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# Antibacterial Effects of Crude Lectin Fraction Bioactive Compound of Red Macroalgae *P. palmata* and *H. glandiforme* from Southern Coast Java Island, Gunungkidul, Yogyakarta, Indonesia

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**ABSTRACT**

Lectins or carbohydrate-binding proteins, are widely distributed in nature and also good candidates in such prospecting of algae. It may have been considered that binding specificity of lectins to some carbohydrates provokes to produce many unique biological activities, including cell agglutination, mitogenic activity, antibacterial activity and antitumor activity. The aim of this study was to determine the antibacterial effect of crude lectins fractions from red macro algae collected from the southern coast of Java island, Gunung Kidul Regency, Yogyakarta Indonesia. The.Crude lectin fraction showed no antibacterial activity against gram-negative bacteria because most of bacteria cells surface contain of mannan glycoprotein and the sample has no specificity to bind this glycoprotein type. Crude lectin fraction tested also have no antibacterial effect aginst gram positife bacteria because only binding specifically to N-glycan specifically as well as O-glycan.

***Keywords:*** *Lectin, algae, antibacteria, Protein, Agglutination.*

## 1. INTRODUCTION

Lectins are a group of proteins that bind to carbohydrates that are not immune and is capable of causing cell agglutination and/or polysaccharide precipitation or glycoconjugate [1]. Hori et al., explains that lectins are one of the many bioactive compounds found in macroalgae [2]. According to [3] Algal lectins have several advantages compared to high-level plant lectins. One of which is lower molecular weight. Haemagglutination activity of algal lectins cannot be inhibited by monosaccharides or oligosaccharides but can be inhibited by glycoproteins. Lectins are able to bind specifically to carbohydrates which result in a variety of unique biological activities such as agglutination, precipitation of polysaccharides, mitogenic activity, antitumor activity and toxicity [2]. This research examined the cytotoxicity and antibacterial effectiveness of the bioactive compounds of Palmaria palmata and Halosaccion glandiforme lectins from the southern coast of Java, Gunung Kidul district, Special Region of Yogyakarta, Indonesia.

## 2. MATERIAL AND METHODS

### *2.1 Materials*

Palmaria palmata and Halosaccion glandiforme red macroalgae were obtained from the coast of Gunungkidul (Krakal Beach, Pok Tunggal Beach and Wedi Ombo Beach), Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, Escherichia coli, Salmonella typhimurium, gram-negative bacteria Vibrio parahaeolyticum and Enterobacter sakazaki.

### *2.2 Extraction process*

This process was carried out using the modified [4] method which included extraction using phosphate buffer saline (PBS) pH 7 for one night at 4oC, precipitation of ammonium sulfate up to 75% saturation, and dialysis.

### *2.3 Testing of hemagglutination activity*

This process was carried out using two types of erythrocytes, native red blood cell (RBC) and trypsin-treated red blood cell (TRBC) with the two fold dilution method at 96-well microtiter v-plate. Observations were made after the incubation process for 1 hour at room temperature by including negative controls. This hemagglutination activity is expressed as a titre, which means the opposite of the highest doubling of dilution which shows positive hemagglutination.

### *2.4 Protein Level Testing*

Protein levels were determined using the BCA Protein Assay Kit (Thermo Scientific TM PierceTM) method using bovine serum albumin (BSA) as standard and carried out with two replications on a 96-well microplate flat bottom. The sample was then incubated for 30 minutes at 37ºC and carried out the UV-vis spectrophotometer at a wavelength of 562 nm.

### *2.5 Inhibition Testing by Sugar and Glycoproteins*

Testing for inhibition of sugar and glycoprotein was carried out using 96-well microtiter v-plate by adding samples to the sugar or glycoprotein that had been added to each well. Observations were made after the incubation process for 1 hour at room temperature.

### *2.6 Antibacterial testing*

Antibacterial testing used microplate 96-well which has been added to Media NB 3.3x, bacterial suspension 5×105 cfu/ml, and Resazurin working solution in each well. Observations were made after the incubation process for 18-24 hours at 37 oC.

