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Characterization and Biochemical Properties of Brown Seaweed *Sargassum Tenerrimum* (*J. agardh*)

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Abstract

The phytochemical analysis of the methanol extract was carried out using UV-Visible spectroscopy, FT-IR spectroscopy and HPLC. The UV-Visible spectrum of the methanol extract of *Sargassum tenerrimum* showed the presence of the compound peaks at absorption 210.2, 241.5, and 610 nm The crude methanol extract of *Sargassum tenerrimum* was passed into FTIR and It was confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds etc. HPLC fingerprint of *S. tenerrimum displayed* seven prominent peaks at the retention time of 5.18, 11.07, 16.223, 18.820, 21.24, 28.49 and 30.09 respectively. The antioxidant potential of *S. tenerrimum* was studied by its Reducing ability and found to have a considerable free radical scavenging activity IC_{50} values (84 ± 0.58 µg/ml) in comparison to the standard ascorbic acid (87.92 ± 0.93 µg/ml).

Keywords
Sargassum tenerrimum, UV-Visible, FT-IR and HPLC.

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INTRODUCTION:

Sargassum tenerrimum is one of the important marine macro algal species belonging to the genus *Sargassum* genera *Phaeophyceae* and a wide range of bioactive properties have been reported (Mizukoshi *et al.,* 1993). It is widely distributed in the tropical and temperate oceans and many parts of Asia. One of the common species of the southern coasts of Tamilnadu, India is reported to be used as animal feed, food ingredients and fertilizer. Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants. They also contain carbohydrates, protein, amino acids, iodine, bromine, vitamins and substance of stimulatory and antibiotic nature. *S. tenerrimum* shows a good amount of flavonoids in support of its antioxidant activity (Meenakshi *et al*., 2009), indicate that this genus is an ideal target for investigating the presence of bio-molecules for various medical and industrial applications.

By keeping the bioactive potential of seaweeds, the present work deals with isolation, characterization and antioxidant activity of polyphenols from brown seaweed *S. tenerrimum*

MATERIALS AND METHODS

Collection of the sample

About 2 kg of fresh seaweed of *S. tenerrimum* collected from intertidal regions of Mandapam coast of Gulf of Mannar (Latitude 9°17' N; Longitude 79°08' E), Tamil Nadu, India and was brought to the laboratory by keeping them in plastic bags with seawater. The brown seaweed belonging to the species were carefully examined, identified and authenticated by the Centre for Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamilnadu.

Preparation of seaweed powder

Fresh seaweed samples were handpicked during low tide and manually cleaned from sand, epiphytes and animal waste. Then the samples were rinsed with sea water to remove associated debris, planktons and loosely attached microorganisms. Morphologically distinct thallus of algae were placed separately in new polythene bags and kept in an ice box containing slush ice and transported to laboratory.

Further, the material was washed thoroughly with tap water to remove the salt on the surface of the samples and the water was drained off from the seaweed and spread on the blotting paper to remove excess water. The shade dried samples were again cleaned with sterile distilled water to remove the remaining salt on the surface of the samples to avoid pumping of the solvent during the extraction process. The seaweed samples were shade dried for 7 days followed by oven drying at 50°C for an hour and ground in an electric mixer for 2 hours. Finally, 650 mg of powdered seaweed sample was obtained and stored in refrigerator (4°C) for further use.

Preparation of the Extract:

Seaweed extract extracted by following the method Manilla, 2009. From the sample (650 mg) 5mg of the sample was weighed and soaked in 10 mL of Methanol and incubated in darkness for about 21 days, the crude extracts were filtered by using muslin cloth and the filtrate extracts were concentrated by rotary vacuum evaporator (>45°C) and then freeze-dried (-4°C) to obtain solid residue and were stored in individual sterile glass container for further use.

Phytochemical Analysis:

The standard procedure was followed to identify the phytochemical constituents present in the Methanol extract of the seaweed *S. tenerrimum* as described by Harborne (1973) and Trease and Evans, (1989).

Tannins: To 200 mg of the seaweed extract 10ml of distilled water was added, and the mixture was boiled and filtered. Drops of $FeCl_3$ was added to the filterate. Blue-black precipitate indicates the presence of Tannins.

