Preliminary Characterization of Crude Lectin Fraction of the Red Alga, *Acrocystis nana* from Wediombo Beach of the Southern Coast of Java Island, Gunung Kidul, Yogyakarta, Indonesia

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Abstract. Lectins, bioactive compounds that found in algae, are bind to sugars or glycoproteins reversibly with high specificity, but are devoid of catalytic activity, and in contrast to antibodies, are not products of an immune response. *Acrocystis nana* is a species of red alga that is collected from the Wediombo beach, coastal region of Gunungkidul, Yogyakarta. Hemagglutination activity of a crude lectin fraction from *Acrocystis nana* was examined using trypsin-treated rabbit erythrocytes and its chemical properties, including its protein content, stability on pH, temperature, and divalent cations were determined as a preliminary characterization phase. Crude lectin of *Acrocystis nana* showed a titer of 2¹² on hemagglutination activity assay and the protein content was 6225.44 µg/ml. Hemagglutination activity of this crude lectin was stable after treatment at various pH from 3 to 10 and its activity was lost by heating at 50°C until 100°C. Moreover, the hemagglutination activity was slightly affected by divalent cations treatment, indicating that the presence of divalent cations may require for its activity, however, further studies are still needed for a more comprehensive understanding about its properties.

1. Introduction

Indonesia is a maritime country with a coastline of 81,000 km [1] and marine area of 5.8 million km² [2]. Wediombo Beach is a beach in the southern coast of Java Island, which locates at 8° 08’ 42.7” S and 110° 35’ 58.1” E. One of the potential of marine resources is algae. Based on the Van Bosse expedition from 1899 to 1900, Indonesia has 555 out of 8642 species of algae in the world (6.42% of all species of algae in the world). The number of red algae species is more than most of other types of algae. *Acrocystis nana* is one of the species of red algae found in Wediombo Beach. It has a shape like human fingers, wrinkled, and clustered like a glove. Its color is yellowish green and the biggest size has a height of 30 cm and a width of 4 cm [2].
One of the bioactive compounds in algae are lectins. Lectins derived from Greek “Legere” which means to pick up or to take. Lectins are proteins that bind to sugar or glycoprotein, non-immune, can agglomerate cells and/or precipitate glycoconjugates. Lectins have at least two binding sites [3]. Lectins specifically recognize and bind some carbohydrates, so it can be a useful tool in some research fields such as immunology, cell biology, membrane structures, and cancer [4]. Lectins are also able to act as anti-viral, anti-cancer, and anti-tumor [5].

With respect to lectins from algae, it has been reported on Praspeptiangga [6] that algal lectins surveys have been conducted from Puerto Rican [7], English [8-10], Japanese [11-13], Spanish [14,15], United States[16,17], Brazilian [4,18,19], and Vietnamese marine algae [20]. Most investigators have detected lectins by agglutination experiments using human and animal erythrocytes and/or other cells [6]. The improvement in the methodologies of both, extraction, and hemagglutination activity assays could increase the number of positive species. In fact, there appears to be coincidence that the rabbit erythrocytes treated with enzyme (trypsin and/or papain) are most suited for the detection of hemagglutination activity in marine macroalgae [13, 18, 21]. Even with the increase in the publications related to algal lectins, biochemical and structural information on algal lectins is still limited [6]. There have been merely 31 lectins from Rhodophyceae and 17 lectins from Chlorophyceae were isolated and characterized up to now [21]. Acrocystis nana has been investigated on its history, nutrition, and benefits to the human body [22]. However, lectin from Acrocystis nana has not been studied yet. Thus, this paper deals with preliminary studies on the chemical properties of crude lectin from the red alga, Acrocystis nana.

2. Experimental

The red alga, Acrocystis nana was collected from Wediombo Beach of the southern coast of Java Island, Gunung Kidul, Yogyakarta, in December 2015. It was packed in plastic bags, and immediately transferred to the laboratory on ice. It was then washed with reverse osmotic water, and kept at -20°C until use. All chemicals used in this study were of an analytical grade.

Crude lectin (salting-out fraction) was prepared by procedures as follow: a hundred grams sample was thawed, cut into small pieces and ground in a mortar, then 2 volumes of 20 mM phosphate buffer pH 7.0 containing 0.85% NaCl (PBS) was added. The powdered alga was then stirred and kept at 4°C overnight. The mixture was centrifuged (10000 rpm, 30 min, 4°C) and to the supernatant, solid (NH₄)₂SO₄ was added to attain a final concentration of 75%-saturation. The mixture was kept overnight at 4°C and centrifuged (10000 rpm, 30 min, 4°C). The precipitates were dissolved in a small volume of PBS and thoroughly dialyzed against the same buffer at 4°C for 8 h with buffer replacement every 2 h [23]. The inner fraction was further centrifuged (10000 rpm, 30 min, 4°C) and the supernatant was recovered as a crude lectin (salting-out fraction).

Hemagglutination activity was determined on a 96-well microtiter V-plate using a 2% suspension (v/v) of trypsin-treated rabbit erythrocytes (TRBC) [12,24]. TRBC was prepared as follow [24]. Rabbit blood was obtained from Bioassay Laboratory of Research and Development Center for Marine and Fisheries Product Competitiveness and Biotechnology (Ministry of Marine Affairs and Fisheries, Jakarta, Indonesia). The blood cells were washed three times with 50 volume of 0.85% NaCl (saline) and suspended in saline to give a 2% (v/v) suspension of native blood cells. A tenth volume of 0.5% (w/v) trypsin in saline was added to a 2% suspension of native blood cells and the mixture was incubated at 37°C for 60 min. After incubation, rabbit erythrocytes were washed four times with saline and a 2% suspension (v/v) of TRBC was prepared in saline [24].

