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A Lectin Isolated From Marine Sponge *Hyatella Cribriformis* Exhibited Potential Antibacterial and Antiproliferative Activities

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ABSTRACT
Lectins are non-immune glycoproteins isolated from marine sponges shown to be many physiological, biological and pharma cological applications. *Hyatella cribriformis,* a marine sponge, yielded a lectin (HCL) that was partially purified using Sephadex G75 gel filtration chromatography. When compared to the markers, the SDS-PAGE analysis of the fraction obtained from *H. cribriformis* produced two bands with molecular weights of roughly 35 kDa and 47 kDa, roughly. Using the well diffusion method, the antibacterial effects of HCL was investigated in several microorganisms. The positive control is gentamycin. Results demonstrated the antibacterial activity of HCL against *S. aureus, P. aeruginosa, K. pneumoniae, and E. faecalis*. The MTT test was used to evaluate the cytotoxicity of HCL against HeLa cell lines at varying doses (50–100 µg/mL). After 24 hours, HCL showed potential cytotoxicity against these cell lines in a dose-dependent manner, with an IC_{50} value of 26.23 µg/mL. In clinical applications, HCL may therefore be a powerful therapeutic agent.

KEYWORDS: Lectins, Gel filtration chromatography, SDS-PAGE, disc diffusion assay, MTT assay

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INTRODUCTION

Lectins, also known as glycoproteins, have the ability to bind to and agglutinate carbohydrates on the surface of erythrocytes (Lam et al., 2011). They possess at least one non catalytic domain that has the ability to reversibly bind specific monosaccharides or oligosaccharides (Peumans, 1995). With many carbohydrate recognition domains, these molecules can agglutinate a variety of foreign particles, such as lymphocytes, yeast, protozoa, and both normal and malignant cells with sugars on their surface. Lectins are essential for controlling host-pathogen interactions, mitogenic stimulations, protein trafficking, tumor metastasis, and fertilization (Sharon, 2004, Paiva, 2010). They have a range of properties, including antiviral, antibacterial, antifungal, anticancer, along with potential therapeutic benefits (Cheung, 2015).

Sponge species are extensively distributed in freshwater and marine environments, making them the oldest metazoan phylum in terms of evolution. They have developed chemical defense mechanisms against other invasive organisms by producing these secondary metabolites (Donia., 2003, Haefner., 2003).

Marine sponges have developed a number of physiologically active substances, such as lectins, which have been the subject of much research in recent decades. Different lectin families, such as galectin, C-type lectins, and tachylectins, have been identified, and other sponge lectins with various biochemical characteristics have been isolated and their structures established (Gardères et al., 2015).

Numerous biological activities, such as cytotoxicity, ichthyotoxicity, anti-inflammatory, antibacterial, anti-HIV, antithrombocyte, and vasodilatory properties, are displayed by marine sponges belonging to the Spongiidae family (Murali et al., 2008). The sponge body of H. cribriformis, a member of this family, is elastic and compressible, and it often has cored primary and uncored secondary fibers (Varsha et al., 2020). Murali et al., (2008) isolated scalarane sesterterpene 24--methoxyscalarolide as well as the known scalaranes 12-epi-deoxoscalarin 1, 12-epi-12-deacetyl-25-deoxyscalarin 2, and scalarolide 3 from this sponge. According to Annamalai et al. (2015), the marine sponge ethyl acetate (EA) fraction of *H. cribriformis* has strong anticancer properties by encouraging tubulin polymerization, which is demonstrated by mitotic arrest and apoptosis induction.

Identifying, isolating, and characterizing a lectin from the marine sponge *H. cribriformis* was the aim of the current investigation.

MATERIALS AND METHODS

Chemicals

Sigma was the supplier of Sephadex-G75 (St. Louis, MO, USA). Himedia (Mumbai) provided the sugars used in this investigation. EDTA, sodium hydroxide, sodium chloride, sodium acetate, and ammonium sulfate were all readily available and of analysis quality.

