Rheological behavior and antioxidant activity of carrageenan extracted from Green seaweed (*Eucheuma cottonii*) using alkaline solution at low temperature

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Rheological Behavior and Antioxidant Activity of Carrageenan Extracted from Green Seaweed (*Eucheuma cottonii*) Using Alkaline Solution at Low Temperature

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Abstract. Seaweed is one of the important biological resources found in coastal and marine regions. One of the main products derived from seaweed is carrageenan. In this work, the carrageenan was extracted from the seaweed using alkaline solution. Two types of bases were used as the extracting solution, namely NaOH and KOH solutions. The extraction results showed that the use of KOH solution resulted in a higher carrageenan yield than that extracted using NaOH solution. It was also found that the carrageenan yield was increasing with the increase of the basic solution volume to mass of the seaweed ratio. The highest carrageenan yield of about 33.4% was obtained using KOH solution at solvent volume to seaweed mass ratio of 45 ml/g. The rheological measurement showed that the rheological behavior fitted best to the Power Law model. Meanwhile, the carrageenan extracted using NaOH solution demonstrated higher antioxidant activity. The highest percentage of DPPH radical scavenging activity of the carrageenan extract was 60.7%, while the IC50 values were in the ranges of 0.2 – 30 ppm indicating a potent antioxidant activity.

Keywords: antioxidant, carrageenan, DPPH, rheology, seaweed

INTRODUCTION

Based on data from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia, Indonesia has a coastline of 95,181 km and is the second longest coastline in the world, with an area of marine waters reaching 5.8 million square kilometers, which is 71% of the total area of Indonesia. As the largest archipelagic country in the world with 17,504 islands, the sea is the life support for the Indonesian nation. Seaweed is one of the important biological resources found in coastal and marine regions. From seaweed, various types of products can be developed and used in various fields, including food, pharmaceuticals, biomedical, cosmetics, paper, and other industry [1]. Seaweed is known as a multicellular marine sea algae which is classified as green (*Chlorophytes*), brown (*Phaeophytes*) and red (*Rhodophytes*) [2].

One of the main products derived from seaweed is carrageenan. Indonesia has become the largest tropical seaweed producer in the world and have around 70,000 families of seaweed. The most widely cultivated species to produce carrageenan is the red macroalgae *Kappaphycus spp*. and *Eucheuma denticulatum* [3]. Carrageenan is basically the cell wall hydrocolloid, a polysaccharides compound which has hydrophilic property and is extracted from *rhodophyte* in neutral or alkaline water under pressure at elevated temperature ranging from 100-140 ºC. Those molecules consisting of sulphated galactans. The carrageenan has the ability to form a gel and increase the viscosity [4].

Carrageenans are polysaccharides which be a part of complex biopolymeric which is widely known as phytocolloids. They are taken from the cellular wall of *Rhodophyceae* (red algae) which mainly come from the genus of *Chondrus*, *Gigartina*, *Euchema*. It was reported that around 250 species have been cultivated to produce carrageenan. Carrageenan has been classified according to number and position of sulphate group contained therein.

The most widely known configurations are kappa, iota and lambda. Their rheological properties are significantly affected by their chemical composition and the configuration of sulphate group inside it [5]. In fact, the carrageenan have a very varied chemical structure which is influenced by several factors, among others algal source, the age of the algae and the isolation processes. The superior physical properties of the carrageenan are utilized for a wide range of applications including as thickening and gelling agents in various structural and functional applications [6].

Several extraction methods have been developed to isolate carrageenan, among others extraction-precipitation process using alkaline water, conventional extraction processes using alcoholic precipitation, extraction processes using tangential ultrafiltration, ultrasound-assisted extraction (UAE) and microwave-assisted extraction [7]. It was reported that deep eutectic solvents method has been successfully extracted k-carrageenan with high yield and had better rheological and physicochemical properties than carrageenan extracted with water in common conventional method [8, 9]. Other extraction methods that are still being developed include ultrasound-assisted extraction [10], ionic liquid-assisted subcritical water extraction [11] and pressurized subcritical water extraction using different kind of solvent such as ethanol, methanol or ethyl acetate, and their mixtures [12].

Since carrageenan extraction from seaweed is mostly performed at a quite high temperature which possibly degrades several important nutritional compounds such as antioxidants, then in this research work, carrageenan was extracted from green seaweed in alkaline water solution at low temperature (60 ºC) under atmospheric pressure. The objective of this research work was to investigate the yield of carrageenan obtained from extraction at low temperatures using KOH and NaOH solution and to observe its rheological behavior and antioxidant activity.

MATERIAL AND METHOD

Seaweed Sample and Chemicals

Green seaweed (*Eucheuma cottonii*) obtained from the beach of Bulukumba, Sulawesi, Indonesia. Chemicals for extraction consisting of sodium hydroxide, potassium hydroxide and potassium chloride were analytical grade from Merck Chemicals. While 2,2-diphenyl-1-picrylhydrazyl (DPPH), a chemical reagent which was used to measure the antioxidant activity of the obtained carrageenan, was also analytical grade and purchased from Merck Chemicals.

