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PENGARUH KONSENTRASI GELATIN DAN GLUTARALDEHIDA PADA KARAKTERISTIK NANOPARTIKEL GELATIN YANG MENGANDUNG EKSTRAK CANTIGI (*Vaccinium varingiaefolium* Miq.) SEBAGAI ANTIOKSIDAN

EFFECTS OF GELATIN AND GLUTARALDEHYDE CONCENTRATIONS ON CHARACTERISTICS OF CANTIGI (Vaccinium Varingiaefolium Miq.*) EXTRACT LOADED GELATIN NANOPARTICLES AS ANTIOXIDANT*

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ABSTRAK

Cantigi adalah tumbuhan endemik daerah sub-alpen Gunung Tangkuban Parahu di Bandung, Indonesia. Penelitian sebelumnya menunjukkan ekstrak etanol daun merah muda memiliki aktivitas antioksidan, namun belum ada informasi bagaimana aktivitas antioksidannya jika dibuat nanopartikel. Tujuan penelitian ini untuk mengetahui pengaruh konsentrasi gelatin dan glutaraldehida terhadap karakteristik nanopartikel gelatin yang mengandung ekstrak Cantigi dan mengevaluasi aktivitas antioksidan nanopartikel tersebut. Daun kantigi diekstraksi dengan cara maserasi menggunakan n-heksan, etil asetat, dan etanol 96%. Ekstrak etanol dikeringkan, dibuat menjadi nanopartikel dengan memvariasikan jumlah gelatin (0,1; 0,2; dan 0,3 g) dan glutaraldehida (0,1; 0,2; dan 0,3 mL), dan dilakukan pada 500 rpm dan 40 ° C selama 3 jam. Nanopartikel dievaluasi ukuran partikel, potensial zeta, morfologi, dan aktivitas antioksidan. Nanopartikel dengan variasi jumlah glutaraldehida memiliki ukuran partikel (PS) 105,9 ± 26,2; 37,1 ± 8,7; dan 32,5 ± 7,4 nm; polydispersity indeces (PI) sebesar 0,508; 0,717; dan 0,563; nilai potensial zeta (ZPV) 0,55; 0,89; dan 0,78 mV; dan aktivitas antioksidan (IC50) sebesar 56,15 ± 0,16; 53,67 ± 0,10; dan 51,57 ± 0,39 ppm. Kemudian, nanopartikel dengan variasi jumlah gelatin memiliki PS 22,5 ± 5,1; 37,1 ± 8,7; dan 83,3 ± 21 nm; PI sebesar 0,604; 0,717; 0,326; ZPV dari 1,27; 0,89; 0,18 mV; dan aktivitas antioksidan 51,58 ± 0,19; 53,67 ± 0,12; dan 55,46 ± 0,04 ppm. Morfologi nanopartikel yang dihasilkan berbentuk bulat. Ekstrak daun cantigi dapat dibuat menjadi nanopartikel gelatin. Semakin kecil konsentrasi polimer yang digunakan dan semakin tinggi konsentrasi glutaraldehida maka semakin kecil ukuran partikel yang dihasilkan dan aktivitas antioksidan meningkat. Aktivitas antioksidan nanopartikel lebih rendah dibandingkan ekstrak (IC50 16,84 ± 0,30 ppm).

Kata kunci: *Vaccinium varingiaefolium* Miq, Cantigi, ekstrak etanol, nanopartikel gelatin, antioksidan

