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EXTRACTION OF FUCOXANTHIN FROM *SARGASSUM WIGHTII*, FTIR SPECTRA AND IT'S ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES

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Keywords:

Sargassum weightii, Antimicrobial Action, Antioxidant activity, FT-IR spectra

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ABSTRACT: *Sargassum wightii*,a seaweed and a marine macroalgae, is known to offer numerous health benefits, and compounds isolated from it are observed as the alternative to conventional treatment as an affordable plant-derived drug. In the present study, the bioactivity of the two fractions from *Sargassum wightii*w as derived using two different solvents, methanol and acetone: methanol. Acetone: Methanol fraction showed better yield. Both the fractions it was tested against both gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. The antioxidant potential was evaluated by the reducing power assay. Fucoxanthin extracted by maceration in methanol: acetone (9:1) showed more excellent antioxidant activity. The partially purified extract was also analysed through the FT-IR spectra. The extract prepared by methanol: Acetone extract performed better than streptomycin, but compared to streptomycin, none of the extracts showed significant antibacterial action at higher concentrations. The FT-IR spectra showed various secondary metabolites and their derivatives in the crude extracts.

INTRODUCTION: Seaweeds, also known as marine macroalgae, are aquatic plants that grow in both fresh and saltwater environments. They are categorised into three primary categories include: green, red, and brown seaweeds [1]. Marine macroalgae are significant due to their roles in aquatic ecosystems, their potential for producing food, oxygen, and bioactive compounds, and their participation in the global carbon cycle [2]. The *Sargassum* genus is a diversified collection of brown seaweeds usually found in tropical and subtropical waters worldwide. It is a member of the *Sargassaceae* family [3].

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One of the members of the genus *Sargassaceae*, *Sargassum wightii*, also known as Wight's *Sargassum*, is found in the Arabian and Indian oceans [4]. The seaweed *Sargassum wightii* is abundant in bioactive substances compressing of, polysaccharides, fucoidan, fucoxanthin, Xanthophyll, terpenoids, etc., [5]. Additionally, it has anti-obesity, anti-cancer, antioxidant, anti-bacterial and anti-inflammatory properties [6].

The *Sargassum* genus is also known to have skin-healing properties. The presence of these properties is attribute of the carotenoid along with *Xanthophyll* pigments, which give *S. wightii* a brown colour [5]. One of the essential carotenoids is Fucoxanthin, which is promoted as a nutraceutical [7]. Fucoxanthin is the marine carotenoid responsible for the brown colour of the seaweed; There are various types of seaweed species, comprising *Undaria*, *Fucus*, *Sargassum* and *Myagropsis*, in

addition to microalgae such as *Phaeodactylum*, *Cyclotella*, *Isochrysis*,, *Nitzschia* and *Prymnesium*, contain, conjugated carbonyl groups and an unusual allenic bond, a 5,6-monoepoxide group in their polyene chain. These compounds possess antioxidant, anti-obesity, anti-inflammatory, and anticancer properties [5, 8]. Many of these attributes are because fucoxanthin can protect against oxidative stress by inhibiting DNA-damaging agents. Further, it showed no toxicity or mutagenicity in mice under the experimental conditions [9]. The extraction yield of Fucoxanthin from various seaweeds depends upon the species chosen, the growth conditions, and the extraction method.

The antibacterial actions of the fucoxanthin have not been explored much except by a few groups, as mentioned. Using the disc-diffusion method, Fucoxanthin isolated from the Himanthalia elongata showed antimicrobial activity against *Listeria monocytogenes*. The fucoxanthin isolated from the *Undaria pinnatifida* demonstrated significant antimicrobial properties targeting gram-positive bacteria. The crude leaf extract from the sargassum weight was shown to be effective against the periodontal pathogen [10].

This study investigates the antimicrobial efficacy of Fucoxanthin, obtained through ultrasonication from *Sargassum* weight, effective against both of gram-positive (GPB) and gram-negative (GNB) bacteria. The assays had been conducted using the microdilution method in a 96-well plate. In addition, the fractions had been characterized using Fourier Transform Infrared Spectroscopy (FTIR), and their antioxidant capacity was assessed. FTIR, and their antioxidant potential was also evaluated.