Carrageenan Extraction

The carrageenan was extracted from the green seaweed (*Eucheuma cottonii*) follow the procedure developed by Bono et al. [13] with modification and through the following steps. At first, the seaweed was washed with water to remove impurities such as sand and salt adhered to the surface. The next step was soaking the seaweed in water at 40ºC for 24 hours to further separate any remaining impurities. After being separated from the soaking water, the seaweed samples were crushed mechanically and then dried in an electrically heated vacuum oven at 60 ºC for 24 hours. After being dried in the oven, the seaweed samples were then again crushed mechanically and sieved using a 80 mesh sieve. The extraction was carried out in a 500 ml flask by putting seaweed sample powder and adding a basic solution (KOH or NaOH) with certain ratios while heating the mixture to maintain the extraction temperature at 60 ºC. During the extraction process, it was stirred continuously. The concentration of the alkaline solution used was 0.2 N. The extraction process was carried out for 1 hour. At the end of the extraction process, the solid formed was separated from the liquid by filtration using a cloth so that the filtrate was totally free from solids. The next step was mixing 100 ml of 3.5% KCl solution into 300 ml of the filtrate in a 500 ml beaker for the gel formation. During the gel formation, continuous stirring was carried out using a magnetic stirrer. After 60 minutes of stirring, the liquid allowed to stand until it formed a gel. The formed gel was washed using distilled water until pH 7 was reached. The washed gel was filtered using a cloth and then dried in a vacuum oven at 60 ºC overnight. The dried carrageenan powder was weighed for yield determination and the dried carrageenan sample was ready to be used for rheological and antioxidant activity analysis. In this study, the varied parameters were the types of alkaline solutions (KOH and NaOH) and the solvent volume to the seaweed mass ratio (30, 35, 40, 45 ml solution/g seaweed) for the extraction process.

Determination of Carrageenan Yield

The yield of carrageenan was calculated from the ratio of dry weight of the carrageenan produced to the weight of the initial fresh seaweed according to Equation (1).

$$\%\, carrageenan\ yield\ = \frac{weight\ of\ the\ dry\ carageenan}{weight\ of\ the\ fresh\ seaweed} x100\% \tag{1}$$

Determination of Rheological Behavior

Rheological behavior was determined by measuring the correlation between shear stress and shear rate using a viscometer Brookfield RVT-98103, Germany. Samples were prepared by adding 2 g of carrageenan powder into 100 ml of distilled water in a beaker then measuring the shear stress and shear rate using a viscometer at 20, 50 and 100 rpm. Observations were made at time intervals of 30 seconds for 120 seconds. During the measurement, the temperature of the solution was maintained at 40 ^{o}C in a water bath, since gelling was likely to occur at room temperature.

DPPH Radical Scavenging Activity Assay

The DPPH method is often used to determine the antioxidant activity of a sample. In principle, when a DPPH solution is mixed with a sample capable of donating hydrogen atoms, radical resuscitation is formed. This reaction removes the purple color which forms the absorption band at 517 nm. So the interaction between the DPPH solution and the sample will cause color reduction [14]. Measurement of the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by the sample followed the procedure performed by Banerjee, et al. [15] with modifications. Sample of solid carrageenan of 0.01 mg and 1 ml 0.1mM DPPH solution were put into a 10 ml volumetric flask. Ethanol is then poured into the flask so that the total volume was 10 ml. The mass of carrageenan solids that were put into the volumetric flask was varied to obtain carrageenan concentrations of 2, 4, 6, 8 and 10 ppm. The solution was shaken until homogeneous conditions were achieved and then allowed to stand for 30 minutes at room temperature. The absorbance was measured at a wavelength of 517 nm using a UV-Visible spectrophotometer. The percentage of radical scavenging activity is calculated according to the following formula.

$$\%\, radical\ scavenging\ activity = \frac{A_o - A_1}{A_o} x\ 100\% \tag{2}$$

where A_o is the absorbance of the control (DPPH solution without sample) while A_1 is the absorbance of the sample (DPPH solution plus sample). Antioxidant activity is also expressed as IC50 which indicates the concentration of sample required to scavenge 50% of the DPPH [16].

RESULTS AND DISCUSSION

Carrageenan Yield

Carrageenans are sulphated galactans which has been widely used for both food and non-food industrial applications as thickeners, gelling, stabilizing, texturing or suspending agents. The main structure of carrageenan is based on an alternating disaccharide repeating sequence of 3-linked -D-galactose and 4-linked -D-galactose. The basic gelling components of carrageenan are kappa and iota disaccharide units, which contain one and two sulfates groups per disaccharide repeat unit, respectively [17]. The carrageenan yield which reflects the mass ratio of carrageenan obtained to the mass of the extracted seaweed expressed in percent was shown in Fig. 1. The higher the yield value, the more economical the seaweed was.

