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# STRUCTURAL CHARACTERIZATION, BIOLOGICAL EVALUATION AND MOLECULAR DOCKING STUDIES OF FUCOIDAN ISOLATED FROM BROWN MARINE ALGAE

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# ABSTRACT

The objective of this present investigation was to evaluate the hemolytic, antiinflammatory, antioxidant, antiproliferative activity and molecular docking studies of fucoidan isolated from brown seaweed Sargassum wightii. Fucodian was isolated from brown seaweed of S. wightii using ethanol precipitation. Functional groups and structural characterization of fucodian was analyzed by Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance spectroscopy, Morphology of the isolated fucoidan was performed by scanning electron microscopy (SEM). The antioxidant properties were determined by DPPH scavenging, nitric oxide scavenging and reducing power assays. The biocompatibility of fucodian was assayed by hemolysis and anti- inflammatory studies and the *in vitro* antiproliferative activity was assayed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) against human lymphoma (U 937) cell lines. The maximum DPPH activity (79.92 $\pm$  0.35), NO scavenging activity (51 $\pm$ 0.28%), reducing ability (230 $\pm$  0.87 in terms of FRAP values) was found at the concentration of 100µg/mL Fucoidan was potent against hemolysis of the erythrocyte in a concentration dependent manner suggesting non- toxic nature. The antiproliferative effect of fucoidan showed a higher percentage of inhibition, that is 50% of the cell death after 48h of incubation was achieved in a dose dependent manner. The fucoidan has revealed a significant biding interaction against human kinase (CK2 formerly known as casein kinase 2) as target protein to uphold antioxidant and anti proliferative activities. These findings indicated that the isolated fucoidan from *S.wightii* found to be a promising candidate for the development of an anti cancer compound for drug delivery applications as well as for further investigations in various cell lines.

Keywords: Fucodian, Hemolysis, Anti-inflammatory, Anti oxidant, Anti proliferative, Docking studies.

# 1. INTRODUCTION

Cancer is a group of major diseases involving abnormal cell growth with the potential to spread to other parts of the body that attacks the modern society today and in the future. Lymphoma is a group of blood cancers that begins in the cell of lymph system; about half of the blood cancers that occur each year are lymphomas or cancers of the lymphatic system. In the year 2020, National Institute of Heath estimated 9.9 % cases of lymphoma cases were diagnosed in the Unites states alone. Chemotherapy, radiotherapy or the combination of the both is currently used for the treatment of lymphoma. However these treatments have numerous drawbacks thus developing natural agents to prevent or suppress the progression of invasive cancers has been recognized recently as an approach with enormous potential [1, 2]. Natural products are considered important for the development of new anticancer lead molecules. Owing to their lower side effects, they have gained considerable attention in the recent past. Among natural products, marine macroalgae (seaweeds) have gained a lot of attention due to its richness in bioactive compounds that could potentially be exploited as functional ingredients in pharmacological applications. Marine algae are excellent candidates for sulfated polysaccharides; the sulfated polysaccharides extracted from marine algae have received considerable attention in the nutraceutical, cosmeceutical, and pharmaceutical fields [3]. Sargassum sps is one of the industrially important brown marine algae. Laminarin, fucoidan, and alginates are naturally occurring biopolymers extracted from marine brown algae. Out of it, fucoidan; a natural component of brown seaweed, has anti-cancer activity against various types of cancer by targeting apoptotic key molecules [4].

Fucoidan is an anionic polysaccharide commonly found in brown seaweeds. The structure of the fucoidan was varied from species to species of brown seaweeds. But its identification is based on fucose and sulfate, along with small quantities of galactose, mannose, xylose and uronic acid [5]. It has been reported that these fucoidans include various biological activities such as antioxidant, anti inflammatory, anti allergic, anti tumor, anti obesity, anti coagulant, anti viral, anti hepatopathy, anti uropathy and anti renalpathy effects [6]. The fucoidan has shown effectiveness in inhibiting the growth of various cell lines as evidenced through *in vitro* assays [7, 8]. Anti tumor activity was exhibited by the fucoidan extracted from the various species of brown seaweed [1, 9, 10].

