Characterization and analysis of the antidiabetic activities of sulphated polysaccharide extract from *Caulerpa lentillifera*

Sofa Fajriah¹, Ilmi Fadhilah Rizki², Ellya Sinurat³

¹ Research Center for Chemistry, Indonesian Institute of Sciences (LIPI)/National Agency and Research Innovation (BRIN), Serpong, Indonesia
² Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok, Indonesia
³ Development Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine and Fisheries, Jakarta, Indonesia

Corresponding author: Ellya Sinurat (ellya_sinurat@yahoo.com)

Received 18 August 2021 • Accepted 12 October 2021 • Published 12 November 2021

Abstract

*Caulerpa lentillifera* is a type of green seaweed that is cultivated in tropical and subtropical areas. The objectives of this study were to determine the characteristics of the sulfated polysaccharides from *C. lentillifera* and evaluate its antidiabetic activity. In the initial process of this study, samples were macerated with ethanol (1:10). Then, the maceration residue was extracted with an accumulator at 75 °C for three hours. The crude extract yield was 4.16% based on weight seaweed. Ion chromatography purification with DEAE-Sepharose resin provided a yield of 14.8% of crude extract. The monomer analysis of *C. lentillifera* from the crude extract and purified extract revealed that galactose monomers were dominant and glucose was a minor component. The total carbohydrate and sulfate contents of purified *C. lentillifera* were higher than those of crude *C. lentillifera*. Bioactivity tests revealed that purified polysaccharides had higher antidiabetic activity against α-glucosidase enzyme than crude ones with IC₅₀ values of 134.81± 2.0 µg/mL. Purified sulfated polysaccharides of *C. lentillifera* could potentially be used as an antidiabetic medication.

Keywords

antidiabetic bioactivity, *Caulerpa lentillifera*, sulfated polysaccharide, alpha-glucosidase

Introduction

Chronic hyperglycemia due to diabetes mellitus can lead to defects in insulin levels (Sharma and Rhyu 2014; Radhika and Priya 2016). Wu et al. (2020) state that diabetes can significantly increase the risk of severity and fatality with SARS-CoV-2 infection. Blood glucose levels can be maintained in the intestine by inhibiting carbohydrate enzymes or delaying glucose absorption (Shirosaki and Koyama 2011). Type 2 diabetes mellitus is the most common type of diabetes worldwide. Cases of diabetes continue to increase annually (Sharma and Rhyu 2014). Seaweed has a varied chemical content and high bioactivity (Sanger et al. 2019; Sharma et al. 2019; Purwaningsih et al. 2020). Caulerpaceae is divided into 75 species based on chlorotic classification and grows in tropical and subtropical regions. *Caulerpa lentillifera* is commonly known as sea grapes. This type of seaweed contains various sulfated polysaccharides and monosaccharides. Sulfated polysaccharides have numerous bioactivities, including antidiabetic, antiviral, antibiotic, anticancer, and immunomodulatory activities (Costa et al. 2010; Imjongjairak et al. 2015; Peng et al. 2019).
The human digestion process occurs in the small intestine under the mediation of α-amylase and α-glucosidase. Carbohydrate hydrolysis causes a sudden increase in postprandial blood concentration. Maltose and isomaltose are produced by the action of α-amylase and are then hydrolyzed by α-glucosidase, a membrane-generated enzyme in the small intestinal epithelium. The enzyme α-glucosidase is a catalyst in the digestion of carbohydrates in the final stages. Therefore, inhibiting α-glucosidase can inhibit the hydrolysis of carbohydrates from suppressing postprandial hyperglycemia (Mwakalukwa et al. 2020). A crude extract obtained by using water from several species of brown seaweed, including Padina sulcata, Sargassum binderi Sonder, and Turbinaria conoides, has been shown to inhibit α-glucosidase activity. Fucoidan is one of the water-soluble sulfated polysaccharides that can inhibit the activity of α-glucosidase and α-amylase differently depending on the harvest period and the targeted enzymes (Sharifuddin et al. 2015). Therefore, identifying sulfated polysaccharides from other species with different monomers capable of antidiabetic bioactivity is necessary. This study aims to characterize the sulfated polysaccharides of C. lentillifera and evaluate their antidiabetic activity.

