Journal Biological Series 2(3): 082-085, July 2019
DOI: 10.15413/jbs2019.0108
ISSN: 2682-5724
©2019 Academia Publishing

Academia Publishing

Research Paper

Platelet anti-aggregation activity of hydrolized and irradiated crude fucoidan from Brown Seaweed (*Sargassum polycystum*)

Accepted 7th June, 2019

Kartiningsih[1,2], Deni Rahmat[2], Partomuan Simanjuntak[3] and Syamsudin Abdillah[4*]

[1]Faculty of Pharmacy, Universitas Pancasila, Indonesia.
[2]Department of Pharmaceutical of Technology, Faculty of Pharmacy, Universitas Pancasila, Jagakarsa, South Jakarta 12640, Indonesia.
[3]Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia.
[4]Department of Pharmacology, Faculty of Pharmacy, Universitas Pancasila, Indonesia.

*Corresponding author. E.mail: syamsudin.abdillah@gmail.com.

ABSTRACT

The platelet anti-aggregation activity of hidrolized and irradiatiated crude fucoidan from brown sea weed (*Sargassum polycystum*) was investigated in this study. The crude fucoidan of the brown seaweed was obtained by maceration using 2% $CaCl_2$ (w/v). The platelet anti-agregation activity test was carried out using the modified LTA method. This study aimed at examining platelet anti-aggregation activity of hydrolyzed and irradiated fucoidan. Thereafter, it was degraded by gamma irradiation in doses of 20, 40 and 60 kGy, respectively. Furthermore, hydrolysis with HCl (4, 6 and 8%) and cellulose enzyme (20, 40 and 60 µl) were also used to degrade fucoidan. The platelet anti-aggregation activity test was carried out using the modified LTA method. The results showed that the lowest molecular weight of degraded fucoidan obtained by gamma irradiation of 20 kGy was 753.16 Da and resulted in activity percentage of platelet anti-aggregation of 75.44%. Molecular weight of fucoidan hydrolyzed by HCl 8% was 714.48 Da, while its platelet anti-agregation activity was 61.91%. In addition, the cellulase enzyme hydrolysis of 60 µl produced degraded fucoidan with molecular weight of 714.67 and its platelet anti-agregation activity was 64.30%. It was concluded that the molecular weight reduction of fucoidan molecules by gamma irradiation demonstrated the highest platelet anti-aggregation activity.

Key words: Brown seaweed, crude fucoidan, HCl hydrolysis, cellulase enzyme, gamma irradiation, platelet anti-agregation

INTRODUCTION

Brown seaweed has various types of species. One of which is *Sargassum* species which contains alginate, laminarin and fucoidan. Fucoidan is a sulfate polysaccharide that can only be found in the leaves of brown seaweed. L-fucose and sulfate are its major contents (Wing, 2012; Atmadja et al., 1996). The platelet anti-aggregation activity can be obtained if the fucoidan has a low molecular weight. Therefore, the molecular weight reduction was initiated to achieve this. This study was carried out by adopting the hydrolysis method with HCl, w cellulase enzyme, and gamma irradiation (Silchenko et al., 2013; Woo et al., 2010; Choi and Kim, 2013; Choi et al., 2014).

The platelet aggregation is one of the factors responsible for blockages in the arterial blood vessels which leads to myocardial infarction or strokes. There are many platelet anti-aggregation drugs such as aspirin, clopidogrel and dipiridamol that are available in the market. However, there are some side effects associated with the prolonged use of these drugs. With regards to these side effects, it was therefore, necessary to develop a drug from natural materials which is safer than the chemically produced drugs (Gross and Weitz, 2009; The Royal Society of Chemistry, 2013).

The structure of fucoidans is similar to that of heparin. Heparin could be used for platelet anti-aggregation. For this reason, fucoidan is believed to possess platelet anti-aggregation characteristics similar to heparin (Woo et al., 2010).

Table 1: Results of identification of functional groups in crude fucoidan using FTIR.