Hence, the present study focused to isolate the fucoidan from *Sargassum wightii* seaweed and to investigate the biocompatibility of fucoidan via hemolytic and anti inflammatory assay. Bioactivity of fucoidan was analyzed through antioxidant assay and an antiproliferative property against human lymphoma (U 937) cancer cell lines was evaluated. Interaction of fucoidan with human kinase (CK2) protein have been chosen for docking studies, endeavored to discuss its potentiality as an anticancer target drug and to provide insights for the utilization of kinase protein against the target by analyzing its inhibition constant and the binding affinity with the amino acids within the proximity of active sites of CK2 protein.

# 2. MATERIAL AND METHODS

# 2.1. Collection of Seaweed Material

The brown seaweed S. wightii was collected from the Mandapam coast in the Gulf of Mannar region, Tamilnadu, India. The collected seaweed was authenticated by Dr. M. Ganesan, Scientist of Marine Algal Research station, CSMCRI (Central Salt & Marine chemicals Research Institute) Mandapam Camp, Tamil Nadu, India. The seaweed was washed thoroughly with tap water and distilled water to remove extraneous materials and shade-dried for 5 days at room temperature. Dried samples were individually cut into small pieces and then blended into a fine powder using the electric mixer then sieved and stored in a sealed bags at 4°C until use.

# 2.2. Isolation of fucoidan from S.wightii

Extraction of fucoidan from brown seaweed *S. wightii* was done by the accompanying methods [11, 12] with some modifications. In brief, 60g of brown seaweed

powder was treated with one litre of 85% ethanol and stirred in a mechanical stirrer at room temperature for 12h, in order to remove pigments, proteins and lipids, the solution was centrifuged at 970g for 10 min. The resultant residue was then washed with acetone and left overnight to dry at room temperature. From these, 10gm of the algal sediment was then collected and mixed with distilled water and placed at 70°C on a hot plate with constant stirring for 1h. The solutions were then centrifuged at 3800g for 10 min to this 1% CaCl<sub>2</sub> was added in order to precipitate alginates. Subsequently the solutions were centrifuged at 3273 g for 10 min to remove impurities. The supernatant was collected and 95% ethanol was added until a final concentration of 70% was reached and stored at 4°C overnight in order to obtain a ethanol-precipitated fucoidan was then filtered to obtain the fucoidan. Fucoidan yield was estimated based on the treatment of obtained sample with 85% ethanol as a percentage of algal dry weight (% dry weight) [10].

# 2.3. Purification of fucoidan [8]

A 0.3g of fucoidan in 30ml of distilled water was heated with 0.75ml of 3.0 M HCl for 3h and then cooled. The resultant mixture was centrifuged at 3273 g for 10min and the supernatant was neutralized with NaOH and poured into 100ml of ethanol. Then the precipitate was dissolved in water and lyophilized.

# 2.4. Chemical Analysis of Fucoidan

The presence of fucose content and sulphate content in the extracted fucoidan was estimated by the phenolsulphuric acid method [13] using L-fucose as standard and by the barium chloride method [14] using sodium sulphate as standard.

#### 2.5. Fourier transform infra red analysis (FT-IR)

FT-IR analysis was performed in Perkin Elmer (spectrum two model) instrument in order to determine the functional groups of isolated fucoidan. The sample were grounded with potassium bromide and pelletized. The spectrum was recorded over the range of 400 to 4000cm<sup>-1</sup>.

# 2.6. Nuclear magnetic resonance analysis (NMR)

A 20mg of the fucoidan was dissolved in 0.75ml of  $D_2O$  (Deuterium oxide) in a NMR tube and the <sup>1</sup>H NMR analysis was carried out using an Bruker 500 MHz Avance III (AV 500) spectrometer.

#### 2.7. Scanning electron microscopy (SEM)

Surface morphology of the fucoidan isolated from *S.wightii* was represented using a scanning electron microscope (Carl Zeiss Evo 18 model).

