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Research Article

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

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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 IC_{50} 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).

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PENSOFT.

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

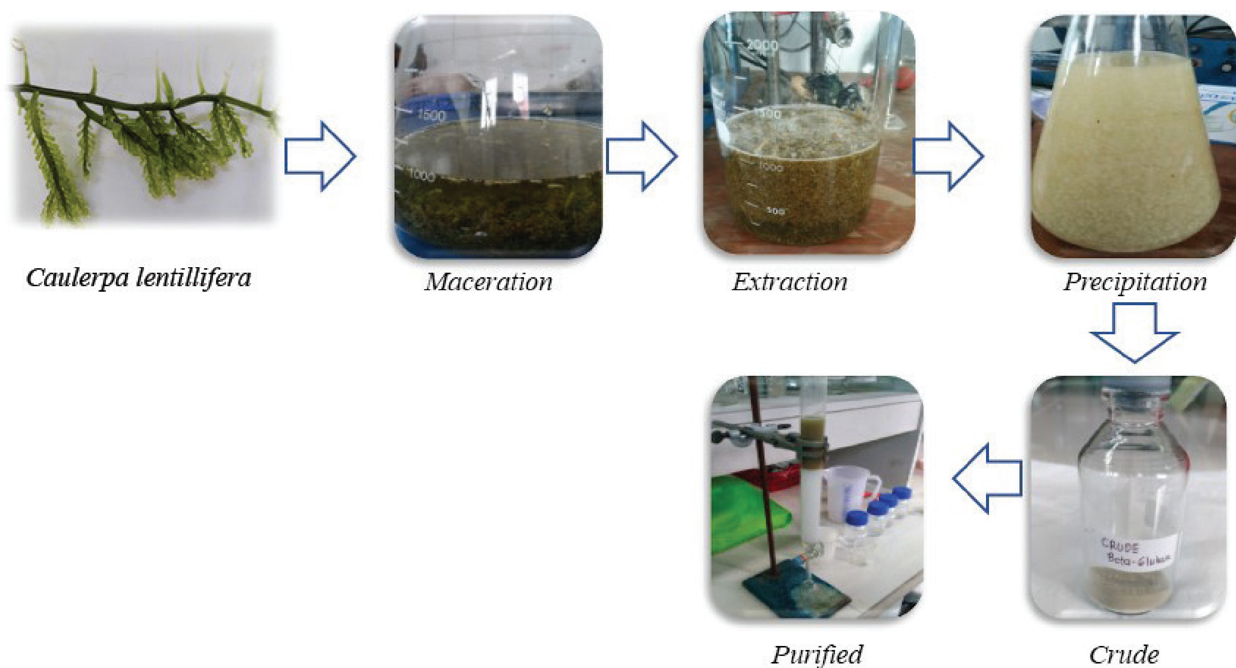


Figure 1. Flow chart of sulfated polysaccharide extraction from *C. lentillifera*.

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–H_2SO_4 method Dubois et al. (1956). Sulfate content was analyzed by using the $BaCl_2$–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 H_2SO_4 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 monomer 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).

^{1}H-NMR analysis

^{1}H- NMR spectra of crude and purified sulfated polysaccharides were recorded with JEOL ECZR500 operating at 500 MHz using D_2O as a solvent using deuterated solvent (δH 4.60) peak of D_2O 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 Na_2CO_3 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):

$$percent\ inhibition = \left(\frac{Abs_{control} - Abs_{samples}}{Abs_{control}}\right) x100\%$$

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 carbonyl 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)