MATERIALS AND METHODS:

Preparation of *Sargassum wightii* Extracts: The extraction was performed using the method developed by Oliyaei N *et al.* [11]. With minor alterations. Two different solvent systems were tried to extract the fucoxanthin; in one of the methods, only acetone was used, while in another, methanol-acetone in the ratio of 9:1 was taken. The desiccated sample was immersed (at a ratio of 1:30 weight/volume) in each of these two solvent systems and placed on a rotary shaker operating at 100 revolutions per minute at room temperature for a duration of 24 hours, while being placed in dark. The extraction process was performed continuously until the solvent became colourless, and the extracts were pooled together and dried.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis: Dried samples had been combined with powdered KBr and compressed into a fine pellet. FTIR spectra of the extracted compounds were measured within at the wave numbers range of 4000 cm^{-1} and 500 cm^{-1} [12].

Antioxidant Activity Analysis: The reducing capacity of fucoxanthin obtained from both methods was assayed in accordance with the process of Husni *et al.,* [13].

The brief, sample of 1 ml solutions with varying concentrations were combined with potassium ferricyanide aqueous solution (2.5 mL, 1%) andpotassium phosphate buffer (2.5 mL, 0.2mol/L, pH 6.6). Mixture was then subjected incubated at temperature 50^{o}C for 20 minutes, subsequent addition of aqueous ferric chloride solution (0.5 mL, 1%) and trichloroacetic acid solution (2.5 mL, 1%) The optical density of the reaction mixture quantified at a wavelength of 700 nm. The reducing power exhibited a direct correlation with the optical density of the reaction mixture.

Antibacterial Activity Determination: The assay used two test strains: *Staphylococcus aureus* and *Escherichia coli*. Streptomycin antibiotic was employed as a positive control. The antibacterial activity of the compounds was assessed using the protocol developed by Sultanbawa *et al.,* with the specified modifications [14].

Each 96-well plate contained single row of wells containing 200µl sterile medium (sterility control). One row contained 50µl bacterial solution with 150µl medium and no extracts (negative control). One row consisted of bacterial cultures with streptomycin dilutions ranging from 10µg/ml to 100mg/ml. The remaining rows contains bacterial cultures with extract dilutions ranging from 10µg/ml to 100 µg/ml. Each 300µl well contained 50µl of inoculum, 2µl of extract, and 148µl of nutrient broth. The plates placed in an incubator set at a temperature of 37ºC for 22 hours. The measurement of optical density was conducted

using a spectrophotometer at a wavelength of 595 nm.

RESULTS:

Extraction Yield: The efficiency of the extraction procedures followed was evaluated by calculating the percentage yield of extracts obtained for each method. Fucoxanthin extracted by maceration using a 9:1 mixture of methanol-acetone showed the highest yield (13.787%), while fucoxanthin extracted using acetone as solvent showed comparatively less yield (0.537%) **Fig. 1.** Thus, the mixture of solvents (9:1 methanol-acetone) proved to be better for the extraction of fucoxanthin.

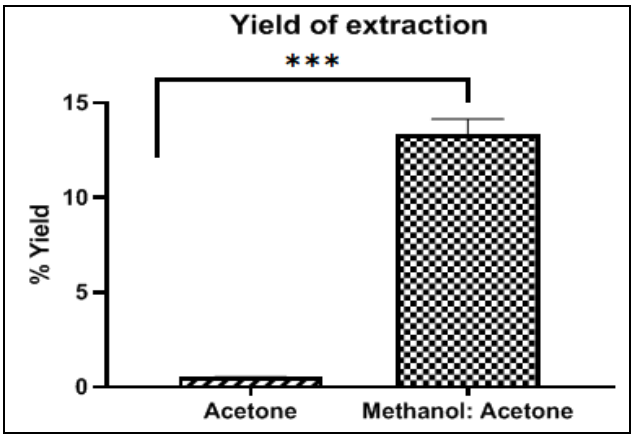

FIG. 1: YIELD OF EXTRACTION OF FUCOXANTHIN FROM *SARGASSUM WIGHTII* BY TWO DIFFERENT SOLVENTS. Methanol: Acetone (9:1) gave a significantly higher yield. P-value was computed using GraphPad Prism software (Version 8) using one-way ANOVA. (Data represents the mean value ± S.D., n=3). (****denote the statistical significant difference ($p < 0.0001$) between the yield of extraction).

Reducing Power Assay: An antioxidant capacity assessment was conducted using a reducing power assay to evaluate the effectiveness of fucoxanthin extracted from *S. wightii*. Ascorbic acid, a standard antioxidant, was employed as positive control. The reducing power of fucoxanthin amplified in a concentration dependent manner as the concentration of fucoxanthin increased from 5 µg/ml to 30 µg/ml. However, the reducing capacity exhibited a lower value compared to that of ascorbic acid **Fig. 2**. Fucoxanthin extracted by maceration in methanol: acetone (9:1) showed more excellent antioxidant activity.

