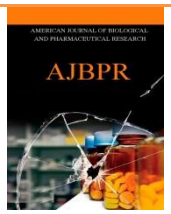
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



EXTRACTION, ESTIMATION AND CHARACTERIZATION OF FUCOIDAN FROM *PADINA TETRASTROMATICA* HAUCK IN MANAPAD COAST, TAMIL NADU, INDIA.

Shri Devi S.D.K.

Department of Botany, Sri Sarada College for Women (Autonomous), Salem – 636 016, Tamil Nadu, India.

Article Info

Received 29/11/2015
Revised 16/12/2015
Accepted 09/02/2016

Key words: -
Seaweeds, *Padina tetrastromatica*, Fucoidan, UV-Vis, HPLC.

ABSTRACT

The present study reports the estimation and characterization of fucoidan extracted from *Padina tetrastromatica* Hauck. collected from Manapad coast, the south east coast of Tamil Nadu, India. The fucoidan was isolated and estimated by Rioux method and screened by UV-Vis spectroscopic and HPLC analysis. The amount of fucoidan present in *Padina tetrastromatica* Hauck. is 19.60% of dry weight. The UV-Vis spectroscopic profile of the fucoidan extract of *Padina tetrastromatica* Hauck. was showed the compounds separated at 400nm, 450nm, 500nm, 550nm, 600nm, 669nm, 700nm, 750 and 800nm with the absorption, 0.414, 0.380, 0.244, 0.040, 0.065, 0.576, 0.032, 0.005 and 0.003 respectively. A sharp peak was observed at 669nm with the absorption 0.576. The qualitative HPLC fingerprint profile of the fucoidan extract of *Padina tetrastromatica* Hauck. was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. One prominent peak was observed and separated at the retention time of 1.500min. The profile displayed the screening and confirmation of fucoidan present in *Padina tetrastromatica* Hauck.

INTRODUCTION

Marine environment possesses a variety of natural products. The main sources of marine natural products are sponges, algae, corals, mollusks, ascidians, micro organisms etc. Seaweeds are the marine macro algae and are not only primary and major producers of organic matter in the marine environment but also exert profound effects on the density and distribution of the inhabitants of the marine environment [1]. Seaweeds have been consumed traditionally in many parts of the world. Seaweeds are characterized by high concentrations of carbohydrates, proteins, fats, phenolic substances, dietary fibre, carotenoid, vitamins and minerals [2]. The seaweeds contain rich excellent sources of bioactive compounds and some valuable medicinal components. The chemical compounds already isolated from algae are providing valuable ideas for the development of new drugs against different diseases such as antibiotics, laxatives, anticoagulants, antiulcer products and suspending agents in radiological preparations [3].

Seaweeds grow abundantly in the Indian coast particularly in the rocky shore regions between high tide and low tide and in the sub tidal region up to a depth where 0.01 % photosynthetic light is available. Rich seaweed beds occur around the south east coast of Tamil Nadu particularly in Manapad coast. Sulphated polysaccharides extracted from brown seaweeds signify the source of marine compounds with potential applications in medicine.

Corresponding Author

SHRI DEVI, SDK
Email:- saishri28@yahoo.com

Over the past decade many studies have demonstrated that sulfated polysaccharides from brown seaweeds possess excellent biological properties. Fucoidan is one of the sulfated polysaccharides produced by brown seaweeds. Several biological activities of fucoidan have been reported. The aim of the present study to isolate, characterize and estimate the amount of fucoidan from brown seaweed *Padina tetrastromatica* Hauck in Manapad coast in the south east coast of Tamil Nadu, India in order to use it as a possible source for new biomedicinal substances to human.

MATERIALS AND METHODS

Collection of Materials

The present study area is Manapad (Lat 8° 22'N; Long 78° 03'E) located in the south east coast of Tamil Nadu, India. The collection of *Padina tetrastromatica* Hauck. (Figure 1) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking.

The collected specimens were washed thoroughly with sea 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 and 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. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were stored in the refrigerator for further use.

