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The Effect of Increasing Solubility with Cosolven and PVP on the Preparation and Characterization of Brown Seaweed Extract (*Sargassum Polycystum*) Nanoparticle as Antioxidants

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Abstract: ***Brown seaweed (Sargassum polycystum) is one of the seaweed groups that has high antioxidant activity. This activity comes fromFucoxanthin, a caotenoid-derivative molecules. The objective of the study is improving solubility of brown seaweed extract in aiming to make a stable and meeting the requirement of nanoparticle characterization test.Brown seaweed was extracted then the solubility of brown seaweed extract was increased by using the cosolventand solid dispersion method. The formula with best solubility test results then be used in the process of making nanoparticles by ionic gelation method by using chitosan as a polymer and sodium tripolyphosphate as a crosslinker agent. Brown seaweed extract was dried by freeze drying method. Then it became powdered brown seaweed extract nanoparticles. From this study, it was obtained that the particle size and the potential zeta of cosolven-based nanoparticle and PVP-based nanoparticles were 226.2 nm and 50.40 mV ; 365.1nm, and 26.56 mV, respectively. The morphology of brown seaweed extract nanoparticles were spherical and amorphous which was supported by TEM and XRD results. Then an antioxidant activity test was carried out by using the ABTS method.IC_{50}values of brown seaweed extract, cosolvent-based and PVP-based nanoparticles were 93.98 ppm, 98.72 ppm,and 97.63ppm, respectively.This study showed that there was significant differences of antioxidant activity betweenbrown seaweed extract, cosolven-based nanoparticle, and PVP-based nanoparticles (one-wayANVA (0.000) < 0.05).***

Keywords: brown seaweed extract, increasing solubility, PVP, cosolvent, nanoparticles, antioxidant.

1. Introduction

Free radicals are molecular atoms or molecules that have unpaired electrons. The unpaired electrons cause highly reactive free radicals which then capture or take electrons from other compounds to neutralize themselves. Accumulation of these damage contributes to various degenerative diseases (1). The negative effects of free radicals on the body can be prevented by compounds called antioxidants. Antioxidants have the ability to give electrons, bind and end free radical chain reactions (2). Brown seaweed is one of the seaweed groups that has the highest antioxidant activity when compared to red algae and green algae. One of the pigments from the carotenoid group that has the potential as an antioxidant is fucoxanthin. The fucoxanthin content (20.95%) of brown seaweed (Sargassumsp) has the potential as an antioxidant and chemopreventive agent because of its ability to reduce free radicals and prevent various degenerative diseases (3). The solubility of fucoxanthin in water is low, so that solubility can be increased by using solubility enhancing methods, namely chemical modification, complexation, cosolven, micellarsolubilization, and solid dispersion (4).

Nanoparticles can be prepared by ionic gelation using chitosan and sodium tripolyphosphate as crosslinker agent. Ionic gelation method is chosen because it is an easy method, has good biocompatibility, and does not require organic solvents (5). The brown seaweed extract nanoparticles formed were then characterized by organoleptic properties, size and size distribution of nanoparticles using Particle Size Analyzer (PSA), morphology of nanoparticles using Transmission Electron Microscopy (TEM) to determine the shape and surface conditions of nanoparticles, Zeta Potential to characterize charge properties nanoparticle surface, and identification of crystal structures with X-ray diffraction. Furthermore, the antioxidant activity of brown seaweed extract and brown seaweed extract nanoparticles (Sargassumpolycystum) was carried out with ABTS damping method (2.2 Azinobis (3-ethylbenzotiazolin) -6- sulfonic acid) (5,6).

2. Experimental

2.1 Materials

Brown seaweed (*Sargassumpolycystum*), Chitosan, Sodium Tripolyphosphate, BP Fucoxanthin, Ethanol 96% P, Glycerin, Propylene Glycol, Tween 80, DMSO 10%, PVP, Glacial acetic acid P, ABTS (2.2 Azinobis (3-ethylbenzotiazolin) -6- sulfonic acid), Ethanol pro analysis, BP Vitamin C, K2S2O8 and pure water.

