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Anticancer Activity of Lectin Extracted from the Saltpan Microalga, *Navicula* sp.

Murugan Anithavani [a], Rayappan Anantha Rajan [b*] and Mariavincent Michael Babu [a]

[a] Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, 629 502, Tamil Nadu, India.
[b] Department of Zoology, Pioneer Kumaraswamy College, Affiliated to Manonmaniam Sundaranar University, Nagercoil, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Haemagglutinins are carbohydrates binding protein applied as tools in cell biology and immunology. In the present study, lectin from selected microalgae *Navicula* sp. was isolated and characterized for biomedical applications. The microalgae were collected from the saltpan water sample. A total of six species were isolated including *Amphora* sp., *Nitzschia* sp., *Navicula* sp., *Scendesmus* sp., *Chlorella* sp. and *Nanochloropsis* sp. by direct plating method using algal isolation medium and characterized. The crude microalgal extracts were screened for the presence of lectin and it was confirmed by haemagglution assay. Among the strains, *Navicula* sp. showed potent haemagglutinin

**Corresponding author: Email: anantharajanr@gmail.com;*

activity. Heamagglutinin from *Navicula* sp. was further partially characterized using chromatography methods. *Navicula* lectin agglutinated animal and human erythrocytes. It showed high specificity for human "O" erythrocytes with the titre value of 32 HA units. The HA activity was stable between pH 7and 8 and showed thermal stability between 30 ºC and 40 ºC ($p<0.01$). The isolated lectin was calcium dependent and HA activity was reduced when exposed to chelators such as EDTA ($p<0.01$). Hemagglutination inhibition assay exhibited the strongest binding specificity towards glucose, sucrose and galactose. The cross-adsorption assay revealed that the *Navicula* sp. Possesses single agglutinin. The purified lectin showed anticancer activity against cervical cancer cell lines.

Keywords: Microalgae; hemagglutination; carbohydrate; lectin.

1. INTRODUCTION

"Haemagglutinins, carbohydrate-binding proteins were isolated from a wide variety of organisms. These haemagglutinins agglutinate normal or transformed cells, including human and animal erythrocytes. Their specificity for carbohydrate structures makes them useful reagents in biochemical, drug, and clinical research. Usefulness of haemagglutinin in various fields of biological research such as cytology, cell biology, immunology and cancer research has caused a growing interest in the discovery and isolation of agglutinins from various new biological sources. As a result, a great number of living organisms have been reported to have haemagglutinins. However, in comparison, there are only limited studies on algal haemagglutinins, in spite of the availability of large number of species and amount of marine macroalgae, as compared to higher plants. In particular, lectins are proteins of nonimmune origin which bind specifically and reversibly to carbohydrates" [1,2]. "Algae are the most diverse organisms in the plant kingdom. Microalgae are microscopic unicellular, photosynthetic organisms. They can be used to produce a wide range of metabolites. Hypersaline ecosystem possesses many unique features different from other aquatic environments. Microalgae act as the sole producer in the saltpan, producing energy by trapping sunlight. Therefore microalgae represent a unique opportunity to discover novel metabolites. Microalgae have for long been used with therapeutic purposes; their systematic screening for biologically active principles began in the 1950s. The increasing interest in marine natural products has led to the discovery of new biologically active compounds and marine algae have been subjected to increasing study for this purpose" [1].

"Biochemical experiments based on agglutinating tests have revealed the presence of haemagglutinin activity in many algal extracts against erythrocytes from several animal species. In most studies this haemagglutinin activity is referred to the presence of proteins or glycoproteins having specificities for carbohydrate structures binding selectively to red blood cells and microorganisms. Lectins have been reported from a large number of algae. Algal lectins are referred to as phycolectins and they differ from plant lectins in a variety of physico-chemical characteristics" [3]. "A few reports also indicated that marine unicellular algae contain haemagglutinins that agglutinate human and animal erythrocytes to varying degrees" [4-6]. Because of their strong haemagglutinating activities, unicellular algae are also a potent source of haemagglutinin in biochemistry and medical applications.