Material and methods

Material

C. lentillifera was obtained from Takalar, South of Sulawesi, Indonesia. This plant was determined at Research Center for Oceanography LIPI with voucher specimen RL-01. Seaweed was dried in a vacuum oven after rinsing thoroughly, dried, and then ground. The ground powder was passed through a 60 mesh. The powder in the container was not allowed to contact air (Jime et al. 2001; Cho et al. 2010).

Extraction of the crude polysaccharide

The extraction process began with the maceration of seaweed powder (100 g) in ethanol at a ratio of 1:10 for 24 h. Then, the maceration mixture was filtered by using a 250-nylon mesh. Next, the solid residue was extracted using water solvent at 70–80 °C with stirring for three h. The solution was filtered, mixed with ethanol at a ratio of 2:1, and then allowed to settle. The mixture was centrifuged at 8000 xg and 4 °C for 20 min. The formed solid particles were dried in a dryer oven at 60 °C and designated as the extract of C. lentillifera, as shown in Fig 1. The yield of sulfated polysaccharide extract was calculated from the dried seaweed. This method was modified from a previous report (Imjongjairak et al. 2015; Sinurat and Kusumawati 2017). In the method Imjongjairak et al. (2015), the milled seaweed was extracted using distilled water (600 mL) at 25–55 °C for 16 hours, whereas the water solvent was removed at 70–80 °C with stirring for 3 hours in this study.

Purification of sulfated polysaccharides

One gram of polysaccharide extract was dissolved in 50 mL and then heated at 80 °C. Sulfated polysaccharides were extracted through ion chromatography by using DEAE-Sepharose 6B-Cl resin in a 2.5 cm-column in diameter and 40 cm in length and eluted with a gradient of NaCl solution from 0.5 M to 2.5 M. The eluent was collected in 10 mL vials; each eluent concentration is obtained five vials and divided into fractions based on absorbance following the modified method of (Sinurat et al. 2015). The
absorbance results showed that three purified fractions, FP1, FPL2 and FP3, were obtained. FP1, FPL2 and FP3 were obtained through elution with NaCl solution at concentrations of 0.5–1.5 M. FP3 required the highest eluent concentration. Ethanol was added to all fractions until a precipitate was formed. The absorbance at 490 nm of the polysaccharide fractions was determined through the phenol−H₂SO₄ method Dubois et al. (1956). Sulfate content was analyzed by using the BaCl₂–gelatine method Dodgson and Price (1962).

**FT-IR Spectra measurements**

The functional groups of crude extract and purified C. lentillifera were identified with infrared spectroscopic analysis using a Perkin Elmer Spectrum One FTIR Spectrometer. FTIR spectra were recorded over the range of 450–4000 cm⁻¹ at room temperature.

**Analysis of monosaccharide composition by HPLC**

The monomer analysis of crude and purified extracts was performed by dissolving 10 mg of samples in 1.5 M TFA at a ratio of 1:1. The mixture was heated at the temperature of 121 °C for 120 min using a heater. The mixture was neutralized with 10% NaOH and then centrifuged at 8000 xg for 20 min. The pH of the mixture was adjusted with 10% NaOH, and solids were precipitated by centrifugation for 20 min at 4 °C. Monomers in the supernatant were characterized using a Prominence-20 HPLC instrument (Shimadzu Protruding-20) with an Agilent Hi-Plex H column; diameter 7.8 mm; length 300 mm; temperature column at 65–85 °C. The samples solution for hydrolysis treatment was spiked with monosaccharide standards (galactose, rhamnose, xylose, glucose, and mannose) at varying concentrations (100–1000 ppm). About 20 µL of each sample was injected into the column with a 0.6 mL/min flow rate for 20 minutes using 0.005 M H₂SO₄ as eluent. The Refractive Index Detector (RID-10A) Merck Shimadzu was maintained at 55°C. D-Glucose ≥ 95.5%, D-xylose ≥ 99%, D-mannose ≥ 99%, D-galactose ≥ 99%, and D-fructose ≥ 99% with concentrations of 100–1000 ppm used as standard monomers. All standards monosaccharides purchased from Sigma-Aldrich (St. Louis, MO, USA) distributor in Jakarta. The method of analysis monomers was modified from a previous report (Sinurat et al. 2015). The difference with method Sinurat et al. (2015) is at varying concentration standards using (1000, 2000,3000, 4000, 5000 ppm).