2.2 Instrument

Analytical scales, glassware, volumetric devices, maserators, rotavapor, Transmission Electron Microscopy, UV-Vis 1800-UV spectrophotometer, rotavapor, freeze dryer SB 6, Particle Size Analyzer, zeta sizer, pH meter, magnetic stirrer, and XRD.

2.3 Methods

Preparation of Brown Seaweed Extract (*Sargassum Polycystum*)
500 g of brown seaweed (*Sargassumpolycystum*) used is washed with water and then drained. Making extracts was done by maceration with 96% ethanol as much as 5 L for 2 x 24 hours with the addition of 5 g of $CaCO_3$ as a neutralizer first.

Preparation of Nanoparticles of the brown seaweed extracts

a) **Cosolven-based nanoparticles:** Chitosan was dissolved in 1% v / v glacial acetic acid to obtain a 1% v / v chitosan stock solution. As many as 100 mg brown seaweed extract was dissolved with cosolven (20 mL PPG, 15 mL glycerin, 15 mL 96% ethanol, 4 mL Tween 80, and 6 mL DMSO 10%). 30 mL of chitosan solution was taken and mixed with a solution of brown seaweed extract. 0.2% w / v tripolyphosphate solution was made in pure water and 15 mL was added by slowly dropping it into a chitosan mixture at a speed of 1 drop / 3 seconds in stirring using a magnetic stirrer with a stirring speed of 250 rpm to form a homogeneous turbidity. The solution is still stirred with a magnetic stirrer stirrer for 30 minutes.
b) **Nanoparticles with PVP:** Chitosan was dissolved in 1% v / v glacial acetic acid to obtain a 1% v / v chitosan stock solution. As many as 100 mg brown seaweed extract was dissolved with 100 mL 96% ethanol and dispersed in 300 mg PVPK-30. 20 mL of chitosan solution was taken and mixed with 70 mL of brown seaweed extract solution. 0.1% w / v tripolyphosphate solution was made in pure water and added 4 mL by slowly dropping it into a chitosan mixture at a speed of 1 drop / 3 seconds in stirring using a magnetic stirrer with a stirring speed of 250 rpm to form a homogeneous turbidity. The solution is still stirred with a magnetic stirrer stirrer for 30 minutes.

2.4 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 *Delsa Nano TM*. The distribution of particle sizewasdetermined 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*. The magnitude of zeta potential was determined based on this test.

Morphology of the nanoparticles using *Transmission Electron Microscopy* (TEM)
The nanoparticle suspension was dropped over the Cu grid (Formvar / Carbon 400 mesh support) and left for 1 minute then excess nanoparticles were absorbed with 41 whatmanpapper filters and left to dry then observed with TEM (FEI type Tecnai G2 Spirit 120 KV).

Powder X-ray Diffraction Analysis (XRD)
The diffractogram of materials and nanoparticles was obtained using an X-ray diffractometer, for example D8 Advance (Bruker, Germany) which was equipped with Cu-Kα X-ray radiation sources at 40 kV and 30 mA. The diffraction angle (2o) is measured at 2-50o with a scanning rate of 2o / minute and a step time of 13.6 seconds.

Antioxidant activity of brown seaweed extract nanoparticles with the ABTS method

a) **Making test solution:** Weigh 25 mg of the sample then dissolved in 25 mL of pro ethanol analysis (1000 ppm), this solution is the standard solution.working solution was made with a concentration of 50 ppm, 75 ppm, 100 ppm, 125 ppm, and 150 ppm in each measuring flask added 1.0 mL of ABTS solution and added with ethanol pro analysis to the mark, then homogenized.
b) Antioxidant activity test, working solutionsand positive control with several concentrations, the absorption was measured using UV-Vis spectrophotometry at a maximum wavelength of 411 nm.

3. Results and Discussion

Examination of Brown Seaweed Extract
Fucoxanthin identification was carried out qualitatively by measuring the maximum wavelength from the fucoxanthin standard compared to the maximum wavelength. From the tests conducted, it is known that thenfucoxanthincomparison standard has a maximum wavelength of 446 nm while brown seaweed extract containing fucoxanthin has a maximum wavelength of 445 nm, this indicates that brown seaweed extract is true containing fucoxanthin.