Lectins have a variety of biological activities and can agglutinate various cells. Since the surface of living cells is covered with a layer of carbohydrates, lectins can easily bind to them, so they can recognize special sugar chain structures depending on the type. "Furthermore, their carbohydrate-binding specificity is usually complex, revealing that they do not bind to simple sugars but have high affinities for complex oligosaccharides, especially those found in animal glycoproteins. Compared to plant lectins, there are only a few reports on the use of marine algal lectins. Many algae also produce antibiotic substances capable of inhibiting bacteria, viruses, and fungi" [7]. The present study was undertaken to identify and characterize hemagglutinin from the microalgae *Navicula* sp. In addition, the anticancer activity was analyzed.

2. MATERIALS AND METHODS

2.1 Collection of Microalgae from Saltpan Water

The isolation of microalgae was carried out from saltpan water sample. The brine water sample was collected from reservoir, condenser, and

crystallizer of puthalam saltpan in Kanyakumari district, India. The water samples were taken from both the surface and middle of the water column. The collected samples were packed in transparent plastic bottles, and marked and placed in an ice bag. The samples were transported to the laboratory for further analysis.

2.2 Isolation and Identification of Marine Microalgae

Microalgae were isolated by agar-plating method which was previously described by Perumal et al. [8]. The culture was grown in a conical flask (500 ml) containing 250 ml of sterile Conway media, and the flasks were maintained at a temperature of 25°C ± 1 °C with a continuous illumination of 1,000 lux with 16:8 light and dark cycle. All species were identified by external morphology using microscope was previously described by Mitra et al. [9].

2.3 Stock Culture Maintenance and Indoor Mass Culture of Isolated Microalgae

The algal stock was maintained in Walne's medium. The cultured flasks were placed under tube lights of 1,000 Lux light intensity. The temperature range was between 28 °C and 30 °C. In 4-5 days, the culture reached log phase and maximum exponential phase was achieved after 10 days.The composition of Walne's medium included macronutrients, such as potassium nitrate – 100 gm; sodium di-hydrogen orthophosphate − 20.0 gm; Ethylene diamine tetra acetic acid (EDTA), sodium salt −45.0 gm; boric acid – 33.48 gm; ferric chloride – 1.30 gm; and manganese chloride −0.36 gm. They were then dissolved in 1 l of distilled water and named as stock solution 'A'. The micronutrients consisted of zinc chloride – 4.2 gm; copper sulphate – 4.0 gm; cobalt chloride – 4.0 gm; and ammonium molybdate – 1.8 gm. All were dissolved in 1 l distilled water. Vitamins such as thiamine (vitamin B1) – 200 mg and cyanocobalamine (vitamin B12) −10 mg were dissolved in 100 ml of water to prepare the stock solution 'C' and stored in a refrigerator. The solutions A, B, and C were prepared in separate reagent bottles. For the preparation of the working solution, 1 ml of solution 'A', 0.5 ml of solution 'B', and 0.1 ml of solution 'C' were individually taken and added in 1 l of filtered and sterilized seawater. The seawater was enriched with the required quantity of Walne's medium. Then, 10–20% of the inoculums of log-phase culture were transferred into the indoor mass culture container with 5 L medium. Finally, the culture container was placed in front of the tube lights of 1,000 lux light intensity with continuous aeration. The temperature range was between 28°C and 30°C.

2.4 Preparation of Algal Sample

100 ml of pure algal culture was taken and centrifuged at 5000 rpm for 20 minutes. The algal pellet was collected, and 2 ml of cold TBS (pH 7.6) was added and homogenize in a glass homogenizer for 10-20 minutes. The homogenate was incubated on the water bath at 60 ^{o}C for 30 minutes. After incubation the homogenate was centrifuged at 10,000 rpm 4 ^{o}C for 20 minutes. After centrifugation, the supernatant was collected and used for hemagglutinin assay.

