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Laminarin (Beta-glucan) of Brown Algae *Sargassum mcclurei*: Extraction, Antioxidant Activity, Lipoxygenase Inhibition Activity, and Physicochemistry Properties

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Abstract: Laminarin is a storage glucan found in almost brown algae and possesses value bioactive. The study focused on the effect of the input factors of the extraction such as the temperature (30, 50, 70, and 90°C), the time (01, 02, 03, and 04 hours), the aqueous to algae ratio (20/1, 30/1, 40/1, and 50/1 (v/w)), and solvent pH (2, 7, and 9) on the function targets (laminarin content, total antioxidant activity, reducing power activity, and lipoxygenase inhibition activity), and the physical chemistry characterization of highest laminarin content was analyzed. The results showed the temperature, the time, the aqueous to algae, solvent pH affected on laminarin content, total antioxidant activity, reducing power activity, and lipoxygenase inhibition activity that got the highest value of 11.98±0.49 mg laminarin equivalent/g DW, 19.66±0.47 mg ascorbic acid equivalent/g DW, 15.55±0.61 mg $FeSO_4$ equivalent/g DW, and 73.04±2.53 µM linoleic acid equivalent/g DW, respectively. The suitable condition of extraction was collected consisting of the temperature of 90°C for 2 hours with the solvent to algae ratio of 40/1 (v/w) in pH solvent of 7. The average molecular weight, viscosity, and sulfate content of laminarin was 505.18 kDa, 14,17±0.29 cPs, and 48.72%, respectively. FTIR spectrum exhibited function groups of laminarin composed of O-H, $-CH_2^-$, C=O, C-O, –C–H, $-O-SO_3^-$, and -CH=CH-(cis).

Keywords: Antioxidant, Extraction, Laminarin, Lipoxygenase, Molecular, Viscosity

1. Introduction

Laminarins are polysaccharides belong to storage glucans of almost brown algae consisting of glucose units with the linkages of ß(1–3) and β(1–6) in a linear chain and branch chain, respectively [1]. Laminarin exists in brown algae according to the forms of soluble and insoluble in water, and the soluble mainly depends on the level of branching in their structure. The structure and content of laminarin are different in each algae species, varies from 0.03 to 6.24% on a dry weight basis [1; 2], for example, *Pelvetia canaliculata* (0.03%) [2], *Ascophyllum nodosum* (5.82%), *Laminaria hyperborea* (6.24%) [1]. Laminarin content also depends on seasons and habitation of brown algae, such as existing in Ireland [1], not in Germany [2], compared in *Ascophyllum nodosum*. Laminarin content of twelve brown algae species in Germany varies from 0.03 to 0.86% on a dry weight basis, except for three species (*Cystoseira tamariscifolia*, *Ascophyllum nodosum*, and *Halidrys siliquosa*) do not contain laminarin [2]. The structure and the content of laminarin decide its bioactive, for example, molecular structure, branch numbers, branch length, and monosaccharide compositions. Almost studies show that laminarin possesses the activity of antioxidant [2], antibacterial [1], anti-resistance to chemical [3], anticancer [4], antitumor [5], and antioxidant activity is interesting more. Almost studies show the content, bioactivity, and structure of laminarin extracting from brown algae in the temperate zone and frigid zone, not tropical zone. The previous studies do not notice on laminarin in brown algae Vietnamese. Species *Sargassum mcclurei* found commonly grown in Vietnam, they are evaluated as a bioactive substance resource and used

as a medicinal plant for a traditional medicine. Thus, the study focused on the extraction of laminarin from brown algae *Sargassum mcclurei* and the evaluation of their bioactive (antioxidant activity, and enzyme lipoxygenase inhibition) and chemical composition.

2. Material and Methods

2.1. Material

Brown algae *S. mcclurei* found commonly grown in Central South of Vietnam, collected in April 2017, cleaned by using the seawater, and transported to the laboratory under 10°C. They were dried until the moisture of 19±1%, ground, and stored in the dark condition at 15°C for further studies.

2.2. Sample Preparation

Brown algae was soaked in aqueous with the aqueous to brown algae ratio (20/1, 30/1, 40/1, and 50/1 (v/w)) in pH (2, 7, and 10) for the time (60, 120, 180, and 240 minutes) at various temperatures (30°C, 50°C, 70°C, and 90°C). After extraction, the supernatant was collected through the membrane Whatman No. 4 and precipitating laminarin by 80% ethanol. The residues (laminarin) were cleaned by 80% ethanol twice and dried for further studies. The experiment design of extraction was according to the method of a run factor and other fixed factors. To evaluate the target functions such as laminarin content, antioxidant activity (total antioxidant, and reducing power), and enzyme lipogenxynase inhibition, 01 g of laminarin was dissolved into 50 water and filtered for further studies, and the analysis of chemical composition and viscosity on the highest antioxidant laminarin.

