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Characterization of galactose rich fucoidan with anticoagulation potential isolated from *Turbinaria decurrens* Bory de Saint-Vincent occurring along the coast of Gulf of Mannar (Pamban), India

N.Shanthi, T.Eluvakkal and K.Arunkumar

Abstract
Fucoidan is a group sulfated hetero polysaccharide found in the cell wall of some members of Phaeophyceae. Two fractions of fucoidan one with low molecular weight (LMW) with > 3500 Da and another with high molecular weight (HMW) with < 3500 Da showed promising anticoagulation activity under *in vitro* APTT assay were isolated from the brown seaweed *Turbinaria decurrens* occurring along the coast of Pamban (Gulf of Mannar), India. Fucoidan isolated using DEAE Cellulose column was characterized by FTIR spectroscopy dominating galactose followed by fucose, mannose and glucose. But more anticoagulation activity was recorded in HMW A1H fraction than LMW fraction isolated from the brown seaweed *Turbinaria decurrens*. The anticoagulation activity was an increased with increase in the sulphate content in the fucoidan.

Keywords: *Turbinaria decurrens*, Fucoidan, DEAE Cellulose, Anticoagulation activity.

1. Introduction
In recent years, novel health has attracted attention on seeking bioactive compounds to expand new drugs and healthy foods [1]. As seaweeds contain various bioactive compounds, they are considered as a valuable resource for nutraceutical and pharmaceutical products [2]. Sulphated polysaccharides of seaweeds are known to exhibit many biological and physiological activities including anticoagulation, antiviral, antitumor, anti-inflammatory and antioxidation [3]. Brown seaweeds are known to synthesize a great variety of sulfated galactans which are found as a major component in their cell wall. In India, among the brown seaweeds, next to *Sargassum,* species of *Turbinaria* occurring abundantly in Gulf of Mannar have been exploited for extracting alginate [4]. A group of pharmaceutical compounds called anticoagulants are used for thrombotic disorders. Heparin, a sulfated polysaccharide is the first compound used clinically for anticoagulation and antithrombotic activities has been extracted from animal sources [5]. On the other hand, Fucoidan, sulfated polysaccharides with anticoagulant activity isolated mainly from brown seaweeds of plant source [6]. The existence of structural similarities between fucoidans and heparin has also been widely reported. Besides the advantage of fucoidan of seaweed is safer because heparin may contain virus of animals that lead to infection during administration. As a safe alternative source, fucoidan gain much attention in the pharmaceutical industry to develop better and safe drugs with low or less side effects [7]. The structures of fucoidan are complex, heterogeneous found variedly among brown algal species [8]. The profound functional properties of the fucoidan are probably due to the presence of sulphate groups in varying amounts and its positions on the macromolecular backbone. Over the recent years, extensive studies have been made on the preparation and characterization of fucoidan in brown seaweeds which contain fucoidan as the second largest amount next to alginate in the cell wall matrix [9]. In the present study, fucoidan isolated from the brown seaweed *Turbinaria decurrens* Bory de Saint-Vincent collected along the coast of Pamban(Gulf of Mannar), India exhibiting *in vitro* anticoagulant activity (APTT assay) characterized by FTIR spectroscopy is reported.

2. Materials and Methods
2.1. Sampling
About 1 kg of healthy brown seaweed *Turbinaria decurrens* Bory de Saint-Vincent was

collected in low tide along the coast of Pamban, Gulf of Mannar, India ($9^0$17'N $79^0$18'E/9.28^0N 79.3^0E) during July 2013. The specimen was washed thoroughly in seawater followed by tap water to remove the macroscopic epiphytes and other extraneous materials. Then they were rinsed with distilled water and air dried in dark for 3 days. Dried specimen was pulverized into fine powder.

2.2. Extraction and purification of fucoidans [10, 11]

Crudes of fucoidan and alginate were successively extracted as mentioned in the Fig. 1. The resulting crude fucoidans (CF-A & B) were separated through DEAE cellulose 52 (SRL, India) ion-exchange chromatography [12] by eluting with increasing morality of NaCl (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 M) until no more sugar was detected by monitoring sugar level in each fraction through phenol sulphuric acid method against standard graph prepared in L- fucose [13]. Based on the sugar content in the successive eluvents, fractions were pooled and dialyzed (MWCO 3500 Da) for 24 hour in distilled water, lyophilized and weighed. Fucoidan content of each pooled fractions was estimated based on the estimated L-fucose level [13]. The amount of sulphate residue was determined by the $BaCl_2$ – gelatin method [14] using Na_2SO_4 as a standard.

