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Research Article

COMPARATIVE ANALYSIS OF ANTI-COAGULANT POTENTIAL OF MARINE MACRO ALGAE

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ABSTRACT

The present study was designed to investigate the anticoagulant property of aqueous extracts of three different marine algae. Sulphated polysaccharides produced by sea weeds form the basis of an economically important and expanding global industry. Fucan (polymer of fucose), ulvan (polymer of rhamnose), galactan (polymer of galactose) are the three important sulfated polysaccharides present in brown algae, green algae, red algae respectively. In this work, sulfated fucan was extracted from *Sargassum sp.* of brown algae, sulfated ulvan was extracted from *Ulva sp.*of green algae, and sulfated galactan was extracted from *Gelidium sp.* of red algae. After extraction, total polysacharrides were estimated in the respective extracts as 33.24mg/ml, 25.6mg/ml, 48.83mg/ml respectively. The sulphate content was found to be 11.13mg/ml, 7.843mg/ml, and 13.3mg/ml respectively. Metachromatic activity was carried out with fucan, ulvan, galactan polysaccharides and the relative metachromatic activity was found to be 0.045, 0.059, and 0.080 respectively. In APTT assay, RCF value for Sargassum is 40.3, Ulva is 5.1 and Gelidium is 159. In PT assay, RCF value for Sargassum is 1.55, Ulva is 1.22 and Gelidium is 2.22. Thus from the results, *Gelidium* (red) extract shown a potential anticoagulant effect followed by *Sargassum* (brown) and *Ulva* (green) algae.

Keywords: *Sargassum sp., Ulva sp., Gelidium sp*., Activated Partial Thromboplastin Time [APTT], *Prothrombin Time* (*PT*), Metachromatic Activity

INTRODUCTION

Seaweeds refer to large marine benthic algae that are multicellular, macrothallic and thus differentiated from most algae that are of microscopic size [1]. Macroalgae are usually classified based on their characteristic phytopigment, such as Rhodophyta (red algae) [10], Phaeophyta (brown algae) and Chlorophyta (green algae) [2,5,6]. These algae are used for the production of biodiesel and ethanol. Similarly, there has been increasing interest in the systematic screening of bioactive compounds from natural resources from marine algae. Some claims about algae include the ability of red algae to improve the immune system, to treat respiratory ailments and skin problems, and cure cold sores. Algae also contain abundant amounts of iodine, an element required by humans and necessary for proper functioning of thyroid. These algae are used traditionally for the treatment of thrombotic disorders in western countries and east Asia [3]. Sulphated polysaccharides are class of compounds having hemi-ester sulphate groups in their sugar units. In marine algae, they occurs as sulphated fucan (brown), sulphated rhamnose (green), sulphated galactan (red) [4]. The objective of the present study was to investigate and evaluate in-vitro anticoagulant properties of aqueous extracts of *Sargassum sp* (brown), *Ulva sp* (green), *Gelidium* sp (red). In addition, total carbohydrate content, sulphate content and metachromatic activity of the crude samples were also estimated.

MATERIALS & METHOD

Collection of species

Marine seaweeds used in this study were collected from Kovalam, Kanyakumari coast, Tamilnadu during the month of June-September, 2011. The collected seaweeds were identified as *Ulva sp.* (green algae), *Sargassum sp.* (brown algae) and *Gelidium sp* (red algae).

Preparation of crude extract

Air dried algal samples were subjected to extraction process using water as solvent. 20g of dried algae was homogenized with 200ml of water and homogenate was boiled for 3 hours at 100°C. Then sample was filtered using filter and filtrate was separated and stored below - 5°C for further use.

Estimation of total carbohydrate

Total carbohydrate was estimated by using phenol – sulphuric acid method with glucose as standard as follows. The standard solution of glucose was prepared with concentration of 1 mg/ml. Various concentration of glucose was taken to construct standard curve of glucose. The crude samples were taken for analysis. 1ml of 4% phenol was added to the sample and mixed well. 5ml of 96%sulphuric acid was added and mixed, and kept for 30 minutes at 30°C and read at A_{460} nm.