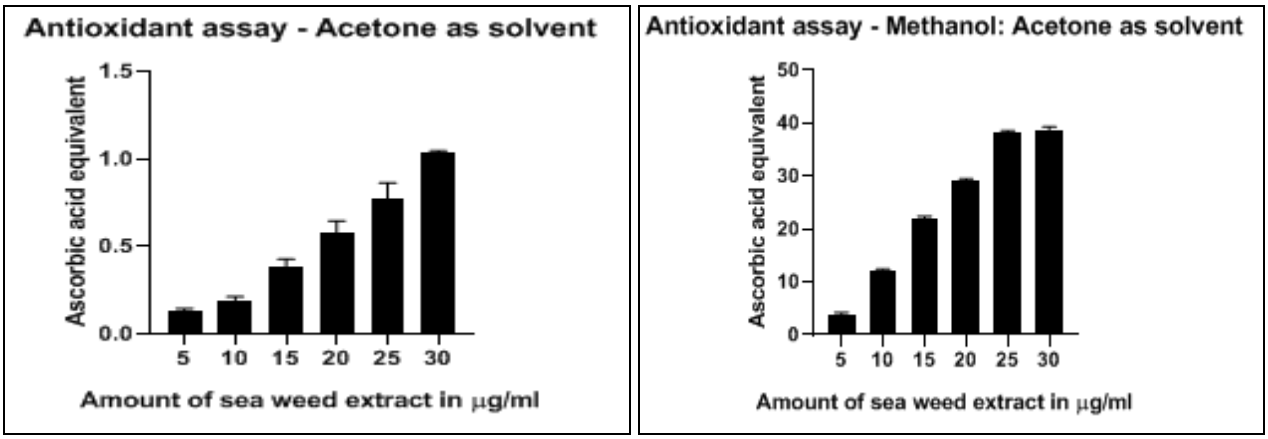

FIG. 2: THE GRAPH SHOWS THE ANTIOXIDANT ACTIVITY OF *SARGASSUM WIGHTII* IN TERMS OF ASCORBIC ACID EQUIVALENT. The methanol and acetone extracts showed more potential at all concentrations.

Antibacterial Activity: The antibacterial activity of fucoxanthin extracted from *S. wightii* was determined with respect to streptomycin on 2 bacterial strains, *E. coli*, gram negative and *S. aureus*, gram positive Fucoxanthin significantly inhibits growth of *E. coli* at higher concentrations. Fucoxanthin extracted from *S. wightii* by maceration using two different solvent systems

showed increasing antimicrobial activity with increasing concentration. Fucoxanthin was more effective than the positive control at lower concentrations, but a higher concentration of streptomycin was more effective than fucoxanthin extracts **Fig. 3.**

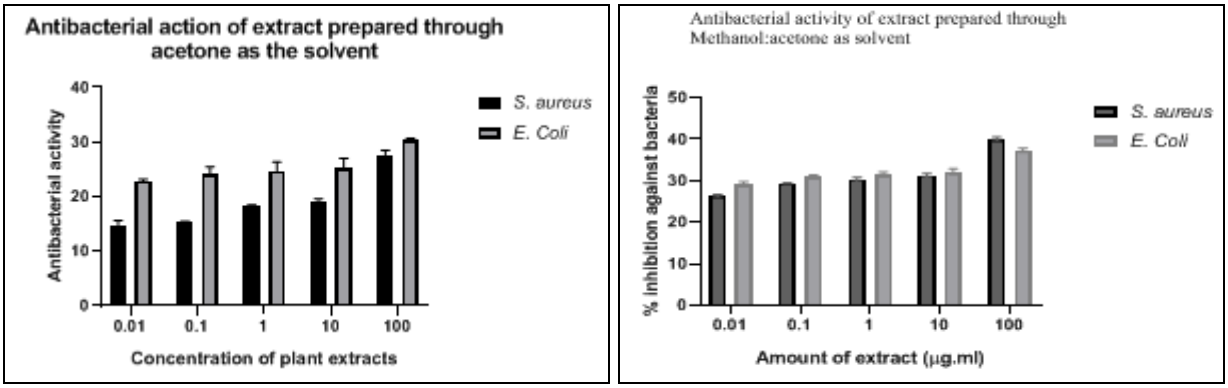

FIG. 3: THE FIGURE DEPICTS THE ANTIBACTERIAL ACTION OF THE TWO EXTRACTS PREPARED THROUGH DIFFERENT SOLVENT. The data represents the mean ± S.D. The data was examined in triplicate. The P value was computed using GraphPad Prism software through a two-way ANOVA analysis, resulting in a value of 0.001 for the comparison between *S. aureus* and *E. coli*.

