International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2017; 5(6): 14-17
Received: 09-09-2017
Accepted: 10-10-2017

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Phytochemical analysis of *Sargassum linearifolium* (Turner) C.Ag. (Brown seaweed) using UV-VIS, FTIR and HPLC

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Abstract
The present study was aimed to explore the phytochemicals of *Sargassum linearifolium* (Turner) C.Ag. collected from Koothankuzhi coast, located in Tirunelveli district, in the south east coast of Tamil Nadu, India. 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 linearifolium* showed the presence of the compounds separated from 200 to 1100nm with the absorption. The crude methanol extract of *Sargassum linearifolium* was passed into FTIR and it confirmed the presence of functional groups such as aromatic nitro compound, amides, alkyl chloride, organophosphorus compounds, amines, sulfonyl chloride, primary amines, ketones, phosphines and aliphatic compound. HPLC fingerprint of *Sargassum linearifolium* displayed seven prominent peaks at the retention time of 2.007min, 2.177min, 2.323min, 2.653min, 2.800min, 3.007min and 3.427min out of ten compounds separated.

Keywords: Brown seaweed, UV-Visible, FTIR, HPLC.

1. Introduction
Phytochemicals are bioactive substances of plant origin. They are also known as secondary metabolites, because the plants that manufacture them may have little need for the defence mechanism [1]. Secondary metabolites are chemically and taxonomically diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [2]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [3]. In the search for phytochemicals that may be of benefit to the pharmaceutical and medicinal industry [4]. At present all over world, there is an increased interest in plant drugs, and this is due to several reasons, specifically, synthetic medicine can be inefficient, abusive or incorrect use of these drugs results in deleterious side effects, whereas drugs obtained from natural plant origin are non-narcotic, having no or fewer side effects and are cost effective [5, 6].
Seaweeds or macroalgae belong to the lower plants that they do not have roots, stems and leaves. Instead, they are composed of a thallus. Some species have gas-filled structures to provide buoyancy. Seaweeds are categorized in to three groups namely the red (Rhodophyceae), green (Chlorophyceae) and brown (Phaeophyceae) algae [7]. Due to various biotic and abiotic pressures faced by these marine algae which influence physiological nature of the cell that leads to the production of several secondary metabolites for their defence. Several of these metabolites are constitutive, which are existing in biologically active forms. Further, there are several reports on various pharmacological activities of different solvent extracts [8, 9]. Within a decade, there were a number of dramatic advances in analytical techniques including UV, FTIR and HPLC that were powerful tools for separation, identification and structure determination of phytochemicals [10]. The aim of this study is to determine the phytochemicals present in the methanol extract of *Sargassum linearifolium* with the aid of UV-Visible spectroscopy, FTIR spectroscopy and HPLC techniques.

2. Materials and method
2.1. Collection of plant materials
The plant materials for the present study were collected from Koothankuzhi (Lat. 8.0883° N, Long. 77.5385° E), located in Tirunelveli district in the south east coast of Tamil Nadu, India, during the month of December, 2015. *Sargassum linearifolium* (Turner) C.Ag. belonging to Phaeophyceae (brown algae) was made during the low tidal and subtidal regions (up to 1m

depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution [11].

2.2. Preparation of extracts
For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8h separately [12].

2.3. UV-Visible spectral analysis
The crude extract of methanol of *Sargassum linearifolium* containing the bioactive compound was analyzed spectroscopically for further confirmation. The crude extracts of *Sargassum linearifolium* were scanned in a wavelength ranging from 200-1100nm using a Shimazdu spectrophotometer and characteristic peaks were detected [13].

2.4. FTIR analysis
FTIR analysis of the methanol extract of *Sargassum linearifolium* was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum [14].

2.5. HPLC analysis
The HPLC analysis of methanol extract of *Sargassum linearifolium* was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Visible detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Visible detector was set at 254nm [15].

Instrumentation
An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), a reverse phase Luna 5µ C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components Methanol:water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27 °C. 20µl of the respective sample and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

3. Result and Discussion
3.1. UV-Visible spectrum analysis
The UV-Visible fingerprint profile of the methanol extract of *Sargassum linearifolium* was selected at the wavelength of 200nm to 1100nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 200, 250, 300, 350, 400, 430, 501, 536, 612, 674, 700, 750, 800, 873, 900, 967, 1000, 1059 and 1100 with the absorption 4.000, 4.000, 4.000, 4.000, 3.957, 4.000, 2.709, 1.786, 1.457, 3.311, 0.968, 0.238, 0.178, 0.242, 0.133, 0.111, 0.114, 0.033 and 0.004 respectively (Fig.1 & Table.1).

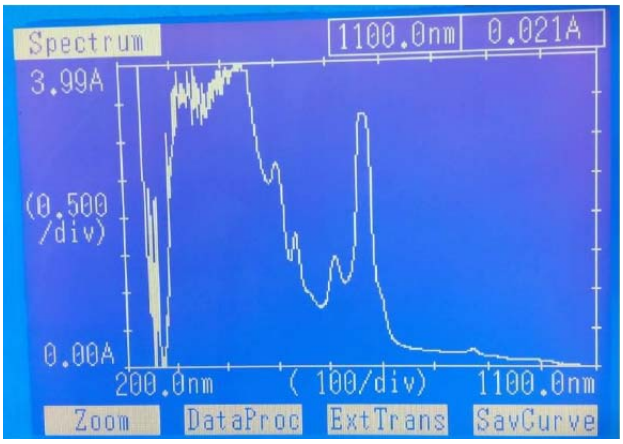

Fig 1: UV-Visible spectrum of methanol extract of *Sargassum linearifolium*

Table 1: UV-Visible spectrum of methanol extract of *Sargassum linearifolium*

Methanol	
Nm	**Abs**
430	4.000
501	2.709
536	1.786
612	1.457
674	3.311
873	0.242
967	0.111
1059	0.033

