

## ANTIBACTERIAL ACTIVITY OF GVL, A LECTIN ISOLATED FROM MARINE ALGAE *GRACILARIA VERRUCOSA*

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### ABSTRACT

Lectins are univalent or polyvalent carbohydrate - binding proteins of non-immune origin that are widely distributed in the marine ecosystem. A lectin was isolated from marine algae *Gracilaria verrucosa* by G-100 gel filtration chromatography. This lectin was named GVL, agglutinated rat, rabbit, goat, chicken and human ABO erythrocytes. The antibacterial activity of this algal lectin was studied using disc diffusion method. GVL exhibited potent antibacterial activity against bacteria such as *E.coli*, *S.pyogenes*, *S. aureus*, *E.faecalis*, *B.subtilis* and coagulase-negative *Staphylococci* (CoNS) by disc-diffusion method. Also, the results were compared with standard antibiotic Ampicillin. These findings indicate that GVL is having therapeutic applications with great importance in clinical microbiology.

**Keywords** – Marine algae, *Gracilaria verrucosa*, gel filtration chromatography, antibacterial activity, disc diffusion method.

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### 1. INTRODUCTION

“Lectins” comes from the Latin word “legere”, which means “to select” according to Boyd [1]. They are glycoproteins that possess the property of binding carbohydrates without



initiating their further modifications through associated enzymatic activity [2]. This ability to recognize and bind reversibly to specific carbohydrate ligand makes them valuable tools in biomedical and glycoconjugate research [3]. Lectins are involved in many biological activities such as host-pathogen interactions, cell-cell communication, induction of apoptosis, cancer metastasis and differentiation, targeting of cells, as well as recognizing and binding carbohydrates [4]. The main applications of lectins include antitumor, immunomodulatory, antifungal, antiviral and anti insecticidal activities [5].

*Gracilaria verrucosa* is a genus of red algae (Rhodophyta) used as a food and widely used for the production of agar and bioethanol [6]. Chemical studies on anti-inflammatory components of *G. verrucosa* led to the isolation of new 11-deoxyprostaglandin, ceramide, keto fatty acids and oxygenated fatty acids [7]. Dayuti *et al.*, [8] studied the phytochemical composition and antibacterial activity of *G. verrucosa* extract against *E. coli* and *S. typhimurium*. *G. verrucosa* extract had affected adipogenesis, reactive oxygen species (ROS) production, and glucose uptake in 3T3-L1 cells [9].

Marine bioresources such as algae produce a great variety of specific and potent bioactive molecules which includes lectins [10]. Among them are the red marine algae *Gracilaria* spp. (*Gracilariaceae*), are good sources of unique lectins [11]. Many red algae species have been reported to have lectins having carbohydrate specificity towards complex glycoproteins or high-mannose N-glycans. These lectin-glycans interactions further trigger many biochemical responses which lead to their extensive use as valuable tools in biomedical research [12].

In the present study a lectin (GVL), was isolated from marine algae *Gracilaria verrucosa* and studied its antibacterial activity.

## 2. MATERIALS AND METHODS

Sephadex-G100 was purchased from Sigma (St. Louis, MO, USA). Mueller-Hinton agar was from Himedia (Mumbai, India). All other chemicals used in this study were of analytical grade commercially available.

### 2.1. Collection of marine algae

*Gracilaria verrucosa* were collected from Vizhinjam beach in Trivandrum, Kerala, India. The

algae were washed thoroughly with distilled water to remove sand and dirt completely and frozen until use.

## 2.2. Gel filtration chromatography

*G. verrucosa* was homogenized in an electric blender in 5mM PBS pH 7.4 (1:10) ratio. GVL precipitated from this supernatant within 0 - 80% Ammonium sulfate was then added with constant stirring on an ice bath. The resultant sample was centrifuged ( $8000 \times g$ , 30 min, 4 °C) in a cooling centrifuge (Eppendorf, 5804R, Germany) and the supernatant was removed. The protein obtained as a pellet in the bottom of the tube was collected and dissolved in minimum buffer. The dissolved pellet was then dialysed in a membrane (Sigma, 25×16mm) with PBS buffer for 3 days with several buffer changes at 4<sup>0</sup>C. The dialyzed sample was then applied to Sephadex G-100 column (1.6×60 cm) gel filtration chromatography. GVL was then eluted with PBS and 2ml fractions were collected. The flow rate was adjusted to 1ml/min and the presence of protein in each fraction was monitored at 280nm with a spectrophotometer (Jasco V630, Germany). The high specific fractions were pooled, dialyzed and checked haemagglutination activity. The sample was then stored for further biochemical studies.