FTIR Analysis: The FT-IR analysis of extracts from *S. wightii* showed peaks similar to the standard fucoxanthin. The distinctive wave frequencies of characteristic functional groups identified in both purified samples were as follows: C–H stretch (2918 - 2929 cm^{-1}), allene (1929cm^{-1}), OH group (3374 - 3421cm^{-1},C=O acetate and conjugated C=O (1634 – 1639cm^{-1}) and trans-distributed –C=C– (1201 – 958cm^{-1}) [15]. However, the peak value obtained at 1929cm^{-1} corresponding to the allenic group of fucoxanthin [15] and is only obtained in the extract obtained by maceration using a mixture of methanol-acetone. The allene stretch is present but not significant in the other extracts **Table 1.** In this study, the extracts prepared by both solvents showed different degrees of antioxidant activities.

TABLE 1: FTIR PEAK VALUES AND FUNCTIONAL GROUPS OF *SARGASSUM WIGHTII* EXTRACTS

Extracts	Peak Value	Functional Group	Functional Group Name	Vibration
Fucoxanthin Maceration (Acetone)	719.96	Unidentified	Benzene derivative	Bending
	1161.37	S=O	Sulfonic acid	Stretching
	1462.78	C-H	Alkane	Bending
	2849.85	C-H	Alkane	Stretching
	2918.42	C-H	Alkane	Stretching
	3411.25	N-H	Amine	Stretching
Fucoxanthin Maceration (Methanol: Acetone; 9:1)	1074.23	S=O	Sulfoxide	Stretching
	1639.91	C=C	Alkene	Stretching
	2358.45	O=C=O	Carbon Dioxide	Stretching
	2919.85	C-H	Alkane	Stretching
	3395.53	N-H	Amine	Stretching

DISCUSSION: In this present study, fucoxanthin was partially purified using maceration as the extraction technique and acetone and methanol acetone as the solvents. The extract obtained using two different solvents showed differences in their FT-IR spectra and antibacterial activities. Several different solvent systems have been employed to extract fucoxanthin from the seaweed previous findings show that fucoxanthin extracts were more effective than other antibiotics like clotrimazole and chloramphenicol [16, 17]. Fucoxanthin exhibited greater efficacy against Gram Positive bacteria (*S. aureus*) compared to Gram negative bacteria (*E. coli*), aligning with obtained results [16, 18, 19]. Acetone and methanol were considered tobe the best solvents to extract antimicrobial components from the different types of seaweed, as described by Cox *et al*., and hence, the utilization of acetone and methanol is justified in this study, with particular emphasis on the use of acetone [20].

In another report, Moubayed NM ET. Alcon ducted the screening study of antimicrobial potency of sea weeds encapsulated from the coast of Saudi Arabia. Seaweed extracts have been found to be more effective against GPB, according to report. This aligns with our result in which it was also noted that *Sargassum weightii* is less efficient against *E. coli* than *S. Aureus* [21]. These might be because of the variance in the composition of the cell walls of GNB and GPB. FTIR chemical analysis reveals the presence of various bioactive components, which are known to have antimicrobial and antioxidant activities. These findings is in agreement with the one reported by.

The Methanol: acetone extracts of *Sargassum weightii* showed more reduced power activity. The reducing power ensures that the antioxidant compounds serve as donor of electron, reducing the oxidized intermediate of the lipid peroxidation process. Additionally, they can act as primary 1^0 and secondary 2^0 antioxidants (Yen and Chen, 1995). The concentration-dependent antioxidant activity was studied about reducing power, providing a comprehensive studying of the antioxidants available in the sample. It had been observed that the reducing power increased with higher concentrations in all the samples [22].

Based on the present studies, *Sargassum* weight can be used as a beneficial antimicrobial agent; however, further studies are needed to purify its secondary metabolites further, and their efficacy can be tested on more microorganisms.

CONCLUSION: The presented study evaluates the antibacterial and antioxidant activity of acetone and 9:1 methanol: acetone extracts of *S. wightii*. Two methods, one conventional (maceration) and the other advanced (ultrasonication), were used to extract fucoxanthin and fucoidan using the stated solvent systems. The extraction yields were significantly low with acetone as solvent. FTIR spectra of the extracted compounds were inconclusive regarding the purity of extracts, indicating the presence of certain other pigments and polysaccharides alongside fucoidan and fucoxanthin. The antioxidant activity of fucoxanthin extracted by maceration was the highest, followed by fucoxanthin extracted by UAE.

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CONFLICT OF INTEREST: All authors declare that they have no conflicts of interest.

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