Estimation of total sulphate

Total sulphate content was estimated by using barium chloride method with sodium sulphate as standard. The standard solution of sodium sulphate was prepared with the concentration of 1mg/ml. Various concentrations of sodium sulphate was taken to construct standard curve. The crude samples were taken for analysis. 2ml of 1mg/ml of barium chloride was added to the sample and mixed well and read at A_{620} nm.

Metachromatic activity

0.25 g of Azur A dye was dissolved in 250 ml of distilled water [11]. Test samples were prepared at different concentrations as 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml, 6mg/ml, 7mg/ml, 8mg/ml, 9mg/ml, 10mg/ml, 11mg/ml, 12mg/ml, 13mg/ml, 14mg/ml, 15mg/ml, 16mg/m, 17mg/ml, 18mg/ml, 19mg/ml, 20mg/ml. 10µl of these samples were mixed with 10ml of Azur A dye. Absorbance was read at wavelength between 400-700 nm. Heparin was used as the standard.

Anticoagulation assay

Blood was drawn from healthy people without history of bleeding disease [8,9]. Nine parts of blood was collected by vein puncture and mixed with one part of 3.8% Tri Sodium Citrate. Blood was centrifuged at 5000rpm at 10mins. Supernatant was collected designated as plasma.

APTT assay

APTT assay was performed by using the standard APTT assay Kit purchased from Diagnostic Enterprises, Himachal Pradesh. 100µl of plasma was mixed with 10µl of solution of different concentrations of sulphated polysaccharides and then 100µl of prewarmed APTT assay reagent was added and incubated for 3 minutes at 37°C and to this 100µl of calcium chloride is added and the time taken for formation of clot was recorded.

PT assay

PT assay was performed by using the standard PT assay Kit purchased from Diagnostic Enterprises, Himachal Pradesh. Prothrombin time (PT) assay was carried out using normal human plasma (100µl) mixed with solution of algal extract (10µl) then after 3 min incubation, clotting was induced by the addition of thrombo reagent (200µl) and clotting time was recorded.

RESULTS AND DISCUSSION

Estimation of Total Carbohydrate

Crude extracts were obtained by hot water extraction method by following the procedure specified in materials and method. The total carbohydrate content of marine algae was found using the procedure specified in materials and methods. From those results it was inferred that, *Ulva* (green algae), *Sargassum* (brown algae), *Gelidium* (red algae) contains about 25.6mg/ml, 33.24mg/ml, and 48.83mg/ml of total carbohydrate respectively.

Estimation of Total Sulphate Content

The total sulphate content of marine algae was found using the procedure specified in materials and method. From those results it was inferred that, *Ulva* (green algae), *Sargassum* (brown algae), *Gelidium* (red algae) contains about 7.843mg/ml, 11.13mg/ml, and 13.3mg/ml of total sulphate respectively.

Metachromatic activity

The metachromatic activities of crude extracts of marine algae were studied. Various concentrations of extracts were taken for metachromatic activity. Heparin was used as standard. Results of metachromatic activity of Heparin, *Ulva sp., Sargassum sp., Gelidium sp were* shown in figure 1,2,3,4 respectively.

The 1mg/ml concentration of Heparin, 18mg/ml concentration of *Ulva sp.,* 20mg/ml concentration of *Sargassum sp.,* 20mg/ml concentration of *Gelidium sp.,* showed high metachromatic activity. At high concentration, a shift in wavelength was recorded. The metachromatic activity was expressed as negative slope of the standard curve of absorbance at 620nm vs sample conc. (mg/ml) and it was found to be 0.897, 0.053, 0.041, 0.072 for heparin, *Ulva sp., Sargassum sp., Gelidium sp* respectively. Relative metachromatic activity of Ulva sp., Sargassum sp., Gelidium sp., was found to be 0.059, 0.045, 0.080.