Alkaloids: 10ml of methanol was added to 200 mg of the seaweed extract, and it was boiled, filtered. To the filtrate 1% HCl followed by few drops of Dragendorff reagent was added, brownish-red precipitate shows the presence of Alkaloids.

Saponins (Frothing test): To 0.5 mg of the seaweed extract added 5 ml of distilled water and shaken vigorously for 2 minutes. Durable foam indicates the presence.

Cardiac Glycosides (Keller-Kiliani test): 1ml of glacial acetic acid and few drops of $FeCl_3$ was added to 2ml of the seaweed extract. The above mixture was treated with Conc.H_2SO_4 formation greenish-blue colour depicting positively.

Steroids (Liebermann-Burchard reaction): 10 ml of chloroform and Acetic anhydride was added in the ratio 1: 1 to 200 mg of the seaweed extract which results in the development of blue-green ring.

Terpenoids (Salkowski test): 2 ml of chloroform ($CHCl_3$) and 3 ml of concentrated sulphuric acid (H_2SO_4) were added to 200 mg of the seaweed extract, reddish brown development signified as terpenoids.

Flavonoids: To 5 ml of dilute ammonia, followed by concentrated H_2SO_4 was added to the seaweed extract formation of deep yellow coloration indicates the presence of flavonoids.

Reducing Sugars: Few drops of Fehling's solution A and B were added to the seaweed extract; an orange red precipitate suggests the presence of reducing sugars.

In vitro Antioxidant assay -Radical Scavenging assay

The free radical scavenging activity of the crude extract from the seaweed *S. tenerrimum* were quantitatively assessed using the DPPH radical method adopted by spectrophotometry. Extracts that exhibited strong antioxidant capacity using the DPPH method proposed by Mensor *et al.*, 2001. Briefly, 0.1mM solution of DPPH in methanol was prepared and 0.1ml of this solution was added to 0.5ml of samples in various concentrations. After 30 minutes, the absorbance was measured at 517nm. The DPPH radical-scavenging activity was calculated.

Percentage of inhibition(I%) was calculated using the formula,

$$\mathbf{I\ \% = (Ac\text{-}As) \times 100 / Ac}$$

Where, Ac is the absorbance of the control and as is the absorbance of the sample.

HPLC Analysis

The methanolic extracts of *S. tenerrimum* were centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No.1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvents. HPLC method was performed

on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. A Hypersil BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL min-1 at ambient temperature (25-28°C). The mobile phase was consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Vis detector was set at 250 nm (Sharanabasappa *et al*., 2007, Mallikharjuna *et al*., 2007).

UV-VISIBLE SPECTROSCPY:

Ultra-Violet-Visible spectroscopy is useful as an analytical technique used to identify some functional groups in molecules, thus aiding in structural elucidation in biomolecules. The methanolic crude extracts of *S. tenerrimum* were scanned in a wavelength ranging from 200-1100 nm using a Shimazdu spectrophotometer and characteristic peaks were detected (John Peter Paul *et al*.,2013).

Fourier Transform Infrared Spectrophotometer (FTIR):

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of the Methanol solvent extracts of the seaweed extract were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm 1 with a resolution of 4 cm through the sample with that passing through the reference cell. The transmitted radiation is detected, and the spectrometer records the absorption spectrum by scanning the wave length of the light passing through the cells.

RESULTS AND DISCUSSION:

From the preliminary phytochemical analysis presence of terpenoids, phenolics, flavanoids and tannins. Our research findings support with the previous study of Bandoniene and Murkovie, 2002 that macroalgae are enriched with antioxidant molecules such as Terpenoids, polyphenols, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavanoids. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria (Cowan,1999). Marine algae have shown to be good source of unsaponofiable, nontoxic sterols that have medicinal value (Rajasulochana *et al*., 2009, Sanchez-Machado *et al*., 2004) anti-inflammatory, anti-feedent and hemolytic effects (Xu *et al*.,2000). The presence of phenolics, alkaloids, flavonoids, steroids and saponins, in the methanolic extracts of *S. tenerrimum* suggest that the seaweeds can be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, anti-feedent, antioxidant, antiallergenic, anti-thrombic, anti-carcenogenic and anti-ulcer agents in the near future.