2.5 Collection of Human and Animal Erythrocytes

Red blood cells of human A, B and O groups and the red blood cells of chicken, goat, cow and rabbit were used for haemagglutinin tests. Blood was collected from healthy human with written consents. Chicken, cow, goat and rabbit blood was collected with suitable anticoagulants from the slaughterhouse. All the blood samples were collected directly in sterile modified Alsevier's medium (anticoagulant) (pH 6.1, 30 mM trisodium citrate, 77 mM NaCl and 114 mM glucose) stored 4 ^{o}C up to 2 weeks.

2.6 Preparation of Erythrocyte Suspension

The freshly collected A,B and O of human, Rabbit, Cow, Goat and Chicken of animal erythrocyte were washed 3 times with 10 volumes of 100 mM Tris-base at pH 7.6 containing 50 mM NaCl and 10 mM $CaCl_2$. To 850 µl of Tris buffer saline solution, 150 µl of centrifuged erythrocyte suspension was mixed well in the same buffer as 1.5% (v/v) cell suspension for agglutination assay.

2.7 Identification of Agglutinin

2.7.1 Haemagglutination assay

Haemagglutination assay was performed in V-bottomed microtiter plates by serial two-fold

dilution of a 25 µl serum sample with an equal volume of TBS-Ca. After dilution 25 µl red blood cell suspension was added to each well and incubated for 1 h at room temperature. Hemagglutination titre was recorded as the reciprocal of the highest dilutions showing positive results. The assays were carried out in duplicates. A control was also maintained with 0.85% NaCl solution with erythrocytes.

2.8 Physico-chemical Characterization of Lectin

2.8.1 pH and thermal stability

Temperature and pH temperature dependence of agglutinin was measured by pre-incubating the algal extract at various temperatures (20 ºC – 90 °C) and pH (3-10) and for 1 h before adding erythrocyte suspension for hemagglutinin assay.

2.9 Cations and EDTA Treatment

To study divalent metal cations (Ca^{2+}, $Mg^{2+}$and Mn^{2+}) dependence on hemagglutination, HA assays were performed in TBS (pH 7.5) with and without these ions at varying concentrations. To study the effect of calcium chelators (EDTA) on the agglutinin, the microalgal extract was pre-incubated at different concentrations (0.01 to 100 mM) of EDTA for 1 hour before adding erythrocyte suspension for HA assay.

2.10 Hemagglutination Inhibition Assay (HAI)

About 25 µl of serially diluted inhibitor (sugars/glycoproteins) solution, 25 µl of the extract was added, mixed and the plate was incubated for 1 hat room temperature. Finally, 25 µl of 1.5% human O erythrocytes suspension was added and incubated for 1 h at room temperature (30 ± 2 ºC). The minimum concentration of the inhibitors required to completely block the agglutination after 1 h of incubation at room temperature (30 ± 2 ºC) was considered as the HAI titer.

2.11 Cross Adsorption Assay

The cross adsorption assay was carried out by mixing 1 ml of erythrocytes and 1ml of crude algal extract. This extract-erythrocyte mixture was incubated at 10 ºC overnight (18 h) with gentle occasional shaking. After centrifugation, the supernatant was tested against selected erythrocytes for hemagglutination assay.

2.12 Purification of Lectin

About 200 g *Navicula* sp. culture was centrifuged at 10,000 rpm for 10 min and the pellet was collected. Then the pellet (15 g) was washed several times with double distilled water. It was macerated carefully in the presence of liquid N_2 and fine powder (5 g) was obtained. It was subjected for the extraction of algal protein using 0.1 M Tris-HCl containing NaCl (0.15 M) using protease inhibitor (phenyl methane sulfonylfluoride, 10 mM). Then the sample was centrifuged at 8000 rpm for 10 min and the clear supernatant was obtained. The separated protein sample was further passed through sephadex G-75 gel filtration column chromatography. The column was equilibrated with 50 mM Tris-HCl and 2 mL fractions were obtained. The eluted fractions were subjected for total protein determination 280 nm. The protein content was analyzed and the fractions were subjected to hemagglutination activity assay. The molecular weight of the lectin was determined using a SDS-PAGE (12% separating gel and 5% stacking gel). It was stained using 0.1% coomassie brilliant blue in methanol and acetic acid (40% and 7%) for 1 h and destained with methanol and acetic acid (40% and 7%).