2.3. Determination of Laminarin Content

Laminarin content was quantified basing on the measurement of the released glucose content after laminarin hydrolysis by the enzyme. 100 µL of the sample added to 100 µL of the enzyme β-glucosidase and kept for 15 min at 40°C, and added to 03 mL of GOPOD (glucose oxidase/peroxidase) reagent and incubated at 40°C for 20 min for measuring the absorbance at 510 nm with laminarin standard from *Laminaria digitata* [6].

2.4. Evaluation of Biological Activity

2.4.1. Evaluation of Total Antioxidant Activity

Total antioxidant activity was determined to base on the color formation of Mo^{5+} and measured at the wavelength of 695nm with ascorbic acid standard as the description in [7].

2.4.2. Evaluation of Reducing Power Activity

Reducing power activity was evaluated to base on the measurement of color formation of Fe^{2+} at the wavelength of 655 nm with $FeSO_4$ standard as in [8].

2.4.3. Evaluation of Lipoxygenase Inhibition Activity

The reaction mixture contained 0.2M citrate-phosphate buffer pH- 9.0, 0.25% Tween 20, 0.125mM linoleic acid, an enzyme solution (57µg protein) and 10 µL algal extract to a final volume of 1ml. 10 µL of aqueous or ethanol was used instead of the extract as a control. The enzyme reaction was carried out in the cuvette and monitored at 234nm until the reaction rate reached a steady state. This wavelength corresponds to the absorption of the hydroperoxides generated by the action of the lipoxygenase on linoleic acid. The percentage inhibition defined by the rate of increase in OD in the absence of algal extract to that measured with the extract [9].

2.5. Determination of Physicochemistry Properties

Molecular weight and fragmentation of laminarin were determined to base on the machine of LC/MS with static phase (Eclipse XDB-C8 5µm, 4.6×150 mm (Agilent)) and a mobile phase (acetonitrile, deion water ((Pure Water System, WP 710), both acetonitrile and deion water composed of 0.1% of formic acid). The gradient of acetonitrile was from 10% to 90% for 20 minutes.

The viscosity determination of laminarin was by using the dissolve of 05 g of laminarin powder into 495 g of aqueous and keeping at 29°C for the viscosity measurement at the rate of 100 rpm.

The analysis of the FTIR spectrum was on the machine Tensor 37 (Brucker, Germany). The spectrum range of 7,500 to 370 cm^{-1} with beamsplitter of standard KBr; the extended spectral range of 15,000 to 370 cm^{-1} with a near and middle infrared detector.

The content of sulfate was quantified according to the $BaCl_2$-gelatin turbidity method, as in [10], based on the absorbance measurement of the released barium sulfate at λ=360 nm with potassium sulfate as standard

2.6. Data Analysis

All experiments were in triplication (n=3). Statistic analysis was by using the software MS. Excel 2010.

3. Resuls and Discussion

3.1. Effect of Extraction Condition

3.1.1. Effect of Extracting Temperature

Extraction temperature affected strongly laminarin content ($p<0.05$), antioxidant activity ($p<0.05$), and lipoxygenase inhibition ($p<0.05$). Laminarin content varied from 1.17±0.04 to 8.49±0.38 mg laminarin equivalent/g DW and got the average value of 5.25±0.25 mg laminarin equivalent/g DW. Total antioxidant activity and reducing power activity was in the range of 2.72±0.22 and 13.1±0.4 mg ascorbic acid equivalent/g DW (Figure 1), and 2.66±0.09 and 12.6±0.34 mg $FeSO_4$ equivalent/g DW (Figure 2), respectively. Lipoxygenase inhibition activity got the value from 51.18±1.28 to 71.77±1.93 µM linoleic acid equivalent/g DW (Figure 2). Laminarin content, total antioxidant activity, reducing power, and lipoxygenase inhibition activity got the highest value in 90°C and increased following the increase of extraction temperature. The average increase in laminarin

content and total antioxidant activity was 2.1 and 1.77 times after the extraction temperature increased to 20°C, corresponding to 2.44 mg laminarin equivalent/g DW and 3.46 mg ascorbic acid equivalent/g DW. Reducing power activity and inhibition of enzyme lipoxygenase was the average increase of 1.76 and 1.12 times, corresponding to 3.31 mg $FeSO_4$/g DW and 6.86 µM linoleic acid equivalent/g DW after the increase of extraction temperature was 20°C. The control of extraction temperature help to the control of laminarin content, antioxidant activity, and enzyme lipoxygenase inhibition activity extracting from species *S. mcclurei*. The extracting temperature in the current study was different from the notice of Spicer et al. [11] on the extraction of laminarin at 40°C under reduced pressure.