**¹H-NMR analysis**

¹H- NMR spectra of crude and purified sulfated polysaccharides were recorded with JEOL ECZR3500 operating at 500 MHz using D₂O as a solvent using deuterated solvent (8H 4.60) peak of D₂O as the reference standard. The experimental properties of this analysis were relaxation delay at 5s, total scan number 24, and temperature at 70°C.

**In vitro α-glucosidase inhibitory activity**

**In the presence of enzymes**

α-glucosidase inhibitory activity was evaluated according to the previously reported method (Dewi et al. 2015), 5 µL of various concentrations of sample in DMSO, 250 µL α-glucosidase (0.124 unit/mL) mixed with 495 µL of phosphate buffer (pH 7.0) and 250 µL of p-nitrophenyl-alpha-D-glucopyranoside. The solution was then incubated at 37 °C for 5 min in a water bath. Next, 250 mg of the α-glucosidase solution was added. The solution mixture was then incubated again at 37 °C for 15 min in a water bath. After incubation, the reaction was stopped through the addition of 1000 µL of 0.2 M Na₂CO₃ solution. Then, the absorbance of the samples was measured with a UV–Vis spectrophotometer at a wavelength of 400 nm.

**In the absence of enzymes**

The procedure used for the blank treatment of quercetin and sample solutions was the same as that used for the treatment with the addition of enzymes, except that enzymes were not added. Instead, 250 µL of dimethyl sulfoxide was added to the samples to compensate for the lost volume. The samples were measured with a UV–Vis spectrophotometer at a wavelength of 400 nm.

α-glucosidase inhibitory activity was calculated as percent inhibition with the following formula (Dewi et al. 2015):

\[
\text{percent inhibition} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{samples}}}{\text{Abs}_{\text{control}}} \right) \times 100\% 
\]

Note: Absorbance control = absorbance value in the control solution; Absorbance samples = absorbance value in the samples solution.

**Results and discussion**

The polysaccharide extraction yield reached 4.16%. The dried deposits from each fraction had weights of 16.5 mg (FP1), 72.7 mg (FP2), and 18.8 mg (FP3). The results for the fractionation of the purified carbohydrate by using ion resin chromatography are presented in Figure 2.

**FTIR analysis**

The spectra of polysaccharides from C. lentillifera are presented in Figure 3. O–H stretching with strong ties was reflected by the presence of a peak at approximately 3435 cm⁻¹ (Saikia and Parthasarathy 2010). The peak at 2920–2935 cm⁻¹ in the FTIR spectra showed C–H stretching. The vibration of the stretching of carbon–yl groups (C=O) in carboxylic acid was found at 1600 cm⁻¹ (Fernando et al. 2017), and S=O stretching at 1400 cm⁻¹ indicated the presence of sulfate. C–OH bonds in polysaccharides in the form of the monomers mannose and galactose were indicated by absorbance at 1150 cm⁻¹ (Balan et al. 2019)
The glycosidic bond in C1–O–C4 was found between 1200 and 900 cm$^{-1}$ (Chandra et al. 2016). The wave region of 810 cm$^{-1}$ to 820 cm$^{-1}$ showed C-6 galactose, which was related to a D-galactose-6-sulphate structure (Sekkal et al. 1995; Pereira et al. 2003). The results of FTIR spectral analysis indicated that the three purified fractions possibly shared a similar component.

**Total carbohydrates and sulfate contents**

The total polysaccharide purification and polysaccharide extraction rate are shown in Figure 4.

Total carbohydrates in crude were 35.63 ±1.28% and purified 40.51 ±1.23% of *C. lentillifera*.

The high total carbohydrate content resulted in high load density due to sulfate bonds. The number of sulfate groups bound to the branch chain of polysaccharides was the key to high bioactivity (Oliveira et al. 2017; Sun et al. 2018). The sulfate bonds in the structure of polysaccharides provided bioactivity to *C. lentillifera*.

**Monosaccharide composition**

Galactose monomers dominated the monomer composition of crude and purified sulfated polysaccharides. The data in Table 1 showed that galactose had a retention time of 9.575 min; compared to the standard galactose, the same retention time of 9,575 minutes and an area of 3.13%. Then glucose had a retention time of 8.988 min compared to glucose standard, the same retention time of 8.988 min, and an area of 1.88%.