2.13 Anticancer Activity of lectin against Cervical Cancer Cell Lines

Human cervical cancer cell lines (HeLa) were purchased from National Centre for Cell Sciences, Pune, India. It was cultured in animal cells culture medium (Dulbecco's modified Eagles medium) supplemented with antibiotics. It was maintained in a humid CO_2 incubator and the viability was tested at various lectin concentrations. The purified lectin was diluted at 6.25 µg, 12.5 µg, 12.5 µg, 25 µg, 50 µg, and 100 µg) in the culture medium and incubated with HeLa cell lines. The anticancer activity was detected by direct microscopic observation and MTT assay method. Etoposide was used as the positive control (10 µl/mL).

2.14 Statistical Analysis

Analysis of variance was performed and the p-value <0.01 was considered statistically significant. Statistical analysis was performed to reveal significance of variation in characterization studies and anticancer activity. The anticancer

activity result was compared with standard (etoposide) and significant level was calculated.

3. RESULTS AND DISCUSSION

3.1 Identification and Screening of Isolated Microalgae

Microalgae are excellent source of novel lectin molecules and algal lectins have several advantages than plant lectins. The most important property of lectins is their ability to bind carbohydrate and agglutinate cells. In this study, saltpan water samples showed the presence of various algae and characterized using a microscope. Agar plate method revealed the presence of algae such as, *Nannochloropsis* sp., *Nitzschia*sp., *Navicula* sp., *Chlorella* sp, *Amphora* sp. and *Scendesmus* sp.

The algae widely distributed throughout the world and under hypersaline conditions (20–300%) diversity is very low. Algal species such as, *Amphora coffeaeformis, Anomoneis, sphaerophora*, *Navicula subinflatoides, Nitzschia communis* and *Nitzschia frustulum* were reported from various environments [10]. The isolated six species were screened for haemagglutination assay to determine the properties of agglutinins in seven different blood viz. hen, cow, goat, rabbit blood and human A, B, O. However, all the microalgal extract were not active on all the blood samples as there was specificity in agglutination as shown in Table 1.

A few reports have indicated that marine unicellular algae contain haemagglutinins that agglutinate human and animal erythrocytes to varying degrees [5,6]. In the present study, four species showed agglutinin activity and other two species viz, *Nannochloropsis* sp. and *Scendesmus* sp. failed to agglutinate all type of erythrocytes. High hemagglutin activity was observed in the extract of microalgae *Navicula* sp. with human B erythrocytes with the titer value of 32 than in the other three microalgal species as in Table 2. Other microalgae species such as *Nitzschia* sp., *Chlorella* sp. and *Amphora* sp. showed the least activity on human and animal erythrocytes. All the four species were failed to agglutinate hen erythrocytes. Sampaio et al*.* [11] reported lectin activity from the crude extract and pure sample from *Ptilotaplumosa* and it was found to be specific towards human blood group B erythrocytes. The earlier report exhibited that most of the microalgal lectins agglutinated the human blood groups and no reported activity for animal erythrocytes. However in the present study *Amphora* sp., *Navicula* sp. and *Nitzshia* sp. agglutinated both human and animal erythrocytes.

A similar result was observed in this study, in which the maximum agglutinating activity was found in *Navicula* sp. against B erythrocyte and the minimum activity was found in *Amphora* sp. in cow erythrocyte. The activity was not observed in the extract of *Nanochloropsis* sp. and *Scendesmus* sp. Because of their strong haemagglutin activities, unicellular algae are also a potent source of haemagglutinin for biochemical and medical applications. Hence a *Navicula* sp. was selected for physico – chemical characterization study. The morphology of *Navicula* sp. was characterized under the light microscope during the exponential growth phase. The cells showed simple morphology with boat shaped diatom. The cells contained within a silica cell wall of two separate valves and brown in colour.