#### 2.8. In vitro antioxidant assays

# 2.8.1. 2 DPPH (2-diphenyl-1-picryl hydrazyl) scavenging activity

Briefly, 0.2mL of sample solution containing varying concentrations (20-100 $\mu$ g/mL) of fucoidan was added to the 1ml of freshly prepared 0.1mM of DPPH-Methanolic solution, shaken vigorously and then incubated in dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 517 nm using a micro plate reader (Epoch Microplate; BioTek) after 30 min of incubation. Butylated hydroxyltoluene (BHT) was used as a positive control. The lower the absorbance of reaction mixture, the higher is the free radical scavenging activity [15]. The percentage of the DPPH scavenging activity is calculated using the following equation:

DPPH Scavenging activity (%) = {(Absorbance of control-Absorbance of sample)/Absorbance of control} x 100

# 2.8.2. Ferric reducing-antioxidant power (FRAP) Assay

The ability to reduce the ferric ions was measured using the method employed by Benzie and Strain [16] with slight modifications. It relies on the principle of reducing property of the antioxidants from ferric 2,4,6-tripyridyls-triazine complex (Fe<sup>3+</sup>- TPTZ) to ferrous deep blue colored complex ((Fe<sup>2+</sup>- TPTZ). The FRAP reagent was freshly prepared before the start of the reaction by adding 10 parts of 300mM sodium acetate buffer (pH 3.6), 1 part of TPTZ in 40mM/L of HCl and 20 mM/L of ferric chloride solution at a ratio of 10:1:1 [17]. Different concentrations (20 -100 $\mu$ g/mL) of fucoidan extract were prepared and 500µl of the prepared extract was incubated with 1000µl of FRAP reagent. After vortexing, the absorbance was measured at 593nm for 0<sup>th</sup> minute. Then after, samples were incubated at 37°C in a water bath and absorbance was measured again after 4 minutes at the same wavelength respectively. Freshly prepared ascorbic acid was used as a standard. The data obtained were expressed in the FRAP values  $(\mu M)/g$  and compared with that of standard ascorbic acid in  $\mu M$ 

FRAP value ( $\mu$ M) = (Change in absorbacne of sample from 0-4 minute/ Change in absorbacne of standard from 0-4 minute) x Frap value of standard ( $\mu$ M)

#### 2.8.3. Nitric oxide scavenging activity

Nitric oxide radical generated from aqueous solution of sodium nitroprusside at physiological pH (7.2) reacts with oxygen to produce stable products (nitrite ions) which was measured by Griess reaction [18]. Briefly, 1 ml of the fucoidan extract with varying concentrations 20 to 100  $\mu$ g/mL) was treated with the 3mL of 10 mM sodium nitroprusside in phosphate buffer. The resultant solution was then incubated at 25°C for 3 h. After 3 h of incubation, 0.5 mL of the incubated solution was withdrawn and mixed with 1 mL of Griess reagent. The mixtures were then incubated for 30 min at room temperature. The absorbance of pink color chromophore produced during diazotization of nitrite was observed at 550 nm on micro plate reader (Epoch Microplate; BioTek). Ascorbic acid (20-100 µg/ml) was used as positive control. The Nitric oxide scavenging activity is calculated by the equation given below:

NO radical scavenging activity (%)={(Absorbance of control-Absorbance of sample)/ Absorbance of control}x 100

# 2.9. Hemolytic activity

Fucoidan extract was tested in human red platelets (HRBCs) to evaluate its hemolytic activity. In brief, 5ml of fresh human blood was collected from a healthy human volunteer and washed threefold with phosphate buffered saline (PBS) to isolate red blood cells and resuspended in 20 ml of PBS. A stock suspension (5% haematocrit) was utilized in all hemolytic assays. RBC suspension of 100µl was treated with equivalent volume of the fucoidan extract at different fixations ranging from 20 to 100µg/ml incubated at 25°C for 30 min. After incubation, the resultant mixture was centrifuged at 1000g for 10 min and the absorbance of the supernatant was estimated at 540nm utilizing a microplate reader (Epoch Microplate; BioTek). RBC treated with 2% Triton X-100 was used as a positive control and RBC with PBS was used as the negative control, respectively, and the hemolysis percentage of RBCs was calculated using the formula [19]:

% hemolysis =  $100 \times \{(OD_{Sample} - OD_{PBS}) / (OD_{Triton X} - OD_{PBS})\}$ 

The effect of Fucoidan on the erythrocyte shape was investigated with optical microscopy.