Wave numbers (cm^{-1})		Reference wave number (cm^1)	Group / structure indication
Fucoidan comparative standard	Crude Fucoidan		
1225.57	1236.58	1280-1210	Sulfate group / bond S=O
1015,54	1088.61	1080-1010	Glycosidic bond (C-O-C) / bond (R-O-SO_3)
-	634.50	610	Fucose

In this research study, the various activities of platelet anti-aggregation of degraded fucoidan from brown seaweed were investigated.

MATERIALS AND METHODS

Collection and identification of plant material

The materials used in this research included brown seaweed (*Sargassum polycystum*) obtained from Garut, West Java. The plant was identified in the Department of Biology, Marine Biology Laboratory, Universitas Indonesia.

Preparation and extraction of the leaf extracts

The brown seaweed powder was macerated using 80% ethanol at a temperature of 20°C for 24 h. The residue obtained was remacerated using $CaCl_2$ at a temperature of 25°C for 6 h. Precipitation occured and the filtrate was collected. The obtained filtrate was concentrated and then dried at a temperature of 35°C for 48 h to obtain a dry extract of the brown seaweed (Wing, 2012).

The characterization of functional groups using Fourier Transform Infrared (FTIR)

One (1) mg of dried brown seaweed extracted was grounded with 200 mg of potassium bromide until an homogeneous mixture was obtained. Furthermore, the sample powder was transformed into a thin tablet at a pressure of 7000 Pa. The transformed tablet was put into a simple pan to produce an infrared spectrum at 4000 to 500 cm ^(-1) wave number (Sawant et al., 2011).

Degradation with gamma irradiation

The concentration of brown seaweed extract used was 20% (20 g of dry powder of brown seaweed with 100 ml distilled water). The extract solution was put into a brown bottle with a diameter less than 15 cm and a height less than 20 cm and was then degraded at a rate of 5.7096 kGy/h. Thereafter, the molecular weight was determined (Choi and Kim, 2013; Choi et al., 2014).

Hydrolysis with hydrochloric acid (HCl)

Five (5) g of dry extract were mixed with 100 ml of concentrated HCl (4, 6, 8% (v / v), respectively. The sample was heated at a temperature of 100°C and then stirred for 1 h. Thereafter, the molecular weight was determined (Saogo, 2016).

Hydrolysis with cellulose enzyme

A total of 2 g of dried extract of brown seaweed were dissolved in a solution of 0.02 M to 100 ml acetate acid at a pH value of 5.5. Three botles were provided and 20 µl, 40 µl, 60 enzymes (110 Ui), respectively added to each botle containing the solution and the mixture was shaken until an homogeneous mixture was obtained. The mixture was incubated at a temperature of 37°C for 12 h. The results obtained were centrifuged and the molecular weight determined (Woo et al., 2010)

In-vitro testing of platelet anti-aggregation activity

The platelet anti-agregation activity test was carried out using the modified LTA method. 1 ml of the PRP samples was added to 3 ml of 0.9% NaCl. Each sample was prepared in concentration of 125, 250, and 500 µg/ml, respectively. In addition, 50 µl of ADP was added to each sample and the absorption measured using a spectrophotometer at a wavelength of 600 nm. The mixture was then incubated at a temperature of 37°C for 20 min. Thereafter, the absorption was measured and the inhibition percentage of platelet aggregation calculated (Dewanto et al., 2011; Bucki et al., 2003; Michelson, 2006).

RESULTS AND DISCUSSION

Based on the FTIR measurement results earlier mentioned, it can be deduced that the comparative material and fucoidan at 1225.57 cm^{-1} and 1223.58 cm^{-1} wave numbers indicates sulfate groups. At 1015.54 cm^{-1} and 1088 cm^{-1} wave numbers, the glycoside bond group was obtained. At 634.50 cm^{-1} wave number, it displays the fucosan group in fucoidan with no comparable glucose groups observed (Table 1). Therefore, the crude fucoidan from brown seaweed (*S. polycystum*) growing in Garut contains sulfate

Table 2: The molecular weight test results with LC MS method.

S/No.	Sample	Molecular weight (Da)
1	BP Fucoidan	237
2	Crude Fucoidan	754,68
3	Crude Fucoidan irradiasi gamma (20, 40, 60 kGy)	753,23
4	Crude Fucoidan hidrolysis HCl (46.8%)	714,48
5	Crude Fukiodan Hidrolisis Enzim Selulose (20, 40, 60 µl)	714,67

Table 3: The results of crude fucoidan platelet anti-aggregation activity before and after molecular weight reduction.