ABSTRACT

Cantigi is an endemic plant of sub-alpine area of Mount Tangkuban Parahu in Bandung, Indonesia. Previous study showed ethanol extract of young red leaves had antioxidant activity, however no information on this activity if changed into nanoparticles. The purpose of this study was to determine the *effects of gelatin and glutaraldehyde concentrations on the characteristics of Cantigi extract loaded gelatin nanoparticles and to evaluate the antioxidant activity of nanoparticles. Cantigi leaves were extracted by maceration using n-hexane, ethyl acetate, and ethanol 96%. The ethanol extract was dried, made into nanoparticles by varying gelatin (0.1; 0.2; and 0.3 g) and glutaraldehyde (0.1; 0.2; and 0.3 mL) amounts, and conducted at 500 rpm and 40 °C for 3 hours. Nanoparticles were evaluated for particle size, zeta potential, morphology, and antioxidant activity. Nanoparticles with glutaraldehyde amount variation had particle sizes (PS) of 105.9±26.2; 37.1±8.7; and 32.5±7.4 nm; polydispersity indeces (PI) of 0.508; 0.717; and 0.563; zeta potential values (ZPV) of 0.55; 0.89; and 0.78 mV; and antioxidant activities (IC50) of 56.15±0.16; 53.67±0.10; and 51.57±0.39 ppm, respectively. Then, nanoparticles with gelatin amounts variation had PS of 22.5±5.1; 37.1±8.7; and 83.3±21 nm; PI of 0.604; 0.717; 0.326; ZPV of 1.27; 0.89; 0.18 mV; and antioxidant activities of 51.58±0.19; 53.67±0.12; and 55.46±0.04 ppm, respectively. Nanoparticle morphology was spherical. Cantigi leaf extract can be made into gelatin nanoparticles; the smaller the concentration of the polymer used and higher the concentration of the glutaraldehyde, the smaller the resulted particle size and increased antioxidant activity. Antioxidant activities of nanoparticles was lower than those of the extract (IC50 16.84±0.30 ppm).*

***Keywords** : Antioxidant, Cantigi, ethanol extract, gelatin nanoparticles, Vaccinium varingiaefolium* Miq.

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INTRODUCTION

Indonesia is a country having a plenty of endemic plants. Cantigi (*Vaccinium varingiaefolium* (Blume) Miq.) is one of the plants. It belongs to Ericaceae family (Sholikhah et al., 2017). Cantigi grows well in the area close to sulphur vents or volcano region, such as Mount Tangkuban Parahu (MTP) having a peak at 2,081 meters above sea level (masl). MTP is a volcanic mountain, about 20 km north of Bandung, Indonesia, or near 6°40′00″ south latitude and 107°37′00″ east longitude. Vaccinium forest is at 1,900 masl, 06°45′40″ S & 107°37′07″E in an area near the crater (Okada et al., 2007).

Cantigi young leaves empirically have been used as anti-aging, anti-inflammation, wound healing, anti-spasmolytic, and anti-hypertension. Previous studies showed that ethanol extract of Cantigi leaves was positive for flavonoids, tannins, quinones, volatile oil, and triterpenoids. It also had strong antioxidant activity (IC_{50}) of 15.62 ppm using DPPH method (Yulyana, 2016).

There are various techniques that can be used to the drug or herbs to enhance the efficiency of drugs like nanoparticles techniques that have been used in many types of medicinal component. It is considered as one of the most effective technique to improve drug delivery system and drug bioavailability. Many studies showed that the technique is applicable to increase the effectiveness of herbal drug properties such as to inhibit foodborne pathogens better inhibition zone compare to the crude, also improve drug delivery system via oral administration (Yongsirasawad et al., 2017). Nanoparticles are solid colloidal particles having the size from 1 to 1,000 nm, but the FDA has standardized the size is less than 100 nm. They can be used to entrap, encapsulate or absorb active agents (drugs, herbs, or other biologically active agents) (Sham et al., 2004).

Nanoparticles have various advantages and unique properties, such as stimulating drug absorption, prolonging drug release, and sustainability. They are also protecting against drug degradation, and making them an ideal candidate for drug delivery system. Certain polymeric materials can be used for the preparation of nanoparticles for drug delivery system offering biocompatible and biodegradable choices (Singh, 2014). Gelatin is a biomaterial having been used for drug delivery. It is produced from animal collagen by partial hydrolysis using acid or alkaline. In terms of biocompatibility and biodegradability, gelatin based nanoparticles are nontoxic, low antigenic, inexpensive, readily available, and provide cross-linking and chemical modification potential. Moreover, gelatin is FDA approved. These make them a reasonable choice for a drug delivery (Ghasemishahrestani et al., 2015).