3.2 Physico-chemical Characterization

3.2.1 pH and thermal stability

The agglutinin activity was highly dependent on the environmental conditions, and the activity was different from one species to another. However, the most studied variables were pH and temperature. The hemagglutinin of the crude extract of *Navicula* sp. was stable at pH 7.0-8.0 in human B erythrocytes and was statistically significant ($p<0.01$) (Table 3) and the activity gradually reduced below 7.0 and above 8.0. At pH 5 and pH 10.0 the haemagglutin activity was completely lost. Kumar et al. [12] stated that the pH impact on *C.sertularioides* has been observed to be effective between 5.0 and 10. Agglutination activity of crude extract of red alga *Acrocystisnana* from southern coast of Java island, Indonesia was stable between the pH 3.0 and 10 [13]. Similar to our study, Malini [14] also reported that the lectin *C. sinuosa* activity was stable at a pH range of 7.0 and 8.0 and no activity was recorded at pH 5.0 and pH 10.0. The crude extract of *Navicula* sp. was tested for the thermostability with respect to its hemagglutinating activity and was stable at temperature range between 20 °C and 40 °C and activity significantly varied ($p<0.01$) (Table 3). The activity was completely lost at higher temperatures 80 °C to 100 °C. The HA activity from *U. pertusa* lectin was not affected exposure to a temperature of 30-70 °C for 30 minutes [15]. Similar to our study, Malini [14] and Kumar et al. [12] also reported that the temperature range of

Table 1. Screening of Saltpan microalgal species for lectin activity

Species	Erythrocytes (Human and Animal)						
	A	B	O	Cow	Goat	Rabbit	Hen
Amphora sp.	+	-	-	+	-	+	-
Nitzschia sp.	-	-	+	-	+	-	-
Navicula sp.	+	+	+	+	-	+	-
Scendesmus sp.	-	-	-	-	-	-	-
Chlorella sp.	-	-	+	-	-	-	-
Nanochloropsis sp.	-	-	-	-	-	-	-

Table 2. Hemagglutin activity of Saltpan microalgae against native human and animal erythrocytes

Algae	Erythrocytes (HA Titer)						
	A	B	O	Cow	Goat	Rabbit	Hen
Amphora sp							
Nitzschia sp	0	0	6	0	8	0	0
Navicula sp	16	32	4	6	0	4	0
Chlorella sp	0	0	8	0	0	0	0

Table 3. Hemagglutination titers of microalgae *Navicula* sp. in relationship to change in pH and temperature

pH (n=5)	HA Titer	Temperature (°C) n=5	HA titer
3	0	20	4
4	0	30	32
5	2	40	32
6	8	50	4
7	32	60	2
8	32	70	2
9	16	80	0
10	0	90	0

extract *C. sinuosa* and *C. sertularioides* lectin in haemagglutination activity was stable between 20 °C and 40 °C. In an earlier report, Sato et al. [16] observed the HA activity from *Oscillatoria agardhii* lectin was affected only by exposure to a higher temperature. Alvarez Hernandez et al. [17] reported HA activity of *C. girajfa* alga was not affected by incubating the sample at 70 °C for 30 minutes and at 80 °C the activity was increased.

3.3 Cations and EDTA Treatment

The agglutinin of *Navicula* sp. was tested with different concentrations of divalent cations including Ca^{2+}, Mg^{2+} and $Mn^{2+}$to assess HA activity (Table. 4). Enhanced HA activity was observed in Ca^{2+} concentration upto10 mm. The titre value of haemagglutination assay was increased to 32 for $Ca^{2+}$with 10 mm concentration ($p<0.01$). Other cations like Mg^{2+} and $Mn^{2+}$was not produced any significant HA activity (Table. 4). Marine lectins are usually identified for their haemagglutination activity by their metal ion dependency. C-type lectins are unique to proteins of carbohydrate recognition and play an important role in innate immunity. It was previously reported that most of the red algae lectins usually do not require divalent cations for haemagglutination activity. Anam et al. [13] reported that the crude lectin extract from *A. nana* requires divalent cations such as Ca^{2+} and Mn^{2+} for its haemagglutinin activity. A few lectins isolated from the red algae such as *Plumaria elegans, P. serrata* [18], *P. filicina* [19], *E. duperreyi* [20], and *V. obtusiloba* [21] and dependent on metals, like Ca^{2+}, Mn^{2+} and Mg^{2+}. Earlier report stated that the red algae does not require divalent cations Ca^{2+}, Mn^{2+} and Mg^{2+} for HA activity, suggesting that this lectin is not a metalloprotein [22]. Thus, metal requirement is not a general characteristic of red algae lectins;