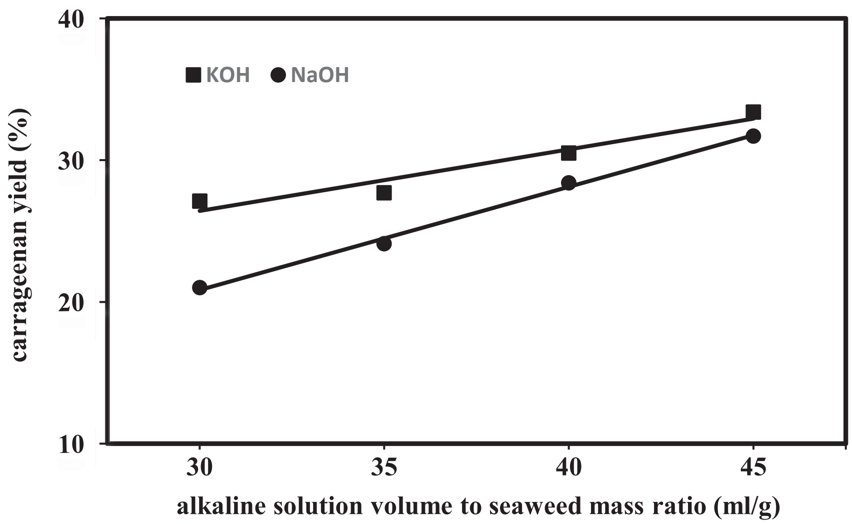


FIGURE 1. Carrageenan yield expressed in percent at various alkaline solution volume to seaweed mass ratio for NaOH and KOH solution.

In this study, extraction was carried out at a fairly low temperature level of about 60 ºC in order to minimize the thermal degradation of antioxidant compounds contained in seaweed. From the Fig 1. It can be clearly seen that the yield of carrageenan increases with the increasing ratio of solvent volume to mass of fresh seaweed. The extraction results also showed that the use of KOH solution produced a higher yield of carrageenan than the NaOH solution on the entire ratio of solvent volume to mass of fresh seaweed used. The highest yield of carrageenan was obtained by extraction with KOH solvent at a solvent volume ratio of 45 ml of KOH/g seaweed solution, which was about 33.4%. This yield is comparable with the yield obtained by Rhein-Knudsen, et al. [18], which obtained carrageenan yield in the range of 13 - 27 (% dry material) in which the seaweed extraction was conducted also using alkaline treatment.

Rheological Behavior

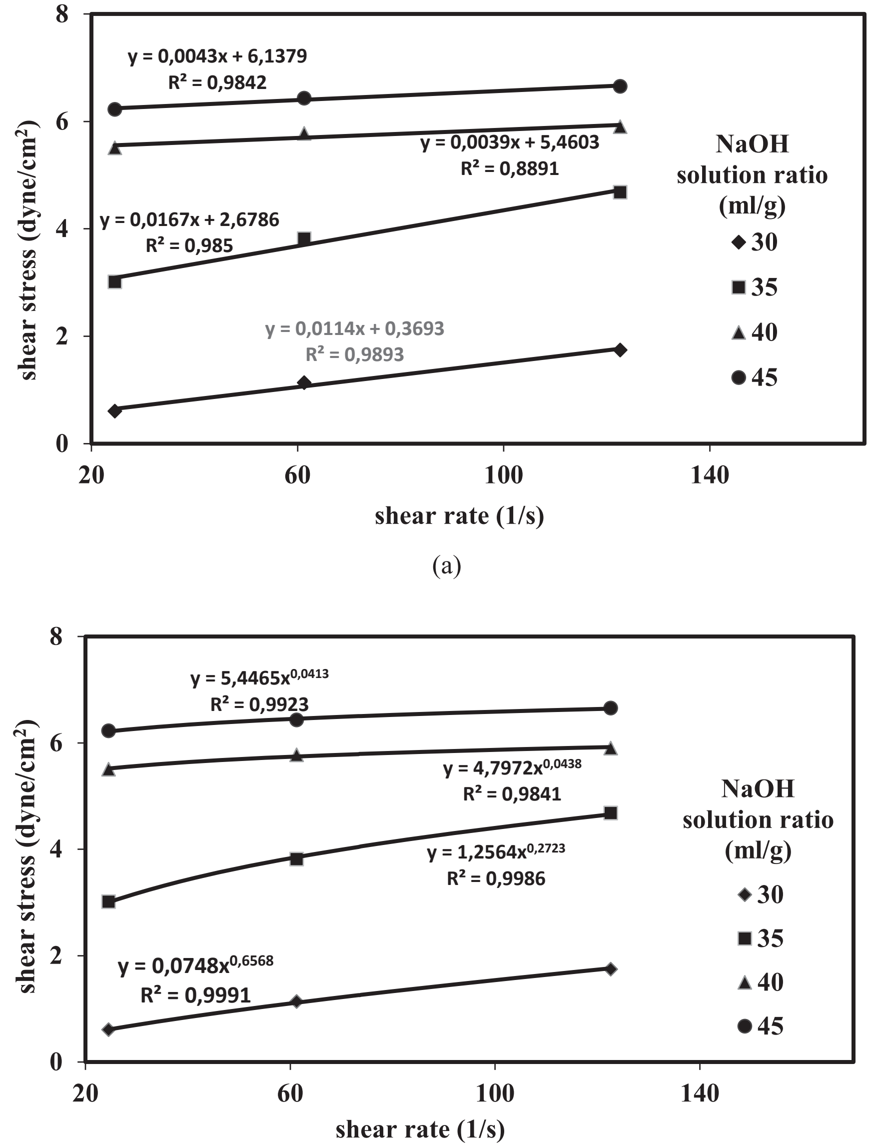
Rheological behavior of the carrageenan represented as correlation between shear stress and shear rate was shown in Fig. 2 and Fig. 3. These results indicated that the carrageenan extracted using KOH solution produced a higher shear stress than carrageenan extracted using NaOH solution. This implied that extraction with KOH solution produced a more viscous carrageenan solution than those extracted using NaOH solution. The ratio of the volume of solvent to the mass of seaweed used in the extraction evidently affected the rheological behavior of the carrageenan produced. The higher the ratio of the volume of the solvent to the mass of the seaweed, the greater the viscosity of carrageenan was indicated by its higher shear stress.

