain breaking antioxidants, function as free- radical acceptors/ scavengers and delay or inhibit the initiation step or interrupt the propagation step of auto- oxidation. Antioxidant activity was tested in the crude pigment. Fucoxanthin is a major carotenoid in brown algae and it has its antioxidant property. Ascorbic acid is used as a standard. Pigment showed 50 % of scavenging activity in 60µg/ml. (fig 7) In the present study the total antioxidant capacity of pigment *Sargassum cinereum* was found to be 60µg/ml showed that 50 %.of scavenging activity. Sudhakar *et al.*, [18], absorbed that the maximum of scavenging activity 57.67 ± 6.65% in *Sargassum longifolium.* In addition, the algal extract showed a strong DPPH radical scavenging activity. The carotenoids, fucoxanthin present in brown seaweeds with its strong antioxidant activity has wide applications in various nutraceutical and pharmaceutical areas as a commercially important bioactive compound [1, 3, and 19].

CONCLUSION

A study has shown that fucoxanthin has strong effects on oxidative antioxidant property. Fucoxanthin have been looked into for its anti – inflammatory, antinociceptive and anticancer effects. Furthermore, the powder form is an appropriate ingredient to enrich nutritional value of other food.

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