Anticoagulation activity

Crude extracts of different concentrations were taken and the anticoagulation activity profile was established by using Activated Partial Thromboplastin Time (APTT) and Prothrombin time (PT) assay.

APTT assay

Crude ulvan from *Ulva sp.,* crude fucan *from Sargassum sp., and* crude galactan from *Gelidium sp* was found to have APTT activity of 153seconds at 20 mg/ml with RCF value as 5.1, 1210 seconds at 20 mg/ml with RCF value as 40.3, 3651 seconds at 20 mg/ml with RCF value as 159. The results have been tabulated in table 1,2.

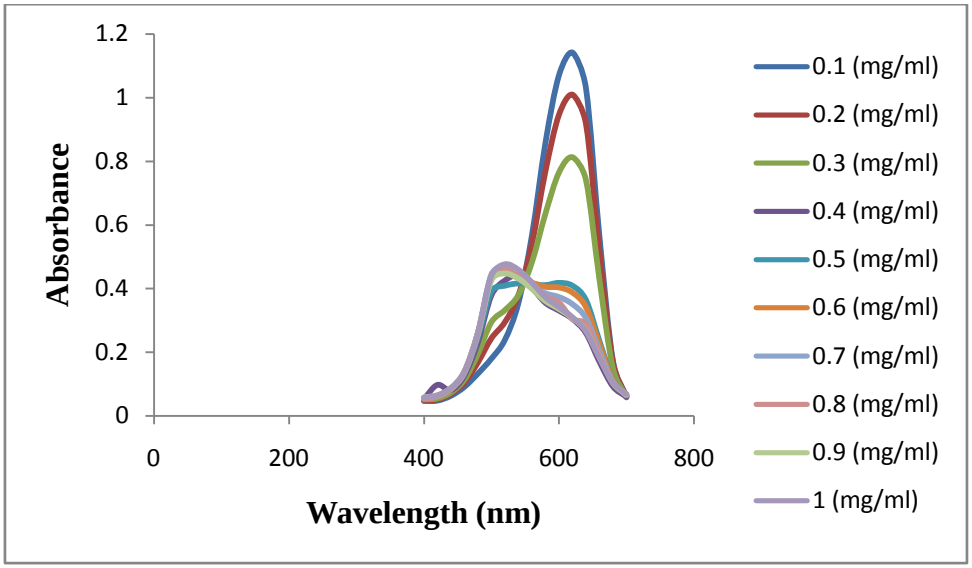

Fig. 1: Metachromatic activity spectrum of heparin.