Fucoidan extraction

Fucoidan was extracted by Rioux method [4]. Dried seaweed was mixed with 1% (w/v) $CaCl_2$ solution (1:30 ratio) and then stirred for 4 hours at 85℃ at 455±5rpm using a stirrer RZR1 (Caframo Ltd. Canada). The supernatant was separated by centrifugation (16,887g, 20 min), and vacuum filtration on Whatman No. 4 filter. The filtered liquid was mixed with 2 volumes of 95% ethanol and 1 volume of 2% (w/v) NaCl and then stirred for 1 hour at room temperature for alcoholic precipitation of fucoidan. This solution was kept at -20ºC for 48 hours. The pellet containing fucoidan was recovered by centrifugation (16,887 g, 12 min). Then, it was resolubillized in 100 ml of fresh deionized water and dialyzed for 48 hr by using membrane of 15 KDa (Sigma, USA) to remove minor constituents and solvents. Fucoidan was recovered by freeze drying and preserved at -20℃ in a sealed tube to keep away from humidity. Yield of fucoidan was calculated as the percentage (%) of dry weight of seaweed.

Fucoidan purity

UV-Visible spectral analysis

The extracted fucoidan was dissolved in deionized water and analyzed UV-Vis spectroscopically for confirmation. The fucoidan extract of *Padina tetrastromatica* Hauck. was scanned in a wavelength ranging from 200-800nm using a Shimazdu spectrophotometer and characteristic peaks were detected. This was repeated thrice for confirmation of exact peak.

HPLC analysis

The purity of the extracted fucoidan was analyzed using HPLC combining Rezex RPM Monosaccharide 50X7.8 mm precolumn (Phenomenex, USA) and Rezex RPM Monosaccharide 300X7.8 mm (Phenomenex, USA). The system consisted of Waters 715 Ultra wisp sample processor (Millipore, USA), LKB Bromma 2150 HPLC pump (LKB, Sweden) and Water 410 differential Refractometer detector (Millipore, USA) linked to Agilent interface analogue 3590E (Agilent technology, USA) with HP Chemstation Rev. A.06.03 software. The mobile phase was 0.2 µm filtered HPLC grade water and the flow rate was 0.6 ml/min.

RESULTS AND DISCUSSION

Preparation and characterization of fucoidan from *Padina tetrastromatica* Hauck.

Fucoidan is one of the sulfated polysaccharides produced by marine seaweeds especially brown seaweeds. Several biological activities of fucoidan have been reported previously. Extraction of 10g dry powder of *Padina tetrastromatica* Hauck. gave the amount of 1.96±0.3 g dry weight (n=3) fucoidan by hot water. It was found that 10g dried weight of *Padina tetrastromatica* Hauck. extract contains 19.6% of fucoidan. The amount of fucoidan was calculated according to the fucose which was measured by the cysteine-sulfuric method.

UV-Vis spectral analysis

The profile of the fucoidan extract of *Padina tetrastromatica* Hauck. was selected at a wavelength of 200nm to 800nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at 400nm, 450nm, 500nm, 550nm, 600nm, 669nm, 700nm, 750 and 800nm with the absorption, 0.414, 0.380, 0.244, 0.040, 0.065, 0.576, 0.032, 0.005 and 0.003 respectively. A sharp peak was observed at 669nm with the absorption 0.576 (Table 1 & Figure 2).

