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Characteristics and Antioxidant Activity of Nanoparticles Chitosan Roselle (*Hibiscus sabdariffa*)

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Abstract

Roselle (*Hibiscus sabdariffa*) contains flavonoids with antioxidant activity. The present study aimed to examine the characteristics and antioxidant activity index (AAI) of roselle extract (RE) after being modified into nanoparticles of chitosan of roselle (NCR). Phytochemical screening indicated that roselle extract contains flavonoids, saponins, tannins, and steroid/ triterpenoid compounds. The NCR was prepared using a bottom-to-up method from a cross-link reaction between chitosan and tripolyphosphate. The ratio of RE: Chitosan: Tripolyphosphate was 2:1:0.1. NCR characterization showed 464.6 nm of particle size, 0.849 of a polydispersion index and -6.0 mV of zeta potential. Antioxidant activity was measured by utilizing the DPPH method (2,2-diphenyl-1-picrylhydrazyl), with a UV-Vis spectrophotometer at a wavelength of 515 nm. IC_{50} data were analyzed using the One Way ANOVA test with a confidence level of 95%. Results showed that NCR had an IC50 value of 22.577 ppm (AAI:2.217), which is classified as a very strong antioxidant. The IC_{50} value of the RE was 77.610 ppm (AAI:0.644), which is categorized as a moderate antioxidant. The results of this study reveal that the modification of roselle extract (RE) into nanoparticles of chitosan of roselle (NCR) was proven to enhance the AAI ($p<0.05$).

Keywords: *antioxidant, nanoparticle, roselle*

1 Introduction

As a tropical nation, Indonesia is granted a large number of plant species, including herbal plants. In Indonesian culture, these herbal plants are commonly utilized to maintain health or even in the traditional medicine [1]. In general, many diseases arise from exposure to free radicals. Free radicals are unstable and always try to take electrons from the surrounding molecules, so free radicals are toxic to biological molecules/cells. Free radicals can interfere with the production of DNA, lipid layers in cell walls, affect blood vessels, and produce prostaglandins and other proteins such as enzymes found in the body [2].

Antioxidants are abundant in natural dietary sources, and the consumption of antioxidants has many potential health benefits [3]. Therefore, exploration of natural materials that can potentially be antioxidants is necessary. According to literature, roselle (*Hibiscus sabdariffa*) has antioxidant activity [4]. Hibiscus sabdariffa L. (Hs, roselle; Malvaceae) has been used traditionally as a food, in herbal drinks, in hot and cold beverages, as a flavouring agent in the food industry and as a herbal medicine [5].

The main constituents of H. sabdariffa relevant in the context of its pharmacological are organic acids, anthocyanins, polysaccharides and flavonoids [6]. Hibiscus sabdariffa and their active constituents play an important role in the prevention of chronic and degenerative disease that are associated with oxidative stress [7]. It is expected that in the form of nanoparticles, the

value of the antioxidant index of roselle can improve [8].

The form of nanoparticles can increase the stability of labile compounds such as antioxidant compounds [9]. Nanochitosan was prepared from chitosan by gelation ionic method [10]. Using the ionic gelation method allows cross-linking to form between chitosan and sodium tripolyphosphate to produce micrometer-sized products that are more stable [11].

The optimum composition of extract roselle: chitosan: STTP ratios were 2:1:0.1, with the optimum formation of chitosan in acetate buffer pH 4 [12]. Antioxidant activity testing can be carried out in vitro with DPPH (2,2 diphenyl-1-picrylhydrazyl). Method DPPH provides reactivity information compound tested with a radical stable. DPPH provides strong absorption at a wavelength of 517 nm with a dark violet color. Radical catcher free causes electrons to become paired which then causes comparable discoloration by the number of electrons taken [13].

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (2) with the loss of this violet color (although there would be expected to be a residual pale-yellow color from the picryl group still present). Representing the DPPH radical by Z • and the donor molecule by AH, the primary reaction is shown in Eq. 1.

$$Z\bullet + AH = ZH + A\bullet \qquad \text{(Equation 1)}$$

where ZH is the reduced form and A • is the free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant [14].