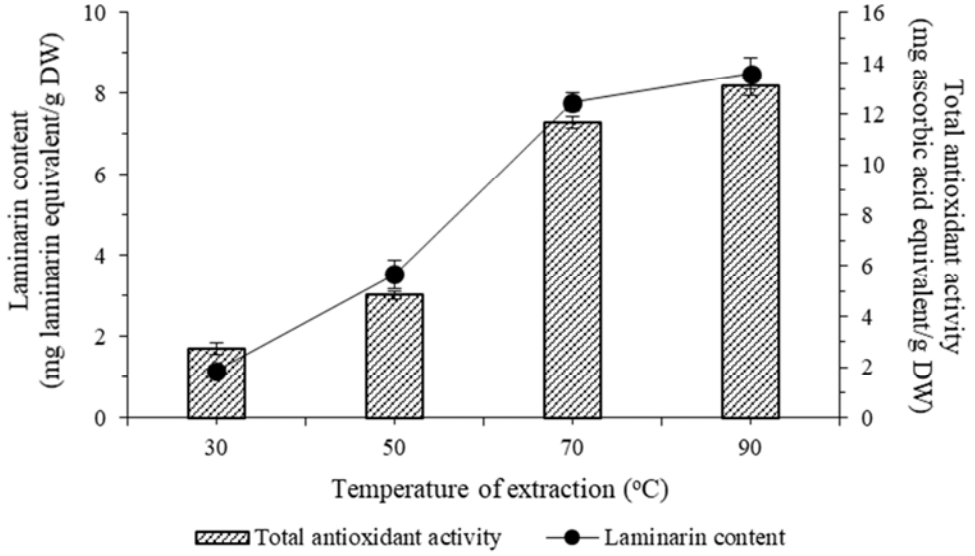

Figure 1. *Effect of extracting temperature on laminarin content and total antioxidant activity.*

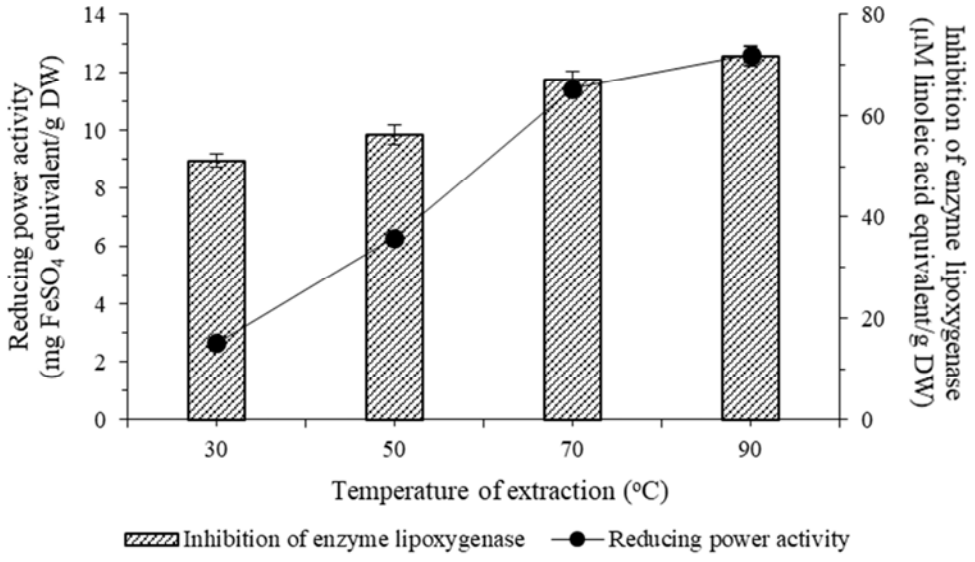

Figure 2. *Effect of extracting temperature on reducing power activity and lipoxygenase enzyme inhibition activity.*

3.1.2. Effect of Extracting Time

Laminarin content, antioxidant activity, and lipoxygenase inhibition activity were strongly impacted by the time of extraction and changed the increasing trend according to the extraction time ($p<0.05$). Laminarin content increased from 8.49±0.38 to 10.58±0.57 mg laminarin equivalent/g DW, corresponding to 19.75% in comparison to the highest laminarin content that occurred in the extracting time of 4 hours (Figure 3). Laminarin content increased by 13.04% for the extracting time of 2 hours, compared to 1 hour. The significant difference in laminarin content only occurred when the extracting time was from 0 to 2 hours. Total antioxidant and reducing power activity varied from 13.10±0.4 to 17.33±0.39 mg ascorbic acid equivalent/g DW (Figure 3) and 12.60±0.34 to 15.24±0.41 mg $FeSO_4$ equivalent/g DW and got the average value of 16.07±0.35 mg

ascorbic acid equivalent/g DW and 14.42±0.37 mg $FeSO_4$ equivalent/g DW (Figure 4), respectively. Lipoxygenase inhibition activity got the highest value of 77.02±2.34 µM linoleic acid equivalent/g DW at the extraction time of 4 hours and was 1.07 times in comparison to the extraction time of 01 hours. Laminarin content, total antioxidant activity, reducing power, and lipoxygenase inhibition activity got the highest value at the extraction time of 04 hours, but the difference in a statistic significance occurred for the extraction time of 2, 3, and 4 hours.