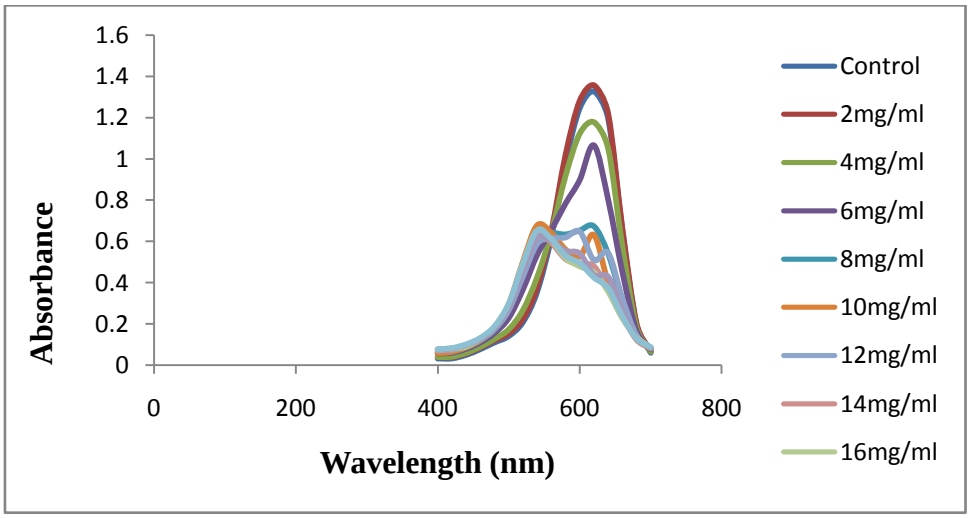

Fig. 2: Metachromatic activity spectrum of *Ulva sp.*

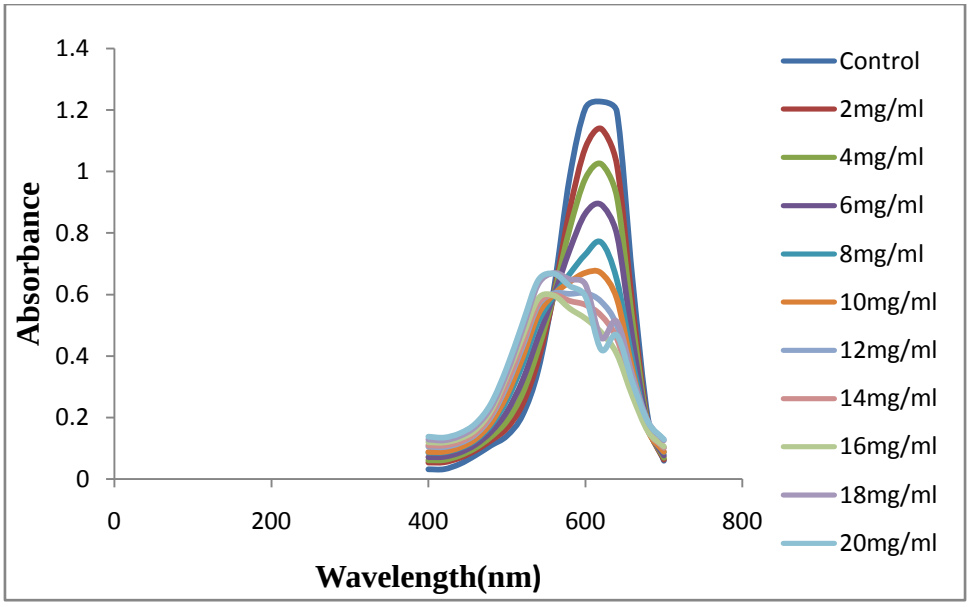

Fig. 3: Metachromatic activity spectrum of *Sargassum sp.*

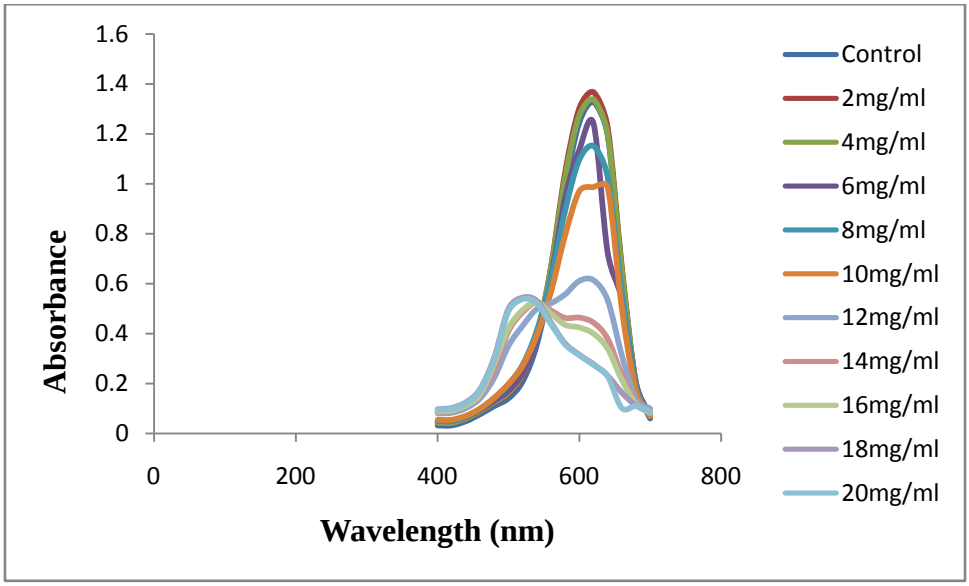

Fig. 4: Metachromatic activity spectrum of *Gelidium sp.*

Table 1: APTT assay of *Ulva sp., Sargassum sp., Gelidium sp.*

Sample (mg/ml)	APTT for *Ulva sp.*	APTT for *Sargassum sp.*	APTT for *Gelidium sp.*
Control	30	30	30
2	60	70	78
4	77	119	108
6	89	166	144
8	73	230	235
10	111	296	451
12	122	315	730
14	129	445	917
16	135	525	1296
18	140	720	1733
20	153	1210	3651

PT assay

Crude ulvan from *Ulva sp.,* crude fucan *from Sargassum sp., and* crude galactan from *Gelidium sp* was found to have PT activity of 22 seconds at 20 mg/ml with RCF value as 1.22, 28 seconds at 20 mg/ml with RCF value as 1.55, 40 seconds at 20 mg/ml with RCF value as 2.22. The results have been tabulated in table 3,4.

Table 2: RCF values of *Ulva sp., Sargassum sp., Gelidium sp.*

Sample (mg/ml)	RCF of *Ulva sp.*	RCF of *Sargassum sp.*	RCF of *Gelidium sp.*
Control	-	-	-
2	2	2.33	3.39
4	2.56	3.96	4.7
6	2.96	5.53	6.26
8	2.43	7.67	10.2
10	3.70	9.86	19.6
12	4.06	10.5	31.7
14	4.30	14.83	39.9
16	4.50	17.5	56.3
18	4.66	24	75.3
20	5.1	40.3	159

Table 3: PT assay of *Ulva sp.*

Sample (mg/ml)	PT for *Ulva sp.*	PT for *Sargassum sp.*	PT for *Gelidium sp.*