2 Method

2.1 Materials

Materials used in the present research were roselle (*H. sabdariffa*) from Kuningan West Java, Indonesia, Mayer reagent (Merck), Dragendroff reagent (Merck), Zn (Merck), hydrochloric acid (Merck), amyl alcohol (Merck), ferric chloride (Merck), Liebermann Buchard reagent (Merck), sodium hydroxide (Merck), sulphatic acid (Merck), acetic acid (Merck), ethanol 98% (Merck), sodium acetate (Merck), aquadest, chitosan (Sigma), sodium tripolyphosphate (Merck), Vit C (Merck), DPPH (1,1 diphenyl-2-picryhydrazil) (TCI), ascorbic acid (Sigma Aldrich) and methanol (Merck).

2.2 Instrumentation

Tools used in the research were digital weighing (Mettler Toledo), beaker glass (Pyrex), volumetric flask (Pyrex), micropipette (Mettler Toledo), rotary evaporator, magnetic stirrer (Mettler Toledo), ultrasonic (Bronson), particle size analyzer (Horiba-SZ-100), Zeta potential analyzer (Horiba SZ-100), pH meter (Mettler Toledo), viscometer (Brookfield), FTIR (Bruker Alpha), and UV-Vis spectrophotometer (PG Instruments).

2.3 Procedure

2.3.1 Roselle Extract (RE) preparation

Firstly, the roselle petals were picked and then washed thoroughly using clean water. Subsequently, the clean petals were dried under the sun for seven days to ensure they were totally dry. Next, the dried petals were pollinated with a milling tool to obtain dry roselle powder. The roselle powder was then sieved using a 60 mesh sieve. Afterward, the sieved powder was macerated with ethanol 96% for three days. The maceration results were then filtered apart from the filtrate and the macerate. Finally, the obtained macerate was evaporated for 30 minutes at 40°C to obtain a thick extract.

2.3.2 Nanoparticles Chitosan Roselle (NCR) preparation

This step began with the preparation of chitosan 0.5% w/v solution by weighing 0.5 grams of chitosan, which was then dissolved into 100 ml of acetate buffer pH 4. Next, the solution was stirred with a magnetic stirrer for 30 minutes until it was homogeneous. At the same time, Sodium Tripolyphosphate (STTP) 0.01% w/v was prepared. 0.01 gram of the STPP was then dissolved in 100 ml of distilled water and stirred with a magnetic stirrer for 30 minutes. The ratio of RE: Chitosan 0.5% w/v: STPP 0.01% w/v (2:1:0.1). In the next step, RE was mixed with chitosan solution and homogenized with a magnetic stirrer for two hours. Then, the STPP solution was added to the chitosan extract solution, homogenized with a magnetic stirrer for four hours and sonicated for 30 minutes.

2.3.3 Antioxydant activity test DPPH (1,1-diphenyl-2-picrylhydrazyl) method

By this stage, 5 mg of DPPH was first weighed. Then, by using a measuring flask, 100 mL of methanol was transferred to a container protected from light. Blanks were made by adding 1 mL of 50 ppm DPPH solution, then 1 mL of methanol was added, and the mixture was incubated for 30 minutes. During incubation, the mixture was kept in a dark place. Subsequently, dilutions of vitamin C were made, with various concentrations of 2, 4, 6, 8, and 10 ppm. Next, each concentration variation was pipetted as much as 1 mL, added 1 mL of DPPH solution, and then the mixture was incubated for 30 minutes. During incubation, the mixture was kept in a dark place. Dilutions of sample RE were made with various concentrations of 15, 20, 25, 30, and 35 ppm, and dilutions of sample NCR were made with variations in the concentration of 15, 20, 25, 30, and 35 ppm. Eventually, each sample and concentration variation were pipetted as much as 1 mL and then added 1 mL of 50 ppm DPPH solution. Inhibition percentage can be calculated using the formula:

$$\%Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} x100\%$$

(Equation 2)

3 Result and Discussion

Roselle is identified scientifically by determination testing as *Hibiscus sabdariffa* L., and belongs to the tribe/family Malvaceae Juss. Phytochemical screening of RE showed the presence of compounds that are frequently reported to have antioxidant activity. The extract contains flavonoids, saponins, tannins, and steroids/triterpenoids (Table 1).