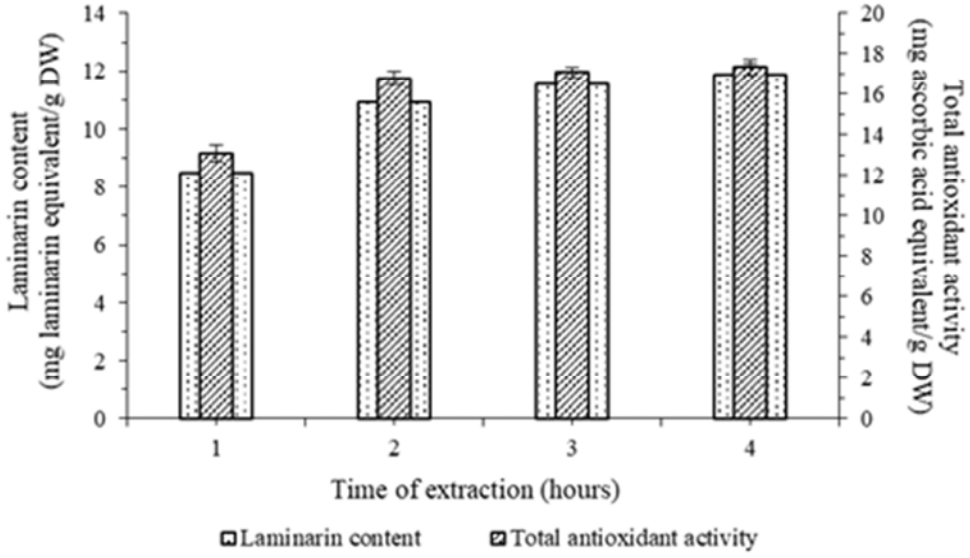

Figure 3. *Effect of extracting time on laminarin content and total antioxidant activity.*

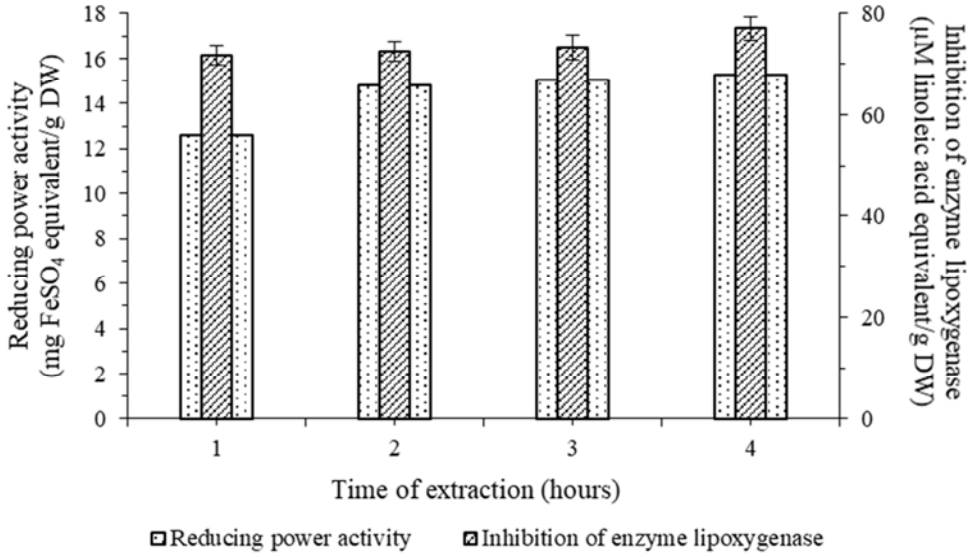

Figure 4. *Effect of extracting time on reducing power activity and lipoxygenase enzyme inhibition activity.*

3.1.3. Effect of the Solvent to Algae Ratio

The solvent to algae ratio affected laminarin content, total antioxidant activity, reducing power activity, and lipoxygenase inhibition activity ($p<0.05$) that got the highest value of 11.98±0.49 mg laminarin equivalent/g DW, 19.66±0.47 mg ascorbic acid equivalent/g DW (Figure 5), 15.55±0.61 mg $FeSO_4$ equivalent/g DW, and 73.04±2.53 µM linoleic acid equivalent/g DW (Figure 6) at the aqueous to algae ratio of 50/1 (v/w). The difference in laminarin content, antioxidant activity, and lipoxygenase inhibition activity did not occur at the aqueous to algae ratio from 40/1 to 50/1 (v/w) ($p>0.05$) but occurred between the aqueous to algae ratio of 30/1 and 50/1 (v/w). The laminarin content, total antioxidant activity, reducing power activity, and lipoxygenase inhibition activity at the aqueous to algae ratio of 20/1 (v/w) was 71.53%, 72.69%, 53.19%, and 80.32%, compared to the aqueous to algae ratio of 50/1 (v/w), respectively, and was 0.78, 0.85, 0.84, and 0.91 times in comparison to the aqueous to algae ratio of 30/1 (v/w). The solvent to algae ratio in the current study was different from the review notice of

SheKhar [1].

