

INTISARI

Lektin merupakan protein non-imun yang mampu mengenali dan mengikat glikan secara spesifik dan reversibel tanpa mengubah strukturnya. Penelitian mengenai lektin masih didominasi dari sumber terestrial, sementara penelitian lektin dari sumber perairan khususnya makroalga masih sangat terbatas. Penelitian ini bertujuan untuk: 1. Melakukan penapisan lektin pada berbagai spesies makroalga dari Pantai Porok, Gunungkidul, Yogyakarta; 2. Mendapatkan data dan informasi profil karakteristik hemagglutinasinya, spesifisitas pengikatan glikan dan stabilitas ekstrak kaya lektin dari berbagai spesies makroalga Pantai Porok, Gunungkidul, Yogyakarta; 3. Mendapatkan data dan informasi spektrum bioaktivitas antibakteri, antitumor dan mitogenik ekstrak kaya lektin makroalga dari Pantai Porok, Gunungkidul, Yogyakarta; 4. Purifikasi parsial lektin dari makroalga terpilih dengan karakteristik dan spektrum bioaktivitas terbaik; 5. Mendapatkan data dan informasi karakteristik dan spektrum bioaktivitas lektin makroalga yang dimurnikan. Sebanyak 21 sampel makroalga dikoleksi dari Pantai Porok, Gunungkidul telah diidentifikasi spesiesnya. Seluruh sampel diekstraksi kandungan proteinnya menggunakan 20 mM PBS, 0,85% NaCl, pH 7,0 dan dipekatkan dengan 75% ammonium sulfat (*salting out fraction / SOF*). Seluruh SOF mengaglutinasi setidaknya 1 jenis eritrosit sebagai indikasi awal kandungan lektin. Lima belas SOF dengan HA pada konsentrasi $\leq 25 \mu\text{g/mL}$ diuji spesifisitas pengikatan glikannya. SOF *Turbinaria ornata*, *Padina crispata*, *Sargassum hystrix*, *Palmaria palmata*, *Chondrophycus intermedius*, *Ceratodictyon* sp. *Sarcodia montagneana*, *Gelidiella fanii*, *Acrocystis nana*, *Hypnea spicifera*; *Ulva lactuca*, *Codium prostatum*, *Codium duthieae*, *Chaetomorpha vieillardii*, dan *Valoniopsis pachynema* memiliki spesifisitas pengikatan pada glikoprotein, namun tidak pada gula sederhana. Mayoritas SOF memiliki spesifisitas pada glikoprotein ikatan-O, fetuin dan *bovine submaxillary mucin* (BSM), serta glikoprotein tipe kompleks dan tinggi mannososa, *bovine thyroglobulin* (BTG) dan *porcine thyroglobulin* (PTG). HA mayoritas SOF turun signifikan pada medium asam, sedikit turun pada medium basa, mulai menurun pada suhu $> 70^\circ\text{C}$, dan tidak memerlukan kation divalent untuk aktivitasnya. SOF *A. nana*, *C. prostatum* dan *C. duthieae* menunjukkan sitotoksitas pada sel karsinoma hepatoseluler manusia (HepG2) dan sel adenokarsinoma prostat (PC3). Hanya SOF *A. nana* yang menunjukkan aktivitas antibakteri terhadap *Escherichia coli*, *Salmonella typhimurium*, dan *Staphylococcus aureus* serta aktivitas mitogenik. Putatif-lektin *A. nana* (ANL) diisolasi menggunakan resin Butyl-Sepharose-XK16, filtrasi fraksi aktif menggunakan *centrifugal filter* dengan *cut off* 100 kDa, dilanjutkan dengan resin DEAE. Analisa SDS-PAGE dengan dan tanpa 2-mercaptoethanol, menghasilkan 1 pita protein tunggal berukuran sekitar 19 kDa. Tahapan isolasi meningkatkan aktivitas spesifik ANL hingga 84,78 kali. ANL mengikat glikoprotein *transferrin human*, BTG, PTG, BSM dan fetuin. HA ANL stabil hingga suhu 70°C , menurun signifikan pada media asam namun stabil pada media basa, dan tidak memerlukan kation divalent. Aktivitas sitotoksik ANL lebih tinggi dibandingkan SOF-nya, dengan mortalitas sel HepG2 sebesar 88,42% pada konsentrasi uji $25 \mu\text{g/mL}$; memberikan respon proliferasi



limfosit terbaik pada konsentrasi uji 0,19 µg/mL, namun memberikan aktivitas antibakteri negatif. Analisa proteomik mencocokkan 1.046 spektra MS ANL dengan pustaka data phylum Rhodophyta Uniprot, menghasilkan identifikasi 382 peptida serta 235 protein. Sebagian besar protein berukuran 10-20 kDa, sebagian besar merupakan *phycobiliprotein*, dan protein dengan Q-skor tertinggi teridentifikasi sebagai Hsp 81-1 *Gracilariopsis chorda*. Putatif-lektin ANL yang mempunyai afinitas tinggi terhadap thyroglobulin dan mucin serta bioaktivitas mitogenik dan antitumor berpotensi untuk dimanfaatkan di bidang bioteknologi dan biomedis.