Collection of *H. cribriformis*

During the busy fishing season, sponges were gathered off the coast of Arogyapuram, which is close to Kanyakumari in Tamilnadu, India. After becoming tangled on the fishing nets (by capture method), the sponges were brought to the lab and promptly frozen until they could be used.

Partial purification of *H. cribriformis* lectin

0.5 mM PBS buffer pH7.4 (1:10) ratio was used in an electric blender to homogenize small pieces of H. cribriformis. A muslin cloth was then used to filter the extract in order to get rid of any sediments or debris. The clear supernatant was then utilized for salting out after centrifuging it at 2000 rpm. Ammonium sulfate was used in the "salting out" process to isolate HCL. Ammonium sulfate (40–80%) was added to the homogenate (10 ml) in small quantities while being continuously stirred in an ice bath. The resulting sample was centrifuged in a cooling centrifuge (Eppendorf, Germany) at 8000 × g for 30 minutes at 4 °C, and the supernatant was extracted. After being collected as a pellet at the tube's bottom, the protein was dissolved in the least amount of buffer. The dissolved pellets were then dialyzed using PBS buffer for three days at 4 ° C with multiple buffer changes in a membrane (Sigma, 25×16mm). The dialyzed material was then submitted to gel filtration chromatography using a Sephadex G-75 column (1.6 x 60 cm). After eluting HCL with PBS, 2 ml of the fractions were recovered. A spectrophotometer (Jasco, Germany) was used to measure the amount of protein in each fraction at 280 nm after the flow rate was adjusted to 1 ml/min. After pooling and dialyzing the high specific fractions, they were preserved for additional biochemical studies.

SDS-PAGE and Coomassie staining

10% SDS-PAGE (sodium-dodecyl sulfate polyacrylamide gel electrophoresis) was performed using Laemmli's technique (1970). Low molecular weight markers were used to calculate the proteins molecular mass (Biorad, USA). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the concentrated protein fractions. Coomassie staining was used to visualize the protein bands in the gel following electrophoresis.

Haemagglutination assay

Serial two-fold dilution was used to measure the haemagglutination activity of HCL, as explained by Sampaio et al., (1998). Human ABO was

isolated from donors who were in good health. Erythrocytes from rats and rabbits were obtained from the University of Kerala's Department of Biochemistry's animal house. In round-bottomed micro titre plates (Tarsons, Mumbai), 10% R.B.C. suspensions were made and utilized to measure the haemagglutination activity. For one hour, the plate was incubated at room temperature. The development of a visible button at the well's bottom suggested that the result would be negative.

Estimation of protein

The protein content of the crude extract and the fraction derived from the gel fraction using bovine serum albumin as a standard was determined using Lowry's technique (1951).

Antibacterial activity

Using the well diffusion method, an *in vitro* antibacterial assay of HCL was conducted against bacteria such *S. aureus, P. aeruginosa, K. pneumoniae,* and *E. faecalis*. The negative control was DMSO, and the positive control was gentamycin. The plates were kept at 36°C in an aerobic environment for 24 hours. Following incubation, the plates were inspected, and the length of the zone that inhibited bacterial growth was recorded (Valgas et al., 2007).

Cell culture

NCCS in Pune, India, provided the cervical (HeLa) cancer cell lines. The cells were kept in DMEM with 100 U/l of penicillin and 10% fetal bovine serum (FBS) added as supplements. In a humified environment consisting of 95% air and 5% CO_2, cells were cultured at 37° C. Periodically, the cells were subcultured.

MTT assay

HeLa cells were used in the MTT experiment to assess the cytotoxicity of HCL on cell line viability (Mosmann, 1983). The following formula was used to determine the viability of the cells.

% viability = (OD of Test/OD of Control) X 100

Statistical analysis

Every experiment was carried out in triplicate (n = 3). The mean ± standard deviation was used to express the data.