Sample concentration (µg/ml)	Brown Seaweed extract (%)	Chloride acid hydrolysis			Hydrolysis cellulase enzyme			Gamma irradiation		
		4	6	8	20	40	60	20	40	60
		Percentage (%)			(µl)			(kGy)		
125	13.76	47.60	48.90	54.72	56.71%	57.34%	59.66%	73.97%	68.18%	67.22%
250	18.45	49.09	52.57	56.80	61.58%	61.87%	62.42%	74.85%	73.84%	68.45%
500	22.19	55.48	56.26	61.91	62.26%	62.83%	64.30%	75.44%	74.15%	72.95%

groups and glucose groups and possesses platelet anti-aggregation features.

From the results obtained from measuring the molecular weight using LC-MS method, it can be deduced that the lowest molecular weight of fucoidan was obtained using gamma irradiation method with dose of 20 kGy (Table 2). The results were collected when crude fucoidan was degraded using gamma irradiation and hydrolysis. Meanwhile, the irradiation with doses of 40 and 60 kGy produced the same molecular weight owing to the saturation of the irradiation dose. The effect of radiation could break the glycoside bond chain in polymer degradation. It results to a decrease in molecular weight due to the random breaking of the polymer chain bonds. The effects of radiation can cause ionization or excitation electrons in atoms and as a result of cutting this chain polymer degradation can occur due to molecular weight reduction in the random termination of polymer chains. Fucoidan with low molecular weight shows platelet antioxidant activity, anti-aggregation and higher anticoagulants.

Table 3 displays the result of platelet anti-aggregation activity of crude fucoidan before and after the molecular weight reduction. The highest value was obtained using crude fucoidan with gamma irradiation degradation method at a dose of 20 kGy resulting in 75.44% of activity percentage as compared to hydrolysis using HCl and cellulase enzymes. This result was obtained using gamma irradiation method in which gamma rays from cobalt-60 with a half-life of 5.26 years, quantum energy 1.17 and 1.33 MeV and cesium-137 with a half-life of 30 years and 0.66 MeV quantum energy were used. From the results of molecular weight examination with the LC-MS method, both HCl hydrolysis of 8%, hydrolysis with cellulose enzymes and irradiation of 20 kGy have molecular weight values that are not much different; however, the strongest platelet aggregation activity is by irradiation of 20 kGy.

Low molecular weight Fucoidan can be made by acidic, enzymatic and radical methods. With the acid method, higher temperature or acidity causes lower molecular weight products, but the sulfate group content is also the bioactivity reduced. It was recently reported that acid hydrolysis tends selective in the desulfatization of the position of the first 2 phase units, while the relationship between glycosidic non-sulfate fukose residues and 4-sulfate residues is cut by acid hydrolysis.

Conclusion

FTIR results showed that crude fucoidan from brown seaweed from Garut, West Java contained sulfate groups. With the reduction in molecular weight of the LC-MS method both 8% HCl hydrolysis, 60 µl cellulose enzyme, 20 kGy irradiation produced molecular weights not much different. Hydrolysis with acids and enzymes is assumed to reduce the sulfate group content as compared to 20 kGy irradiation which has platelet aggregation activity. The highest platelet anti-aggregation activity of degraded fucoidan was 75.44% which was close to the activity of clopidogrel as positive control (76.53%).

RECOMMENDATIONS

The findings in this research work confirmed the platelet anti-aggregation activity of hidrolyzed and irradiatiated crude fucoidan from brown sea weed (*S. polycystum*). Therefore, to take the work to a beneficial level, the following recommendations were made:

1) Further research work should be to molecular mechanism of crude fucoidan for platelet anti-aggregation activity;
2) The crude fucoidan should be explored for the *in vivo* platelet anti-aggregation activity.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Ministry of Research, Technology and Higher Education Republic of Indonesia (Kemenristekdikti) for the funding support of the research project (Hibah Penelitian Tim Pasca Sarjana).