HPLC Analysis

The qualitative HPLC fingerprint profile of the fucoidan extract of *Padina tetrastromatica* Hauck. was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The fucoidan extract prepared by cold extraction was subjected to HPLC for the separation and identification of fucoidan present in the *Padina tetrastromatica* Hauck. One prominent peak was observed and separated at the retention time of 1.500min. The profile displayed the screening and confirmation of

fucoidan present in *Padina tetrastromatica* Hauck (Figure 3).Fucoidan is one of the sulfated polysaccharides produced by many brown seaweeds. Several biological activities of fucoidan have been reported in the past few decades. For example, a complex sulfated polysaccharide from the algae *Fucus vesiculosus* was found to inhibit HIV *in vitro* and had a synergistic effect with azidothymidine [5]. Fucoidan from the brown seaweed *Adenocystis utricularia* was reported to inhibit the action of the type I & II herpes simplex viruses [6]. Some extracts from marine algae also have antibacterial properties [7]. Fucoidan from the brown seaweed *Sargassum thunbergii* has shown anti-tumour activity [8] and the inhibition of tumour metastasis of the rat mammary adeno carcinoma cell has also been reported [9]. Moreover, low molecular weight fucoidan isolated from *Ascophyllum nodosum* showed an anti-proliferative effect on both normal and malignant cells including fibroblasts, sigmoid colon adeno carcinoma cells and smooth muscle cells [10-11]. The inhibitory effect appears to depend on the sulfate content of the fucoidan [12]. It has been proposed that the inhibition of metastasis from some tumours are caused by sulfated polysaccharides interfering with the passage of tumour cells across the capillary wall. Furthermore, an over sulfated polysaccharide prevents the tube formation of Human Umbilical Vein Endothelial cells whereby over sulfated fucoidan induced Plasminogen activator inhibitor-1 on the cell surface [13]. The Heparan Sulfate (HS) molecules are required for binding to the high affinity receptors with tyrosine kinase activity. In addition, fucoidans are also reported to be involved in the adhesion, attachment and spreading of certain human melanoma cell lines [14].

Table 1. UV-Vis spectral analysis of *Padina tetrastromatica* Hauck. with nm and Absorption

nm	400	450	500	550	600	**669**	700	750	800
Abs	0.414	0.380	0.244	0.040	0.065	**0.576**	0.032	0.005	0.003

nm - nanometer
Abs- Absorption

Figure 1. Natural Habit of *Padina tetrastromatica* Hauck

Figure 2. UV-Vis spectral analysis of *Padina tetrastromatica* Hauck.

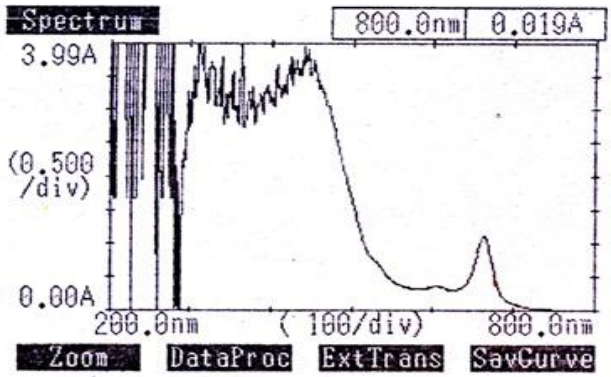
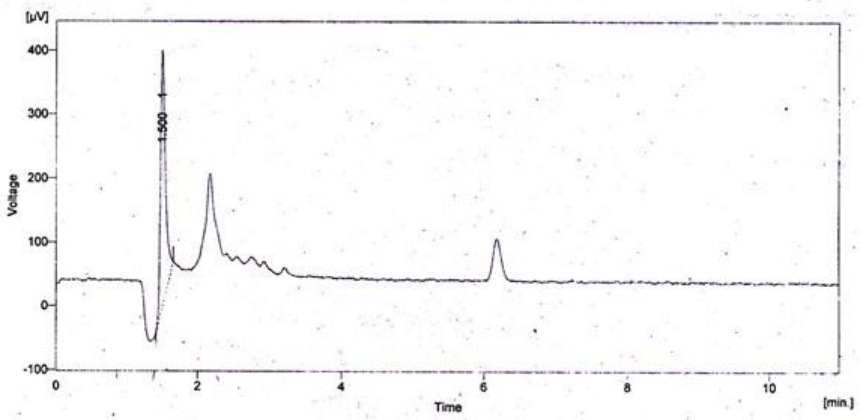


Figure 3. HPLC analysis of *Padina tetrastromatica* Hauck.



