International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.06, pp 190-195, 2018

Preparation of nanoparticles of the extract of the extract of brown seaweed (*Sargassum polycystum*) and *in vivo* anti-platelet testing

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Abstract : Brown seaweed (*Sargassum polycystum*) contains fucoxanthin and flavonoids where platelet anti-aggregation activities are found. Brown seaweed is macerated in 96% ethanol. The formation of nanoparticles can improve the stability and activity of brown seaweed extracts. This research was conducted to determine the ratio of the platelet anti-aggregation effect between extracts and nanoparticles of brown seaweed. The nanoparticles were made using the method of ionic gelation by mixing 1% chitosan solution with 0.1% brown seaweed extract solution which were then reacted with 0.1% NaTPP and 1% tween80. Those nanoparticles were characterized by an average particle size of 294.9 nm; the average zeta potential by 38.2 mV; and spherical and irregular particle morphology. The platelet anti-aggregation activities differ significantly in bleeding time and coagulation time between the extracts and the nanoparticles of brown seaweed. The percentages of increases in the bleeding time and the coagulation time with a dosage of extracts by 400 mg/kg BW were 23.53% and 30.55%, respectively; while the percentages of increases in the bleeding time and the coagulation time with a dosage of nanoparticles by 400 mg/kg BW were 61.15% and 63.50%.
Keywords : brown seaweed extracts, nanoparticles, fucoxanthin, chitosan, sodium tripolyphosphate, anti-aggregation.

Introduction

Indonesia is the world's largest archipelagic state, with a land area of only 1.9 million km2, then 75% of Indonesia's territory consists of oceans, comprising 3.1 million km2 of territorial waters and 2.7 million km2 of the exclusive economic zone (EEZ) (1). Plenty of Indonesia's marine biodiversity has been utilized. One of the marine resources which has already been utilized is macroalgae, aka seaweed. There are three types of seaweed, namely red seaweed (rhodophyta), green seaweed (clorophyta), and brown seaweed (phaeophyta). Brown seaweed type *Sargassum* contains fucoidan (2), flavonoids (3), alginate, protein, vitamin C, tannin, iodine, phenol, and pigments, namely fucoxanthin, xanthophylls, β-carotene, chlorophyll, and chlorophyll derivatives (4).

DOI= http://dx.doi.org/10.20902/IJCTR.2018.110624

Findings of the research by Dewanto into brown seaweed type *Sargassum duplicatum* from the waters of Mengumen, Kebumen in vitro suggest that the platelet anti-aggregation value of acetosal as the positive control is equal to 48.49%, the n-hexane fraction with a dosage by 500 µg/mL is equal to 55.51%, and the ethyl acetate fraction with a dosage by 500 µg/mL is equal to 52.10% (3).

The application of nanotechnology in pharmacology offers several benefits, among others, to increase the solubility of a compound, to increase effectiveness and efficiency of orally and intravenously administered drugs, to reduce the dosage of treatment, and to increase absorption (5).

Nanoparticles were prepared using the ionic gelation method, by mixing chitosan solution with brown seaweed extract solution, then they were reacted with sodium tripolyphosphate solution. The resulting nanosuspension was dried using the freeze drying method to obtain chitosan nanoparticles of the brown seaweed extracts which are physically and chemically more stable. To this end, medicines made from natural ingredient derived from brown seaweed (*Sargassum polycystum*) is developed to examine the effect of platelet anti-aggregation that can prolong the bleeding time and the coagulation time of brown seaweed extracts and brown seaweed extract nanoparticles.

Experimental

Analytical scales, macerator/stirrer, magnetic stirrer, Scanning Electron Microscope (SEM), particle sizer, zeta potential, freeze dryer, vaporizer cup, rotavapor, pestle, mortar, stirrer, cotton, filter paper, 1.0 ml syringe, oral feeding tube, sterile lancet/razor, holder, surgical scissors, mouse cage, mouse scales, and glassware

Materials

Brown seaweed (*Sargassum polycystum*), 96% ethanol, calcium carbonate, chitosan, glacial acetic acid, sodium tripolyphosphate, tween 80, pure water, and ascardia tablets

Method

Preparation of nanoparticles of the brown seaweed extracts

Chitosan was dissolved in 1% v/v glacial acetic acid to obtain 1% v/v chitosan stock solution. As many as 100 mg of brown seaweed extracts was dissolved in 100 mL of 96% ethanol. 30 mL of chitosan solution was mixed with 70 mL of brown seaweed extract solution. 0.1% w/v tripolyphosphate solution was prepared in pure water and 16.5 mL of it was added by slowly dripping it into the mix of chitosan and brown seaweed extracts while stirring this mix using a stirrer. 0.1% v/v tween 80 solution was prepared in pure water and 5 mL of it was added to the nanoparticle suspension. The solution was stirred using a magnetic stirrer for 30 minutes.