#### 2.10. Antiinflammatory activity

# 2.10.1. Inhibition of protein denaturation method

Protein denaturation assay was done according to the method of Mizushima and Kobayashi [20] and Sakat et al.

[21] with some modifications. 5ml of reaction mixture consisted of 0.5ml of 1% bovine serum albumin, 4.50ml of phosphate buffered saline and 0.1ml of Fucoidan extract. The reaction mixture was mixed well and incubated in a water bath for 10min at 37°C, and then the reaction mixture was heated at 51°C for10 min. After cooling, the turbidity was measured at 660 nm. Diclofenac sodium salt was used as control. The percentage inhibition of denaturation was calculated using the following formula:

% Inhibition of protein denaturation= 100- {(A<sub>1</sub>-A<sub>2</sub>)/A<sub>0</sub>} x 100

# 2.10.2. Membrane stabilization

An anti-inflammatory activity for fucoidan was done by membrane stabilization method [22]. Five milliliters of whole blood were collected from a healthy human volunteer and mixed with equal volume of Alsever's solution subjected to centrifugation at 1000g for 10 min. The supernatants were discarded and the RBC pellets further washed thrice with isosaline and resuspended in water. Various concentrations of formulations from 20 to 100µg/ml were prepared to which 250µl of RBC was added along with 0.5ml of PBS and 1ml of hypo saline, which was subjected to incubation at 37°C for 30min. The suspension was then centrifuged at 3000 rpm for 5 min and measured at 540nm using microplate reader (Epoch microplate spectrophotometer; BioTek). Antiinflammatory assay was further determined by the formula:

% stabilization = (OD sample-OD control) x 100

# 2.11. MTT-Based Antiproliferative Assay

The cytotoxic effect of the isolated fucoidan against human lymphoma (U 937) cancer cell lines was assessed using 3-(4, 5 dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT)[23]. The cells were seeded in 96-well plates in 100µl of medium containing 10% fetal bovine serum (FBS) solution at a plating density of 1×10<sup>5</sup> cells/well. Well-plates were incubated at 37°C, 5% CO<sub>2</sub> and 95% air, and 100% relative humidity 48 h prior to the addition of the sample. After incubation, fucoidan sample at various concentrations (20-100  $\mu$ g/l) were added and the well-plates were incubated at 37°C for 48 h. After 48 h, 50 µl of MTT solution (5 mg/ml, dissolved in distilled water) was added to each well and further incubated for 4 h. After the media were removed, 100µl of dimethyl sulphoxide (DMSO) were added to each well to solubilize the formazan crystals and

their absorbance was measured at 570 nm using a micro plate reader. The experiments were conducted in triplicates. The morphological changes for the cells treated with and without fucoidan were observed and photographed under inverted animal cell culture microscope (LABOVERT-FS model) under 40 x objective. The Percentage of cell viability and cell inhibition was calculated as follows:

Cell Viability (%)= (OD value of treated cells/ OD value of control cells) x 100

Cell inhibition (%)= 100-Cell viability

# 2.12. Molecular Docking Study

Fucoidan isolated from brown seaweed S.wightii was exploited for molecular docking studies to determine the chemical interaction of fucoidan with targeted protein responsible for lymphoma cancer. The crystal structure of target protein kinase CK2 catalytic activity enzyme (lymphoma cancer protein RCSB PDB ID: 5N1V) was retrieved from PDB database (https://www.rcsb.org). The UniProt ID of this target protein was taken from the Uniprot database (https://www.uniprot.org/). Ligand molecule structure was drawn by Chem Draw 12.0 software. The synthesized 2D compound structures were changed into mol format that was further converted into the 3D the structure by Auto Dock software was utilized for docking process, all molecular docking calculations were performed on Auto Dock-Vina software. Cocrystallized ligands, waters and co-factors were removed before preparing protein for docking. To calculate Kollman charges and polar hydrogen's, the Auto Dock Tools graphical operator interface was used.