The standard galactose curve equation showed that the crude and purified polysaccharide samples had galactose contents of 598.99 ±6.4 ppm and 886.63 ±8.5 ppm, respectively. The chromatograms showed that the crude polysaccharides contained glucose monomers at a concentration of 380.31 ±4.2 ppm. By contrast, glucose was present at trace amounts of 0.33% in the purified sulfated polysaccharides. This amount was so small that it was considered non-existent. Research has shown that sugar components in sulfated polysaccharides play a role in bioactivity.

Another study reported that the constituent monomers of *C. lentillifera* from the South China Sea consisted of xylose, galactose, glucose and glucuronic acid (Tian et al. 2019). Mannose, galactose, glucose and xylose are the constituent monomers of sulfated polysaccharides in the extracts of *C. lentillifera* from Phetchaburi Province, Thailand (Chaiklahan et al. 2020). This result suggested that the constituent monomers of sulfated polysaccharides are similar across species.
Pharmacia 68(4): 869–875

Figure 4. Analysis of total carbohydrate and sulfate of C. lentillifera.

'H-NMR analysis

'H-NMR spectroscopy was performed for the structural analysis of mono-, oligo- or polysaccharides (Figure 5). The 'H-NMR carbohydrate spectrum showed that polysaccharide content was in the range of 3–4 ppm.

'H-NMR was also used to identify sugars and specific sugars (Duus et al. 2000). In Figure 5, the peak within the 1–2 ppm range indicated CH₃ binding, which was predicted to belong to the monomer rhamnose. Then, the peak in the 3–4 ppm region indicated a chemical shift in the H atom bound to the O–C that indicated polysaccharides. Furthermore, the peak in the area of 5–6 ppm indicated the presence of anomeric sulfate protons (Bush 1988; Yao et al. 2021)

In vitro-α-glucosidase inhibitory activity

This test aimed to determine the inhibitory effect of sulfated polysaccharides on the enzyme α-glucosidase. The test results were interpreted as IC₅₀ values, which indicated the dose of the sulfated polysaccharide needed to inhibit 50% of enzyme activity. The antidiabetic activity was analyzed by using the principle of the inhibition of the enzyme α-glucosidase. Then, the percentage of inhibition was determined by using a UV–Vis spectrophotometer at a wavelength of 400 nm. The percent inhibition by purified extracts increased relative to that by crude extracts as the concentrations of the extracts were varied from 200, 100, and 50 to 25 µg/mL. In the antidiabetic activity test, the percent inhibition was calculated by obtaining the difference between the absorbance of the control and the sample, then divided by the absorbance of the sample and multiplied by 100%. The percent inhibition results shown in Figure 6 are based on the standard and sample absorbances.

The data obtained showed that the purified extracts had higher percent inhibition than the crude extracts from C. lentillifera (Figure 6). The graph clearly showed that increasing the concentration of the sample would increase

| No | Name       | Retention time | Area    | % Area | Height | Int Type | Peak Type |
|----|------------|----------------|---------|--------|--------|----------|-----------|
| 1  | Unknown    | 6.829          | 1939764 | 94.99  | 89817  | bV       | Unknown   |
| 2  | Glucose    | 8.988          | 38304   | 1.88   | 2855   | VV       | Found     |
| 3  | Galactose  | 9.579          | 64004   | 3.13   | 4366   | VB       | Found     |

| Tabel 1. Composition of monomer constituents of a crude sulfated polysaccharide extract from C. lentillifera. |

| No | Name | Retention time | Area    | % Area | Height | Int Type | Peak Type |
|----|------|----------------|---------|--------|--------|----------|-----------|
| 1  | Unknown | 6.831          | 1913942 | 94.92  | 89190  | bV       | Unknown   |
| 2  | Glucose | 8.767          | 6713    | 0.33   | 447    | VV       | Unknown   |
| 3  | Galactose | 9.577          | 95705   | 4.75   | 6460   | VB       | Found     |

| Tabel 2. Composition of monomer constituents of purified sulfated polysaccharides of C. lentillifera. |

| No | Name | Retention time | Area    | % Area | Height | Int Type | Peak Type |
|----|------|----------------|---------|--------|--------|----------|-----------|
| 1  | Unknown | 6.831          | 1913942 | 94.92  | 89190  | bV       | Unknown   |
| 2  | Glucose | 8.767          | 6713    | 0.33   | 447    | VV       | Unknown   |
| 3  | Galactose | 9.577          | 95705   | 4.75   | 6460   | VB       | Found     |