Measurement results of brown seaweed extract nanoparticles
The formation of cross linking or is influenced by the addition of chitosan and NaTPP by ionic glass method. The principle of this method is the occurrence of ionic interactions between the amino groups of positively charged chitosan and negatively charged polyanions forming intramolecules.

Characteristics of the Nanoparticles	cosolven-basednanoparticle	PVP-based nanoparticles
Particle Size (nm)	226,2	365,1
Polydispersity Index	0,870	0,523
Zeta Potential (mV)	50,40	26,56

The results of particle size obtained on nanoparticles with cosolven and nanoparticles with PVP respectively 226.2 nm and 365.1 nm met the requirements of 10-1000 nm. This is due to the formation process using the ionic glass method. In addition, the particle size is also influenced by the addition of tween as a stabilizer used to prevent aggregated particles. The results of the Polydispersity Index (IPD) on nanoparticles with cosolven and nanoparticles with PVP obtained values of 0.870 and 0.523 which addressed the value of meeting the requirements, namely 0-1.

Based on the results of the zeta potential analysis, the potential zeta value of the nanoparticles with cosolvene was obtained using 0.2% NaTPP of 50.4 mV, while the

nanoparticles with PVP using 0.1% NaTPP gave a zeta potential yield of + 26.56 mV. The potential zeta value good is above the value (+/-) 25 mV, because the particle that has the above zeta potential (+/-) 25 will have a rejecting force between particles that have the same charge. While particles that have a low potential zeta will tend to be aggregated with particles with the same charge. Positive potential zeta value is caused by an amino group (NH_3 +) from chitosan with a group (O-) from Na TPP which is a polyanion compound so that it forms a cross-linked bond.

The results of *Transmission Electron Microscopy* (TEM) brown seaweed extract nanoparticles

Figure 1: *Transmission Electron Microscope* (TEM) of Cosolvent-based Nanoparticles

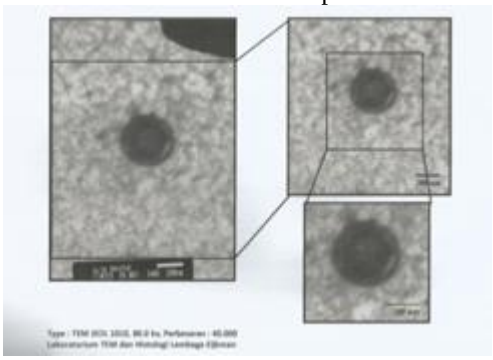

Figure 2: *Transmission Electron Microscope* (TEM) of PVP-based Nanoparticles

Morphology of brown seaweed extract nanoparticles was examined using the*Transmission Electron Microscope* (TEM) with a magnification of 40,000X. The results showed that the morphology of the particles produced by spherical ionic gelation method. This shows that the nanoparticles are well formed, the nanoparticles with the inside are darker than the surrounding environment, indicating that the nanoparticles absorb sufficient amounts of brown seaweed extract. Morphological measurement results showed that the morphology of brown seaweed extract nanoparticles with PVP was able to absorb brown seaweed extract better than brown seaweed extract with cosolven nanoparticles characterized by black spheres found on the inside.

Nanoparticle XRD measurement

Sample	Crystalline	Amorphous
Chitosan	74,7%	25,3%
NaTPP	50,24%	49,76%
PVP	52,11%	47,89%
Brown seaweed extract	59,55%	40,45%
Nanoparticles with PVP	49,87%	50,13%