Kata kunci: Lektin, hemagglutinin, makroalga, *Acrocystis nana*, Rhodophyta, Protein pengikat-glikan

ABSTRACT

Lectins are non-immune proteins that are capable of recognizing and binding glycans in a specific and reversible manner, without altering their structural integrity. The majority of lectins research is focused on terrestrial sources, with limited investigation of aquatic-derived lectins, particularly macroalgae. The objectives of this research are: 1. To Conduct lectin screening on various macroalgae species from Porok Beach, Gunungkidul, Yogyakarta; 2. To obtain data and information on the profile of hemagglutination characteristics, glycan binding specificity and stability of lectin-rich extracts from various species of macroalgae from Porok Beach, Gunungkidul, Yogyakarta; 3. To obtain data and information on antibacterial, antitumor and mitogenic bioactivity spectra of lectin-rich extracts of macroalgae from Porok Beach, Gunungkidul, Yogyakarta; 4. To partially purify of lectins from selected macroalgae with the best characteristics and bioactivity spectrum; 5. To obtain data and information on the characteristics and bioactivity spectrum of purified macroalgae lectins. Twentyone macroalgae samples collected from Porok Beach, Gunungkidul have been identified to the species level. All samples were extracted for its protein content using 20 mM PBS, 0.85% NaCl, pH 7.0, and concentrated with 75% ammonium sulfate (salting out fraction/SOF). All SOFs demonstrated indication of lectin content, as evidenced by the hemagglutination activity (HA) at least on one type of erythrocyte. Fifteen SOFs with HA at concentrations of ≤ 25 $\mu\text{g/mL}$ were characterized for their glycan binding specificity. SOF of *Turbinaria ornata*, *Padina crispata*, *Sargassum hystrix*, *Palmaria palmata*, *Chondrophycus intermedius*, *Ceratodictyon* sp., *Sarcodia montagneana*, *Gelidiella fanii*, and *Acrocystis nana*, *Hypnea spicifera*, *Ulva lactuca*, *Codium prostatum*, *Codium duthieae*, *Chaetomorpha vieillardii*, and *Valoniopsis pachynema* exhibited binding specificity to glycoproteins, but not to simple sugars. The majority of SOFs demonstrated specificity for O-linked glycoproteins, fetuin, and bovine submaxillary mucin (BSM), as well as complex and high-mannose type glycoproteins, bovine thyroglobulin (BTG), and porcine thyroglobulin (PTG). The HA of the majority SOFs exhibited a significant decrease in acidic medium, a slight decrease in alkaline medium, a gradual decline at temperatures exceeding 70°C, and divalent cations independent. SOFs of *A. nana*, *C. prostatum*, and *C. duthieae* demonstrated cytotoxicity on human hepatocellular carcinoma cells (HepG2) and prostate adenocarcinoma cells (PC3). Only SOF of *A. nana* demonstrated antibacterial activity against *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* and mitogenic activity to human lymphocyte. The putative lectin of *A. nana* (ANL) was isolated using Butyl-Sepharose-XK16 resin, followed by filtration of the active fraction using a 100 kDa cut-off centrifugal filter and subsequent purification using DEAE resin. A single protein band of approximately 19 kDa was observed in the SDS-PAGE analysis. The isolation step resulted in an 84.78-fold increase in the specific activity of ANL. ANL has been demonstrated to bind human transferrin glycoprotein, BTG, PTG, BSM, and fetuin. The ANL is stable at temperatures up to 70°C, exhibits a significant decrease in activity in acidic media, and is stable in alkaline media and

divalent cations independent. The cytotoxic activity of ANL was observed to be higher than that of its SOF, with HepG2 cell mortality reaching 88.42% at a test concentration of 25 µg/mL. At a test concentration of 0.19 µg/mL, ANL exhibited the most favourable lymphocyte proliferation response. However, it should be noted that ANL did not demonstrate any antibacterial activity. A total of 1,046 MS spectra of ANL were matched with the Uniprot data library of the Rhodophyta phylum, resulting in the identification of 382 peptides and 235 proteins. The majority of the identified proteins were within the 10-20 kDa range, predominantly comprising phycobiliproteins. The protein with the highest Q-score was determined to be Hsp 81-1 of *Gracilariopsis chorda*. ANL putative lectins that demonstrate high affinity for thyroglobulin and mucin, as well as mitogenic and antitumor bioactivities, have the potential to be utilized in biotechnology and biomedicine area.

Keywords: Lectin, hemagglutinin, macroalgae, *Acrocystis nana*, glycan-binding protein