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Various methods, including simple w/o emulsion method, reverse phase preparation technique, inverse miniemulsion technique, coacervation technique, desolvation technique, two-step desolvation technique, and nanoprecipitation technique have been used to prepare gelatin nanoparticles (Khan, 2014). Moreover, there are some studies using these methods to produce gelatin nanoparticles containing different bioactive molecules, including plant extracts (Ghasemishahrestani et al., 2015; Song et al., 2019; Kesornbuakao et al., 2018). Although all of these methods have several advantages, they still have limitations (Drew et al., 2017).

The objective of this study was to determine the effects of gelatin and glutaraldehyde concentrations on the characteristics of Cantigi extract loaded gelatin nanoparticles and to evaluate the antioxidant activity of nanoparticles.

MATERIALS AND METHODS

Materials

Young red Cantigi leaves used were collected from MTP, north Bandung, West Java, Indonesia; and identified at Pusat Penelitian Biologi, Lembaga Ilmu Pengetahuan Indonesia, Cibinong Science Center, Cibinong, Bogor, Indonesia. Solvents used for maceration (hexane, ethyl acetate, and ethanol) were pro analytical grade from Mallinckrodt Baker (USA). DPPH (2,2-diphenyl-1-picrylhydrazyl) and vitamin C were from Sigma-Aldrich Chemie (Steinheim, Germany). Gelatin type B was from Nitta Gelatin India Ltd.

Preparation of Extract

Dried Cantigi leaves were powdered, extracted by maceration methods using hexane, and then the residual solid part was extracted using ethyl acetate. The residual solid part of the ethyl acetate extraction was then extracted using ethanol 96%. Finally, ethanol extract was concentrated and dried by vacuum rotary evaporator (Yulyana, 2016).

Antioxidant Activity

DPPH method

DPPH (2.2-diphenyl-1-picrylhydrazyl) radical is a compound which is relatively stable and commercially available. The method was carried out based on Desfrey modification (Desfrey, 2017). 10 mg of Cantigi extract was weighed and dissolved in 10 mL methanol (as stock solution of 1000 ppm). A series of concentrations of 5, 10, 15, 20, 25 µg/mL were made by diluting the stock solution, then 1,0 mL of 0.4 mM DPPH in methanol was added to each concentration. The total volume was 5 mL by addition of ethanol. Each concentration was homogenized and incubated at 37^oC for 30 minutes, then measured at 517 nm. DPPH method was used to measure the antioxidant activities of the positive control (vitamin C) and the Cantigi extract loaded gelatin nanoparticles as well.

Preparation of Cantigi Extract Loaded Gelatin Nanoparticles

Gelatin nanoparticles were prepared using desolvation method as described by Coester et al. with slightly modification (Coester et al., 2000). Gelatin (100, 200, and 300 mg) was dissolved into 25 ml of purified water at 40^o C. Cantigi extract (100 mg) was dissolved into co-solvent (ethanol 96%, purified water, glycerin, DMSO). The solution color was pale red. Then the extract solution was added into the gelatin solution. The mixture was mixed at 1000 rpm. Pluronic F-68 (300 mg) as the stabilizer then was added without stopping mixing. The pH was adjusted to 2.00 ± 0.05 using 1N HCl. Aceton of 37.5 mL was then added, the color changed to orange. After 10 minutes, 0.2 mL of 25% glutaraldehyde (0.1; 0.2; and 0.3 mL) was added as the crosslinker. The mixing was continued for 3 hours. Finally, the nanoparticles formed were freeze dried.