In this research work, two rheological models were observed for their suitability with the experimental data, namely the Bingham model and the Power Law model, in which the correlation of the rheological models were expressed in equation (3) and (4), respectively [19].

$$\tau = \tau_y + \mu_p \gamma \tag{3}$$

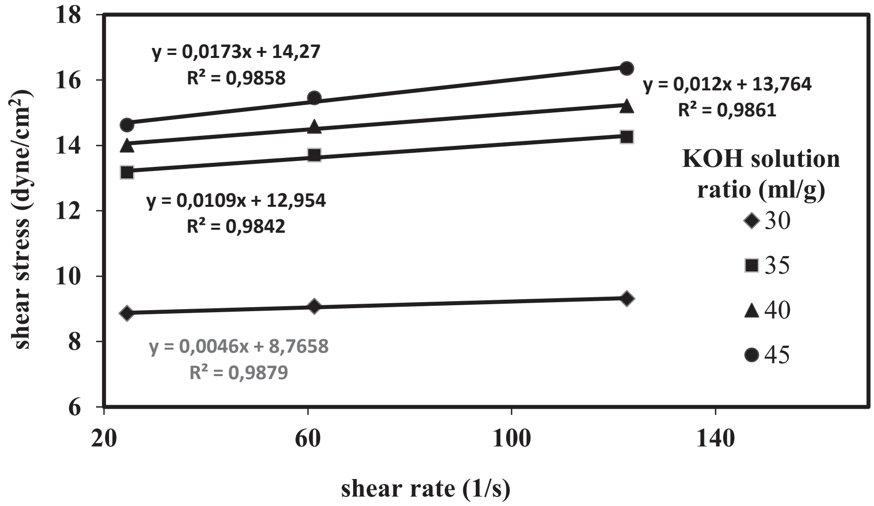
$$\tau = \kappa \, \gamma^n \tag{4}$$

where τ: shear stress (Pa), γ: shear rate (s^{-1}), τ_y: yield stress ($kg/m.s^2$), μ_p: liquid plastic viscosity (kg/m.s), κ: consistency index of fluid ($Pa.s^n$), n: flow behavior index (dimensionless).



(b)

FIGURE 2. Plot of rheological model of the carrageenan extracted using NaOH solution at various solution ratio, experimental values were indicated by the solid marker, while rheological model was represented by the solid line. (a) Bingham model; (b) Power Law model



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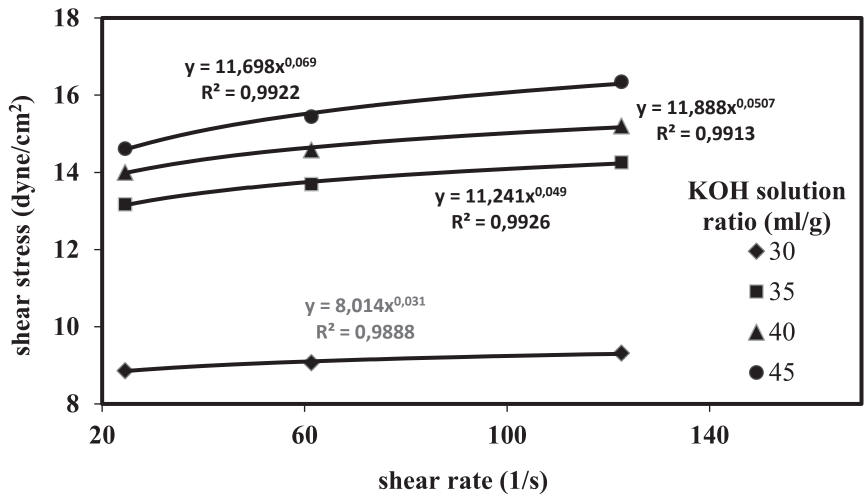


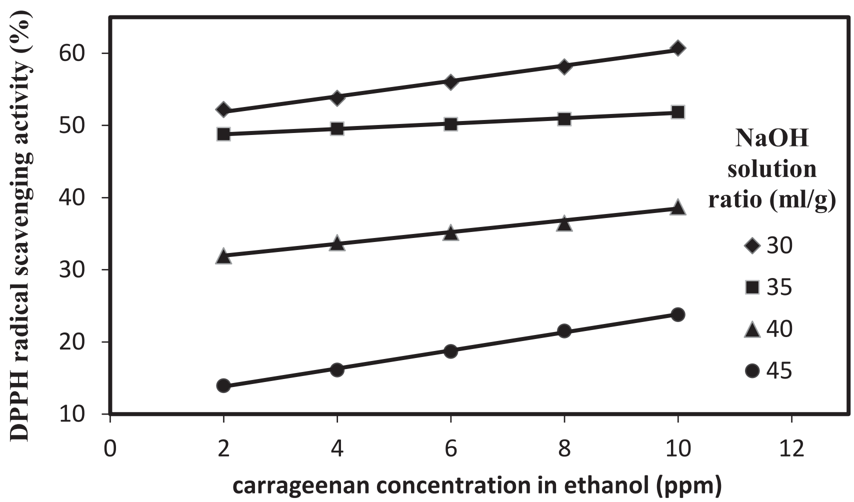
(b)