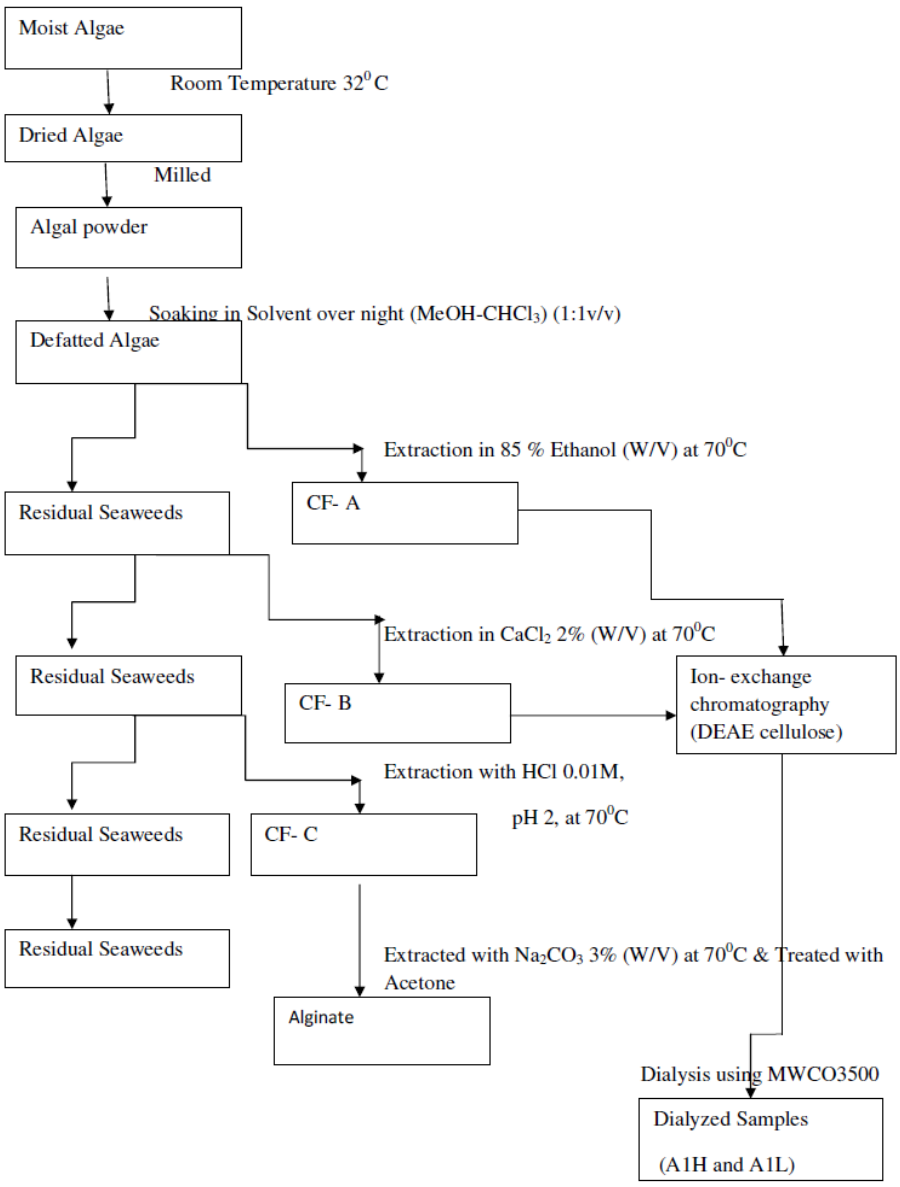

Fig 1: Flow chart for extraction of fucoidan from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Manner (Pamban), India.

2.3. *In vitro* coagulation assay (APTT assay) [15]

Blood samples were collected from voluntary healthy persons in a vial containing 3.8 % sodium citrate (9:1). Blood samples were centrifuged for 20 minutes at 4000 rpm and the plasma (90 µl) was mixed with purified fucoidan fractions (10 µl) and incubated for 1 minute at 37^0 C, then 100 µL of APTT reagent was added to the mixture and incubated for 5 minutes at 37 ^{0}C. Thereafter, clotting was induced by adding 0.025 mol/L $CaCl_2$ (100 µl) and clotting time was recorded.