Figure 5. NMR analysis of crude (a) and purified (b) sulfated polysaccharides from C. lentillifera.
the inhibition capability of the extract. As shown in Figure 5, the pure extract with a concentration of 200 ppm had an inhibition rate of 76%, whereas the crude extracts had an inhibition rate of 38%. The IC50 values of both samples were calculated by using the linear regression equation. The IC50 values of the crude and purified extracts were 253.49 ± 3.0 and 134.81 ± 2.0 µg/mL, respectively. These results showed that the purified extract had a more significant potential to inhibit 50% of α-glucosidase activity than the crude extract of C. lentillifera.

The α-glucosidase enzyme had amino acid residues that were predicted to be its active sites. These amino acids were glycine, methionine, aspartic acid, isoleucine, asparagine, tryptophan, lysine, serine, phenylalanine, leucine, arginine, alanine, proline, glutamine, histidine, valine, and glutamic acid. A compound targets the active site of α-glucosidase for inhibition. Quercetin, which is used as a standard in α-glucosidase enzyme inhibition testing with IC50 value 2.07 ± 0.0 µg/mL (Dewi et al. 2018), is one of the flavonoid compounds that can inhibit the α-glucosidase enzyme in vitro.

**Conclusions**

Sulfated polysaccharide extracts from C. lentillifera presented various characteristics and peaks at wave numbers that indicated the presence of sulfate ester groups. The monomer that constituted the sulfated polysaccharides of C. lentillifera included galactose, which was included in the composition of D-galactose-6-sulfate. The in vitro α-glucosidase enzyme inhibition test showed that the purified sulfated polysaccharide had higher activity than the crude sulfated polysaccharide.

**Acknowledgements**

We wish to thank to Research Center for Chemistry, National Agency and Research Innovation (BRIN) for providing a financial support through National Priority of Traditional Medicine at Engineering Science Deputy (26/A/DT/2021).

**References**

Balan V, Mihai CT, Cojocaru FD, Uritu CM, Dodi G, Botezat D, Gardikiotis I (2019) Vibrational spectroscopy fingerprinting in medicine: From molecular to clinical practice. Materials 12(18): 1–40. https://doi.org/10.3390/ma12182884

Bush CA (1988) High resolution NMR in the determination of structure in complex carbohydrates. Bulletin of Magnetic Resonance 10(3): 73–95. https://www.weizmann.ac.il

Chaiklahan R, Srinorasing T, Chirasuwan N, Tamtin M, Bunngag B (2020) The potential of polysaccharide extracts from Caulerpa lentillifera waste. International Journal of Biological Macromolecules 161: 1021–1028. https://doi.org/10.1016/j.ijbiomac.2020.06.104

Chandra A, Talari S, Martinez MAG (2016) Advances in Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. Applied Spectroscopy Reviews 52(5): 456–506. https://doi.org/10.1080/05704928.2016.1230863

Cho M, Yang C, Kim SM, You S (2010) Molecular Characterization and Biological Activities of Water-soluble Sulfated Polysaccharides from Enteromorpha prolifera. Food Science and Biotechnology 19(2): 525–533. https://doi.org/10.1007/s10068-010-0073-3

Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Câmara RBG, Nobre LTDB, Costa MSSP, Almeida-Lima J, Farias EHC, Leite EL, Rocha HAO (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. Biomedicine and Pharmacotherapy 64(1): 21–28. https://doi.org/10.1016/j.biopha.2009.03.005

Dewi RT, Tachibana S, Fajriah S, Hanafi M (2015) α-Glucosidase inhibitor compounds from Aspergillus terreus RCC1 and their antioxidant activity. Medicinal Chemistry Research 24(2): 737–743. https://doi.org/10.1007/s00044-014-1164-0