FIGURE 3. Plot of rheological model of the carrageenan extracted using KOH solution at various solution ratio, experimental values were indicated by the solid marker, while rheological model was represented by the solid line. (a) Bingham model; (b) Power Law model

Referring to the value of the determinant coefficient generated from the two models, it demonstrated that rheological behavior of the carageenan was more fitted to the Power Law model. This proved that the carrageenan extract in water formed a non-Newtonian fluid. While in general, by investigating all of rheological behavior presented in the figures, it showed that the magnitude of the shear stress was not very sensitive to the changes in shear rate. This was of an advantage of this product related to energy consumption due to the occurrence of shear forces in the process using this product.

Antioxidant Activity

There was growing an interest in antioxidants due to the need to prevent cell damage in the human body due to the activity of free radical compounds. Although the human body was able to produce its own antioxidants, additional antioxidants were still needed because the antioxidants in the body were often unable to neutralize all the existing free radical compounds. Antioxidants were also needed to prevent the deterioration of fats and other food ingredients. For that purpose, natural antioxidants were preferred compared to synthetic antioxidants because they tend not to cause adverse side effects [20]. Thus, utilizing seaweed as an alternative source of natural antioxidants seems to be a promising prospect. At the same time, there has been a growing need of a reliable analysis method for determining these antioxidant activities derived from food and biological system [21]. The DPPH method which is the most widely used method to measure the antioxidant activity was going to be applied in this research work. In principle, the stable free radical diphenylpicrylhydrazyl (DPPH) reagent was mixed with a substance that has the ability to donate a hydrogen atom, leading to the loss of the violet color due to the occurrence of reduction reaction [22].



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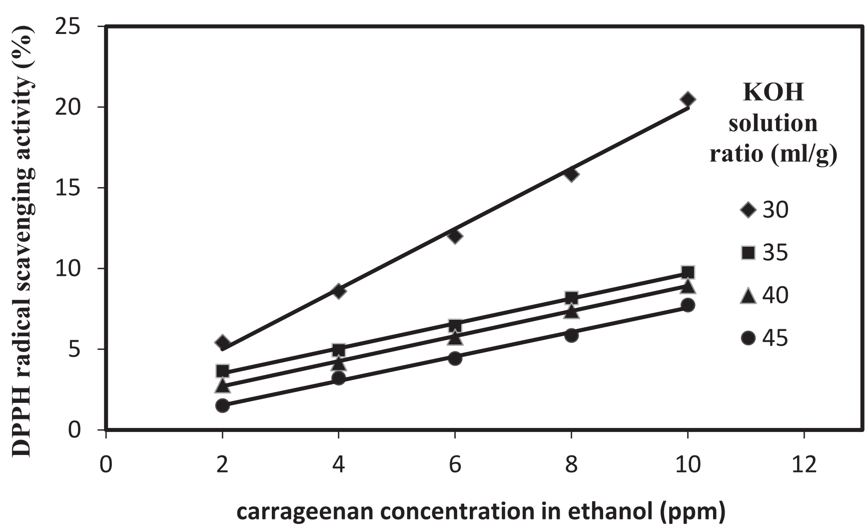

(b)

FIGURE 4. Antioxidant activity of the carrageenan extract expressed as percent DPPH free-radical scavenging activity. (a) extraction using NaOH solution; (b) extraction using KOH solution.

The results of the DPPH radical scavenging activity analysis on all seaweed extracts were presented in Fig. 4. It demonstrated that carrageenan extracted with NaOH solvent produced a higher percentage of DPPH radical scavenging activity than those extracted with KOH solvent. The highest value of percent DPPH radical scavenging activity obtained was about 60.7%, i.e. which was obtained from sample of 10 ppm carrageenan extracted using NaOH solution at solvent volume to seaweed mass ratio of 30 ml/g. Meanwhile, the highest percent DPPH radical scavenging activity extracted using KOH solution was about 20.5%. It was reported that the type of solvent used in the extraction could dramatically affect the types and number of extracted components which in turn affected the antioxidant activity [23].

Antioxidant activity was also commonly expressed as IC50, which was the concentration of samples capable of scavenging 50% of DPPH [24]. The IC50 value was inversely proportional to the antioxidant activity. Samples with higher IC50 had lower antioxidant activity. The results of the antioxidant activity analysis expressed as IC50 of *Eucheuma cottonii* extract was shown in Table 1. It showed that the lowest IC50 value was 0.2 ppm, which was achieved in the extract obtained using NaOH solution. This indicated that carrageenan extracted using NaOH solvent demonstrated higher antioxidant activity. The carrageenan extracted using KOH solution were most probably contained more impurities compared to those extracted using NaOH solution indicated by their higher yield.