## 3. RESULT AND DISCUSSION

### *3.1 Protein Level Testing*

The results of a research conducted by [5] showed that the average level of red algae protein was above 1 mg/ml, which was in accordance with the results of the two samples which were more than 1 mg/ml, so a 10x dilution was needed to obtain the absorbance results because salting-out fraction of the two red algae sample was thought to have a very high protein content. [6] explains that different protein concentrations indicated differences in the natural conditions of the compound, the extraction method used, sample particle size, storage conditions and time, extraction time, and ratio of the amount of solvent to the number of samples [6]. In addition, the red algae species that are used also greatly influence the levels of protein obtained (Table 1).

**Table 1.** Protein level of salting-out fraction

| No. | Names of Species | Protein Level (µg/ml)* |
|---|---|---|
| 1. | *Palmaria palmate* | 8.123,470 |
| 2. | *Halosaccion glandiforme* | 6.225,438 |

*10x Dilution Results

Testing of hemagglutination activity was carried out using two types of erythrocytes, RBC and TRBC. Both samples showed higher hemagglutination activity when tested using TRBC compared with RBC, which was 26 for Palmaria palmata samples and 213 for Halosaccion glandiforme samples. According to [7], sometimes lectins do not show interactions with carbohydrates due to the presence of steic obstructions so that the enzymatic initial treatment of erythrocytes is needed for hemagglutination testing. In addition [8] states the treatment of erythrocytes with protease enzymes is often used to remove glycoprotein fragments from RBC membranes, this is what then allows an increase in sensitivity to testing of hemagglutination activity resulting in a higher value (Table 2).

**Table 2.** Hemagglutination Activity in Rabbit Erythrocytes

| No. | Names of Species | RBC | | TRBC | |
|---|---|---|---|---|---|
| 1. | *Palmaria palmate* | $2^3$ | $2^3$ | $2^6$ | $2^6$ |
| 2. | *Halosaccion glandiforme* | $2^9$ | $2^9$ | $2^{12}$ | $2^{14}$ |

*RBC: red blood cell

*TRBC: trypsin-treated red blood cell

Lis and Sharon [9] explains that lectins are proteins that have two or more sides of carbohydrate binding so they are called di- or polyvalent which causes lectins to be able to bind mono- or polysaccharides and glycoproteins specifically. The ability of lectins to cause agglutination of erythrocytes can be inhibited if there is a bond between lectins and sugar which binds to it specifically so that the lectin is no longer able to form

cross bonds on the sugars found in the erythrocyte cell wall. Table 3 below presents data on the inhibition of hemagglutination activity of salting-out fraction Palmaria palmata and Halosaccion glandiforme by sugar and glycoprotein.

Lectins from macroalgae in general cannot be inhibited by monosaccharide activity [10] [11]. However, the above results showed that monosaccharides and disaccharides were able to inhibit the salting-out fraction activity of both samples with concentrations of more than 100 µg/ml. In addition, the Halosaccion glandiforme sample showed a low

**Table 3.** Inhibition of Hemagglutination by Monosaccharides, Disaccharides, and Glycoproteins

| No | Glucose atau Glycoprotein | Minimum Inhibitory Concentration (µg/ml) | |
|---|---|---|---|
| | | *Palmaria palmate* | *Halosaccion glandiforme* |
| 1 | Monosaccharides and Disaccharides (mM) | >100 | >100 |
| 2 | Glycoprotein (µg/mL) | | |
| | *N-Glycan* | | |
| | *Complex type* | | |
| | *Transferin human* (Tf) | >2,000 | >2,000 |
| | Asialo *transferin human* (aTf) | >2,000 | >2,000 |
| | *High-mannose type* | | |
| | *Yeast mannan* | >2,000 | >2,000 |
| | *Hybrid type* | | |
| | *Thyroglobulin from bovine thyroid gland* (BTG) | >2,000 | 1,000 |
| | Asialo *thyroglobulin from bovine thyroid gland* (aBTG) | 2,000 | >2,000 |
| | *Thyroglobulin from porcine thyroid gland* (PTG) | >2,000 | 125 |
| | Asialo *thyroglobulin from porcine thyroid gland* (aPTG) | 2,000 | 250 |
| | *O-Glycan* | | |
| | *Mucin from bovine submaxillary gland* (BSM) | >2,000 | >2,000 |
| | Asialo *mucin from bovine submaxillary gland* (aBSM) | 250 | 125 |
| | *N/O-Glycan* | | |
| | *Fetuin from fetal bovine serum* (Fe) | >2,000 | >2,000 |
| | Asialo *fetuin from fetal bovine serum* (aFe) | >2,000 | >2,000 |