Table 1: Phytochemicals present in Methanolic extract of *Sargassum tenerrimum*.

S.No	Particulars	Methanolic extract
1	Alkaloids	+
2	Flavonoids	++
3	Tannins	+
4	Saponins	-
5	Phenolics	-
6	Steroids	-
7	Terpinoids	++
8	Cardiac glycosides	
9	Reducing Sugars	++

DPPH -Radical Scavenging activity

The free radical scavenging potential of *Sargassum tenerrimum* was studied by its reducing ability against DPPH, and found to have a considerable free radical scavenging activity indicating by their IC50 values (84±0.58µg/ml) in comparison to the standard ascorbic acid (87.92±0.93µg/ml) represented in Fig 1.

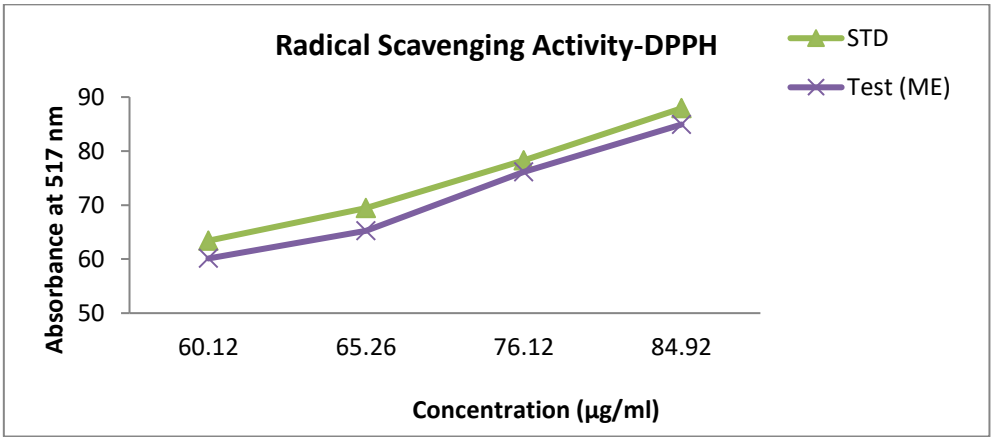

Figure 1: Radical Scavenging Activity of Sargassum *tenerrimum*

Results from antioxidant assays showed that the seaweed extracts possess antioxidant activity. The DPPH test provides information on the reactivity of the test compounds with the stable free radicals, when the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces, and the DPPH solution is decolorized as the colour changes from deep violet to light yellow. The degree of reduction in the absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The Methanolic extract of *S. tenerrimum* appeared to be as potent as Ascorbic acid which is used as the standard.

Many phenolic compounds from seaweeds have demonstrated high antioxidant activity like stypodiol, isoepitaondiaol, terpenoids etc. (Nahas *et al*, 2007). Polyphenols constitute important antioxidant molecules of plant origin. Polyphenolics like catechin, epicatechin and gallate showing antioxidant activity has been isolated from *Halimeda* sp. (Devi *et al.,* 2008). Phlorotannins isolated from *Sargassum pallidum* and *Fucus vesiculosus* have also shown significant antioxidant properties (Ye *et al*. 2008; Díaz-Rubio *et al*, 2011). Souza *et al.*, (2012) isolated sulfated polysaccharides by aqueous extraction from the red seaweed *Gracilaria birdiae* and observed that the slimy substance exhibits moderate antioxidant properties as measured by DPPH free-radical scavenging effect.

UV–Vis absorption analysis of *Sargassum tenerrimum* extract

The UV–Vis absorption spectrum was recorded to depict the efficacy of *S. tenerrimum* seaweed extract. The UV–VIS absorption spectrum of the *S. tenerrimum* seaweed extract is shown in fig 2. One of the essential requirements for a best performing sensitizer is broad and intense absorption in the visible and near-IR region of the solar spectrum (Nazeeruddin *et al*., 2001). The absorption spectrum of the *S. tenerrimum* seaweed extract showed a broad absorbance, with absorption peaks at 231.7, 261.3 nm. These absorption peaks can be associated with the characteristic absorption of flavonoid and chlorophylls (Lai *et al*., 2008, Kushwaha *et al*.,2013). The spectra for flavonoids typically lie in the range of 190-800 nm (Neha and Jyoti, 2013).