TABLE 1. Antioxidant activity expressed as IC50

Solution Ratio (ml/g seaweed)	IC50	
	Extraction Using NaOH Solution	Extraction Using KOH Solution
30	0.2	26.1
35	5.3	62.2
40	24.1	62.9
45	30.9	64.7

CONCLUSION

Extraction of carrageenan from green seaweed was successfully carried out using an alkaline solution at a fairly low temperature of 60 ºC. It was thus believed that the thermal degradation of the antioxidants in the extract could be minimized. The yield of carrageenan reached 33.4% when KOH solution was used, and this was quite comparable with the previous study. It was also found that the carrageenan yield increased with increasing the solution volume to seaweed mass ratio. Rheological behavior of carrageenan extract followed the Power Law model, and it was found that the shear stress was not very sensitive to the changes in shear rate. Based on the result of the antioxidant activity assay, it was found that the carrageenan extract obtained using NaOH solution had a higher antioxidant activity than those obtained using KOH solution. The percentage of DPPH radical scavenging activity of carrageenan extract obtained with NaOH solution reached 60.7%, which was 3 times higher than the corresponding value obtained with KOH solution. This result indicated that the type of the alkaline solvent used for the extraction greatly affected the yield and the antioxidant activity of the resulting carrageenan. Meanwhile, the IC50 values of the NaOH extract were in the range of 0.2 – 30 ppm indicating a potent antioxidant activity.

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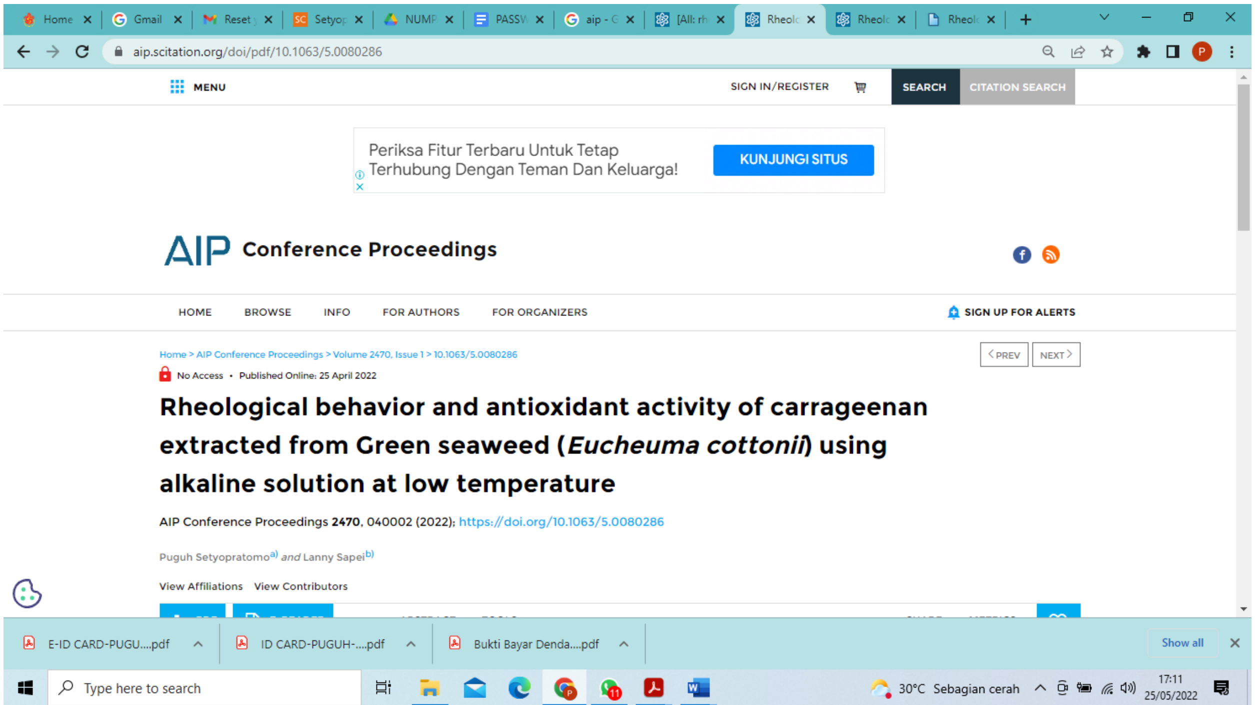

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Puguh Setyopratomo and Lanny Sapei

