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Preparation Of Nanoparticles Containing Soursop (*Annona Muricata* L.) Leaves Extract Using Gelation Ionic Method And Determination Of Its Antioxidant Activity

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ABSTRACT

Annona muricata L. is a member of annonaceae family and its leaves are known as antioxidant and anticancer medicinal herbal. The advantage of nanoparticle application is to increase the penetration of the active compound into the biological system. The aim of this study was to compare the antioxidant activity of the extract, powder extract and powder nanoparticle extract. The simplicia was macerated using ethanol 70% and concentrated using rotavapor. The viscous extract was prepared into nanoparticles by ionic gelation method using poly acrylic acid as the nanoparticles polymer forming and calcium chloride as crosslinking. Afterwards, nanoparticle suspensions were dried by spray drying to get nanoparticle powder. Antioxidant activity was investigated by *in vitro* study of radical scavenger DPPH. In this study, the nanoparticles had a particle size of 558.4 nm and a zeta potential of 27.07 mV. In addition, the particle morphology of nanoparticles was spherical. The results of antioxidant activity showed that IC_{50} of Vitamin C, *soursop (A. muricata)* leaves extract, powder extract, powder nanoparticles were 3.78 ppm; 24.62 ppm; 52.08 ppm; and 46.88 ppm, respectively. The comparison of the antioxidant activity of the extract, the powder of extract, and the powder of the nanoparticles which was tested by one-way ANOVA showed that there were significant differences among all samples.

Keywords : *Annona muricata* L., nanoparticles, gelation ionic method, antioxidant

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INTRODUCTION

Annona muricata, also known as sirsak, soursop, graviola and guanabana, is an evergreen plant which distributed in tropical and subtropical regions. The fruits of A. *muricata* are commonly used to prepare juice, syrups, candies, beverages, ice creams and shakes. Different parts of A. *muricata* are usually contributed in ethnomedicinal activities, and indigenous communities in Africa, South America, and Indonesia extensively use this plant in their folk medicine. There was found numerous study of its biology activity, including anticancer, hepatoprotective, anticonvulsant, anti-arthritic, antimalarial, antiparasitic and antidiabetic activities. Phytochemical studies reveal that annonaceous acetogenins are the major constituents of A. *muricata*. More than 100 annonaceous acetogenins have been isolated from leaves, barks, seeds, roots and fruits of A. *muricata.* (1). Annonaceous acetogenin is known to have a potent anti-cancer activity (2)((3).

Particle diffusion through the intestinal mucosal barrier is restricted by the viscoelastic and adhesive properties of the mucus gel layer, preventing their penetration to the underlying absorptive endothelial cells. To overcome this natural barrier, we can develop nanoparticles which have a remarkable ability to cleave mucoglycoprotein substructures responsible for the structural and rheological properties of mucus (4).

In this research we developed nanoparticle extract by ionic gelation method using poly acrylic acid as the nanoparticles polymer forming and calcium chloride as crosslinking. In this study we compared the antioxidant activity of the extract, powder extract and powder nanoparticle extract. Antioxidant testing method is based on the ability of the extract in reducing free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as measured using a UV-Visible spectrophotometer (5).

MATERIAL AND METHODS

Plant materials :

The voucher specimen was dry leaves of A. *muricata* which collected from Bogor and determined in Center for Plant Conservation Botanic Gardens, Bogor, Indonesia.

Chemical materials :

2,2-diphenyl-1-picrylhydrazil (DPPH) (Sigma Aldrich), poly (acrylicacid) (PAA) (Sigma-Aldrich), calcium chloride, ascorbic acid, dimethyl sulfoxide (DMSO), propylenglycol.

Instruments:

Shimadzu UV-Visible Spectrophotometer, spray dryer (Buchi Press-II), Malvern Particle sizer, Scanning Electron Microscopes (SEM).