#### 3. RESULTS AND DISCUSSION

Fucoidan is а naturally occurring sulphated polysaccharide extracted from the marine brown seaweeds, which contains major components of L-fucose and sulfate ester groups [8]. It has a wide range of biomedical and pharmacological properties. Many researchers have reported that the fucoidan isolated from various seaweeds showed strong inhibitory effect against different types of tumor cell lines [24]. Therefore, the fucoidan has become a focus of considerable attention to perform our work for the determination of antiinflammatory, hemolytic, cancer prevention and cytotoxicity. In the present study, the yield percentage of fucoidan isolated from S.wightii was about 6.25±0.34%. The chemical composition of fucoidan was summed in Table 1 contains 27.47±0.12 % of sulphate content

and  $31.76\pm0.23\%$  of fucose (carbohydrate) content respectively.

position of fucordan from <i>s.wighth</i>			
Parameters Percentage (%			
Yield	6.25±0.34		
Sulphate	ate 27.47±0.12 %		
Fucose	31.76±0.23%		

Table 1: Yield percentage and chemical composition of fucoidan from *S.wightii* 

#### 3.1. FT-IR analysis

The FT-IR analysis of fucoidan isolated from *S.wightii* is depicted in fig.1. The major absorption band at 3436cm<sup>-1</sup> indicates the presence of O-H stretching.



Fig. 1: FT-IR spectra of fucoidan isolated from *S.wightii* 

The band observed at 2925cm<sup>-1</sup> and 2854cm<sup>-1</sup> was characterized for the absorption of polysaccharide corresponds to C-H stretching vibrations pyranoid ring or C-6 group of fucose and galactose units [25]. The strong absorption band at 1636cm<sup>-1</sup> indicates the C=C stretching vibration of uronic acid. The band at 1410, 1320 and 1170 cm<sup>-1</sup> indicated the presence of S=O stretching vibrations sulphate, sulphonate, sulphonamides, which was a characteristic component for fucoidan and sulphated polysaccharides in marine seaweed [8]. In addition to this peak at 1410cm<sup>-1</sup> also signifies a C-H vibration of the polysaccharide units. Absorption band at 1272cm<sup>-1</sup> was assigned to the C-O stretching vibrations. Strong absorption band at 1041cm<sup>-1</sup> and 843 cm<sup>-1</sup> was attributed to the presence of  $\alpha$ glycosidic linkages.

# 3.2. <sup>1</sup>H NMR analysis

The proton NMR spectrum of fucoidan isolated from *S.wightii* is shown in fig. 2. It is used to evaluate the anomercic grouping and the sulfation arrangement in polysaccharides. The intense signals arise from 4.79 and 4.22ppm attributed to the presence of  $\alpha$ -L-fucose and 3-linked  $\alpha$ -L-fucose, considered to be the principal component of fucoidan. The detection of signal at 4.61ppm at 1[H] indicates the presence of 3-linked d-galactopyranosyl. Signal at 3.73 at 4 [H] and 3.30 at 2[H] represent to 2, 3- linked  $\alpha$ - $\beta$ -Mannose and  $\beta$ -D-Xylose respectively. The structural similarity features were observed by various researches in fucoidan of different varieties of brown seaweed [26, 27].



Fig. 2: <sup>1</sup>H NMR Spectra of fucoidan isolated from S.wightii

# 3.3. SEM analysis

The SEM image of fucoidan was recorded and depicted in fig. 3. The morphology of fucoidan seems to be further widespread surface area with overlapping/ aggregation of oval shaped particles and the surface of the particles are smoother in appearance.



Fig. 3: Scanning electron microscopic image of fucoidan isolated from *S.wightii* 

# 3.4. Determination of antioxidant activity

# 3.4.1. DPPH scavenging activity

Antioxidants synthesized naturally from seaweed and other natural products are non toxic, eco-friendly and more potential than the synthetic antioxidant. The activity of antioxidant is determined by the presence of bioactive compounds such as phenolics and flavonoids. Bioactive compounds scavenge DPPH via the hydrogen donating power, thus reducing DPPH to DPPH-H.

DPPH scavenged effectively by antioxidants in this study, the DPPH scavenging activity or inhibition percentage of isolated fucoidan was compared with the synthetic antioxidant Butylated hydroxyltoluene (BHT) shown in fig.4. The results revealed that the isolated fucoidan possessed a DPPH scavenging activity and showed higher scavenging effects of 80% at  $100\mu$ g/ml compared to the scavenging effects of BHT showed 90% at  $100\mu$ g/ml respectively (Fig. 4). Since the DPPH radical scavenging activity of isolated fucoidan is considerable and had the strongest inhibiting activity towards DPPH radical, they could be used as substitutes to replace synthetic antioxidants, which have been reported to be carcinogenic at high doses.



