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PHYTOCHEMICAL ANALYSIS OF CHLOROFORM EXTRACT OF *SARGASSUM LINEARIFOLIUM* (TURNER) C.AG. (BROWN SEAWEED) USING UV-VIS, FTIR AND HPLC

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ABSTRACT

The present investigation was carried to determine the phytochemicals of *Sargassum linearifolium* (Turner) C.Ag using UV-Visible spectroscopy, FTIR spectroscopy and HPLC. The UV-Visible spectrum of chloroform extract of *Sargassum linearifolium* (Turner) C.Ag. showed the peaks at the nm of 325, 412, 537, 610, 668, 882, 962, 1015 and 1059 with the absorption 1.573, 2.783, 0.593, 0.502, 1.150, 0.181, 0.108, 0.099 and 0.030 respectively. The FTIR spectrum was revealed the presence of amides, aliphatic nitro compounds, organo phosphorus compounds, ether, amines, aromatic nitro compounds, benzene ring in aromatic compound, aromatic nitro compound, primary amines, aldehyde, aliphatic compound and primary amides in chloroform extract of *Sargassum linearifolium*. The qualitative HPLC fingerprint profile displayed thirteen compounds at different retention times. The profile displayed seven prominent peaks at the retention times of 1.637min, 2.360min, 2.670min, 2.803min, 3.017min, 3.267min and 3.433min followed by six moderate peaks at the retention time of 4.003min, 4.293min, 5.970min, 7.210min, 8.673min and 9.773min.

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INTRODUCTION

Seaweeds are scientifically termed marine macro algae literally meaning large algae. Seaweeds are relatively simple photosynthetic plants with unicellular reproductive structure, non vascular filamentous and thalloid plants [1]. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrients, pigments and chemical composition. Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae [2]. More than 2400 marine natural products have been isolated from seaweeds. The Southern Coast of India bears luxuriant growth of seaweeds [3].

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals known as the secondary metabolites that have protected human from various diseases [4]. Seaweeds also contain a range of unique phytochemicals not present in terrestrial plants. As such, edible seaweeds may be the only relevant dietary source of some of these factors. A wide range of studies have described the phytochemical of edible seaweeds. This capacity is endowed by the presence of sulphated polysaccharides, polyphenolic compounds and antioxidant enzymes [5]. Over the past several decades seaweeds have been used by humans as medicine and food as a fresh source. Seaweeds are reservoirs of carotenoids, pigments, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B_1, B_{12}, C, D and E [6]. Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a source of bioactive compounds with immense medicinal potential [7]. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity [8, 9]. Due to the biological interest, the aim at this present investigation was to determine the phytochemical analysis of the chloroform extract of *Sargassum linearifolium* (Turner) C.Ag using UV-Visible spectroscopy, FTIR spectroscopy and HPLC analysis.

MATERIALS AND METHOD

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 [10].

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 [11].

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 [12].

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 [13].

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 [14].

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).

RESULTS AND DISCUSSION

UV-Visible spectrum analysis

The UV-Visible spectrum of the chloroform 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 325, 412, 537, 610, 668, 882, 962, 1015 and 1059 with the absorption 1.573, 2.783, 0.593, 0.502, 1.150, 0.181, 0.108, 0.099 and 0.030 respectively (Figure-1 & Table-1).

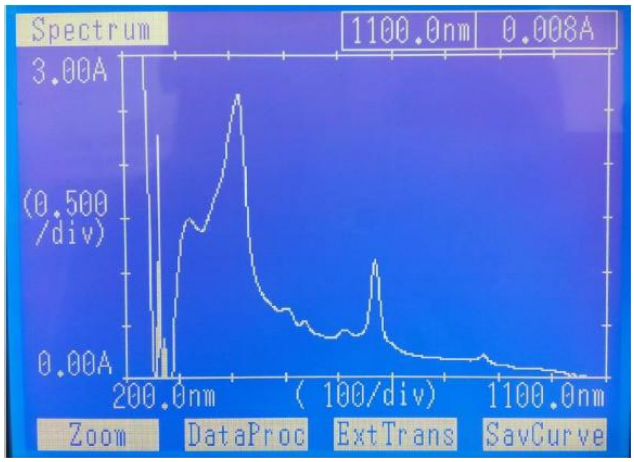

Figure 1: UV-Visible spectrum of chloroform extract of *Sargassum linearifolium.*

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

Chloroform	
Nm	**Abs**
325	1.573
412	2.783
537	0.593
610	0.502
668	1.150
882	0.181
962	0.108
1015	0.099
1059	0.030