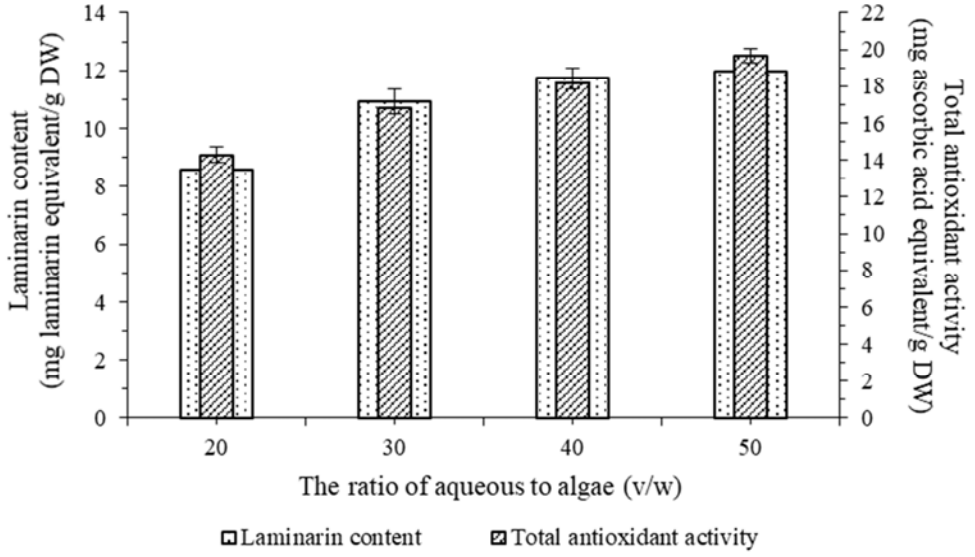

Figure 5. *Effect of the solvent to algae ratio on laminarin content and total antioxidant activity.*

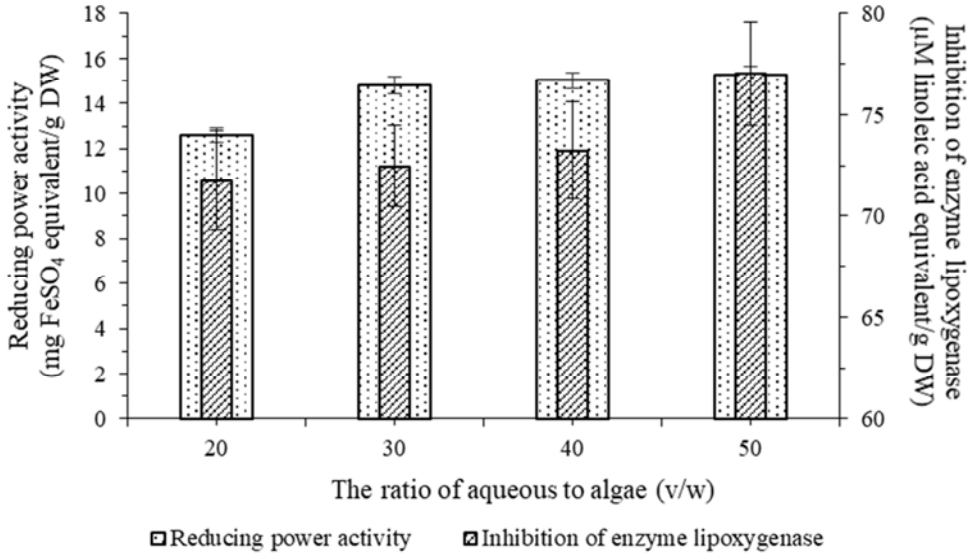

Figure 6. *Effect of the solvent to algae ratio on reducing power activity and lipoxygenase enzyme inhibition activity.*

3.1.4. Effect of the Solvent pH

The difference in solvent pH caused the significant difference of laminarin content, antioxidant activity, and lipoxygenase inhibition ($p<0.05$). Laminarin content at solvent (pH 2) was 74.09% and 61.35%, compared to solvent (pH 9) and solvent (pH 7), corresponding to 7.35±0.26 mg laminarin equivalent/g DW. Antioxidant activity and lipoxygenase inhibition activity got the highest value at solvent (pH 7) and the lowest value at solvent (pH 2) (Figure 7). The activity of total antioxidant, reducing power, and lipoxygenase inhibition was 11.96±0.42 mg ascorbic acid equivalent/g DW, 9.84±0.37 mg $FeSO_4$ equivalent/g DW, and 50.39±1.85 µM linoleic acid equivalent/g DW at solvent (pH 2), corresponding to 0.81%, 0.85%, and 0.95% in comparison to solvent (pH 9) (Figure 8). The solvent pH using for the extraction of laminarin depended on brown algae species and laminarin structure, as in the notice of Shekhar et al. [1].

