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Phytochemical Screening and Isolation of Fucoxanthin Content of *Sargassum ilicifolium*

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ABSTRACT

Brown algae (Phaeophyceae) from west coast of Maharashtra was extracted for their phytochemical and fucoxanthin content. The major pigment in brown algae is fucoxanthin, it is one of the most abundant carotenoids in nature. Fucoxanthin has a unique chemical structure because it has a bond alenat and 5.6 monoepoxide within the molecule. Electrospray Ionization (ESI) of LCMS was investigated for the determination of molecular weight. In the positive ion mode monomer, dimmer and higher order adduct were also detected. The molecular weight of compound fucoxanthin is 659.43 by LCMS. In the present study, Sargassum ilicifolium shows subsidiary components like carbohydrates, alkaloids, glycosides, saponins, tannins and terpenoids. Dried powder of brown alga was analyzed for fluorescence study which results in prospective source of bioactive compounds.

Keywords: *Fucoxanthin, LCMS, Sargassum ilicifolium, Phytochemical Screening.*

INTRODUCTION

Seaweeds are important marine resources exploited for their commercial value as the source of phycocolloids such as agar, agarose, algin and carrageenan, besides their use as food, source of enzymes, dyes, drugs and growth promoters etc. Fucoxanthin is the main pigment found in brown algae which is most abundance carotenoids in nature[1]. It has been observed that this oxygenated carotenoid is a very effective inhibitor of cellular growth and promotes apoptosis in human cancer cell lines[2,3]. Moreover, this pigment possesses anti-inflammatory[4], antidiabetic[5] and antioxidant activities[6]. The brown colour of the phaeophyceae results from the dominance of the pigment fucoxanthin which masks the other pigments (including chlorophyll a and c, beta-carotene and other xanthophylls[7]. Seaweeds have rich sources of novel and potentially bioactive primary and secondary metabolites[8]. Considering the chemical and immense pharmacological properties of brown algae, the present study was carried to analyze the qualitative phytochemical constituents of methanolic extract of *S. ilicifolium*.

MATERIALS AND METHODS

The *S. ilicifolium* was collected from west coast of Maharashtra. Seaweed samples were picked collected and immediately washed with sea water to remove the foreign particles, sand particles and epiphytes, stored in polythene bags and immediately transported to the laboratory.

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To remove the salt on the surface of the sample it was washed thoroughly with the tap water. Then the seaweeds were spread on blotting paper to remove excess water and dried.

Preparation of extract

10grams of air dried powder of *S. ilicifolium* was extracted in 50 ml solvent viz., chloroform, acetone, pet-ether, benzene and aqueous for 24 hours on a rotary shaker at a constant speed (200 strokes per minute). The extract was filtered through a Buckner's funnel using Whatman No.1. filter paper and the filtrate was collected (crude extracts).

Fluorescence analysis

The powdered materials were tested with different reagents such as chloroform, benzene, methanol, aqueous, 50% sulphuric acid, 1N HCl, ethanol, NaOH, HNO_3, acetone, pet ether and toluene. The crude extracts were examined under visible and UV light and changes in colour were recorded. Fluorescence analysis method was carried out as per Pandurangan *et al.,*[9].

Phytochemical screening

The different extracts were tested for alkaloids, carbohydrates, glycosides, steroids, flavonoides, flavones, tannins, terpenoides, phenols, coumarins, anthroquinone and phlobatannin. Phytochemical screening of *Sargassum* was carried out according to standard procedures by Harborne[10].

LC MS analysis

LC MS is an advantageous technique, which combines the chromatographic separation by liquid chromatography with the detection by mass spectrometry (MS). In a general way, the molecules present on the sample are converted into a gas phase ionic species by the addition or removal of electrons or protons[11]. The purification of fucoxanthin method was adopted from Noviendri *et al.,*[12] with little modification.

RESULTS AND DISCUSSION

The fluorescence analysis of different extracts of *S. ilicifolium* is recorded in table 2.

Table 1: Powder Characteristics of *Sargassum ilicifolium.*

S. No.	Characteristics	Properties
1.	Colour	Greenish Brown
2.	Odour	Fishy
3.	Texture	soft

Table 2: Fluorescence analysis of powdered form of *Sargassum ilicifolium*.

Solvents	Ordinary	UV Light
Chloroform	Green	Yellow
Benzene	Green	Faint Red
Methanol	Faint green	Faint Red
Aqueous	Yellowish	Faint Red
H_2SO_4	Black	Faint Red
1N HCl	-	Faint Red
Ethanol	-	Yellow
NaOH	Faint brown	Yellow
HNO_3	Orange	Yellow
Acetone	Faint green	Orange
Pet ether	Faint green	Orange
Toluene	Brown	Red

Table 3: Phytochemical constituents present in different extract of *Sargassum ilicifolium.*