*Titre Before Treatment:
*Palmaria palmata* : $2^2$
*Halosaccion glandiforme* : $2^3$

**Table 4**. Antibacterial Test Results Salting-out fraction of red Algae

| No. | Bakteri | *Minimum Inhibitory Concentration* (µg/ml) | | |
|---|---|---|---|---|
| | | *P. palmata* | *H. glandiforme* | Kontrol* |
| **Bakteri Gram Positif** | | | | |
| **1.** | *S.aureus* | >10,000 | >10,000 | 3.125 |
| **2.** | *B.subtilis* | >10,000 | >10,000 | 3.125 |
| **Bakteri Gram Negatif** | | | | |
| **3.** | *V.parahaeolyticum* | >10,000 | >10,000 | 6.25 |
| **4.** | *E.sakazaki* | >10,000 | >10,000 | 6.25 |
| **5.** | *E.coli* | >10,000 | >10,000 | 1.953 |
| **6.** | *S.typhimurium* | >10,000 | >10,000 | 3.125 |

*Positive control used chloramphenicol

concentration of inhibition by several hybrid type N-glucan glycoproteins namely PTG of 125 μg/ml and asialo PTG of 250 μg/ml, and O-glucan i.e. asialo BSM of 125 μg/ml, whereas Palmaria palmata samples were inhibited by glycoprotein O-glycans namely asialo BSM of 250 μg/ml (Table 3). Based on these data, higher inhibitory activity is shown in the form of asialoglycoprotein. According to [11], removal of sialic acid from glycoproteins causes a better ability to inhibit lectin activity. This is because ligands from glycoproteins can be formed by removing sialic acid from glycoproteins [2].

According to [13], the interaction of lectins and bacteria can occur based on the presence of extracellular glucans which makes the carbohydrate binding side (lectins) play an important role in the interaction of lectins with bacteria. The interaction of lectin with carbohydrates on the cell surface of bacteria results in bacteriostatic occurrence, which may be due to changes in the flow of nutrients in these microbes.

The cell wall of gram-positive bacteria consists only of teichoic acid and peptidoglycan layers [9]. Peptidoglycan consists of disaccharides and peptides (DSP) structures that repeatedly bind to form a glycosidic bond (β-1.4 bond between MurNAc and GlcNac) and peptide bonds, usually between the terminal D-alanine carboxyl group in one DSP unit and an amino group on other DSP units [15]. The two samples of salting-out fraction that were tested were known to only have hybrid type O-glycans and O-glycans specificity on glycoproteins and did not show specificity to GlcNAc so they were unable to bind to cell walls of gram-positive bacteria. The test showed that the red macroalgae salting-out fraction tested was not able to inhibit the growth of gram-positive bacteria.

Gram-negative bacteria have cell walls consisting of outer membranes, porins, lipopolysaccharides, lipoproteins, peptidoglycan layers and periplasmic parts [14]. According to [16] cell walls in gram-negative bacteria are generally composed of mannan polymers that have mannose monomers. From the tests carried out, it was ascertained that all samples did not have an antibacterial effect when tested at a concentration of 10,000 μg/ml. This is because in all salting-out fractions tested, it does not have the specificity to be inhibited by yeast mannan. The absence of specificity on glycoprotein N-glycans of this type of high mannose causes lectins not to have a bacteriostatic effect on gram-negative bacteria because they are unable to bind sugar to the cell surface of gram-negative bacteria.

## 4. CONCLUSION

The results of testing of hemagglutination activity using TRBC Halosaccion glandiforme (214) was higher than Palmaria palmata (26). The hemaglutination activity of Palmaria palmata was able to be inhibited by PTG, aPTG, and aBSM, while Halosacccion glandiforme in hemagglutination activity was inhibited by aBSM glycoprotein with low concentration. The crude fraction of lectins tested did not have an antibacterial effect on gram-negative bacteria and gram-positive bacteria.

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