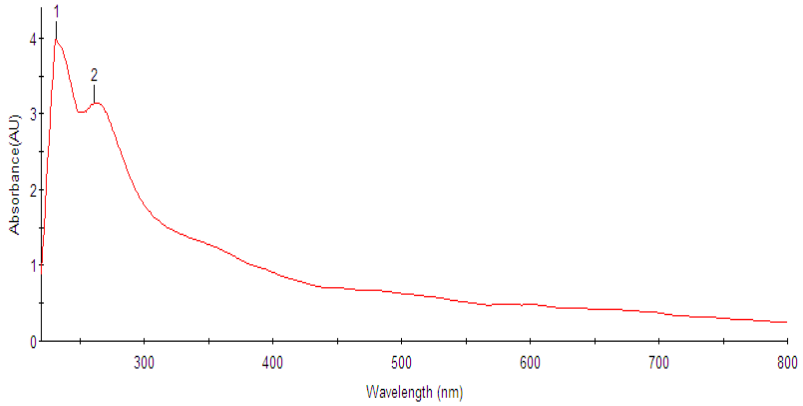

Figure 2: UV-Visible spectrum of methanol extract of *Sargassum tenerrimum*

Table 2: UV-VIS Peak Methanoic values of *S. tenerrimum*

S.NO	PEAK (nm)	Absorption (nm)	Compounds
1	231.7	3.99	Phenol and Flavonoid (Neha and Jyoti, 2013)
2	261.3	3.15	

FTIR analysis of *Sargassum tenerrimum*extract

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The crude methanolic extract of *S. tenerrimum* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis showed different peaks at 3437.06, 2095.70, 1639.20, 1417.82, 698.29, 661.99 and 469.98 cm^{-1} respectively. It confirmed the presence of functional groups such as amides, phosphorus compound, alcohols, phenols and halogen compounds etc were shown in Table.; Fig.

The absorption bands stretched in the region between 1100 and 1000 cm^{-1} are corresponding to the C–H bending or C–O or C–C vibrations of carbohydrates (Li *et al*., 2004) and polysaccharides (Nakamoto, 1986) present in the *S. tenerrimum* extract. The band recorded around 881 cm^{-1} is due to the aromatic C–H out-of plane vibration, which indicates the existence of aromatic ring pigment compound (Figueira *et al*., 1999). The weak absorption band centered at 698.29, 661.99 cm^{-1} can be ascribed to the C–H bending vibration, which is also confirmed the presence of carbohydrates. The strong and broad absorption band positioned at 3437.06 cm^{-1} can be attributed to the free O–H and N–H stretching vibrations of the amino acids (Rao, 1963). The existence of chlorophyll groups is confirmed through the C–H stretching vibrations at 2976, 2928 and 2895 cm^{-1} (Socrates, 1994).

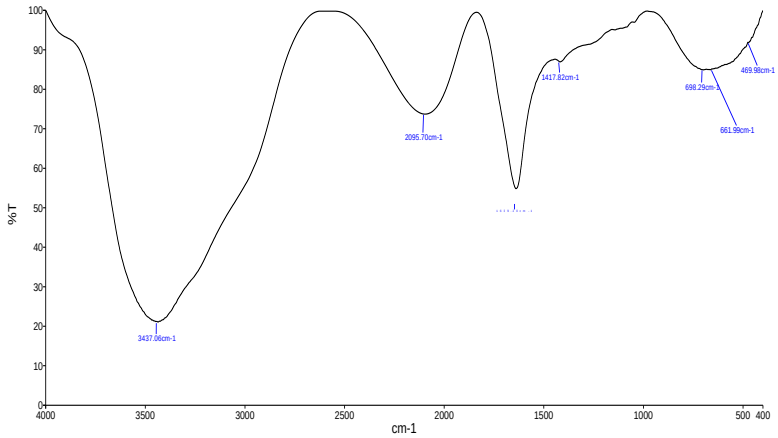

Figure 3: Identification of Functional group- FT-IR

Table 3: Identification of Functional group- FT-IR

S.NO	PEAK (nm)	I.E	FUNCTION GROUP	COMPOUND
1	3437.06	S	N-H stretching O-H stretching	Polysaccharides Amino acids
2	2095.70	S	C-H symmetry	Aliphatic compounds
3	1639.20	S	N=O asymmetric Stretching (Nitrate)	Ester, Pectin
4	1417.82	W	O-H bending	Aromatics
5	698.29	W	C=S stretching	Sulfates
6	661.99	M	C-S Stretching	Sulfides
7	469.98	W	S-S Stretching	Disulphides