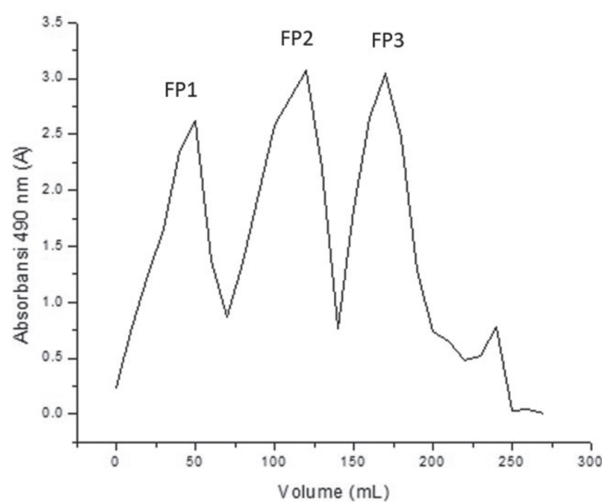


Figure 2. Analysis of the purified fractions of *C. lentillifera* on DEAE Sepharose. Noted: FP 1 fraction 1; FP 2 fraction 2; FP 3 fraction 3.

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.

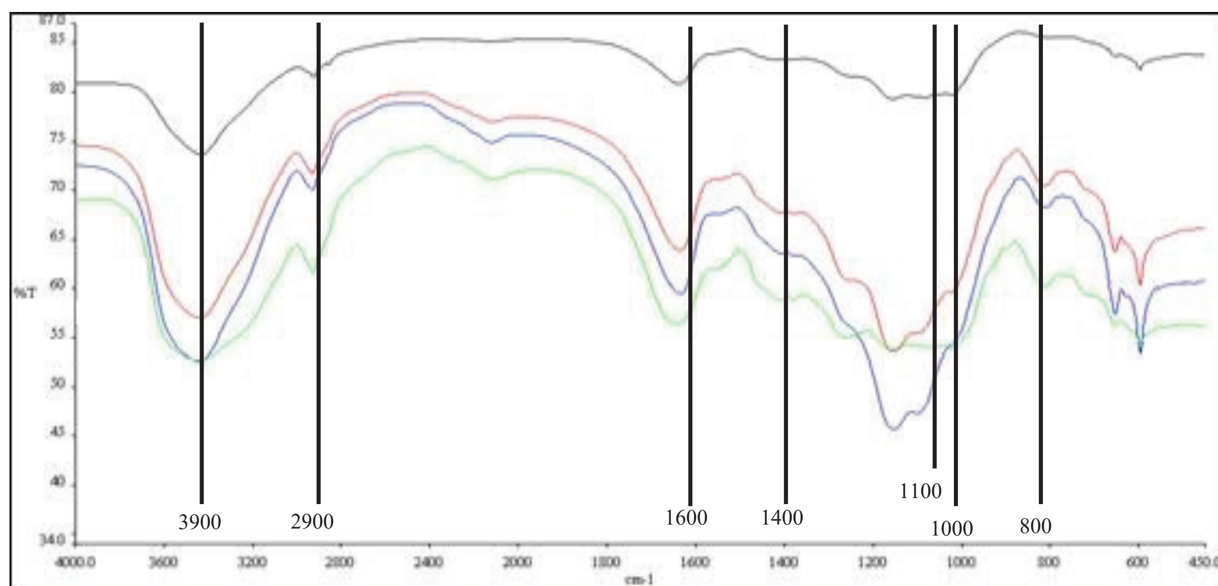


Figure 3. FTIR spectra of polysaccharides *C. lentillifera;* the black band (crude extract); green band (FP1 part); red band (FP2 part); blue band (FP3 part).

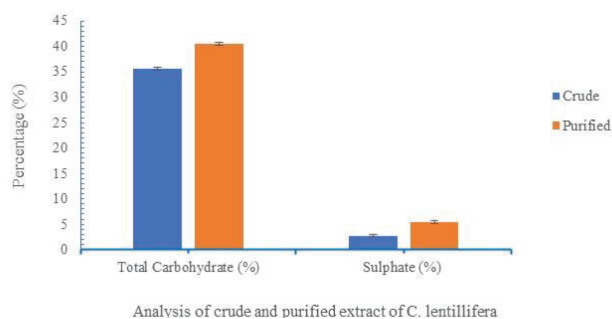


Figure 4. Analysis of total carbohydrate and sulfate of *C. lentillifera*.

1H-NMR analysis

1H-NMR spectroscopy was performed for the structural analysis of mono-, oligo- or polysaccharides (Figure 5). The 1H-NMR carbohydrate spectrum showed that polysaccharide content was in the range of 3–4 ppm.