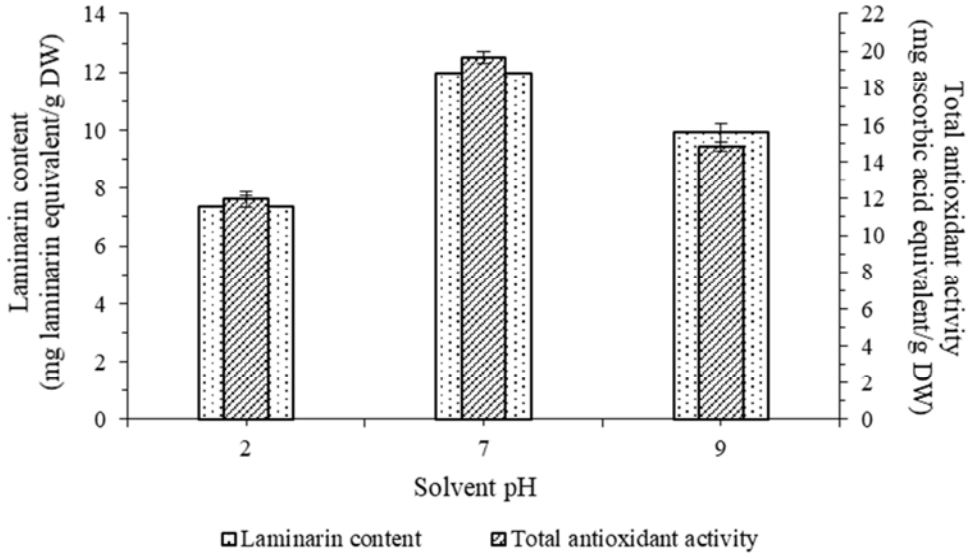

Figure 7. *Effect of solvent pH on laminarin content and total antioxidant activity.*

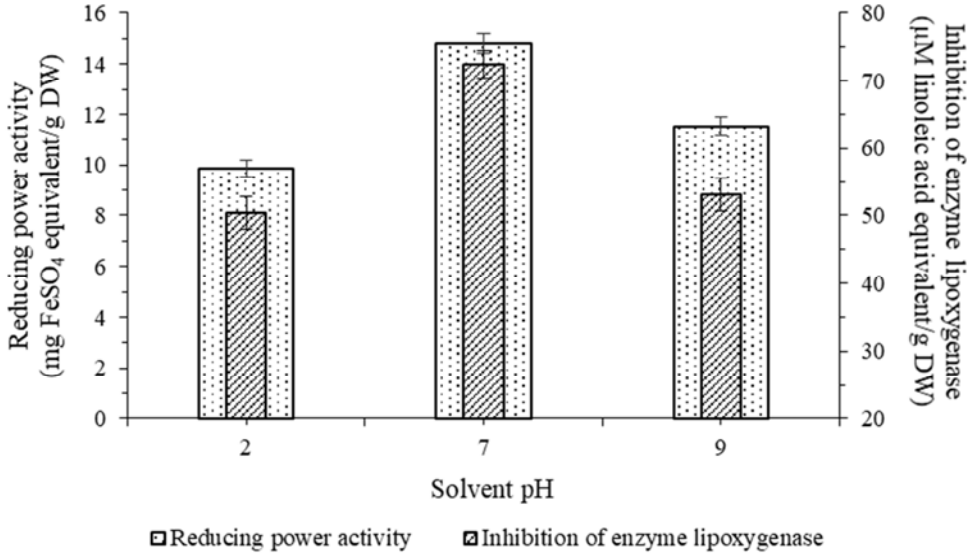

Figure 8. *Effect of solvent pH on reducing power activity and lipoxygenase enzyme inhibition activity.*

3.2. Laminarin Characterization

The average molecular weight and the viscosity of laminarin ware 505.18 kDa and 14.17±0.29 cPs, respectively. The average molecular weight was lower than alginate. LC/MS spectrum showed 07 fragments in laminarin structure consisting of 292.0837, 380.0946, 481.6256, 759.1812, 962.2458, 1138.2717, and 1517.3714 (m/z) (Figure 9). The sulfate content of laminarin (48.72%) was higher than the notice of Chen-Feng [5]. The retention time was suitable to the notice of Spicer et al. on laminarin extracting from Laminariales and Fucales [11].

FTIR spectrum exhibited function groups of laminarin characterization, for example, characteristic absorption peak at 3427.67, 1637.70, 1038.33, 1417.26, 1237.34, and 688.05, corresponding to the group of O-H, C=O, C-O, –C–H, $-O-SO_3^-$, and -CH=CH-(cis), respectively, that belonged to the vibration of O-H stretching, the stretching of symmetry and asymmetry, C-O stretching, –C–H bending, S≡O stretching, and -CH=CH-(cis) bend. The characteristic absorption peak at 2922.60 and 2854.87 was the vibration of C-H stretching belonging to $-CH_2-$ group or $–CH_3$ group (Figure 10). FTIR showed ring structures in laminarin molecular and stretching of different groups. The results exhibited the impact of the temperature, time, and physical chemistry factors caused by the change of chemistry characterization of laminarin.