I.E: Intensity estimation; S= Strong; M = Medium; W=Weak

HPLC profile of methanolic Extract *Sargassum tenerrimum*

The qualitative HPLC fingerprint profile of Methanol extract of *S. tenerrimum* were selected at a wavelength of 250nm due to the sharpness of the peaks and proper baseline. Methanol extract of *S. tenerrimum* was subjected to HPLC for the isolation and identification of constituents seven compounds

were separated at different retention time viz.,5.18,11.07,16.223,18.820,21.24,28.49 and 30.09 respectively. The profile displayed one prominent peak at a retention time of 5.18 min and some moderate peak were also observed at the retention time 11.07,16.223 and 18.820 min respectively (Table.4; Fig.4).

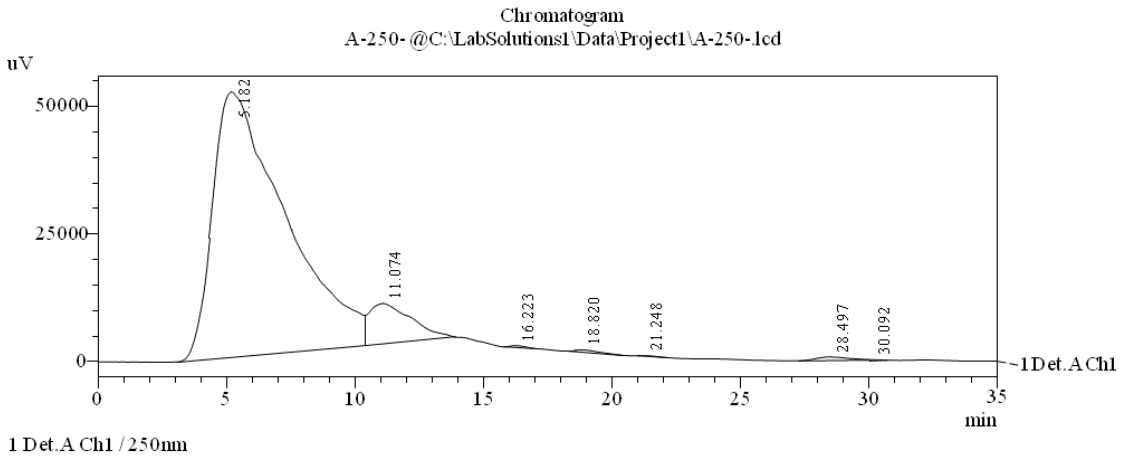

Figure 4: HPLC profile of methanol extract of *Sargassum tenerrimum*

Table 4: HPLC profile of methanol extract of *Sargassum tenerrimum*

Peak	Ret. Time min	Area mVs	Height [mV]	Area %	Height %
1	5.182	10171588	51945	90.822	84.19
2	11.074	901362	7863	8.048	12.74
3	16.223	15941	381	0.142	0.61
4	18.820	34506	508	0.308	0.82
5	21.248	6181	126	0.055	0.20
6	28.497	65852	702	0.588	1.13
7	30.092	4037	171	0.036	0.27
Total		11199467	61696	100.00	100.00

CONCLUSION:

The present study revealed that the brown seaweed *S. tenerrimum* is a good source of possessing phytochemicals. The phytochemicals were characterized using UV-Visible spectrum, FTIR and HPLC profile which showed the presence of secondary metabolites. The antioxidant potential of the methanolic extracts also showed remarkable scavenging activity. Recently, a number of researches have been reported on the phytochemistry of seaweeds across the world. Thus, the present study on methanolic extract of *S. tenerrimum* shows a new research platform which can help the manufacturers for identifying and Selecting seaweed as raw material for drug production.

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