Table 1. Phytochemical testing result

Sample	Testing	Result
Roselle Extract	Alkaloid	+
	Saponin	+
	Tannin	+
	Flavonoid	+
	Steroid/Triterpenoid	+
	Quinone	-

Description: + indicates the sample contained in the compound

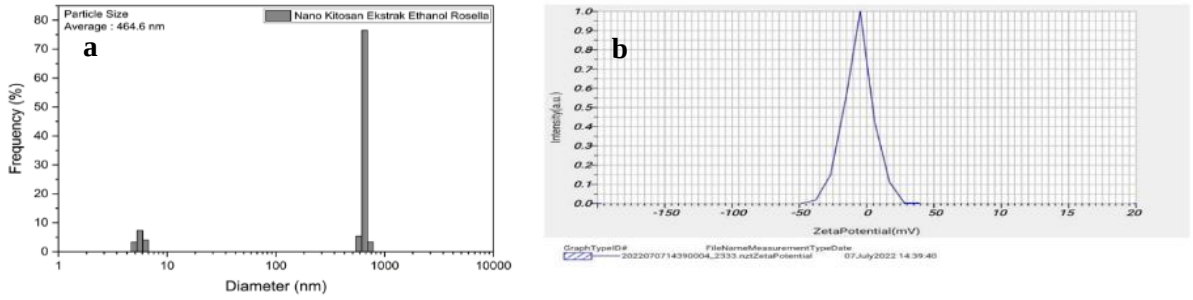

Figure 1. The particle size (a) and zeta potential (b) of NCR

The NCR had a particle size 464.6 nm and polydispersity index of 0.849. The NCR is already in nanoparticle size, but the polydispersity index value shows that the particles formed still have a wide size range, but seen in the graph, they already have a uniform average size in a large portion around 400-500 nm. The measurement of nano-size was obtained by cross-link method, and when the RE and chitosan-STTP were mixed, there should be no heating assistance at all. On the other hand, low-temperature heating can damage the glass ion layer formed from the cross-link reaction. Afterwards, they were stirred with a stirrer to help the dissolving process.

The zeta potential value of NCR shows a value of -6.0 mV, this shows the less stable nature of NCR, which is still easy to form aggregates with fellow particles to form aggregates that have larger particle sizes. Based on the literature, this can be caused by the number of cations from STTP which is less, causing the anion of chitosan to still not be stabilized in the cross-link bond reaction (Figure 1b).

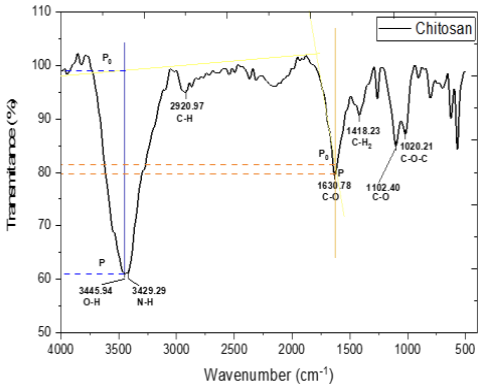

Figure 2. FTIR spectrum of chitosan and deacetylation degree (%DDA)

FTIR characterization was performed to determine the functional groups of chitosan, STTP, and NCR formed. Interactions due to cross-linking between NCR-chitosan-STTP can be seen through the shift in the wave number and intensity of each functional group. From the spectrum in Figure 2, it can be seen that the chitosan characteristic was at 3445 cm^{-1}, whereas N-H and O-H groups were almost close together. At a wavelength of 1630, a characteristic of chitosan was observed, i.e., stretching vibrations on the C-O group. At the wavelength in the spectrum, we could obtain the absorbance and calculate the % degree of deacetylation. The degree of deacetylation showed the value of the loss of free amino groups produced after the deacetylation process. The higher the percentage of the DDA value, the more amine groups in the chitosan molecule, and it also indicates a more reactive molecule (Figure 3).