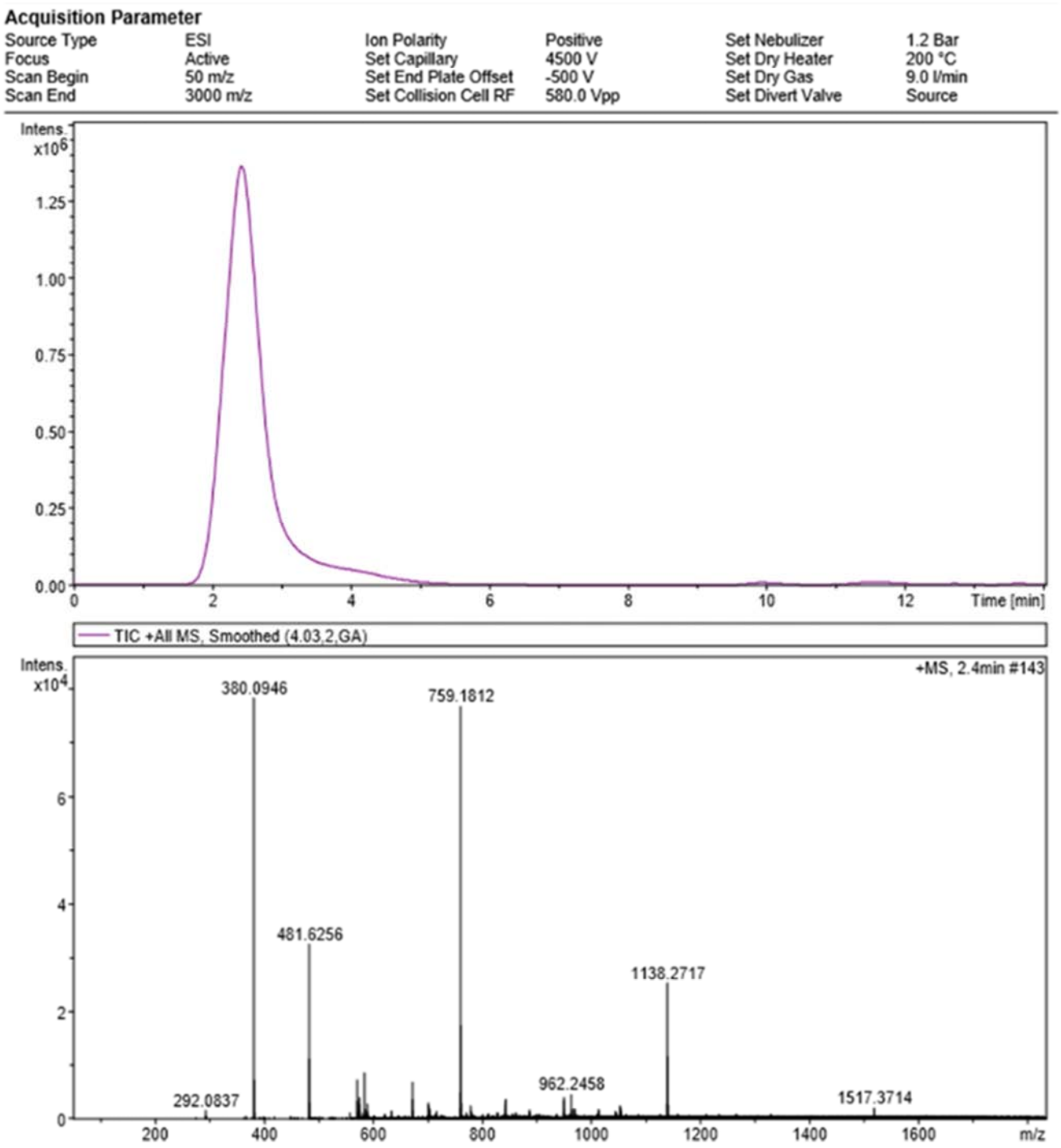

Figure 9. *LC/MS spectrum of laminarin.*

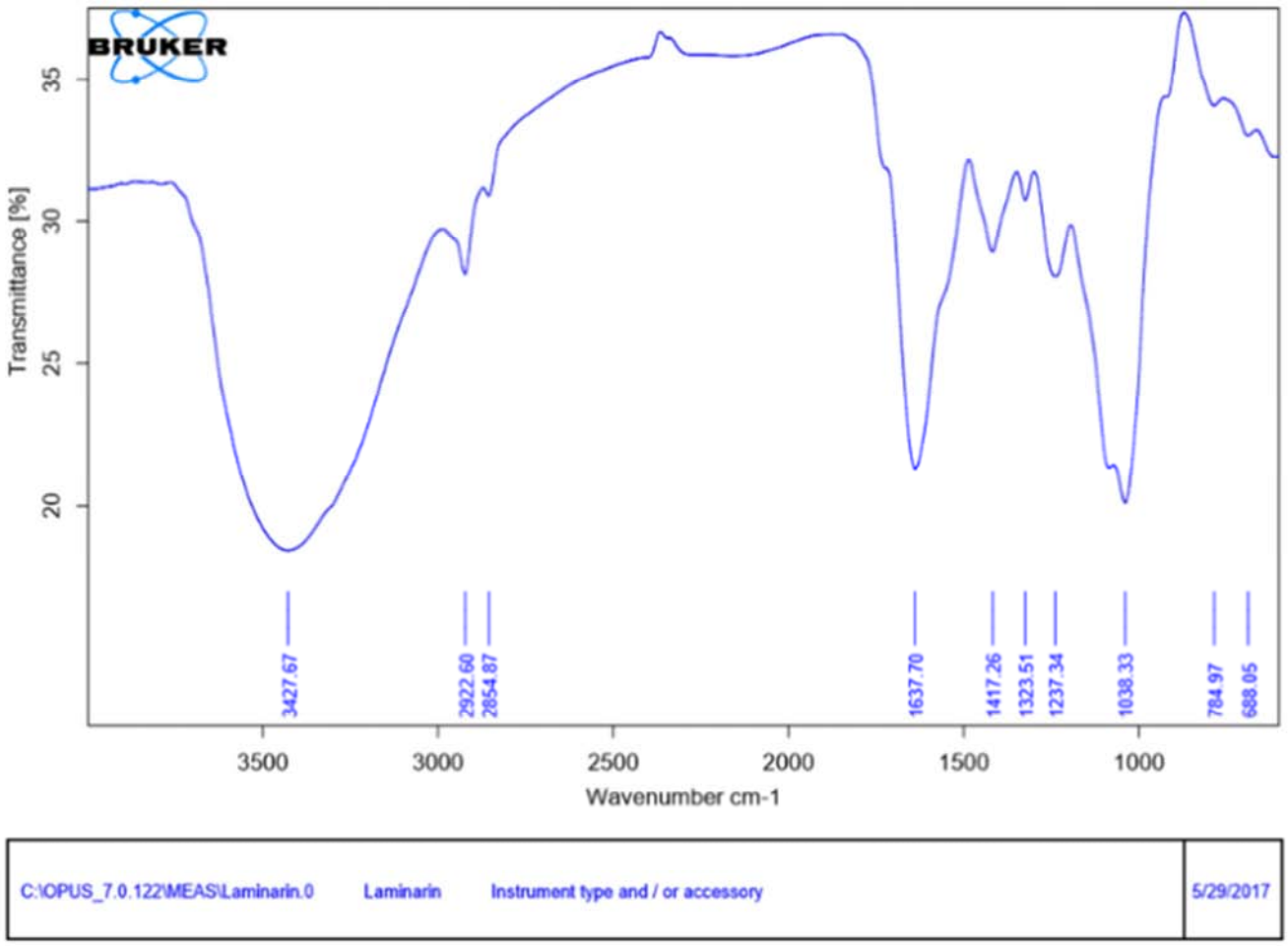

Figure 10. *FTIR spectrum of laminarin.*

Table 1. *Functional groups characterization of laminarin on FTIR spectrum*

Group	Vibration	Peak (cm^{-1})		
		Laminarin [5]	**Laminarin sulfate [5]**	**Laminarin in the current study**
O-H	The vibration of O-H stretching	3370	3441	3427.67
-CH_2-	The vibration of C-H stretching	2924	2978	2922.60, 2854.87
C=O	The stretching vibration of symmetry and asymmetry	1641	1649	1637.70
C-O	The vibration of C-O stretching	1043, 1076	1070	1038.33
–C–H	–C–H bending	1453.36–1385.50/cm		1417.26
-O-SO_3-	The vibration of S≡O stretching	-	1258	1237.34
-O-SO_3-	The vibration of C-O-S stretching	-	816	
-CH=CH-(cis)	The bend of -CH=CH-(cis)			688.05

The vibration of -OH stretching and C-H stretching in -CH_3 or -CH_2 groups occurred at the peak of 3370 cm^{-1} and 2924 cm^{-1}, respectively (Table 1). The stretching vibrations peaks of S=O and C-O-S exhibited the sulfate groups in the sugar molecules of laminarin, for example, 1258 cm^{-1} and 816 cm^{-1}, respectively.

4. Conclusion

Laminarin of brown algae *Sargassum mcclurei* grown commonly in Vietnam possessed antioxidant activity and lipoxygenase inhibition activity, got the highest value of 11.98±0.49 mg laminarin equivalent/g DW, 19.66±0.47 mg ascorbic acid equivalent/g DW, 15.55±0.61 mg $FeSO_4$ equivalent/g DW, and 73.04±2.53 µM linoleic acid equivalent/g DW, respectively. The suitable condition of extraction for activity laminarin was at 90°C for 2 hours in aqueous (pH 7) with the aqueous to algae ratio of 40/1 (v/w). Laminarin had the average molecular weight and the viscosity corresponding to 505.18 kDa and 14,17±0.29 cPs, respectively. Active laminarin composed of 7 fragments (292.0837, 380.0946, 481.6256, 759.1812, 962.2458, 1138.2717, and 1517.3714 (m/z)) with sulfate content of 48.72%. Laminarin of brown algae *Sargassum mcclurei* is a potential in the

application into functional foods and pharmaceutics.

Acknowledgements

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