RESULTS AND DISCUSSION

Ammonium sulphate was used to salt out the crude extract of *H. Cribriformis* (HCL) in order to isolate the lectin. HCL was measured at saturation levels between 40 and 80%. Following the haemagglutination test, these fractions were dialyzed and purified using gel filtration in a Sephadex G-75 column. The absorbance was measured at 280 nm after the eluents were gathered in 2 ml fractions (n-50) in Eppendorf tubes. The 24th and 27th fractions of HCL showed peaks (Fig-1). Table 1 displayed the HCL yield both prior to and during purification. The haemagglutinating activity of the fractions with high absorbance was examined. Following the haemagglutination test, the eluted fraction was put through electrophoresis. Low molecular weight proteins are represented by the bands that were obtained. Therefore, lectin from *H. Cribriformis* is represented by the band with a molecular weight between 35 and 47 kDa (Fig-2). HCL agglutinated all type of erythrocytes tested such as human ABO and rabbit erythrocytes (Table 2). Similarly, structural characteristics and biological activity of lectins obtained from marine sponges that have been reported by Gomes Filho et al., (2014) and Gardères et al., (2015).

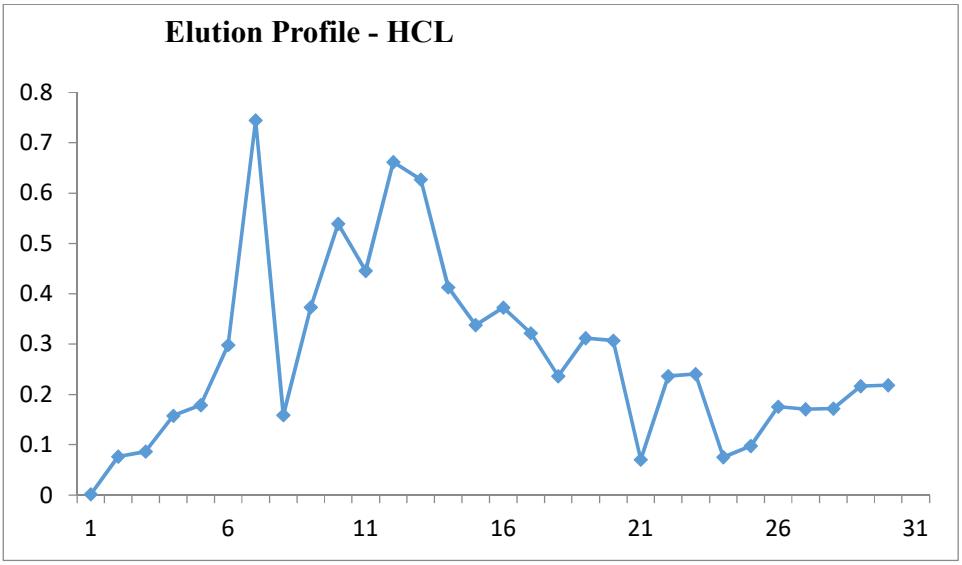

Figure 1: Purification of HCL

Table 1: Purification of HCL

Sample	Total protein(mg/ml)	Yield of protein (%)
Crude	0.623	100
HCL	0.320	51.36

Table 2:

HCL	Haemagglutination titre
A+	128
B+	2
O+	2
Rat	16
Rabbit	4

It was discovered that HCL had the strongest antibacterial action against *K. pneumoniae* when compared to control gentamycin when tested against four distinct bacterial strains, including *E. faecalis, P. aeruginosa, K. pneumoniae* and *S. aureus*. Agglutination and possible antibacterial action against both Gram-positive and Gram-negative bacteria have been demonstrated by sponge lectins. Indeed, several lectins can interact with bacterial lipopolysaccharides (LPS) or the extracellular matrix of microorganisms, as well as recognize and bind to glycans on cell surfaces and cell wall polysaccharides (Miki etal., 2012, Purish etal., 2013). Additionally, the capacity of sponge lectins to bind certain carbohydrates in bacterial cells may be utilized to create novel antimicrobial drugs, according to Gardères (2015). Characterization of the structure of a galectin from the marine sponge *Aplysina lactuca* (ALL) that works in concert with antibiotics to combat bacteria were studied by Duart etal., (2023).