Characteristics of the nanoparticles

Determination of the distribution of particle sizes

A total of 100.0 µL of the nanoparticle suspension was dispersed in 50.0 mL of aquadest and measured using particle sizer *DelsaNanoTM*. Experiments were undertaken using titration three times (*triplo*). The distribution of particle sizes was determined based on this test.

Determination of zeta potential

A total of 100.0 µL of the nanoparticle suspension was dispersed in 50.0 mL of aquadest and measured using particle sizer *DelsaNanoTM*. Experiments were undertaken using titration three times (*triplo*). The magnitude of zeta potential was determined based on this test.

Morphology of the nanoparticles nanopartikel using scanning electron microscopy (SEM)

Scanning Electron Microscope (SEM) was used in morphological observation and determination of nanoparticle sizes. This method is an efficient way to get the image of the specimens' surface. This microscope functions by emitting electrons onto the specimens' surface. Information about the surface of the particles can

be obtained by introducing a probe into the pathway of the electron beam that comes into contact with the particles' surface. Information can also be carried by the probe that captures electrons in the tunnel between the surface of the specimen particle and the probe tip or a probe that captures the thrust between the surface and the probe tip.

Experimental Animals

The experimental animals used were 32 male DDY mice aged 2-3 months with a weight ranging from 25 to 35 grams

Anti-platelet Activity Testing

Determination of the Bleeding Time

Mice were put into the holder. The end of the mice's tail was cleaned using 70% alcohol then the tail of the mouse was injured 2 cm away from the end of the tail to a depth of 2 mm using a razor blade. The blood dripping was absorbed by filter paper. The interval between the time when blood firstly dripped and the time when it stopped dripping is the bleeding time (6,7,8,9).

Determination of the Coagulation Time

Blood samples were taken by cutting the end of the mice's tail using surgical scissors. Blood that came out was dripped on the glass object and measured the time when fibrin threads were formed was measured. Coagulation time is defined as the time when blood firstly dripped until fibrin threads were formed for the first time (9).

Results and Discussion

Results of the Examination of Brown Seaweed Extract Nanoparticles

Brown seaweed extracts with phytochemical filtration contain flavonoid compound and using the spectrophotometric method with a maximum wavelength of 445.0 nm, they contain fucoxanthin compound.

Results of the Measurement of the Particle Size of Brown Seaweed Extract Nanoparticles

Formation of cross-links is influenced by the addition of chitosan and NaTPP using the ionic glass method. The principle of this method is the existence of ionic interactions between the amino groups of chitosan that are positively charged and polyanion that are negatively charged which later form intramolecules. The particle size obtained by 294.9 nm meets the requirement, i.e. ranging from 10 to 1000 nm. This is because the formation process was undertaken using the ionic glass method. In addition, the size of the particles was also influenced by the addition of tween as a stabilizer used to prevent particles from aggregating (Table 1). The Index of Polydispersity (IPD) obtained was equal to 0.416 and this value approaches zero, thus it can be concluded that the deviation of size uniformity in measurement is getting increasingly small and the nanoparticles generated have relatively the same size.

Table 1. Particle Size of Brown Seaweed Extract Nanoparticles

	Particle size (nm)
Mean	294,9
Polydispersity index	0,416

Table 2. The Zeta Potential Measurement of Brown Seaweed Extract Nanoparticles

No.	**Zeta Potential (mV)**
1.	38,6
2.	39,4
3.	36,5
Mean	38,2

Results of the Zeta Potential Measurement of Brown Seaweed Extract Nanoparticles

The value of potential zeta obtained is equal to 38.2 mV, which indicates that the particles in the nanosuspension are well distributed and prevent from aggregating. The potential zeta has a positive value because the amino groups (NH_3^+) of chitosan and the (O^-) groups of NaTPP which are polyanion compound form a cross-link (Table 2).