Characterization of Cantigi Extract Loaded Gelatin Nanoparticles

Nanoparticle size distribution and zeta potential measurements

Mean particle size and zeta potential of the nanoparticles were determined using Zeta Sizer Nano Malvern (Zen 3600, UK). The mean diameters and polydispersity index (PI) values were obtained at 90° angle in 10 mm diameter cells (Subara, 2019).

Scanning electron microscopy (SEM)

Morphologies characterization by scanning electron microscopy

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Field emission scanning electron microscopy (FESEM JEOL, JSM 6700F Model) was used to observe the size and shape of the nanoparticles. In brief, a small amount of the nanoparticles was dried, mounted on aluminum plates, and pasted with double sided copper tapes. The samples were sputtered with a thin layer of gold and placed on the packet chamber at an accelerating voltage of 10 kV (Subara, 2018)

RESULTS AND DISCUSSION

Cantigi (*Vaccinium varingiaefolium* (Blume) Miq has young leaves with color of red and old leaves with color of green (Figure 1). The red ones had stronger antioxidant activity (IC_{50} of 16.84 ppm) compare to the green ones (IC_{50} of 45 ppm) (Lupita ,2019). This activity was close to the previous study [3]. The pH of ethanol extract of Cantigi leaves was acidic (pH 2.87). This might be caused by acid and sulphur content as the plants grow well close to the crater and as showed by unreported study. Moreover, the solubility of the extract in many solvents tent to be low, except in DMSO (Table 1). In general, the solubility of plant extract in many solvents decrease after drying, and DMSO or co-solvent can be used to overcome this problem.

Figure 1. Cantigi plant with young leaves (red) and old leaves (green)

Table 1. Characteristics of ethanol extract of Cantigi leaves

Parameter	Result
Organoleptic	Color: Dark red Odor: Specific for Cantigi extract Physical form: Viscous
pH	2.87 $\pm$ 0.02
Water content	5.46 $\pm$ 0.24
Solubility	1:100 in Water 1:100 in Ethanol 96% 1:100 in Glycerin 1:100 in Acetone 1:10 in DMSO

Table 2 shows 6 formulas that were studied. The first three formulas studied the effects of various concentrations of gelatin on the characteristics of the ethanol extract loaded gelatin nanoparticles, while the rest formulas studied the effects of various concentrations of glutaraldehyde on the characteristics of the ones. The results showed that nanoparticles with gelatin amounts variation had PS of 22.5±5.1; 37.1±8.7; and 83.3±21 nm; PI of 0.604; 0.717; 0.326; ZPV of 1.27; 0.89; 0.18 mV; and antioxidant

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activities of 51.58±0.19; 53.67±0.12; and 55.46±0.04 ppm, respectively. Then, the nanoparticles with glutaraldehyde amount variation had particle sizes (PS) of 105.9±26.2; 37.1±8.7; and 32.5±7.4 nm; polydispersity indices (PI) of 0.508; 0.717; and 0.563; zeta potential values (ZPV) of 0.55; 0.89; and 0.78 mV; and antioxidant activities (IC50) of 56.15±0.16; 53.67±0.10; and 51.57±0.39 ppm, respectively. The results can be seen on Table 3 and Table 4. This the first report on gelatin nanoparticles loaded with Cantigi extract.

Table 2. Formula (F) of Cantigi ethanol extract loaded gelatin nanoparticles

Ingredients	F1	F2	F3	F4	F5	F6
Gelatin	100 mg	200 mg	300 mg	200 mg		
Purified water	25 Ml					
Cantigi extract	100 mg					
Ethanol 96%	10 mL					
Purified water	5 Ml					
Glycerin	15 mL					
DMSO	7 Ml					
Acetone	37.5 Ml			37 Ml		
Pluronic F-68	300 mg					
Glutaraldehyde	0.2 mL			0.1 mL	0.2 mL	0.3 Ml
Total volume	100 Ml					