The MTT assay was used to examine the cytotoxicity of HCL against HeLa cell lines at different doses (10–100 µg/mL). These cell lines were susceptible to dose-dependent cytotoxicity from HCL after 24 hours (IC50 = 26.23 µg/mL) (Fig-3). These lectins cause apoptosis, cytotoxicity, and tumor growth suppression when they interact with the malignant cell surface's altered glycosylation pattern (Pajic et al., 2002). HeLa and FemX cells are also toxically affected by a sponge lectin from *H. crater* (Kabir et al., 2013). This characteristic is also seen in lectins from *C. varians* (CvL) and *C. apion* (CaL) against HeLa and K562 leukemic cells [28, 29].

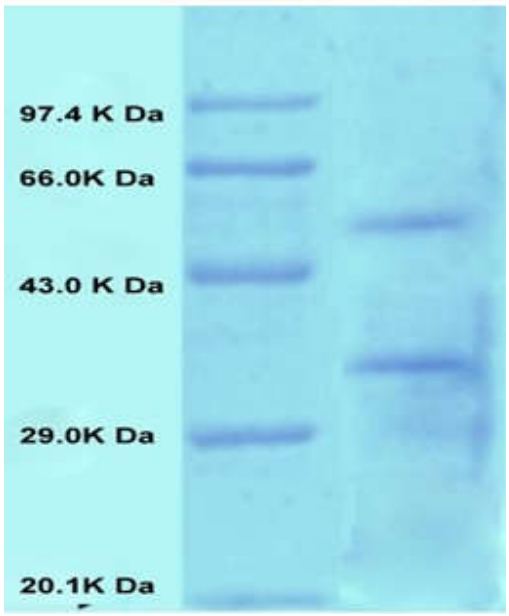

Figure 2:

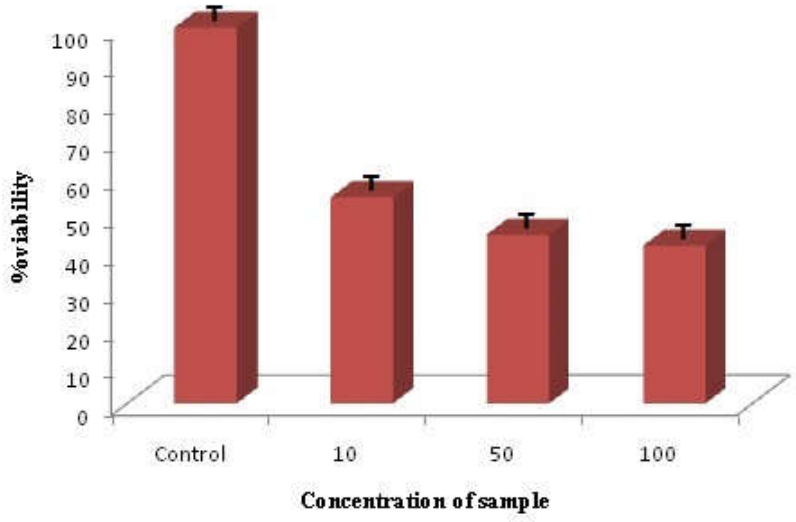

Figure 3:

Table 3:

Name of bacteria	Percentage inhibition of bacterial growth (HCL-100 µL) (mm) (mean ± SD)	Gentamycin's percentage of bacterial growth inhibition (20mg / mL)(mm) (mean ± SD)
E. faecalis	1.2 ± 0	3.7 ± 0
P. aeruginosa	1.2 ± 0	3.7 ± 0
K. pneumoniae	1.2 ± 0	2.9 ± 0
S. aureus	1.6 ± 0.2	4 ± 0.2

Purification and the mechanism of action of HCL against HeLa cell lines require further investigation. In the future, *H.cribriformis* lectins will be useful in the production of anti-cancer medications and as antibiotics. Because lectins are so specific, they hold great promise for treating infections—as long as the dosage is kept well below the hazardous limits. Because of their numerous physiological, biological, and pharmacological applications, lectins derived from marine creatures have emerged as a rapidly expanding subject in the life sciences.

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