CONCLUSION

From the present study, it was concluded that *Padina tetrastromatica* Hauck. is the best source of synthesizing fucoidan. It was estimated 19.60% of fucoidan present in *Padina tetrastromatica* Hauck. Fucoidan extracted from *Padina tetrastromatica* Hauck. was characterized by UV-Vis spectroscopic and HPLC analysis. UV-Vis spectroscopic analysis showed the exact peak at 669nm with the absorption 0.576. A prominent peak was observed at the retention time of 1.500min by HPLC analysis. The results obtained in this present study support that *Padina tetrastromatica* Hauck. contain fucoidan which has a lot of biological activities.

ACKNOWLEDGEMENT

The author is thankful to the University Grants Commission for providing the financial assistance through Minor Research Project (Ref. No. MRP-5609/15 (SERO/UGC) sanctioned January, 2015.

CONFLICT OF INTEREST

The author declares that she has no conflict of interest.

REFERENCES

1. Ganga RB, Sambasivarao E, Prayaga MP, Praneeth VS and Mallikarjuna RT. (2011). *In vitro* antibacterial activity and preliminary phytochemical screening of three algae from Visakhapatnam coast, Andhra Pradesh, India. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(4), 399-401.
2. Bocanegra A, Nieto A, Blas B and Sanchez FJ. (2003). Diets containing a high percentage of Nori or Konbu algae are well accepted and efficiently utilized by growing rats but induce different degrees of histological changes in the liver and bowel. *Food and Chemical Toxicology* 41, 1473-1480.
3. Davis TA, Volesky B and Mucci A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, 37, 4311-4330.
4. Ruperez P. (2002) Mineral content of edible marine seaweeds. *Food Chem.*, 79, 23-26.
5. Sugawara I, Itoh W, Kimura S, Mori S and Shimada K. (1989). Further characterization of homopolysaccharide as anti-HIV agent. *Experientia*, 45, 996-998.
6. Ponce NMA, Pujol CA, Damonte EB, Flores ML and Stortz CA. (2003). Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structure studies. *Carbohy. Res*, 338, 153-165.
7. Rao PS and Perekh KS. (1989). Antibacterial activity of Indian seaweed extracts. *Bot. Mar*., 25, 577-582.
8. Zhuang C, Itoh H, Mizuno T and Ito H. (1995). Antitumour active fucoidan from the brown seaweed, Umitoranoo (*Sargassum thunbergii*). *Biosci. Biotech. Biochem*, 59, 563-567.
9. Coombe DR, Parish CR, Ramshaw JA and Snowden JM. (1987). Analysis of the inhibition of tumour metastasis by sulphated polysaccharides. *Int. J. Cancer*, 39, 82-84.
10. Vischer P and Buddecke E. (1991). Different action of heparin and fucoidan on arterial smooth muscle cell proliferation and thrombospondin and fibronectin metabolism. *Eur. J. Cell. Biol*, 56, 407-414.
11. Ellouali M, Durand P and Jozefonvicz J. (1993). Antitumour activity of low molecular weight fucans extracted from brown seaweed *Ascophyllum nodosum*. *Anticancer Res*, 13, 2011-2020.
12. Haroun BF, Ellouali M, Sinquin C and Boisson VC. (2000). Relationship between sulfate groups and biological activities of fucans. *Thromb. Res*., 10, 453-459.
13. Soeda S, Kozako K, Iwata K and Shimeno H. (2000). Oversulfated fucoidan inhibits the basic fibroblast growth factor-induced tube formation by human umbilical vein endothelial cells: its possible mechanism of action. *Biochim. Biophys. Acta*, 14, 127-134.
14. Ginsburg V and Roberts DD. (1988). Glycoconjugates and cell adhesion: the adhesive proteins laminin, thrombospondin and bon Willebrand's factor bind specifically to sulfated glycolipids. *Biochem*, 7, 16-21.