3.2. FTIR analysis
The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra red radiation. The methanol extract of *Sargassum linearifolium* 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 527.49, 612.36, 744.47, 1040.52, 1214.11, 1384.79, 1642.27, 1714.6, 2359.74 and 2851.56cm^{-1}. It was confirmed the presence of functional groups such as aromatic nitro compound (NO_2 deformation), amides (SO_2 deformation), alkyl chloride (C-Cl stretch), organophosphorus compounds, amines (C-C-N bending), sulfonyl chloride (SO_2 antisym stretch), primary amines (NH_2 deformation), ketones (C=O stretch), phosphines (P-H stretch) and aliphatic compound (CH antisym and sym stretching) respectively (Fig.2 & Table.2).

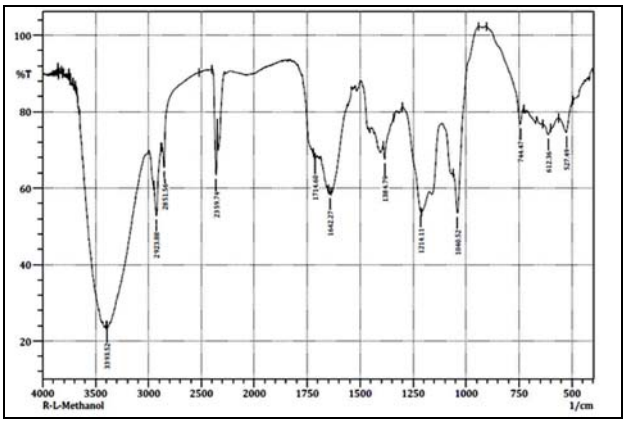

Fig 2: FTIR Spectrum of methanol extract of Sargassum linearifolium

Table 2: FTIR peak value of methanol extract of *Sargassum linearifolium*

Peak Value	Functional group	Assignments
527.49	NO2 in aromatic compound	NO2 deformation
612.36	C=O in amides	SO2 deformation
744.47	C-Cl alkyl chloride	C-Cl stretch
1040.52	P-O-C in organophosphorus compounds	P-O-C antisym stretch
1214.11	C-C-N in amines	C-C-N bending
1384.79	SO2 in sulfonyl chloride	SO2 antisym stretch
1642.27	NH2 primary amines	NH2 deformation
1714.6	C=O in ketones	C=O stretch
2359.74	PH in phosphines	P-H stretch
2851.56	CH3 and CH2 in aliphatic compounds	CH antisym and sym stretch

3.3. HPLC analysis

The qualitative HPLC fingerprint profile of the methanol extract of *Sargassum linearifolium* was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanol extract prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Sargassum linearifolium.* Ten compounds were separated at different retention time of 0.997min, 2.007min, 2.177min, 2.323min, 2.653min, 2.800min, 3.007min, 3.247min, 3.427min and 5.957min respectively. The profile displayed seven prominent peaks at the retention time of 2.007min, 2.177min, 2.323min, 2.653min, 2.800min, 3.007min and 3.427min, followed by three moderate peaks were also observed at the retention time of 0.997min, 3.247min and 3.427min (Table-3 & Figure-3).

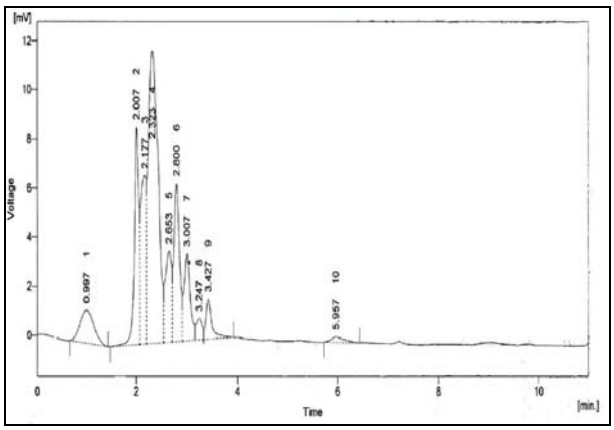

Fig 3: HPLC profile of methanol extract of *Sargassum linearifolium*

Table 6: HPLC profile of methanol extract of *Sargassum linearifolium*

	Reten. Time [min]	Area [mV.s]	Height [mv]	Area [%]	Height [%]	W05 [min]
1	0.997	26.768	1.349	6.2	3.0	0.32
2	2.007	52.838	8.859	12.2	19.5	0.10
3	2.177	53.115	6.867	12.2	15.1	0.14
4	2.323	162.171	11.927	37.3	26.2	0.26
5	2.653	31.993	3.714	7.4	8.2	0.17
6	2.800	51.383	6.457	11.8	14.2	0.14
7	3.007	30.717	3.551	7.1	7.8	0.15
8	3.247	7.294	0.870	1.7	1.9	0.17
9	3.427	13.974	1.640	3.2	3.6	0.11
10	5.957	4.022	0.235	0.9	0.5	0.25
	Total	434.275	45.469	100.0	100.0	

4. Conclusion

The present study revealed that the brown seaweed *Sargassum linearifolium* is a good candidate of possessing the phytochemicals. The phytochemicals were characterized using UV-Visible spectrum, FTIR and HPLC profile which showed the presence of secondary metabolites. More studies are necessary to isolate the bioactive compounds and predict the bioactivities further for the knowledge and promote the exploitation of the marine brown algae. The results of the present research offer a platform of using methanol extracts of *Sargassum linearifolium* as alternative for various diseases.

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