Table 2. The percentage of deacetylation degree (%DDA)

Wave length	P_0	P	Abs	% DDA
3445	99.12225	61.14567	0.2098	96.0079
1630	81.64883	79.5812	0.0111	

The % DDA obtained met the requirements of chitosan which could be used as food ingredients which was standard for food needs is above 70%, and also be used as cosmetic and medicinal ingredients, which was above 90%.

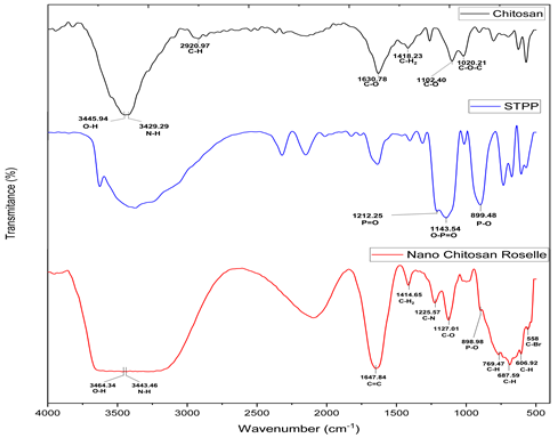

Figure 3. FTIR spectrum of chitosan-STTP-NCR

From the results of the FTIR spectrum, it can be seen that the O-H group in chitosan was at a wavelength of 3445 cm^{-1}, and then at NCREE, it shifted to 3464 cm^{-1}, with a higher intensity. The N-H group was at wave number 3461, which indicates the N-H stretching secondary amide. There was also a stretching P-O aliphatic group from STTP at a wave number of 898 cm^{-1}.

Table 3. Physical testing result

Sample	Testing	Result
Nanochitosan Roselle	Color	Dark chocolate
	Form	Thick liquid
	Odor	Etanol-acetate
	pH	4.79
	Viscosity	1435 cP
	Density	1.183 g/mL
	Zeta potential	-6.0 mV

The results of physical testing showed that the NCR was a dark brown viscous liquid. It had a high density, acidic pH, and odor characteristic similar to acetate and ethanol (Table 3).

3.1 Antioxydant Activity

Table 4. Antioxidant activity result (DPPH method)

Sample	IC_{50} ppm	AAI	Category
RoselleExtract	77.610	0.644	Moderate
Nanochitosan Roselle	22.577	2.217	Very strong
Vitamin C	5.787	8.641	Very strong

The results of statistical analysis showed that all samples have significantly different IC50 values ($p < 0.05$), with vitamin C as a positive control (Table 4). The antioxidant activity of Nanochitosan Roselle is better than Roselle Extract, as indicated by a lower IC50 value. This means that efforts to enhance antioxidant activity by modifying extract preparations into nanoparticle preparations are effective to be done. Such modification can increase the surface area of the extract so that the contact between antioxidant compounds and free radicals will be wider, and it will also increase free radical scavenging reactions, which eventually leads to a decrease in the effects of free radicals. In addition, in the preparation of nanoparticles, antioxidant compounds are more stable. This can be associated with the electrostatic interaction between the positively charged amine group of chitosan, which is protonated under acidic conditions and the oxygen atoms in the flavonoid compounds to stabilize this interaction.

4 Conclusion

Modifying roselle extract (RE) into nanoparticles of chitosan roselle (NCR) with cross-link reaction chitosan-STTP was proven to enhance its antioxidant activity, which was tested in vitro using the DPPH method in this study.

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