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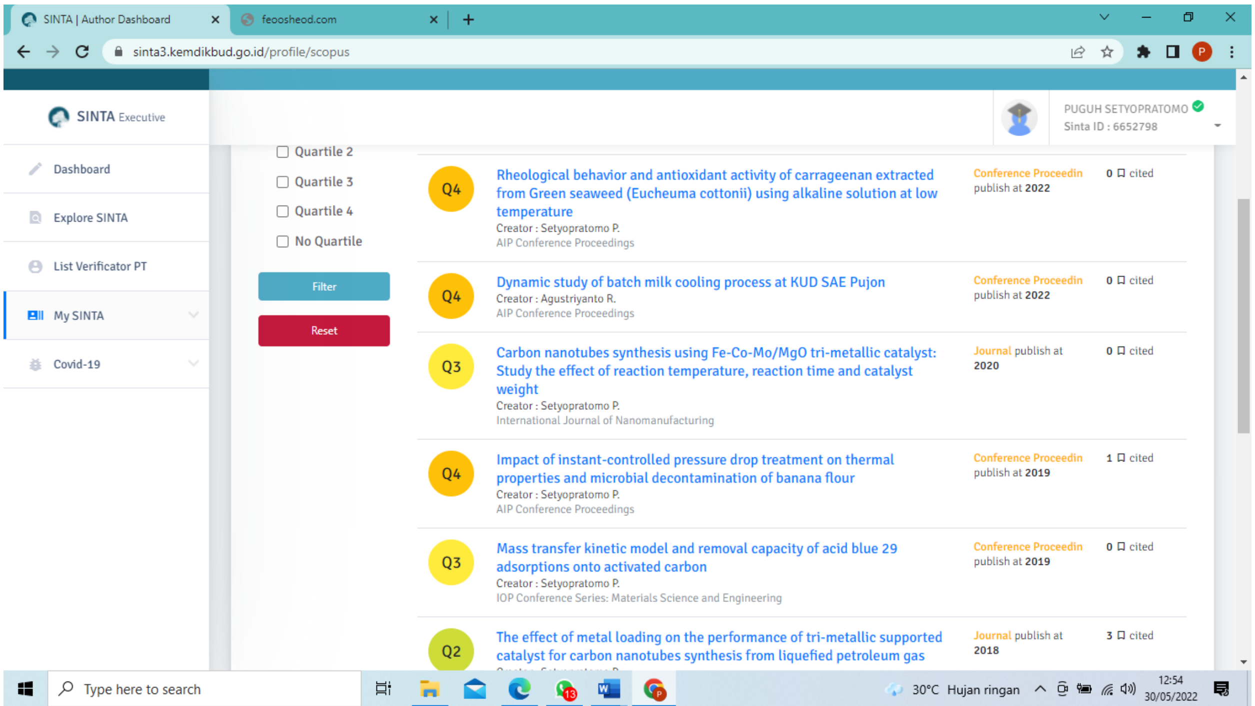

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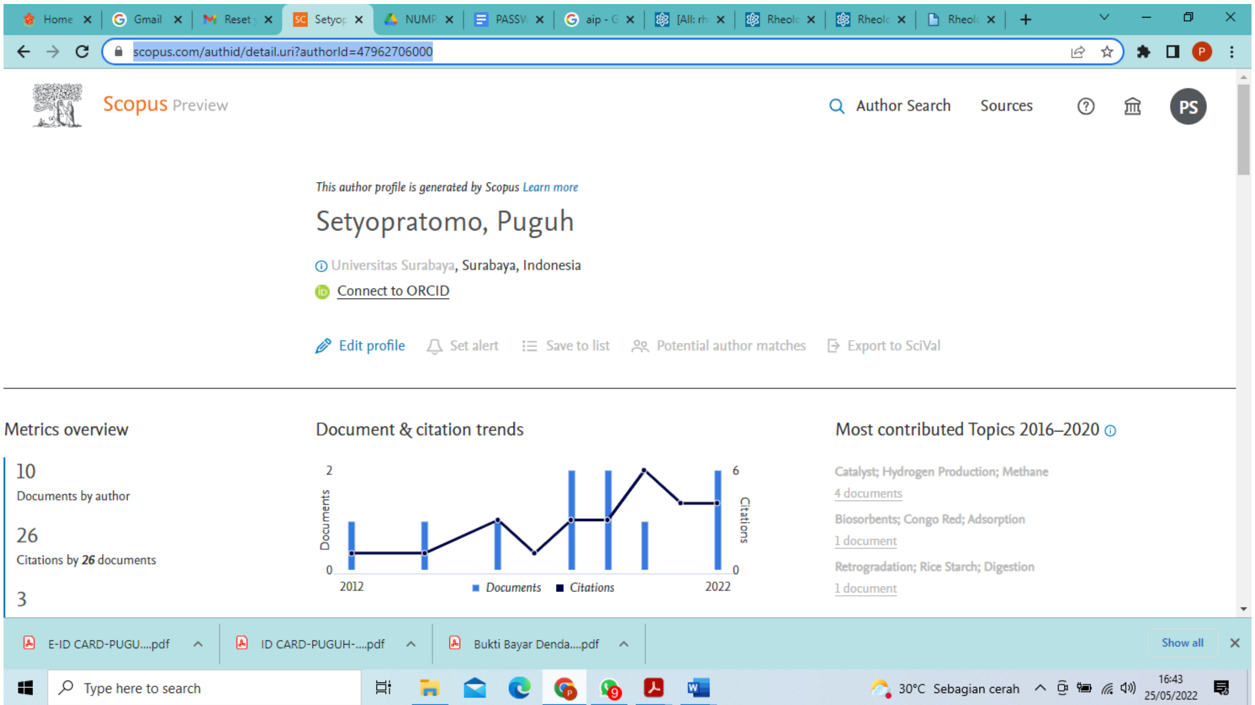