Control	18	18	18
2	18.5	18.72	20
4	19	22	26
6	19.3	24	28
8	19.8	24.8	30
10	20	25.1	32
12	20.4	25.7	33
14	20.6	26	34
16	21.1	26.3	36
18	21.4	27	38
20	22	28	40

Table 4: RCF values of *Ulva sp., Sargassum sp., Gelidium sp.*

Sample (mg/ml)	RCF of *Ulva sp.*	RCF of *Sargassum sp.*	RCF of *Gelidium sp.*
Control			
2	1.02	1.04	1.11
4	1.05	1.22	1.44
6	1.07	1.33	1.55
8	1.1	1.37	1.66
10	1.11	1.39	1.77
12	1.13	1.42	1.83
14	1.14	1.44	1.88
16	1.17	1.46	2
18	1.18	1.5	2.11
20	1.22	1.55	2.22

Thus in the clotting cascade, ulvan, fucan, galactan may blocks the intrinsic pathway by inhibiting factors XII, XI, X, IX, VIII, prothrombin which identified by the results of APTT assay. It also blocks extrinsic pathway by inhibiting factors X, V, prothrombin which was identified through PT.

CONCLUSION

In this research work, sulphated polysaccharides were extracted from marine algae. Total carbohydrate content and total sulphate content were estimated. Based on that different concentration of samples are prepared, tested for metachromatic activity and anti-coagulation assays were carried out. *Gelidium sp.* showed very high anti-coagulation activity. It extended blood clotting time up to 3600 secs with respect to APTT and 40 secs with respect to PT.

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