Fig. 4: DPPH scavenging activity of fucoidan isolated from *Sargassum wightii* compared to standard BHT

# 3.4.2. Ferric reducing-antioxidant power (FRAP) Assay

FRAP assay is based on the ability of antioxidants present in the fucoidan extract to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of TPTZ complex. The results (Fig. 5) shows the FRAP values of the fucoidan isolated from *S.wightii* at varying concentrations (20, 40, 60, 80, 200 $\mu$ g/ml) with ascorbic acid as standard. The maximum reducing ability at 100 $\mu$ g/ml for fucoidan and ascorbic acid were found to be 230 $\pm$ 0.54 and 315 $\pm$ 0.21 respectively. The above findings states that the reducing ability of fucoidan showed a dose dependent antioxidant activity.



Fig. 5: Ferric reducing antioxidant activity of fucoidan isolated from Sargassum wightii against standard ascorbic acid.

### 3.4.3. Nitric oxide scavenging activity

Nitric oxide (NO) is a potent cellular mediator involved in numerous physiological functions of the body such as smooth muscle relaxation, inhibition of platelet aggregation, neuronal signaling and regulation of cell mediated toxicity. The NO generated from sodium nitroprusside directly reacts with oxygen to produce nitrite. Fucoidan showed a moderate nitric oxide scavenging between 20 to  $100\mu$ g/ml in a dose dependent manner (Fig. 6). Fucoidan extract exhibited the maximum percentage of inhibition of  $63\pm0.51\%$ increased with an increasing concentration of the extract. Ascorbic acid, the natural antioxidant was used as a control for comparison.



Fig. 6: NO Scavenging activity of fucoidan isolated from *Sargassum wightii* against standard ascorbic acid

#### 3.5. Hemolytic activity

Fig. 7 depicted the anti-hemolytic activity of fucoidan on human RBC. Rupturing of RBC cells is known as hemolysis. In our study, fucoidan extract maintained the stability of human red blood cell membrane by preventing the hemolysis. Hemolysis percentage found to be increased with increase in concentration. At a concentration of  $100\mu$ g/ml, 88% of hemolysis was prevented. Structure morphology of RBC is depicted in fig 8.

#### 3.6. Anti inflammatory assay

# 3.6.1. Inhibition of protein denaturation

It was observed that fucoidan extract exhibited concentration-dependent inhibition of denaturation of protein, where the inhibition of the denaturation of the BSA increased with the increase in concentration of the extract, thus the highest inhibition of denaturation activities were observed at  $100\mu$ g/ml (60%) as shown in fig. 9.



Fig. 7: Hemolytic activity of fucoidan



Fig. 8: Optical microscopy images of RBC treated with (a) PBS (b) Fucoidan at 20µg/ml (c) Fucoidan at 100µg/ml





#### 3.6.2. Membrane Stabilization assay

Anti inflammatory action is one of the significant strategies for treating diseases. Since, the human red blood cells (HRBC) membrane is similar to lysosomal membrane components. Prevention of saline induced human RBC membrane lysis was considered as a measure of anti inflammatory activity of fucoidan. HRBC membrane stabilization by fucoidan revealed the anti inflammatory activity of fucoidan and the results are shown in fig 10. At higher concentration level ( $100\mu$ g/ml) it showed about 80.5% of antiinflammatory activity compared to 90% lysis induced in control, diclofenac sodium was used as control.