2.4. Characterization of fucoidan by FTIR spectroscopy

The qualitative investigation of the purified fucoidan was done by

FTIR spectroscopy (Shimadzu, Japan) described by [16]. The spectra were recorded between 4000 and 400 cm^{-1} wave number and the trembling was recorded as a graphic representation.

3. Result and discussion

In this investigation, results on fucoidan extracted in successive method separated through DEAE Cellulose column exhibiting *in vitro* anticoagulant activity (APTT assay) characterized by FTIR spectroscopy isolated in brown seaweed *Turbinaria decurrens* Bory de Saint-Vincent collected along the coast of Pamban (Gulf of Mannar, India) are presented.

3.1. Yield as well as monosugars and sulphate content of crude fucoidan

In the present study, recorded yield of crude fucoidans CF-A and CF-B from the brown seaweed *Turbinaria decurrens* were 0.59% and 0.70%, respectively. The fraction CF-C yields 2.33 % alginate. Various monosugar composition and sulphate level in the crude fucoidans are presented in the Fig. 2a and 2b. As reported by [17], the relative content and composition of fucoidan in brown seaweeds varied depending on species, harvest season, age of the plant and extraction methods adapted. In the specimen of brown seaweed *Turbinaria decurrens* collected during July 2013, both crude and fractionated fucoidans constituted same types of monosugars (fucose, glucose and mannose) dominating galactose followed by fucose and equal amount of glucose and mannose. Sulphate content recorded was higher in fraction CF-A than CF-B. Whereas purified fractions obtained from both crude fucoidan fractions CF-A and CF-B separated through anion-exchange DEAE-cellulose column was contained relatively high sulphate (Fig. 2b).

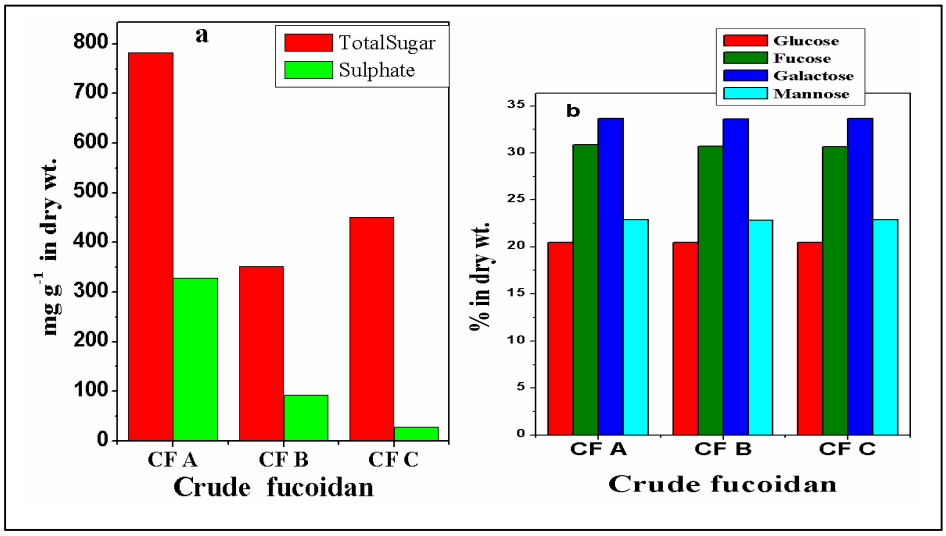

Fig 2: Total sugar, sulphate (a) and monosugar constituents (b) of various crude fucoidans extracted from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Mannar (Pamban), India.