S.No.	Phytochemical parameter	Water extract	Chloroform	Acetone	Pet ether	Benzene
1.	Alkaloids	+	+	-	-	-
2.	Carbohydrates		+	+	-	-
3.	Glycosides	+	+	-	-	-
4.	Steroids	-	-	+	-	-
5.	Flavonides	-	-	-		-
6.	Flavones	-	-	-	-	-
7.	Saponins	+	-	+	+	+
8.	Fixed oil and Fat	+	-	-	-	-
9.	Tannins	+	-	-	-	-
10.	Terpenoids	-	+	+	-	-
11.	Proteins	-	-	-	-	-
12.	Amino acids	-	-	-	-	-
13.	Phenols	-	-	-	-	-
14.	Coumarins	-	-	-	-	-
15.	Anthroquinone	-	-	-	-	-

Note: '+' active compound present
'-'active compound absent

The results of the preliminary phytochemical investigation of different extracts of *Sargassum ilicifolium* are summarized in **Table 3.** The aqueous extract of *Sargassum ilicifolium* contains alkaloids, saponins, tannins and phlobatannins. Phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, terpenoids and saponins are found to be high in aqueous extract of *Sargassum.* It is clearly evident from the table 3, that other phyto constituents like coumarins, phenols and anthraquinones were totally absent. Seaweeds are low in fats but contain vitamins and bioactive compounds such as terpenoids and sulfated polysaccharides, they have natural anti-oxidant which is not found in land plants[13,14]. Literature survey showed that seaweed contain large amount of polysaccharides but less amount of proteins and amino acids[15]. Studies on brown seaweed *Sargassum wightii* showed the presence of flavonoids and steroids[16,17]. The total phenol content of edible Irish brown seaweed, *Himanthalia elongate* was found to be at higher level[18]. These results suggest that presence of primary bioactive metabolites of commercial importance which acts on the precursors for the synthesis of secondary metabolites.

Fig.1: Structure of Fucoxanthin

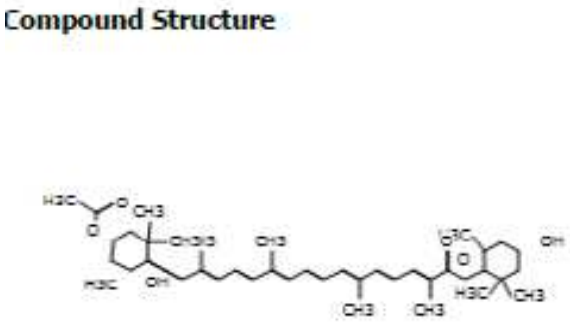
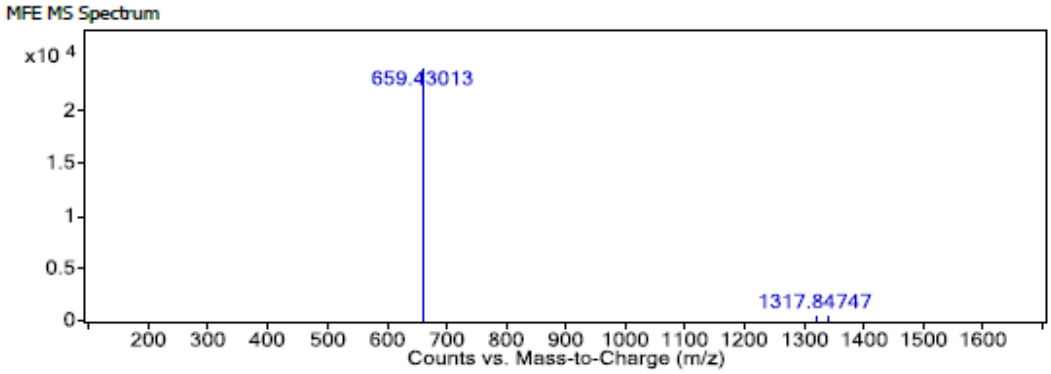


Table 4. Compounds fractions fucoxanthin ion molecule present in the *S.ilicifolium*

S.No.	Ion mass (m/z)	Allegations of molecular ion fragments
1.	659.43013	(M+H)+
2.	660.43488	(M+H)+

From the obtained data, the first peak is the molecular weight of 659.43 fucoxanthin with m/z while the second peak is the molecular weight of 660.43 m/z which is thought to occur dehydro fucoxanthin removal of water and a third peak 1317.84 m/z is the moment analysis there is the addition of Na ions (which is attached to the ion H^-) and the fourth peak of Na atoms there is an abundance of isotopes with a molecular weight of 1339.83 m/z. In this report a pure fucoxanthin can be detected by LCMS m/z having molecular wt 659.43013(fig.2). Garside and Riley[18] reported a molecular wt. of fucoxanthin pure compounds having a molecular wt. of 658 consists of a group of molecules acetyl and two hydroxyl groups.

Fig. 2: Mass spectrum of Fucoxanthin



CONCLUSION

Fucoxanthin pigment content from *S. ilicifolium* is 659.43. The marine environment hosts a wide range of bio-resources that have tremendous potential phytochemicals. From the above study, it may be concluded that extract of *Sargassum* shows the presence of various phytochemicals and their bioactive compounds may be utilized for bio-efficacy and bioactivity.

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