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 FTIR results of chloroform extract of *Sargassum linearifolium* showed different peaks at 518.82, 617.18, 654.79, 1034.74, 1153.35, 1229.54, 1370.33, 1512.09, 1547.77, 1642.27, 1735.81, 2938.35 and 3335.66cm^{-1}. It was confirmed the presence of functional groups such as either (C-C-C bend), amides (C=O bend), aliphatic nitro compounds (NO_2 deformation), organophosphorus compounds (P-O-C antisym stretch), ether (C-O-C stretch), amines (C-C-N bending), aromatic nitro compounds (NO_2 syn stretch), benzene ring in aromatic compound (ring stretch), aromatic nitro compound (NO_2 syn stretch), primary amines (NH_2 deformation), aldehyde (C=O stretch), aliphatic compound (CH antisym) and primary amides (NH_2 antisym stretch) respectively (Figure-2 & Table-2).

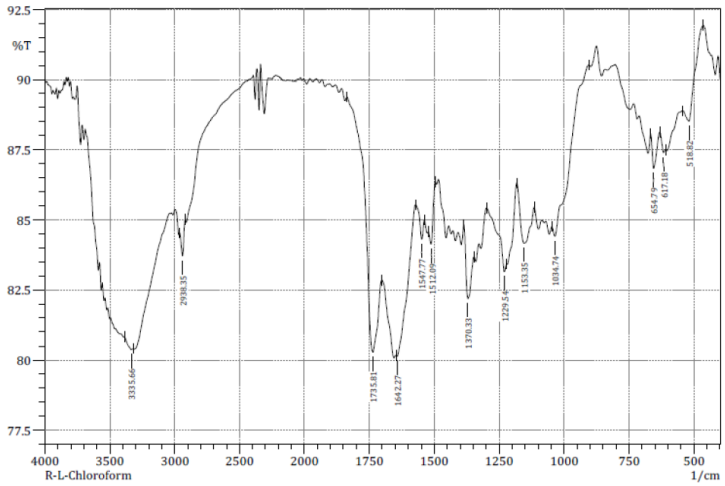

Figure 2: FTIR Spectrum of chloroform extract of *Sargassum linearifolium.*

Table 2: FTIR peak value of chloroform extract of *Sargassum linearifolium.*

Peak Value	Functional group	Assignments
518.82	C-O-C in ether	C-O-C bend
617.18	C=O in amides	C=O out of plane – bend
654.79	NO_2 in aliphatic nitro compounds	NO_2 deformation
1034.74	P-O-C in organophosphorus compounds	P-O-C antisym stretch
1153.35	C-O-C in ethers	C-O-C stretch
1229.54	C-C-N in amines	C-C-N bending
1370.33	NO_2 in aromatic nitro compounds	NO_2 sym stretch
1512.09	Benzene ring in aromatic compound	Ring stretch
1547.77	NO_2 in aromatic nitro compounds	NO_2 sym stretch
1642.27	NH_2 in primary amines	NH_2 deformation
1735.81	C=O aldehyde	C=O stretch
2938.35	CH_3 and CH_2 in aliphatic compound	CH antisym and sym stretching
3335.66	NH_2 in primary amides	NH_2 antisym stretch

HPLC analysis

The qualitative HPLC fingerprint profile of the chloroform extract of *Sargassum linearifolium* was prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Sargassum linearifolium*. Thirteen compounds were separated at different retention time of 1.637min, 2.360min, 2.670min, 2.803min, 3.017min, 3.267min, 3.433min, 4.003min, 4.293min, 5.970min, 7.210min, 8.673min and 9.773min respectively. The profile displayed seven prominent peaks at the retention time of 1.637min, 2.360min, 2.670min, 2.803min, 3.017min, 3.267min and 3.433min followed by six moderate peaks were also observed at the retention time of 4.003min, 4.293min, 5.970min, 7.210min, 8.673min and 9.773min (Figure-3 & Table-3).