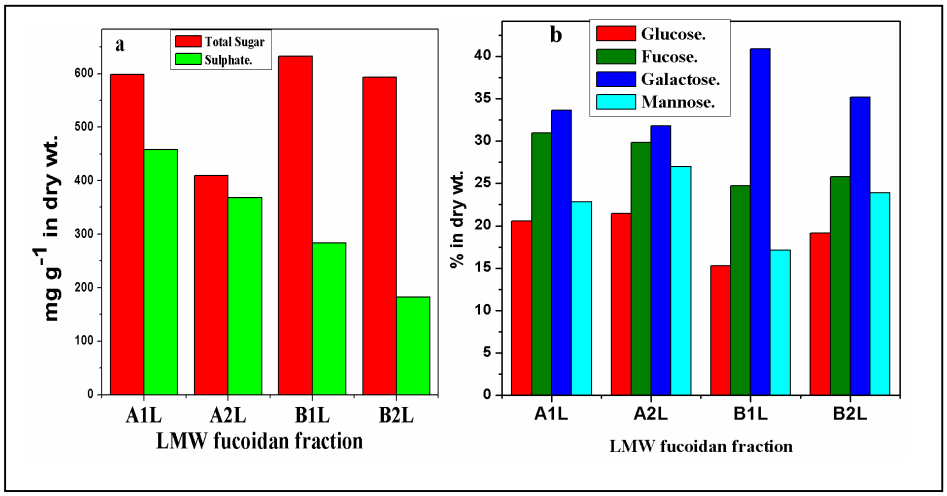

Fig 3: Total sugar, sulphate(a) and monosugar constituents(b) of purified low molecular weight(LMW) fucoidans (< 3500 Da) separated on DEAE cellulose column extracted from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Mannar(Pamban), India.

3.2. Monosugars and sulphate content of fractionated fucoidans

Fucoidans with 50-1,00,000 Da are considered as potential anticoagulants and fractions with higher than 8,50,000 Da usually showed low anticoagulant activity [18]. In the present study,

fucoidan fractions isolated from the brown seaweed *Turbinaria decurrens* were separated and purified as > 3500 Da considering as LMW (low molecular weight) and above 3500 Da as HMW (high molecular weight). Both types of fucoidans were analysed for various composition monosugar and sulphate as well as anticoagulant activity. Yield and composition of monosaccharides in LMW fucoidan fractions present in the Fig. 3a and 3b showing a high amount of galactose followed by fucose and equal quantity of glucose and mannose as recorded in its crude and also in fractions HMW fucoidan (Fig. 4a and 4b).Whereas sulphate content was higher in the fractions of HMW fucoidan than LMW fucoidan fractions. This high amount of sulphate content recorded in the fucoidan isolated from the brown seaweed *Turbinaria decurrens* considered advantageous because sulphate group in the fucoidan is responsible for high biological activity which is increased with increasing sulphate content in the fucoidan [19]. Fucoidan fraction A1H with HMW showed maximum anticoagulation activity by in the APTT assay among the fucoidan fractions isolated (Fig. 5) was found to be a potential anticoagulant.

additional

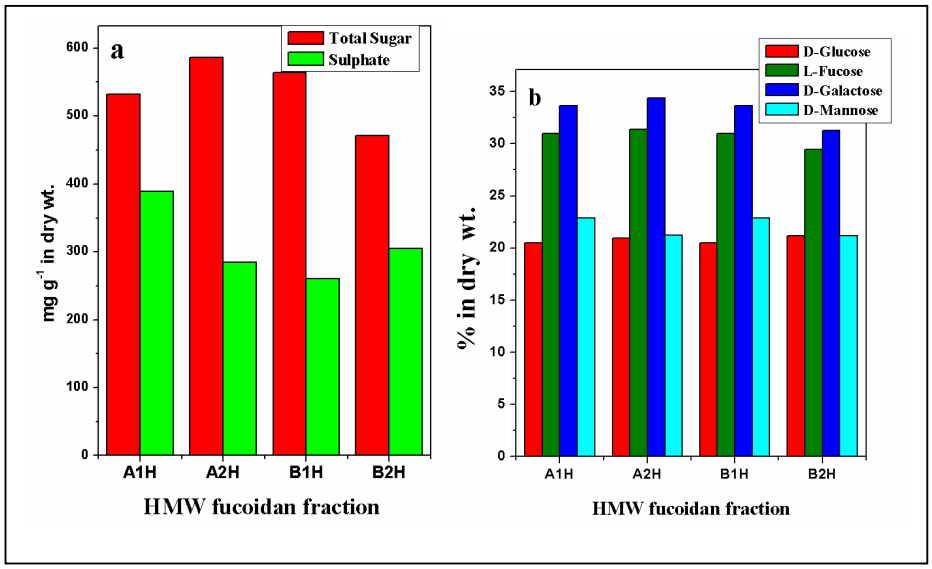

Fig 4: Total sugar, sulphate(a) and monosugar constituents(b) of purified high molecular weight(HMW) fucoidans (> 3500 Da) separated on DEAE cellulose column extracted from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Mannar(Pamban), India.