Fig. 10: Anti inflammatory activity of fucoidan by HRBC membrane stabilization

# 3.7. Anti-proliferative effect of fucoidan on human lymphoma cancer cells

To evaluate the bioactivity of fucoidan as a anticancer agent, an anti-proliferative effect against the lymphoma cancer cell line (U 937) and normal cells of green monkey kidney cells (vero cells) was assessed, an MTT assay was performed to measure the cell viability following the treatment of U 937 lymphoma cancer cells with fucoidan over the range of 20 -100 $\mu$ g/ml for 48h.U937 lymphoma cancer cell proliferation was prominently inhibited in concentration dependent manner. Fig. 11 and 12 clearly demonstrate the anti-proliferative effect and percentage of inhibition of U937 cells. Percentage of cell viability reduced with the increased concentration of fucoidan, indicate that the rate of cell proliferation is reduced. 50% of the cells

were inhibited at a concentration of  $100\mu$ g/ml over a period of 48h, whereas 70% of live cells were observed in the control. The cytomorphological changes of fucoidan treated with U 937 cells and vero cells were shown in fig 13. The control cells remains in normal and uniform morphology whereas the cells treated with fucoidan showed some morphological changes where the cells was disturbed and shrink due to the absorption and reaction of fucoidan extract with the cells.



Fig. 11: Anti proliferative activity of fucoidan against human lymphoma cells (U 937) and Vero cells (African green monkey kidney cells).



Fig. 12: Percentage inhibition of fucoidan against human lymphoma cells (U 937) and Vero cells



Fig. 13: Morphological observation of cancer cell lines under inverted microscope (40x): (a) Vero control (b) Vero treated (c) U 937 control (d) U 937 treated upon 48h of incubation

#### 3.8. Molecular Docking Study

The docking investigations revealed the interactions of fucoidan with CK2 target protein 5N1V. The binding affinity energy values and their RMSD values (rmsdl.b and rmsdu.b) were given respectively (Table 2). Additionally, the inhibition constants for fucoidan have been calculated and listed by using Ki=exp( $\Delta$ G/RT) equation, where,  $\Delta$ G, R and T are the docking binding

energy, gas constant  $(1.9872036 \times 10^{-3} \text{ kcal/mol})$  and room temperature (298.15 K), respectively.

Inhibition constant (ki) gives information that ligand interacts with a substance of the enzyme and ki is the measure of ligand binding affinity to protein at the same time. If Ki is smaller, the lesser amount of medication needed to inhibit the activity of that enzyme. From the results, it was found that fucoidan showed the highest binding affinity (-6.2 Kcal/mol) interacted with VAL-116 (H-bonding) and HIS -160 (H-bonding) pockets (Fig.13).

Table 2: AutoDockVina results of the bindingaffinity and RMSD values of different poses in5N1V inhibitor of FU compound

Mode	FU -5N1V		
	Binding affinity	rmsdl h	rmsdu b
	(kcal/mol)	THISULD.	misdu.D
1	-6.1	0.000	0.000
2	-5.8	13.277	15.478
3	-5.6	27.763	28.770
4	-5.6	24.963	28.770
5	-5.6	29.621	32.798
6	-5.6	17.677	20.170
7	-5.5	28.698	31.680
8	-5.5	17.805	20.397
9	-5.3	2.822	4.298

Inhibition Constant: 46.825µM



Fig. 13: Binding interactions of fucoidan ligand with target protein 5N1V

# 4. CONCLUSION

The use of natural and plant based anticancer products is a significant tool to fight against the cancer cells owing to their fewer or no side effects. The present investigation provides extensive evaluation of in vitro hemolytic, anti inflammatory, antioxidant, anti proliferative properties and molecular docking of fucoidan isolated from brown seaweed S.wightii. The isolated fucoidan was identified through FT-IR and NMR. Our results showed that the isolated fucoidan exhibited a significant antioxidant activity. Further its hemolytic activity and anti inflammatory properties in HRBC model showed appreciable result by making it potential candidate for pharmacological applications. Fucoidan exposed the anti proliferative property at 100µg/ml concentration induces about 50% of cell death in human lymphoma cancer cells. The molecular docking studies on CK2 protein kinase illustrated the binding interactions of fucoidan with target protein and the results are supported with that of antioxidant and anti proliferative activities. On the basis of above findings, it has been proved that the fucoidan isolated from S.wightii brown seaweed possess biocompatible, significant antioxidant and anti proliferative potential against human lymphoma cancer cells which was in support with the analysis of docking , thus making it a suitable candidate to identify its anticancer potential for future applications. In addition, future research is essential to isolated many secondary metabolites to explore the scientific shreds of evidences by performing in vivo studies.

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#### **Conflicts of interests**

The authors declare no conflict of interest.

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