Dewi RT, Darmawan A, Mulyani H, Lotulung PDN, Minarti, Megawati (2018) α-Glucosidase Inhibitory Effect of Sulochrin From Aspergillus
terreus and Its Brominated Derivatives. Malaysian Journal of Science 37(1): 70–81. https://doi.org/10.22452/mjs.vol37no1.5

Dodgson KS, Price RG (1962) A note on the determination of the es ter sulphate content of sulphated polysaccharides. The Biochemical Journal 84: 106–110. https://doi.org/10.1042/bj084106

Dubois M, Gilles K, Hamilton J, Rebers P, Smith F (1956) Colorimetric Method For Determination of Sugar and Related Substances. Anal. Chem. 28(3): 350–356. https://doi.org/10.1021/ac60114a017

Duus JO, Gotfredsen CH, Bock K (2000) Carbohydrate Structural Determination by NMR Spectroscopy: Modern Methods and Limitations. Chemical Reviews 100(12): 4589–4614. https://doi.org/10.1021/ cr990302n

Fernando IPS, Samaraweka KKA, Lee WW, Kim H, Kim E, Gunasekara UKDSS, Abeytunga DTU, Silva EDDx, Lee H, Ieon Y (2017) FTIR characterization and antioxidant activity of water soluble crude polysaccharides of Sri Lankan marine algae. ALGAE 32(1): 75–86. https://doi.org/10.4490/algaes.2017.32.12.1

Hounsell EF, Feeney J, Scudder P, Tang PW, Feizi T (1986) H-NMR studies at 500 MHz of a neutral disaccharide and sulphated di-, tetra-, hexa- and larger oligosaccharides obtained by endo-β- galactosidase treatment of keratan sulphate. European Journal of Biochemistry 157(2): 375–384. https://doi.org/10.1111/j.1432-1033.1986.tb09679.x

Imjongjairak S, Ratanaikanokchai K, Laohakunjit N, Pason P, Waemonkul R (2015) Biochemical characteristics and antioxidant activity of crude and purified sulfated polysaccharides from Gracilaria fisheri. Bioscience, Biotechnology, and Biochemistry 80(3): 524–532. https://doi.org/10.1007/s10529-015-11033-4

Mwakuluka R, Amen Y, Nagata M, Shimizu K (2020) Postprandial hyperglycemia lowering effect of the isolated compounds from olive mill wastes - An inhibitory activity and kinetics studies on α-glucosidase and α-amylase enzymes. ACS Omega 5(32): 20070–20079. https://doi.org/10.1021/acsomega.0c01622

Oliveira C, Ferreira AS, Novoa-Carballal R, Nunes C, Pashkuleva I, Hounsell EF, Feeney J, Scudder P, Tang PW, Feizi T (2000) Carbohydrate Structural Determination by NMR Spectroscopy: Modern Methods and Limitations. Chemical Reviews 100(12): 4589–4614. https://doi.org/10.1021/ cr990302n

Peng Y, Song Y, Wang Q, Hu Y, He Y, Ren D, Wu L, Liu S, Cong H, Zhou H (2019) In vitro and in vivo immunomodulatory effects of fucoidan compound agents. International Journal of Biological Macromolecules 127: 48–56. https://doi.org/10.1016/j.ijbiomac.2018.12.197

Perera L, Sousa A, Coelho H, Amado AM, Ribeiro-claro PJA (2003) Use of FTIR, FT-Raman and 13 C-NMR spectroscopy for identification of some seaweed phycocolloids. Biomolecular Engineering 20: 223–228. https://doi.org/10.1016/S1389-0344(03)00058-8

Purwaningsih S, Santoso J, Handharyani E, Setiawati NP, Deskawati E (2020) Artificial rice from Gracilaria sp. as functional food to prevent diabetes. IOP Conference Series: Earth and Environmental Science, 414(1): 012017. https://doi.org/10.1088/1755-1315/414/1/012017

Radhika D, Priya R (2016) Assessment of Anti-Diabetic Activity of Some Selected Seaweeds. European Journal of Biomedical and Pharmaceutical Sciences 2(6): 151–154.