## 2.3. Haemagglutination assay

Haemagglutination studies of GVL were carried out using human ABO, chicken, goat and rabbit erythrocytes in a 96-well microtitre plate (Tarsons products, Mumbai). Purified GVL (100µL) was serially diluted into the successive wells with PBS. Then 10% erythrocyte suspension was added to all the wells and the plate was incubated for 30 min at 37°C. Agglutination was recorded visually, formation of a mat at the bottom of the plate while button formation indicates a negative reaction [13]. The haemagglutinating activity was expressed as a titre, the reciprocal of the highest dilution that showed positive results [14].

## 24. Microorganisms

The bacterial strains were provided by the Department of Biotechnology, University of Kerala, Kerala, India. The bacterial strains used in the study include- Gram-positive (*B. subtilis*, *S. aureus* and *S. pyogenes*) and Gram- negative (*E.coli*, coagulase-negative *Staphylococci* (CoNS), *E.faecalis*, *B.subtilis* and *C.tetani*).

## 2.5. Antibacterial activity

Antibacterial activity of GVL was tested as described by [15]. After preparation of the media, 10 ml of sterile nutrient broth was aseptically inoculated with the test culture organisms and

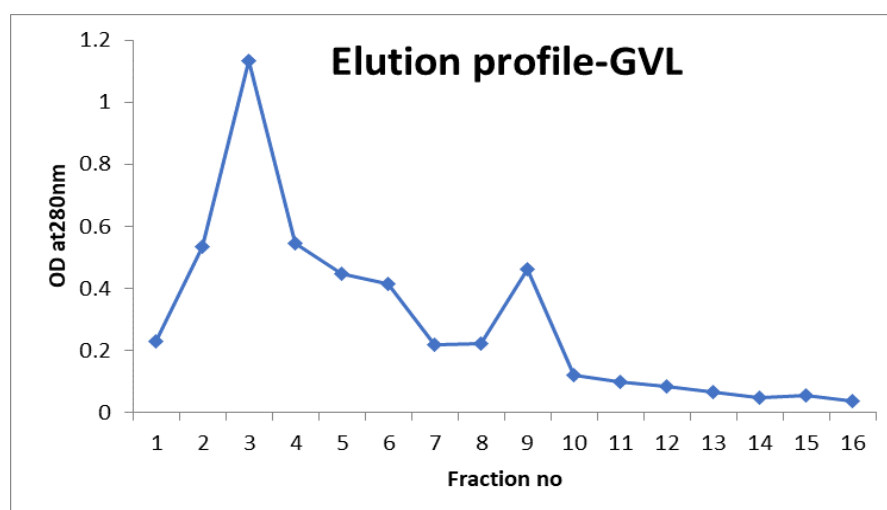
incubated at  $35 \pm 2^\circ\text{C}$  for 18 hours. After incubation, the test cultures were applied on air-dried nutrient agar plates using a sterile glass spreader. Using clean forceps, the sterile discs loaded with the lectin was placed on the surface of Mueller Hinton Agar plates seeded with the test bacterial strains. Ampicillin discs as the positive and PBS as the negative control were used. The plates were then incubated at  $35 \pm 2^\circ\text{C}$  for 24 hours. The zone of bacterial growth inhibition was observed and its diameter was measured in millimeters (mm).

## 2.6. Statistical analysis

All the experiments were performed in triplicate ( $n = 3$ ). The data were expressed as mean  $\pm$  standard deviation.