however, a few lectins are metalloproteins. In the present study microalgae *Navicula* sp. are Ca^{2+}, so this *Navicula* sp. are metalloprotein. Hemagglutinin activity of the *Navicula* sp. extract was tested with different concentrations of the calcium chelator (EDTA). The highest activity was observed up to 0-1.0 mm concentration (Table 4) was observed. EDTA at higher concentrations reduced the HA but lower concentrations was not affected HA activity ($p<0.01$). Melo et al*.* [21] studied agglutination activity of the lectin from *Vidalia obtusiloba* was completely affected by treatment with 5 mM EDTA. The ions such as calcium and manganese improved HA activity.

3.4 Hemagglutinin Inhibition Assay

Sugar binding specificity of hemagglutinin lectin from *Naviculasp*. was examined by hemagglutination inhibition tests using various carbohydrates to human B Erythrocytes. In this assay melibiose, fructose, dextrose, arabinose, xylose, did not inhibit the agglutination activity. Of all the sugars tested, galactose, sucrose and glucose showed the highest inhibitory potency with a HA titer of 32. The sugar specificity of lectin varied significantly ($p<0.01$) (Table 4). Hemagglutinating activity of purified lectin of *Vidalia obtusiloba* was inhibited by simple sugars such as N-acetylgalactosamine (3.12 mM) [18,23]. Similar observations of inhibition by mucin on red algal lectins were reported from *Ptilotafilicina* J. Agardh [19], *Enantiocladia duperreyi* (C. Agardh), *Falkenberg* [20], *Ptilota serrata* Kützing [19] and *Pterocladiella capillacea* Santel & Hommers [24].

3.5 Cross Adsorbtion Assay

Cross adsorption assay was carried out to identify the nature of the agglutinin to determine single or multiple factors (Table 6). The present results revealed that the microalgae *Navicula* sp. contain a single agglutinin.

3.6 Purification of Lectin from the Alga

The *Navicula* sp. lectin was purified using a gel filtration chromatography. Hemagglutination titer of microalga was extremely high than other fractions. The hemagglutination activity was tested and presented Hemagglutination unit (HU/mL). The active fraction 12 showed purified protein with 45 kDa (Fig. 1). Silva et al. [25] purified lectin from *Tetradesmus obliquus* using gel filtration chromatography and the molecular weight was 78 kDa. The molecular weight of lectin varied based on sources. For example, lectin isolated from *Spirogyra* exhibited decreased molecular weight than *Tetradesmus obliquus*. The molecular weight of purified lection of *Spirogyra* was 56 kDa [24].

3.7 Anticancer Activity of the Lectin Purified from *Navicula* sp.

The anticancer activities of lectin toward HeLa cell lines were detected in our study and the result was depicted in Fig. 2. In our study, anticancer activity was low at lowest dose of lectins and increased activity was observed at higher lectin supplemented microtiter plates containing HeLa cell lines. At higher concentrations, algal lectin significantly induced apoptosis in HeLa cell lines and was dose dependent ($p<0.01$). “Lectin is a carbohydrate-binding protein that effectively recognizes specific cells such as cancer cells by binding to cell-surface polysaccharides. It has been reported that tumour cells show glycosylation patterns, making them distinguishable from non-cancerous cells. Consequently, lectin has been recognized as a good anticancer agent” [26]. Barre et al. [27] characterized mannose specific lectin from microalgae and reported anticancer and antiviral activities. Likewise, Saad et al. [28] were assessed antitumour and antiviral activities of a novel lectin from *Oscillatoria acuminate* MHM-632 MK014210 and the characterized lectin exhibited dose dependent activity.