Saikia BJ, Parthasarathy G (2010) Fourier Transform Infrared Spectroscopic Characterization of Kaolinite from Assam and Moghalaya, Northeastern India. Journal of Modern Physics 1(4): 206–210. https://doi.org/10.4236/jmp.2010.14031

Sanger G, Ranung LK, Dumongilala LJ, Kaseger BE, Montolalu LADY (2019) Phytochemical constituents and anti-diabetic activity of edible marine red seaweed (Halymenia durvilae). IOP Conference Series: Earth and Environmental Science 278(1). https://doi. org/10.1088/1755-1315/278/1/012069

Sekkal M, Dincib V, Legrandh P, Huenneb JP (1995) Molecular Investigation of the glycosidic linkages in several oligosaccharides using FT-IR and FT Raman spectroscopies. Journal of Molecular Structure 349(95): 349–352. https://doi.org/10.1016/0022-2860(95)08781-P

Sharifuddin Y, Chinn YX, Lim PE, Phang SM (2015) Potential bioactive compounds from seaweed for diabetes management. Marine Drugs 13(8): 5447–5491. https://doi.org/10.3390/md13080547

Sharma A, Koneri R, Jha DK (2019) A Review of Pharmacological Activity of Marine Algae in Indian Coast. International Journal of Pharmaceutical Sciences and Research 10(8): 3540–3549. https://doi.org/10.13040/IJPSR.0975-8232.10(8).3540–49

Sharma BR, Rhyu D (2014) Anti-diabetic effects of Caulerpa lentilifera: Stimulation of insulin secretion in pancreatic β-cells and enhancement of glucose uptake in adipocytes. Asian Pacific Journal of Tropical Biomedicine 4(7): 575–580. https://doi.org/10.12980/APJT.4.2014APJT.14-0091

Shi H, Ruan L, Yan X, Yao D, Xu X (2014) The role of Liptopernaia vannamei p38 in white spot syndrome virus infection. Developmental and Comparative Immunology 44(1): 180–185. https://doi.org/10.1016/j.dci.2013.12.005

Shirasaki M, Koyama T (2011) Laminaria japonica as a food for the prevention of obesity and diabetes. Advances in Food and Nutrition Research 1(64). https://doi.org/10.1016/B978-0-12-387669-0.00015-6

Sinurat E, Kusumawati R (2017) Optimization of Crude Fucoidan Extraction Methods from Brown Seaweed Sargassum binderi Sonder. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan. JPB Kelautan Dan Perikanan 12(2): 125–134. https://doi.org/10.15578/jpbkp.v12i2.388

Sinurat E, Peranginangin R, Saepudin E (2015) Purification and Characterization of Fucoidan from The Brown Seaweed Sargassum binderi sonder. Squalen Bulletin of Marine & Fisheries Postharvest & Biotechnology 10(2): 79–87. https://doi.org/10.15578/squalen. v10i2.133

Sun Y, Gong G, Guo Y, Wang Z, Song S, Zhu B, Zhao L, Jiang J (2018) Purification, structural features and immunostimulatory activity of novel polysaccharides from Caulerpa lentillifera. International Journal of Biological Macromolecules 108: 314–323. https://doi.org/10.1016/j. ijbiomac.2017.12.016

Tapotuban AM (2018) Komposisi Kimia Rumput Laut (Gracillaria fisheri) dari Perairan Kei Maluku dengan Metode Pengeringan Berbeda. Jurnal Pengolahan Hasil Perikanan Indonesia 21(1): 13–23. https://doi.org/10.17844/jphpi.v21i1.21257

Tian H, Liu H, Song W, Liu X, Yin L (2020) Polysaccharide from Caulerpa lentillifera: extraction optimization with response surface methodology, structure and antioxidant activities. Natural Product Research 35(22): 3417–3425. https://doi.org/10.1080/14786419.2019.191 705077

Wu J, Zhang J, Sun X, Wang L, Xu Y, Zhang Y, Liu X, Dong C (2020) Influence of diabetes mellitus on the severity and fatality of SARS-CoV-2 (COVID-19) infection. Diabetes, Obesity and Metabolism 22(10): 1907–1914. https://doi.org/10.1111/dom.14105

Yao HY, Wang QJ, Yin JY, Nie SP, Xie MY (2021) A review of NMR analysis in polysaccharide structure and conformation: Progress, challenge and perspective. Food Research International 143: e110290. https://doi.org/10.1016/j.foodres.2021.110290