Methods :

Extraction

After collection, the leaves of A. *muricata* were air dried (moisture free), grinded to powder. Amount 200 g the dried powdered leaves of A. *muricata* were macerated with 2 litre of EtOH 70% in four times. The extract was concentrated using vacuum rotavapor (45^{o}C, 35 rpm, 175mmHg), here in after called an extract. Afterwards, 500 mg dry extract were dissolved in the mixture consisting of 10 ml of 70% ethanol, 15 ml of propylene glycol, and 0.5 ml of DMSO and aquadest up to 100 mL. The solution was then dried using spray drier with inlet temperature of 190 ^{0}C and outlet temperature of 90 ^{0}C, here in after called a dry extract.

Nanoparticles

The extract was made nanoparticles suspension with poly acrylic acid (PAA) and calcium chloride. PAA solution was used at a concentration of 0.05% (in water) in ad pH with NaOH to pH 8 and 0.1% calcium chloride solution in water. The viscous extract of *soursop (A. muricata)* leaves was diluted with 10 mL of

ethanol, 15 mL of propylene glycol, 0.5 mL of DMSO and aquadest up to 100 mL. After the extract was soluble in the mixture of solvents, PAA solution was added to the extract solution in the final concentration of 0.05%. Afterwards, 0.1% $CaCl_2$ was added drop wise under permanent stirring for 30 minutes at a speed of 400 rpm to form a suspension of nanoparticles.

The stability of nanoparticles was observed for 5 days in term of color, turbidity and sediment. Evaluation of nanoparticle included particles size using a Malvern Particle sizer and examination of the zeta potential using a Malvern zeta potential measuring device.

Furthermore, the nanoparticles suspension was dried using spray drier with inlet temperature of 190 ^{0}C and outlet temperature of 90 ^{0}C. The evaluation of the dry powder of nanoparticles *soursop (A. muricata)* included organoleptic, moisture content and examination of dried nanoparticle morphology using Scanning Electron Microscopes (SEM).

Antioxidant activity test

Antioxidant activity in the extract, the dried extract, and the dried nanoparticles soursop (A. *muricata)* leaves were determined by reacting free radical compound (DPPH) solution in methanol with the sample solution in various concentrations. The solution of extract was prepared in the concentration of 1000 ppm, aliquot extract were pippeted in 5.0 ml volumetric flask to get different concentrations (15 – 75 ppm) of extracts, then added 1.0 ml DPPH radical solution and adjust with methanol. The reaction mixture was shaken and then allowed to stand for 30 minutes at room temperature. After 30 minutes, the absorbance was recorded at 515 nm. The experiment was triplicates and ascorbic acid served as positive control. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. The percentage of inhibition was calculated using following formula:

$$\text{The percentage of inhibition} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank Absorbance}} X\ 100\%$$

IC_{50} value was determined using the linear equation of the graph of inhibition percentage versus sample concentration. IC50 is the concentration required to reduce 50% absorption intensity of DPPH solution.

RESULTS AND DISCUSSION

The obtained soursop extract was viscous, black and has distinctive odor, and bitter taste. The rendement yielded was 15.02% and content of water was 7.93% $\pm$ 0.28. Nanoparticles suspension were observed for the stability, particle size, and potential zeta. The results of nanoparticles suspension evaluation can be seen in Table 1.

Table 1. Results of the evaluation of the nanoparticle suspension soursop (A. *muricata)*

Evaluation	Nanoparticle Suspension
Color	Yellow
Opacity	Transparent
Sediment	none
Particle size	558.4 nm
Potential zeta	27.07 mv

A suspension is said nanoparticles if the particles have a size of 10-1000 nm. The examination of particles size in the suspension showed the particle size requirements are met as shown in table 1. The preparation method used affects the obtained results. This study used an ionic gelation method with stirring using a magnetic stirrer in order to get a solution with a particle size in nanometer in diameter.

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The particle size distribution is expressed in the polydispersity index. The requirement range of polydispersity index is between 0 to 1. Polydispersity index value close to 0 indicates a homogenous dispersion. While the polydispersity index with values greater than 0.5 indicate a high heterogeneity. The nanoparticle has a polydispersity index of about 0.094, thus the nanoparticle showed a relatively homogeneous dispersion.