Table 4. Effect of cations and EDTA on the hemagglutinin activity of the microalgae *Naviculasp*

Concentration in mM (n=5)	HA Titer			
	Ca^{2+}	Mg^{2+}	Mn^{2+}	EDTA
0	4	0	0	32
0.1	16	0	0	32
1	32	0	0	32
10	32	0	0	8
100	4	0	0	8

Table 5. Hemagglutinatin inhibition activity of the saltpan microalgae *Navicula* sp. in various sugars

Sl.No.	Carbohydrate	HAI Titer
1	Melibiose	-
2	Mannose	6
3	Glucose	32
4	Maltose	8
5	Fructose	-
6	Glucosamine	8
7	Rhamnose	16
8	Dextrose	-
9	Arabinose	-
10	Xylose	-
11	Sucrose	32
12	Galactose	32

Table 6 Hemagglutination titer of microalgae *Navicula* sp. after adsorption with different erythrocytes

Erythrocytes (n=5)	HATitre					
	A	B	O	Cow	Goat	Rabbit
None	16	32	4	6	0	4
A	0	0	0	0	0	0
B	0	0	0	0	0	0
O	0	0	0	0	0	0
Cow	0	0	0	0	0	0
Goat	0	0	0	0	0	0
Rabbit	0	0	0	0	0	0

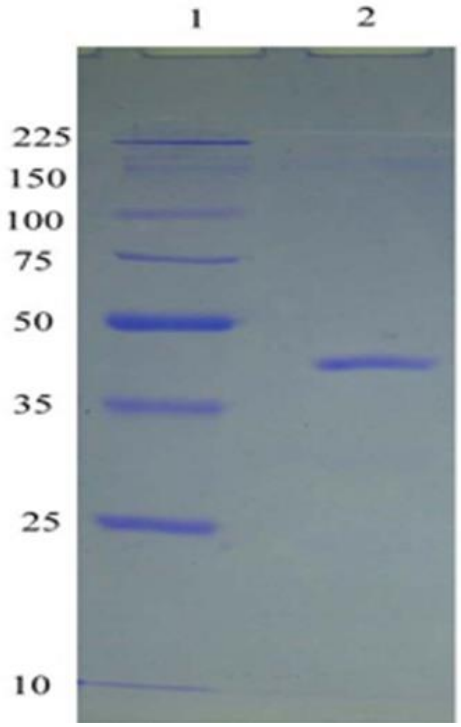

Fig. 1. SDS-PAGE analysis of protein from the algal extract of *Navicula* sp. The algal protein was purified and the molecular weight was determined using 12% SDS-PAGE

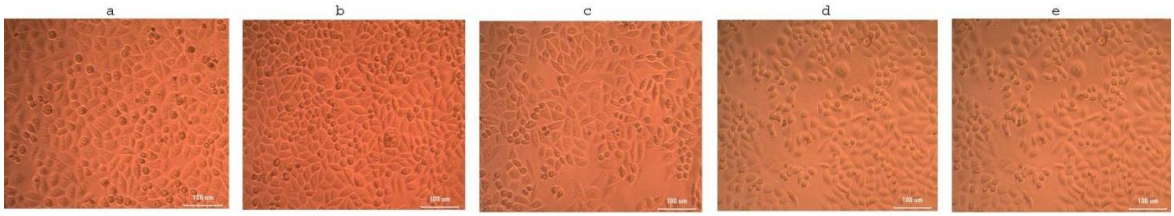

Fig. 2. Anticancer activity of algal lectin against HeLa cell lines. Lectin was added at various concentrations and anticancer activity was determined

4. CONCLUSION

The anticancer property of lectin was isolated and characterized from the saltpan Microalga, *Navicula* sp. The anticancer property of microalgal lectins has been reported for few decades. Lectins isolated from *Navicula* sp. In this study, the isolated lectin showed potential anticancer activity against cervical cancer cell lines. Hence, the outcome of this research work conclusively established that microalgal cultures with high lectin producing characteristics are present in saltpan environment. They need to be characterized and exploited commercially for various biotechnological applications. The isolated protein can be applied for further single-molecule interaction studies due to its bioactivity mechanism, which would be worth conducting in the future.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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