REFERENCES

Atmadja WS, Kadi A, Sulistyo, Rachmaniar S (1996). Pengenalan jenis jenis rumput laut indonesia. Puslitbang Oseanografi-LIPI;. Pp.120-53.

Bucki R, Pastore TJJ, Giraud F, Sulpicejand JC, Janmey PA (2003). Flavonoid inhibition of platelet procoagulant activity and phosphoinositide synthesis. J. Thrombosis and Haemostasis. *1*(8): 1820–1828. https://doi.org/10.1046/j.1538-7836.2003.00294.x

Choi J, Gu LS, Jong HS, Cho M, Cheon LP (2014). Effect of gamma irradiation on the structure of fucoidan. Radiation Physics and Chemistry. 100: 54–58. https://doi.org/10.1016/j.radphyschem.2014.03.018

Choi J, Il, Kim HJ (2013). Preparation of low molecular weight fucoidan by gamma-irradiation and its anticancer activity. *Carbohydrate Polymers*. *97*(2): 358–362. https://doi.org/10.1016/j.carbpol.2013.05.002

Dewanto, Mutripah S, Iskandar Y, Intan Barliana M (2011). Potensi Rumput Laut Coklat Jenis Sargassum duplicatum yang berasal dari Perairan Menganti-Kebumen sebagai Antiagregasi Platelet. pp.1–14.

Gafurjon T, Jung ML, Heon-Sub S, Tae-Hoo Y, Ibrokhim YA (2011). Low Molecular Fucoidan and its Macromolecular Complex with Bee Venom Melittin. Scientific Research. 2: 298-303.

Gross PL, Weitz JI (2009). New antithrombotic drugs. *Clinical Pharmacology and Therapeutics*. https://doi.org/10.1038/clpt.2009.98

Michelson AD (2006). Evaluation Of Platelet Function By Flow Cytometry. *Pathophysiology of Haemostasis and Thrombosis*. *35*(1–2): 67–82. https://doi.org/10.1159/000093547

Saogo, Krisye M (2016). Hidrolisis Rumput Laut Coklat *Sargassum* sp (CP 02) dengan Kapang Laut RN dan Aktivitas Hidrolinat Sebagai Inhibitor Tirosinase. Departemen Teknologi Hasil Perairan Fakultas Perikanan dan Ilmu Kelautan Institu Pertanian Bogor.

Sawant SD, Baravkar AA, Kale RN (2011). FT-IR spectroscopy: Principle, technique and mathematics. Int. J. Pharma. Bio Sci. *2*(1): 513–519.

Silchenko AS, Kusaykin MI, Kurilenko VV, Zakharenko AM, Isakov VV, Zaporozhets TS, Zvyagintseva TN (2013). Hydrolysis of fucoidan by fucoidanase isolated from the marine bacterium, formosa algae. Marine Drugs. 211(7): 2413–2430. https://doi.org/10.3390/md11072413

The Royal Society of Chemistry (2013). Fucoidans from Brown Seaweed: An Update On Sctructures, Extraction Technics and Use of Enzyme as Tools for Structural Elucidation.

Wing W (2012). Extraction, Characterization and Antioxidant Activity of Fucoidan from New Zealand *Undaria pinnatifida* (Harvey) Suringar (tesis). New Zealand: Auckland University of Technology.

Wing W (2012). Extraction, Characterization and Antioxidant Activity of Fucoidan from New Zealand *Undaria pinnatifida* (Harvey) Suringar (tesis). New Zealand: Auckland University of Technology.

Woo JK, Yean-Kyoung K, Mi-Kyung J, Hye RM, et al (2010). Anticoagulating Activities of Low-Molecular Weight Fuco-Oligosaccharides Prepared by Enzymatic Digestion of Fucoidan from the Sporophyll of Korean *Undaria pinnatifida*. Pharmacal Research. 33(1): 125-131

Cite this article as:

Kartiningsih, Rahmat D, Simanjuntak P, Abdillah S (2019). Platelet anti-aggregation activity of hydrolized and irradiated crude fucoidan from Brown Seaweed (Sargassum polycystum). J. Biol. Ser. 2(3): 082-086.

Submit your manuscript at
http://www.academiapublishing.org/journals/jbs