Zeta potential values can be positive or negative. Those getting close to 0 causes the formed particles have the greater possibility to attract other particles and can lead to aggregation (unstable suspension). Based on the results of zeta potential, the resulting nanoparticles had a negative value which was quite far from 0 causes a repulsive force among particles. The zeta potential of nanoparticles suspensions were negative because the nanoparticles were derived from the polymer which is negatively charged. Thereby, the possibility of aggregate formation and deposition of nanoparticles dispersed in the suspension system is reduced and nanosuspension become stable.

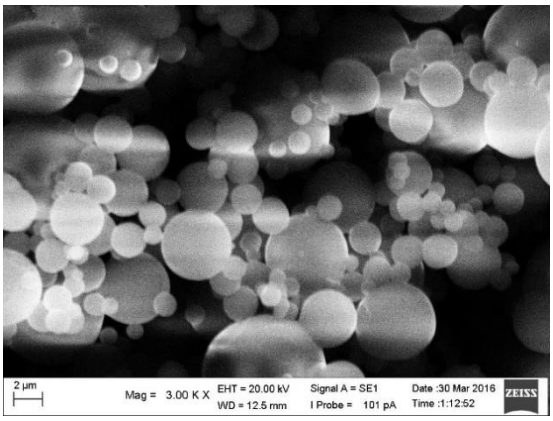

Figure 1. Morphology of dry powder of nanoparticles using *Electron Microscope* (SEM)

The nanoparticles suspension extract was dried using spray drier to obtained dry powder of nanoparticles. The evaluation of nanoparticle powder can be seen in Table 2. Morphology of dry powder nanoparticle is shown in Figure 1. It's demonstrating that the powders were spherical as the very small drops from the nozzle cause the nanoparticles join each other to form bigger particles.

The antioxidant activity in this study using DPPH free radical reduction in visible spectrophotometry at a wavelength of 515 nm. As a comparison, it was made dry powder from soursop leave extract using the same drying method and ascorbic acid serves as standard. The result of antioxidant activity can be seen in Figure 2. The antioxidant activity of the nanoparticles was higher than that of the extract as the nanoparticle could interact more effectively compared to the extract and the drying process on both extract and nanoparticles can lower their activity. This demonstrates that the nanoparticle preparation increases the antioxidant activity. The nanoparticle provides more spaces for the interaction with other substances including free radicals.

Table 2. The evaluation of Nanoparticles soursop (A. *muricata*) leaves extract

Evaluation of Nanoparticles	Examination
Color	Pale yellow
Odor	Extract like
Taste	Bitter slightly sweet
Powder properties	coarse, higroscopis
Water content	5.11%±1.29

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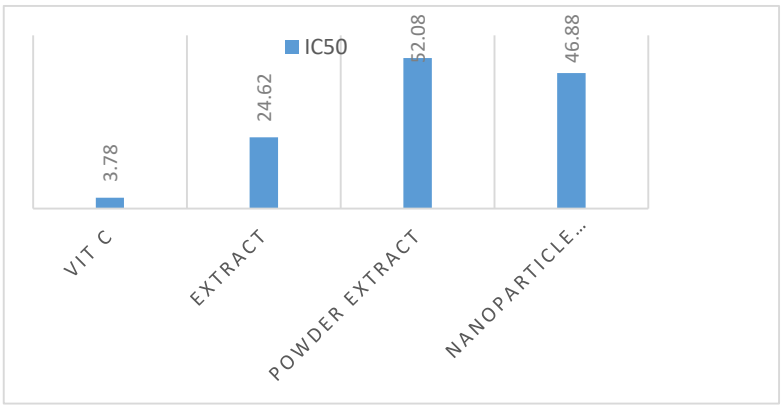

Figure 2. *IC50* Values of Test Sample

CONCLUSIONS

Soursop *(A. muricata*) leaves can be formulated into nanoparticles using PAA / $CaCl_2$ with IC50 value comparatively higher compared to the dry powder of extract without nanoparticles formulation.

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