Table 3. Particle size, polydispersity index, and zeta potential of ethanol extract loaded gelatin nanoparticles

Formula (F)	Particle size (nm)	Polydispersity index	Zeta potential (mV)
F1	22.5 ± 5.1	0.604	1.27
F2	37.1 ± 8.7	0.717	0.89
F3	83.3 ± 21	0.326	0.18
F4	105.9 ± 26.2	0.508	0,55
F5	37.1 ± 8.7	0.717	0,89
F6	32.5 ± 7.4	0.563	0,78

Table 4. Antioxidant activities (IC_{50}) of ethanol extract loaded gelatin nanoparticles and crude extract

Sample	IC_{50} (ppm)
Crude extract	16.84 ± 0.23
F1	51.58 ± 0.19
F2	53.67 ± 0.12
F3	55.46 ± 0.04
F4	56.15 ± 0.16
F5	53.67 ± 0.10
F6	51.57 ± 0.39
Vitamin C	2.74 ± 0.02

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The above results showed that Cantigi leaf extract can be made into gelatin nanoparticles; the smaller the concentration of the polymer used and higher the concentration of the glutaraldehyde, the smaller the resulted particle size and increased antioxidant activity. In general, the particle sizes were less than 100 nm, and these complied with the FDA standard. Polydispersity indices were between 0 and 1. These meant that the dispersities were still good. Unfortunately, the zeta potentials of the gelatin nanoparticles were too low, close to zero. These could mean that the stability of the gelatin nanoparticles would be affected. But, the use of surfactant such as Pluronic F-68 had enhanced the stability of the gelatin nanoparticles indicated by no precipitation and no change in color for 30 days (Figure 2). Nanoparticle morphology was spherical (Figure 3). This morphology was still in accordance with many previous studies on gelatin nanoparticles (Khan, 2014).

Day 1 (no precipitation) Day 30 (no precipitation)

Figure 2. Physical stability of ethanol extract loaded gelatin nanoparticles of F1, F2, and F3 at room temperature.

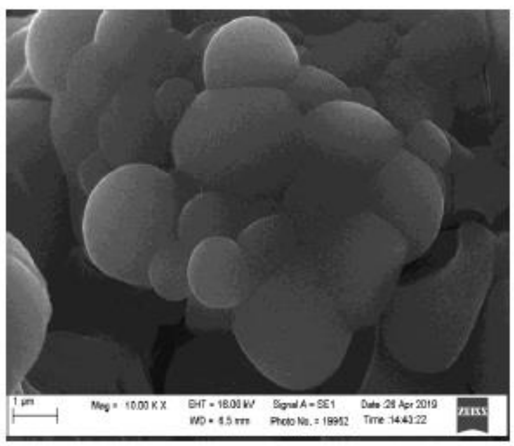

Figure 3. The morphology of the ethanol extract loaded gelatin nanoparticles

The antioxidant activities of the gelatin nanoparticles were lower than the activity of the crude extract. This can be explained as follows. After the gelatin nanoparticles were formed, the extract was entrapped or encapsulated in the nano structure. This influenced the release of the extract to be slower. Also, the change in structure of active compounds into less active or inactive ones might lead to decrease the activity. Moreover, the functional groups of the extract might change because of intra or inter crosslinking process during manufacturing of the gelatin nanoparticles. The crosslinker used such as glutaraldehyde could cause this intra or inter chemical reaction with gelatin molecule (Khan, 2014; Yongsirasawad et al., 2018).

CONCLUSIONS

Based on the results, it can be concluded that cantigi leaf extract can be made into gelatin nanoparticles. The smaller the concentration of the polymer used and higher the concentration of the glutaraldehyde, the smaller the resulted particle size and increased antioxidant activity. Antioxidant activities of the gelatin nanoparticles were lower than those of the extract (IC_{50} 16.84±0.23 ppm).

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CONFLICT OF INTEREST

It is stated that no conflict of interest in this study.

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