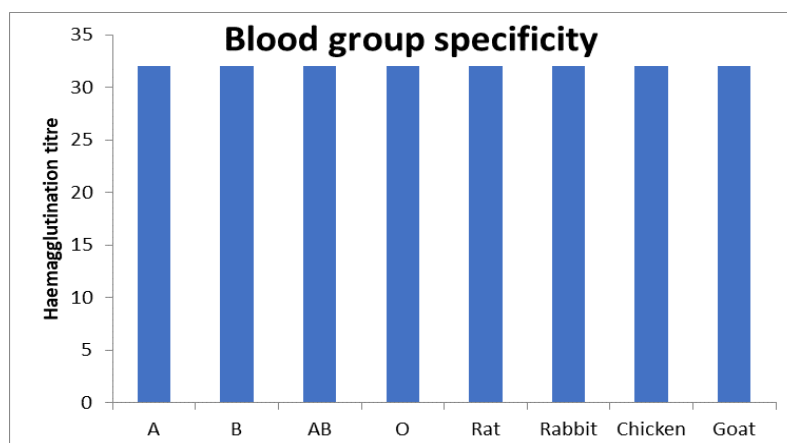
## 3. RESULTS AND DISCUSSION

GVL was isolated from a marine sponge by (0-80%) ammonium sulphate precipitation followed by gel filtration chromatography. The elution profile of GVL after gel filtration chromatography is shown in figure 1.



**Fig.1.** Elution profile of GVL

The lectin was previously isolated and studied from *G. Verrucosa* by Kanoh *et al.*, [16]. It is a dimer (MW 27,000 and MW 23,000); its molecular weight (MW) and isoelectric point are 49,000 and 3.8, respectively. GVL agglutinated all types of erythrocytes tested such as human ABO, rat, chicken, goat and rabbit erythrocytes (Fig-2).



**Fig.2.** Blood group specificity of GVL

The lectin-induced agglutination of cells has originally served as the most common assay to detect and quantify lectin activity in a variety of organisms [17,18]. Ravirajan *et al.*, [19] screened twenty- one seaweeds collected from the Gulf of Mannar Biosphere Reserve for haemagglutinin activity in nine different types of blood and found that they were not active on all blood samples there was the specificity differed greatly in the native and papain treated erythrocytes. Thus, the majority of the seaweeds screened are a potential source of haemagglutinin as their extracts agglutinate one of the blood groups tested.

GVL showed high antibacterial activity against *E.coli* and *B. subtilis* ( $25 \pm 5$ mm) and moderate activity against coagulase-negative *Staphylococci* (CoNS), *E.faecalis* *S. aureus* and *S. pyogenes* (Table-1).

**Table 1.** Antibacterial activity of GVL

Name of bacteria	Percentage inhibition of bacterial growth(mm) (mean $\pm$ SD)	Percentage inhibition of bacterial growth Ampicillin (250 $\mu$ g)(mm)
<i>B. subtilis</i>	$25 \pm 5$	$22.67 \pm 1.15$
<i>S. aureus</i>	$10.67 \pm 1.15$	$24 \pm 1.73$
<i>S. pyogenes</i>	$11.33 \pm 1.15$	$14 \pm 1.73$
<i>E.coli</i>	$25 \pm 5$	$27 \pm 1.73$
coagulase-negative <i>Staphylococci</i> (CoNS)	$11.33 \pm 1.15$	$11.33 \pm 1.15$
<i>E.faecalis</i>	$10.67 \pm 1.15$	$11.33 \pm 1.15$
<i>C.tetani</i>	$0 \pm 0$	$0 \pm 0$

GVL does not exhibit any activity against *C.tetani*. Our results are in agreement with certain previous reports, which mention the greater activity of lectin towards Gram-negative and Gram-positive microorganisms [20,21]. Many human pathogens utilize cell surface glycans as either receptors or ligands to initiate adhesion and infection [22-28]. Lectins of different carbohydrate specificities can promote growth inhibition or the death of bacteria. Thus, lectins can be utilized for the detection, typing and control of bacteria and fungi that cause damage to plants and humans [29]. Antibacterial activity on Gram-positive and Gram-negative bacteria occurs through the interaction of lectin with components of the bacterial cell wall including teichoic and teichuronic acids, peptidoglycan and lipopolysaccharides [30,31]. Lectins, as a key member of pattern recognition receptors, could function as phagocytosis receptors, soluble opsonins, and agglutinins to mediate the recognition of microbes [32].

#### 4. CONCLUSIONS

The growing applications of lectins underscore the potential economic importance of the discovery of novel species for biotechnology. A lectin from marine sponge *G. Verrucosa* (GVL) was partially purified by gel filtration chromatography. GVL exhibited a strong antibacterial response against the bacteria tested.

#### 5. ACKNOWLEDGMENTS

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