Determination of protein content using the BCA protein assay reagent kit by Pierce Biotechnology (2002). The standards used in this test is a bovine serum albumin (BSA). A total of 25 mL of the lectin put into 96-wells microtter flat-plate, added 200 mL of working reagent (reagent A: B 50: 1), incubated (37°C, 30 min), the absorbance at λ 562 nm.

Effects of pH, temperature, and divalent cations on hemagglutination activity were examined as follows [24]. To examine the effect of pH, 500 µl each of a test solution was dialyzed against 100 mL of a 50 mM buffer of various pH values between 3 and 10 at 4°C for overnight, and then dialyzed
against PBS pH 7.0 with buffer replacement every 2 h. Fraction after dialyzed with PBS was assayed for hemagglutination activity. To determine the effect of temperature, 500 µl each of a test solution was incubated at various temperatures from 30°C to 100°C for 30 min and subjected to hemagglutination activity assay after cooling. To examine the effect of divalent cations, 500 µl of a test solution was dialyzed against 50 mM EDTA in PBS at 4°C overnight and the inner fraction was measured for hemagglutination activity. Additionally, an equal volume of 20 mM CaCl₂ or 20 mM MgCl₂ in saline was added to the inner fraction, kept for 2 h at room temperature, and then measured for hemagglutination activity [24].

3. Results and Discussion
Crude lectin (salting-out fraction) from red alga Acrocystis nana show a titer of 2¹² on hemagglutination activity assay, while the crude extract’s titer was 2⁹ (table 1.). The volume of crude extract and salting-out fraction were 201 ml and 11.5 ml, respectively, therefore, values of total hemagglutination activity (THA) of crude extract and salting-out fraction were 102,912 and 47,104, respectively (table 1.). Protein content of crude extract was 513.28 µg/ml, while salting-out fraction’s protein content was 6,225.44 µg/ml (table 1.).

Hemagglutination activity of this crude lectin was stable after treatment at various pH between 3 and 10 (Fig. 1a.) and the activity of crude lectin was not changed at 30°C, but decreased at 40°C, and it was inactivated as incubation temperature exceeded 50°C (Fig.1b.). Hemagglutination activity of salting-out fraction was unchanged after dialyzed against EDTA in PBS, however the presence of divalent cations, such as Ca²⁺ and Mg²⁺ slightly change the activity.

| Table 1. Hemagglutination activity and protein content of crude lectin from Acrocystis nana |
|---------------------------------|--------|---------|---------------|
| Acrocystis nana                 | Volume (ml) | HA⁽ᵗ⁾  | THA⁽ᵗ⁾         | Protein Content (µg/ml) |
| Crude extract                   | 201    | 2⁹     | 102,912        | 513.28                  |
| Salting-out fraction            | 11.5   | 2¹²    | 47,104         | 6,225.44                |

⁽ᵗ⁾HA, hemagglutination titer, the reciprocal of the highest two-fold dilution exhibiting positive hemagglutination; THA, total hemagglutination titer (volume x HA).

Figure 1 (a) shows that effect of pH on hemagglutination activity of crude lectin from red alga Acrocystis nana and titer of 2¹ was used for preliminary characterization of crude lectin Acrocystis nana. Hemagglutination activity of this crude lectin was slightly decreased (2²) on pH 3.0 to 5.0 and pH 8.0 to 10.0, however, titer was stable (2³) at pH 6.0 and 7.0. It may suggested that the hemagglutination activity of crude lectin from the red alga, Acrocystis nana was stable against the influence of pH, as reported for some algal lectins [25].

Figure 1. Effect of pH on hemagglutination activity of crude lectin from Acrocystis nana
Effect of temperature on hemagglutination activity is shown in Fig. 1b. Titer of 2^2 was stable at a temperature of 30°C, while at 40°C temperature treatment, the crude lectin’s hemagglutination titer has decreased insignificantly, therefore it might be considered that hemagglutination activity at a temperature of 30°C to 40°C was still stable. However, the activity of this crude lectin was lost at temperature treatment of 50°C to 100°C, suggesting this crude lectin was not thermostable. In several lectin studies from purified red macroalgal lectins reported that some of them are thermostable [12, 25]. Meanwhile, according to [26] the activity of lectins that isolated from the marine sponges were stable up to a temperature of 60°C. Moreover, Pterocladia capillaceas is one example of red algal lectins that was unstable on high temperature treatment, as its activity was lost at 60°C [15]. Further, effect of divalent cations on hemagglutination activity of crude lectin from *Acrocystis nana* was also determined in this study. Titer of 2^2 was also used for this analysis (data not shown). Dialysis against EDTA and addition of divalent cations, such as Ca^{2+} and Mg^{2+}, slightly affect the hemagglutination activity, indicating that the activity of crude lectin from the red alga *Acrocystis nana* may required divalent cations for their hemagglutination.

4. Conclusion
Hemagglutination activity, total hemagglutination activity, and protein content of crude lectin from the red alga, *Acrocystis nana* was 2^2, 47,104 and 6,225.44 µg/ml, respectively. The activity of this crude lectin was stable after treatment at various pH, and the crude lectin lost its activity when heating temperature was exceeded 50°C. Moreover, the hemagglutination activity was slightly affected by divalent cations treatment, indicating that the activity may depend on the presence of divalent cations. However, further studies are still needed for a more comprehensive understanding about its properties.

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