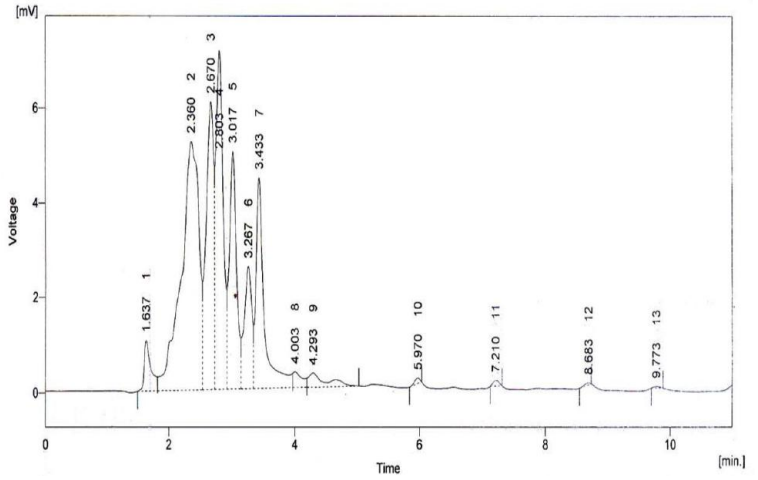

Figure 3: HPLC profile of chloroform extract of *Sargassum linearifolium.*

Table 3: HPLC profile of chloroform extract of *Sargassum linearifolium.*

	Reten.Time [min]	Area[mV.s]	Height [mv]	Area [%]	Height [%]	W05 [min]
1	1.637	8.057	1.061	2.4	3.3	0.10
2	2.360	103.019	5.235	31.0	16.1	0.27
3	2.670	50.883	6.060	15.3	18.7	0.15
4	2.803	57.231	7.138	17.2	22.0	0.14
5	3.017	40.167	5.010	12.1	15.4	0.13
6	3.267	21.355	2.570	6.4	7.9	0.16
7	3.433	40.825	4.445	12.3	13.7	0.11
8	4.003	3.351	0.333	1.0	1.0	0.23
9	4.293	5.841	0.298	1.8	0.9	0.20
10	5.970	0.694	0.115	0.2	0.4	0.10
11	7.210	0.813	0.120	0.2	0.4	0.12
12	8.683	0.289	0.041	0.1	0.1	0.09
13	9.773	0.242	0.033	0.1	0.1	0.13
	Total	332.767	32.460	100.0	100.0	

CONCLUSION

From the present study, it was concluded that UV-Visible spectrum, FTIR and HPLC analysis can be used as effective tool in identifying the phytochemicals. It also suggested that *Sargassum linearifolium* is the richest sources of phytochemicals which can be isolated and further screened for different kinds of biological activities depending on the therapeutic uses. Further work will be conducted the isolation and characterization of active principles responsible for the biopotential. The presence of various functional groups and phytocompounds in *Sargassum linearifolium* conform that it acts as a most important source of drugs against various ailments.

REFFERENCE

1. Chapman, V.J. The marine algae of Fiji. Rev. Algol. 1971, 10:164-171.
2. Domettila, C., Joselin, J., Jeeva, S. Phytochemical analysis on some south Indian seaweeds. Journal of Chemical and Pharmaceutical Research, 2013, 5(4):275-278
3. Sahoo, D., Sahu, N., Sahoo, D. Seaweeds of Indian Coast, New Delhi: A.P.H. Publishing Corporation, 2001, Pp.283.
4. Savithramma, N., Linga Rao, M., Ankanna, S. Screening of traditional medicinal plants for secondary metabolites. Int. Jour. Res. Parm. Sci., 2011, 2(4):643-647.
5. Keyrouz, R., Abasq, M.L., Bourvellec, C.L., Blanc, N., Audibert, L., ArGall, E. Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. Food Chem. 2011, 126(3):831-836.
6. Skulberg, O.M. Microalgae as a source of bioactive molecules-experience from cyanophyte research. Journal of Applied Phycology. 2000, 12(3-5):341-348.
7. Shyamala, V., Thangaraju, N. Screening of phytochemical and antibacteriial activity of three different seaweeds from Gulf of Mannar, Tamil Nadu. Phykos, 2013, 43(1):32-38.
8. Smith, A.J. Medicinal and pharmaceutical uses of seaweed natural products: A review. Journal of Applied Phycology, 2004, 16:245-262.
9. Craig, W.J. Health-promoting properties of common herbs. Am J Clin Nutr.. 1999; 70:491S-499S.
10. John Peter Paul, J., Amster Regin Lawrence, R. Preliminary phytochemical profile of *Sargassum linearifolium* (Turner) C.Ag. in Koothankuzhi coast, Tirunelveli district, Tamil Nadu, India. Acta Velit, 2016, 3(2):28-32.
11. John Peter Paul, J. Screening of phytochemicals of chloroform extract of *Oxystelma esculentum* R.Br. International Journal of Medicinal Chemistry and Analysis, 2017, 7(2):48-51.
12. John Peter Paul, J. and Shri Devi, S.D.K. (2013). Phytochemical Screening of *Padina Tetrastromatica* Hauck, *American Journal of PharmTech Research*, 3(5):214-222.
13. John Peter Paul, J., Yuvaraj, P. Phytochemical analysis of *Padina distromatica* Hauck. Indo American Journal of Pharmaceutical Research, 2013, 3(7):5290-5297.
14. John Peter Paul, J., Shri Devi, S.D.K. Phytochemical studies on *Padina pavonica* (L.) Thivy ex Taylor. Indo American Journal of Pharmaceutical Research, 2013, 3(6):4452-4459.