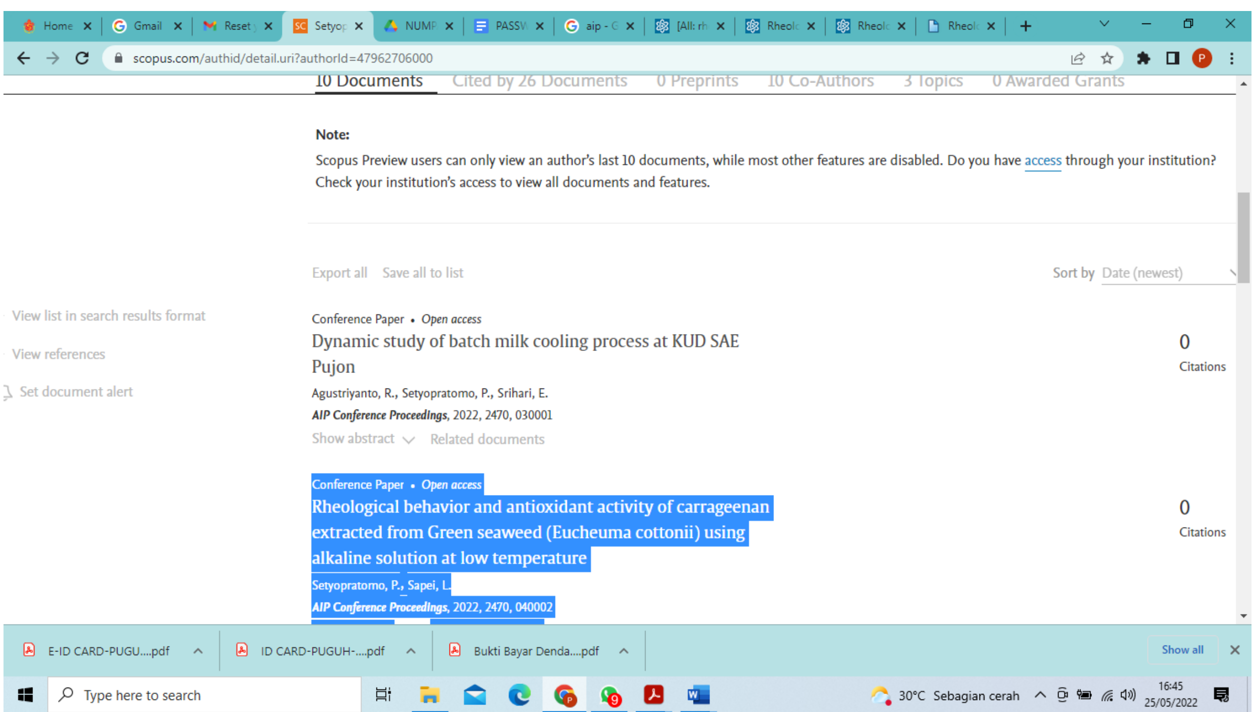

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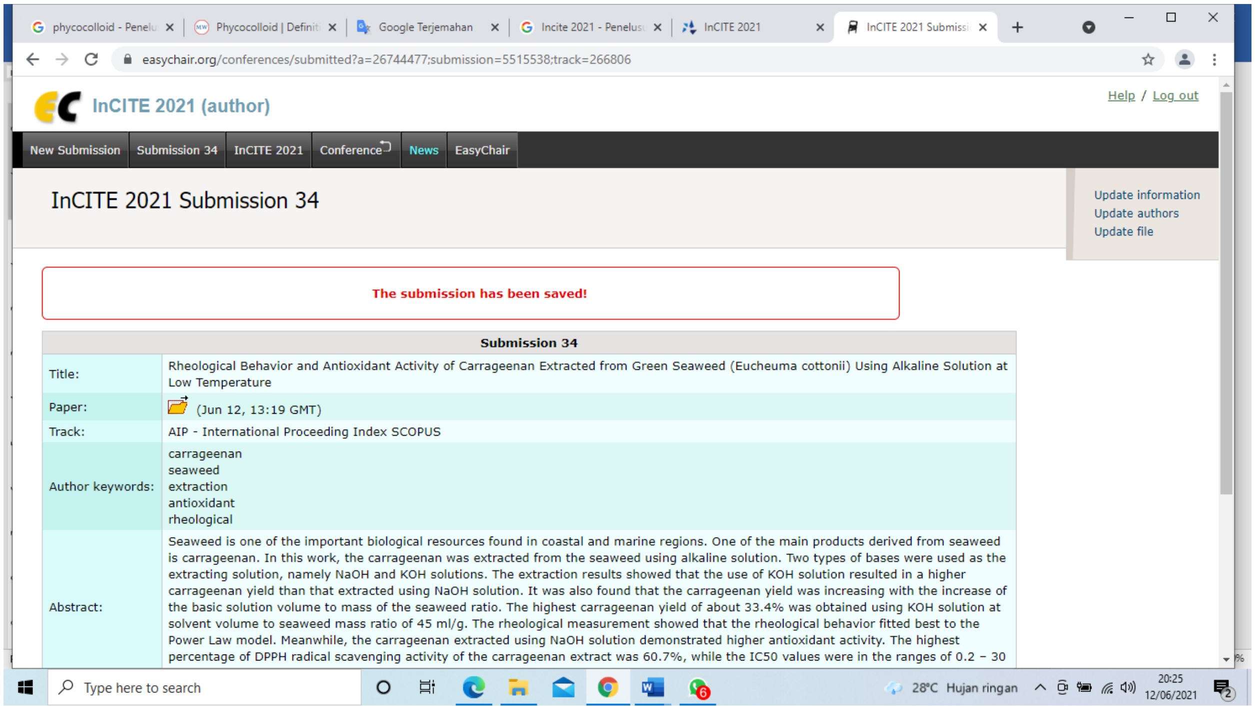

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