Examination Results Using Scanning Electron Microscopy (SEM) of Brown

Morphology of brown seaweed extract nanoparticles was examined using the Scanning Electron Microscope (SEM) with 1000x and 2000x magnification. Research findings suggest that the morphology of the particles generated using the ionic gelation method was spherical. In the absence of the addition of NaTPP, morphology of the particles will be in the form of membrane sheets. Therefore, the addition of NaTPP which determines the size of the particles makes the resulting nanoparticles have spherical shape. In addition, this spherical shape is also influenced by optimization of the concentration of chitosan and NaTPP (Figure 1)

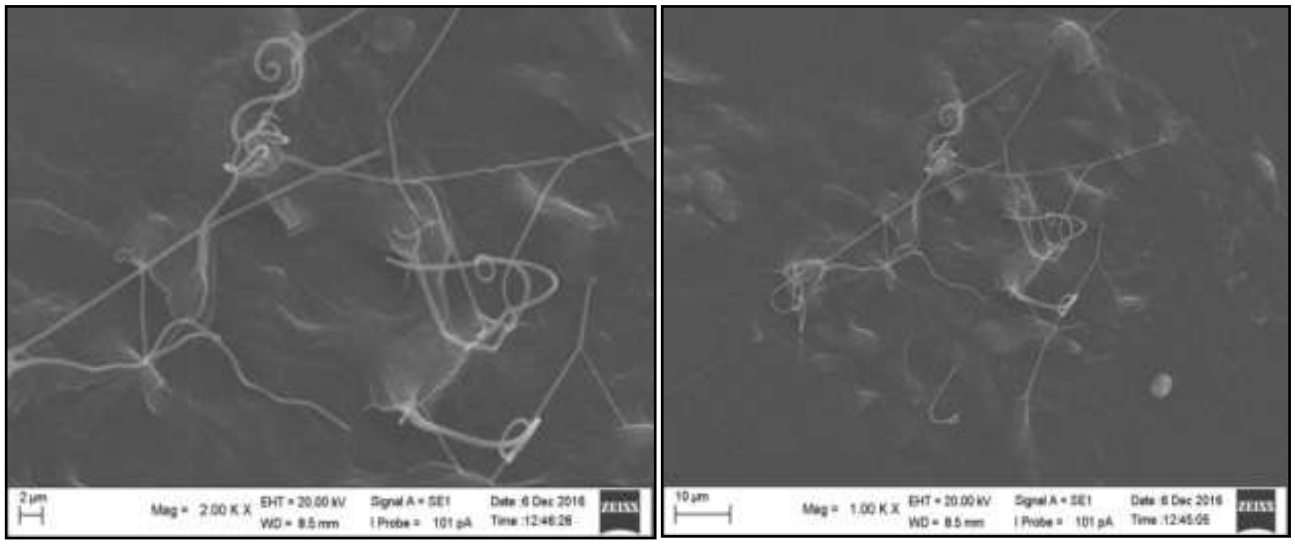

Figure 1. Scanning Electron Microscopy (SEM) of Brown Seaweed Extract Nanoparticles

Based on the percentage of the increase in the bleeding time and the coagulation time, it is revealed that the nanoparticles of brown seaweed have higher platelet anti-aggregation activities than those of the extracts of brown seaweed. The nanoparticles of brown seaweed extracts have shorter bleeding time than that of the positive control group, i.e. acetosal, with a dosage of 80 mg/kg BW, meaning that the ability of the nanoparticles of brown seaweed extracts with a dosage of 100, 200, or 400 mg/kg BW is less effective than that of the positive control, i.e. acetosal, with a dosage of 80mg/kg BW. The nanoparticles of brown seaweed extracts with a dosage by 100 and 200 mg/kg BW have shorter coagulation time than that of the positive control group, i.e. acetosal, with a dosage of 80 mg/kg BW, meaning that the ability of the nanoparticles of brown seaweed extracts with a dosage of 100, or 200 mg/kg BW is less effective than that of the positive control, i.e. acetosal, with a dosage of 80mg/kg BW (Table 3).

Table 3. The Bleeding Time and Coagulation Time Measurement

Group	Mean bleeding time			Mean coagulation time		
	Day 0	Day 8th	Δ%	Day 0	Day 8th	Δ%
Extract Dose 100mg/kgBW	72,5±6,24	73,5±6,24	1,38	87,75±6,31	100,75±5.64	14,81
Extract Dose 200mg/kgBW	84±8,81	94,5±7,81	12,5	93,75±7.34	110,5±10,2	17,87
Extract Dose 400mg/kgBW	89,25±8,45	110,25±9,35	23,53	86,75±8,34	113,25±9,24	30,55
Nanoparticle Dose 100mg/kgBW	82,25±7,23	116,5±10,24	41,64	100,5±9,56	123,75±9,45	23,13
Nanoparticle Dose 200mg/kgBW	115,5±10,24	138,5±9,56	19,91	99±9,50	130,75±10,25	32,07
Nanoparticle Dose 400mg/kgBW	104,25±9,45	168±10,23	61,15	97,25±8,34	159±9,56	63,50