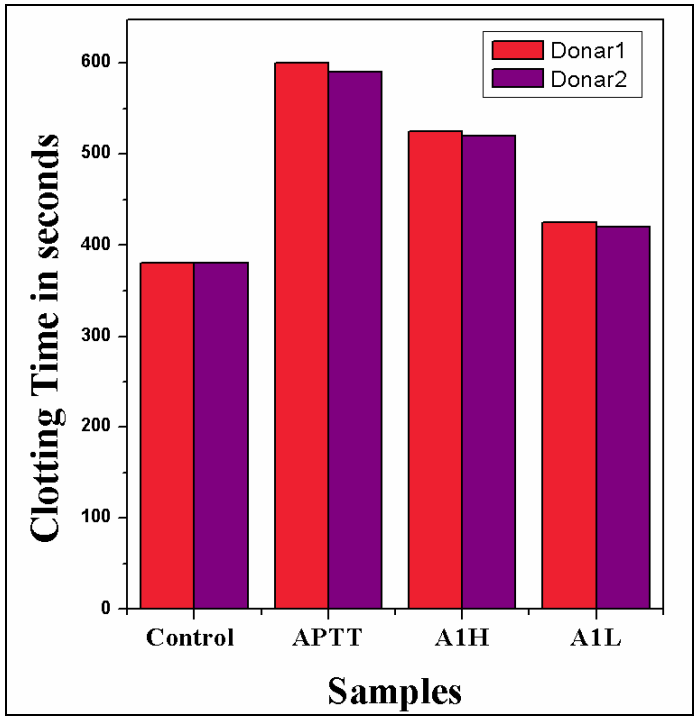

Fig 5: *In vitro* anticoagulation assay of fucoidan fractions AL1(LMW) and AH1(HMW) isolated from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Mannar(Pamban), India

3.3. Anticoagulation activity of purified fucoidans

Although heparin have strong anticoagulation activity and widely been used for anticoagulation for hemodialysis patients, it shows some side effects like hemorrhage, osteoporosis etc. Sometime contaminated virus become pathogenic because heparin is obtained from animal sources. Therefore, fucoidans isolated from seaweeds are safe and suitable alternative to heparin [20]. The structure and molecular weight of fucoidan varied depending on species of seaweeds and also display variation in bioactivities including anticoagulation [21]. The two sulphated galactose rich fucoidan fractions (A1L & A1H) isolated from *Turbinaria decurrens* were recorded difference in the sulphate content with fraction A1H contained high sulphate exhibiting more anticoagulation activity than A1L. This result shows that the anticoagulant activity of the fucoidan was related with its sulphate content.

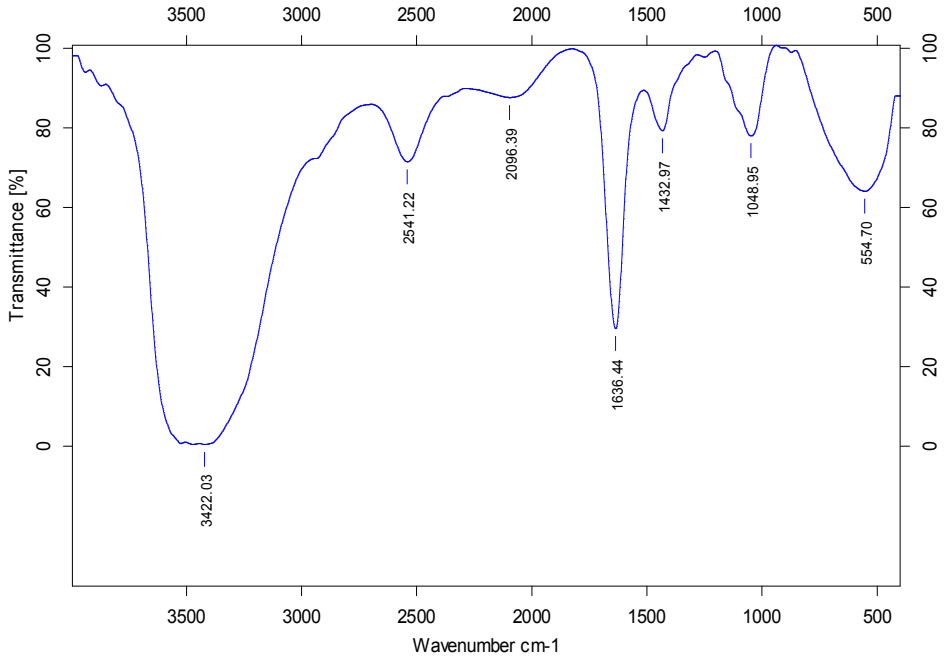

Fig 6: FTIR spectra of purified fucoidan fraction A1L isolated from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Mannar (Pamban), India.