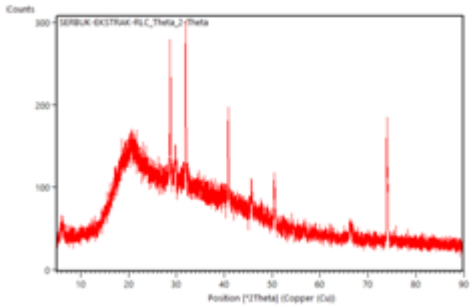

Figure 3: Results of XRD examination of brown seaweed extract

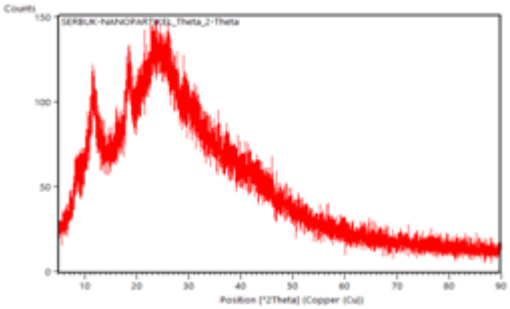

Figure 4: Results of XRD nanoparticle with PVP

This shows that the manufacture of nanoparticles can indicate an increase in amorphous form of the extract so that it can increase the solubility of brown seaweed extract.

Antioxidant Activity Test Results of Brown Seaweed Extract and Nanoparticles Brown Seaweed Extract with ABTS Method

Sample	*IC_{50}(ppm)*
BP Vitamin C	2,76
Brown Seaweed Extract	93,98
Cosolvent-based Nanoparticles	98,72
PVP-based Nanoparticles	97,63

The difference in antioxidant activity between brown seaweed extract nanoparticles and cosolven against brown seaweed extract with PVP nanoparticles was indicated by the results of data processing using one-wayANVA test at a real level = 0.05 indicating that IC_{50}brown seaweed extract, brown seaweed extract nanoparticles using cosolven and brown extract nanoparticles using PVP have a significant difference, this is indicated by p-value (0.000)<0.05 so that H_0rejectedis and H_1 is acceptedwhich means there is significant difference between each antioxidant activity.

4. Conclusions

1) Brown seaweed extract (Sargassumpolycystum) can be made into nanoparticles using solubility enhancing techniques, namely cosolven and PVP through ionic gelation method using 1% chitosan polymer and sodium tripolyphosphate as cross-linker to meet the requirements of nanoparticle physical quality with particle size on nanoparticles cosolven 225.2 nm and nanoparticles with PVP 365.1 nm. The results of zeta potential values on nanoparticles with cosolven 50.40 mV and nanoparticles with PVP 26.56 mV and morphological results of

particles that have spherical shape and have an amorphous structure.

2) Based on the results of the paired one-way ANVA statistical analysis, it shows the results of p-value (0.000) < 0.05 so that there is significant difference between each antioxidant activity of brown seaweed extract (*Sargassumpolycystum*) at 93.98 ppm, brown seaweed extract nanoparticles (*Sargassumpolycystum*) and cosolvent at 98.72 ppm with extract nanoparticles brown seaweed (*Sargassumpolycystum)* with PVP of 97.63 ppm.

References

[1] Liochev, S.I. Reactive Oxygen Species and the Free Radical Theory of Aging. Free Radical Biology and Medicine. 2013 ; 60(1): 1-4.
[2] Halliwell, B. Free Radicals and Antioxidant Updating a Personal View, Nutrition Review. 2012; 70: 257-265.
[3] Miyashita K, "Anti-Obesity Theraphy by Food Component: Unique Activity of Marine Carotenoid Fucoxanthin, 2014. (1). p 1-4.
[4] Saffoon, N., Jhanker, Y.M. dan Huda, N.H. Dissolution Profile of Ibuprofen Solid Dispersion Prepared with Cellulosic Polymers and Sugar by Fusion Method. Stamford Journal of Pharmaceutical Sciences, 2011; 1:31-37.
[5] Ranjit K and Baquee AA. Nanoparticles: An overview of preparation, characterization, and application. Int Res J Pharm 2013; 4(4): 47-53.
[6] Ozygen M., Reese, Neil R., Artemio Z., et al. Modified 2,2-Azino-bis-3ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method To Measure Antioxidant Capacity of Selected Small Fuits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2-Diphenyl-1-picrylhydrazil (DPPH) Methods. Journal of Agricultural and Food Chemistry. 2006: 1151-1157.