The mechanisms underlying the effect of prolonged bleeding time in the addition of 96% ethanol extracts and nanoparticles of brown seaweed (*Sargassum polycystum*) are estimated to be chained by the work mechanisms of the compounds found in brown seaweed, namely fucoidan and flavonoids. Fucoidan compounds can prevent platelet aggregation induced by thrombin. Thrombin can activate platelets through the G-protein receptors, namely protease-activated receptors-1 (PAR-1). Inhibition of PAR-1 will lead to inhibition of platelet activation. Flavonoid compounds can prevent platelet aggregation by inhibiting the activities of the enzyme *cyclooxygenase* so as to reduce the synthesis of thromboxane A2. Favonoid compounds can inhibit the various stages of the formation of atherosclerosis, endothelial damage, leukocyte activation, adhesion, aggregation, and platelet secretion.

Conclusions

1. Nanoparticles of the extracts of brown seaweed (*Sargassum polycystum*) made from chitosan-NaTPP polymers using the ionic gelation method meet the physical quality requirements for nanoparticles. On the average, the particle size is 294.9 nm, the average zeta potential is 38.2 mV, and in terms of morphology, the resulting nanoparticles are spherical.
2. The nanoparticles and the extracts of brown seaweed with a dosage of 100, 200, and 400 mg/kg BW differ in bleeding time and coagulation time. Research findings suggest that the extracts and nanoparticles of brown seaweed with a dosage by 100, 200, and 400 mg/kg BW can prolong the bleeding time and the coagulation time in mice. The longest bleeding time and coagulation time are found in the nanoparticles of brown seaweed extracts with a dosage of 400 mg/kg BW, i.e. by 61.15% and 63.50%, respectively.

References:

1. Hon Yip W, Joe LS, Mustapha WAW, Maskat MY, Said M. Characterisation and Stability of Pigments Extracted from Sargassum binderi Obtained from Semporna, Sabah. Sains Malaysiana. 2014;43(9):1345–54.
2. Dewanto, Mutripah S, Iskandar Y, Intan Barliana M. Potensi Rumput Laut Coklat Jenis Sargassum duplicatum yang berasal dari Perairan Menganti-Kebumen sebagai Antiagregasi Platelet. 2011;1–14.
3. Chelucci RC, Dutra LA, Elisa M, Pires L, Regina T, Melo F De, et al. Antiplatelet and Antithrombotic

Activities of Non-Steroidal Anti-Inflammatory Drugs Containing an N-Acyl Hydrazone Subunit. molecules. 2014;2:2089–99.

4. Peng J, Yuan J, Wu C, Wang J. Fucoxanthin , A marine carotenoid present in brown seaweeds and diatoms : metabolism and bioactivities relevant to human Health. Marine Drugs. 2011;1806–28.
5. Zhang H, Tang Y, Zhang Y, Zhang S, Qu J, Wang X, et al. Fucoxanthin : A promising medicinal and nutritional ingredient. Evidence-Based Complementary and Alternative Medicine. 2015.
6. Zhao X, Dong S, Wang J, Li F, Chen A, Li B. A Comparative study of antithrombotic and antiplatelet activities of different fucoidans from *Laminaria japonica*. Thrombosis Research. Elsevier Ltd; 2012; 771-778.
7. Zhu Z, Zhang Q, Chen L, Ren S, Xu P, Tang Y, et al. Higher specificity of the activity of low molecular weight fucoidan for thrombin-induced platelet aggregation. Thrombosis Research. Elsevier Ltd; 2010;125(5):419–26.
8. Yadav, Hemant K.S., Nagavarma B V N, Ayaz A, Vasudha L.S., Shivakumar H.G, (Review Article) Different Techniques For Preparation Of Polymeric Nanoparticles, Asian Journal of Pharmaceutical and Clinical Research, Vol. 5, Suppl 3, 2012;16-23.
9. Pathak Y, Thassu D. Drug Delivery Nanoparticles Formulation and Characterization. Vol. 191. New York: informa healthcare; 2009.
10. Chelucci RC, Dutra LA, Elisa M, Pires L, Regina T, Melo F De, et al. Antiplatelet and Antithrombotic Activities of Non-Steroidal Anti-Inflammatory Drugs Containing an N-Acyl Hydrazone Subunit. molecules. 2014;2:2089–99.
11. Bucki R, Pastore J., Giraud F, Sulpice JC, Janmey PA. Flavonoid inhibition of platelet procoagulant activity and phosphoinositide synthesis. Journal Thrombosis Haemostasis. 2003;1820–8.

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