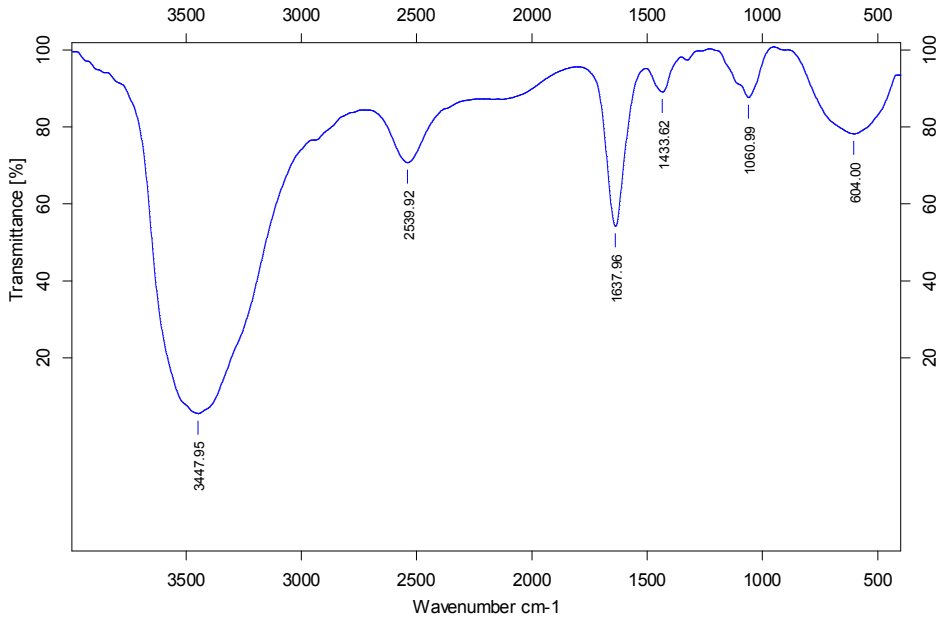

Fig 7: FTIR spectra of fucoidan fraction A1H isolated from the brown seaweed *Turbinaria decurrens* collected along the coast of Gulf of Mannar (Pamban), India.

3.4. Characterization of fucoidan by FTIR spectroscopy

Monosaccharide types, glucosidic bonds and functional groups present in the structure of polysaccharides including fucoidan can be analysed using FTIR spectroscopy [22]. Fucoidan showed bands

in the regions of 1036.60, 1080.05 and 1139.21 confirming that it is an acidic polysaccharide. The bands between 1603 cm^{-1} and 1030 cm^{-1} corresponded to the glycosidic linkage stretch vibration of C–O–C and C–O–H. In addition, the signals close to 1609 cm^{-1} and 1420 cm^{-1} are due to the asymmetric and symmetric stretch vibration of C-O-O of uronic acid [23, 24]. The spectra obtained from the wave number 400-4000 cm^{-1} gave structural information of the isolated compounds [25]. The purified galactose rich sulphated fucoidan fractions A1L and A1H exhibiting anticoagulation activity were characterized using FTIR spectra (Fig. 6 and 7). Both fractions showed peak in region of 3600-3000cm^{-1}, a broad bond centered at 3422.03 and 3447.95 cm^{-1} assigned to hydrogen bonded O-H stretching vibration, the weak signal at wave length 2541.22 and 2539.92 cm^{-1} indicated the presence of C-O-C and asymmetric stretching of carboxylate vibration at 1636.44 and 1637.96 cm^{-1}. The band at 1432.97 and 1433.62 cm^{-1} assigned to C-OH. The weak band at 1048.95 and 1060.99 cm^{-1} indicate the presence of S=stretching vibration of sulphate group. The band at 1037.21 cm^{-1} assigned to C=O stretching vibrations.

4. Conclusion

It is concluded that fucoidan extracted by successive method isolated through DEAE cellulose column from the Gulf of Mannar brown seaweed *Turbinaria decurrens* was dominating galactose followed by fucose and equal amount of mannose and glucose. Fucoidan fractions with low molecular weight > 3500 Da and high molecular weight < 3500 Da were containing same proportion of monosugar as like crude fucoidan obtained in *T. decurrens* whereas sulphate is rich in the fraction of high molecular mass which exhibit high anticoagulation activity.

5. Acknowledgement

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6. References

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