1H-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 CH_3 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 IC_{50} 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

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	6.829	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 2. Composition of monomer constituents of purified sulfated polysaccharides of *C. lentillifera*.

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

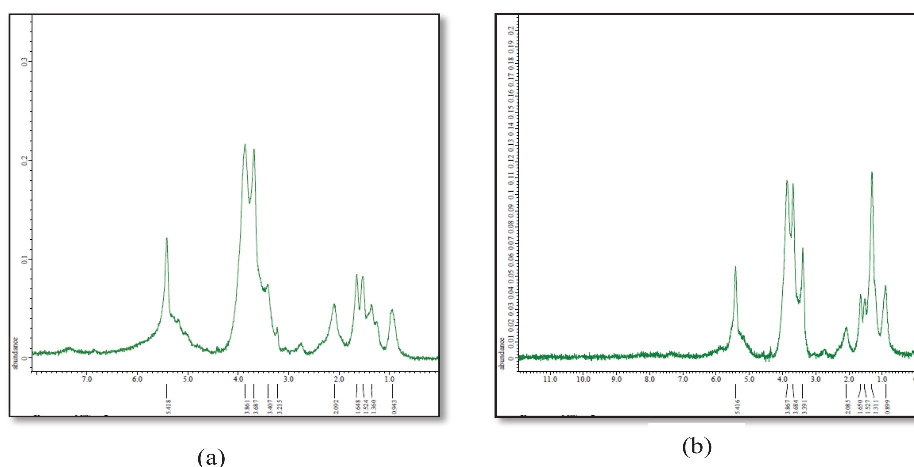
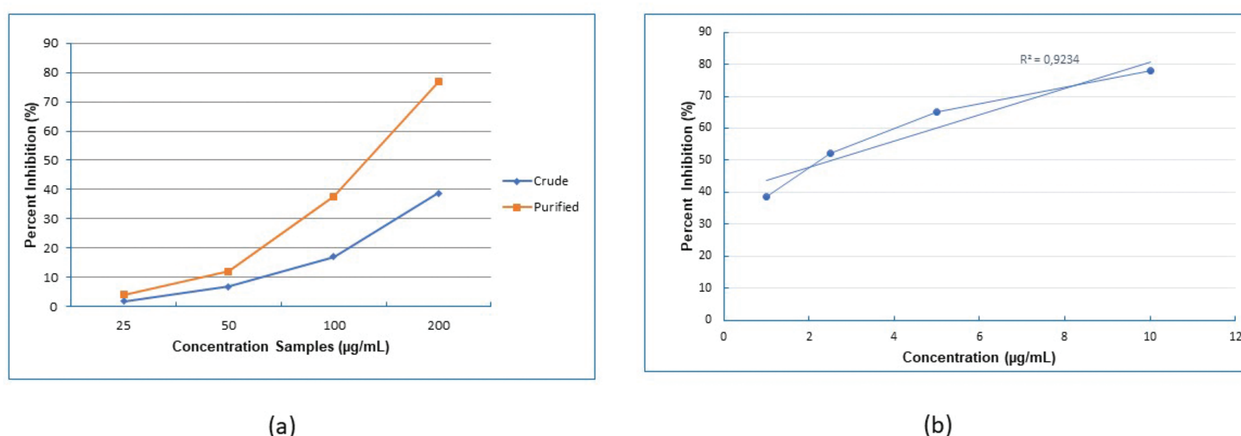


Figure 5. NMR analysis of crude (a) and purified (b) sulfated polysaccharides from *C. lentillifera*.



(a) (b)

Figure 6. Analysis of the antidiabetic activity of (a) sulfate polysaccharides from *C. lentillifera* (b) quercetin.

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 IC_{50} values of both samples were calculated by using the linear regression equation. The IC_{50} 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 IC_{50} value 2.07± 0.0 μg/mL, is one of the flavonoid compounds that can inhibit the α-glucosidase enzyme *in vitro* (Dewi et al. 2018).

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.

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