

Synthesis Nanoparticles of Chloroform Fraction from *Kaempferia rotunda* Rhizome Loaded Chitosan and Biological Activity as an Antioxidant

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ABSTRACT

The main objectives of this research are to synthesize chitosan nanoparticles of chloroform fraction of *K. rotunda*, to characterize the products, and to conduct a biological test on these products as an antioxidant. Chloroform fraction of *K. rotunda* was loaded on chitosan nanoparticles and then was prepared by ionic gelation of chitosan with sodium tripolyphosphat (Na-TPP) in various compositions. Characterization of the products were investigated for particle size, zeta potential, and morphology by Scanning Electron Microscopy (SEM). The biological activity of the products as an antioxidant was tested by the DPPH method. Results of this study showed that the nanoparticle can be synthesized at the concentration ratio of 10: 1 for chitosan/Na-TPP. The size were in the range of 172 to 877 nm, with a zeta potential of + 28.06 to + 38.03 mV. The nanoparticle was cylinders in shape and smooth in surfaces. The antioxidant activity of chitosan nanoparticles of chloroform fraction of *K. rotunda* showed less activity compared with the previous fraction.

Keyword : Nanoparticles; *K. rotunda*; antioxidant

INTRODUCTION

Kaempferia is a genus, belonging to the family of Zingiberaceae. This plant grows in Southeast Asia, India, Sri Lanka, Indonesia, and Southern China. *Kaempferia* is interchangeable with *Boesenbergia* genus according to Baker¹. The plants grows naturally in damp, shaded parts of the lowland or on hill slopes, as scattered plants or thickets. There are economically important species among the plant of Zingiberaceae, which are perennial rhizomatous herbs, contain volatile oil and other important compounds of enormous medicinal values¹. This research was focused on *Kaempferia rotunda* known as kunci pepet or kunir putih in Indonesia. This plant has been traditionally used to treat abdominal pain, sputum laxative, wounds, and diarrhea colic disorder. Many researchers reported that this plant has biological activity as antioxidant², antimutagenic³, and anticancer^{4,5}. The previous study showed that solubility of chloroform fractions of *K. rotunda* is relatively low³. Therefore, cytotoxic activity of this fraction in fighting against the T47D cell of breast cancer by *in vitro* and *in vivo* is also low. In order to increase the solubility of this fraction, nanoparticle technology is applied in this research. The main objectives of this research are then to synthesize nanoparticle chitosan of chloroform fraction of *K. rotunda*, to characterize this product, and to conduct biological test as an antioxidant.

In recent years, nanotechnology begins to grow in the fields of engineering, medicine, electronics, optics and biomedicine⁶. Nanotechnology is the study of particles in

the size range of 1-1000 nm. Currently, nanotechnology is widely used in chemistry and medicine, so as to facilitate works on the molecular and cellular levels. In the field of pharmaceuticals and medical, nanotechnology development has been achieved and applied in human life. The application of nanotechnology in medicine is especially to cure disease and repair damaged tissues such as bone, muscle, and nerves. The use of nanotechnology in the pharmaceutical field has many advantages such as increasing the solubility of the compound, reducing drug doses, and increasing absorption. Nanotechnology has been able to manipulate drugs to reach a target with a right dose, therefore many researchers have used it to heal some serious diseases such as tumors, cancer and HIV⁷. These nanoparticles are expected to be adsorbed in an intact form in the gastrointestinal tract after oral administration⁸. Polymeric nanoparticles from biodegradable and biocompatible polymers are good candidates for being drug carriers to deliver medicine. Several studies have reported the manufacturing of nanoparticles. For example, Dustgani⁹ conducted a study on the manufacturing of nanoparticles of chitosan as a matrix for dexamethasone. Also, the synthesis of nanoparticles of chitosan as a matrix for glycyrrhizinate was reported by Wu Y⁸. Furthermore, Kim¹⁰ made the chitosan nanoparticles as a retinol matrix. Nanoparticle products derived from natural materials such as curcumin were used for the treatment of cancer. Many products of nanoparticle were developed and used in clinical treatments^{11, 12}.

The synthesis of nanoparticles can use several methods

Table 1: Synthesis of nanoparticle products of chloroform fraction of *K. rotunda* loaded Chitosan

Code Formula	Sample (g)	Chitosan (g)	Na-TPP (%)	Yield (X ± SD) g	Particle size (nm)	% nano and microparticles	Zeta potensial (mV)	Physical properties
A1	1	0.1	0.02	0,72 ±0.02	1981-2976	Microparticle (100%)	2.33	Solid, white yellow
A2	1	0.2	0.02	0.39 ±0.02	172-877	Nanoparticle (100%)	28.06	Solid, white yellow
A3	1	0.3	0.02	0.39 ±0.03	259-2269	Nanoparticle (64,2%); Microparticle (35.8%)	40.30	Solid, white yellow
A4	1	0.4	0.02	0.44 ±0.05	259-1318	Nanoparticle (65%); Microparticle (35%)	34.20	Solid, white yellow
B1	1	0.1	0.01	0.38 ±0.03	150-877	Nanoparticle (100%)	38.23	Solid, white yellow
B2	1	0.1	0.03	0.77 ±0.08	3905-4472	Microparticle (100%)	2.75	Solid, white yellow
B3	1	0.1	0.04	0.74 ±0.04	3409-3905	Microparticle (100%)	2.44	Solid, white yellow

Table 2: The inhibition activity (IC₅₀) of nanoparticle of chloroform fraction of *K. rotunda* loaded chitosan and positive control as antioxidant

Sample Code	IC ₅₀	Note
A1	792.76	Less active
A2	425.63	Less active
A3	973.12	Less active
B1	200.00	Less active
Chloroform fraction of <i>K. rotunda</i>	25.20	active
Positive control (Ascobat acid)	3.77	Very active

such as ionic gelation, emulsification, coacervation or precipitation, and spray drying methods¹³. Ionic gelation method involves connecting a cross between polyelectrolyte in the presence of ion-pair multivalent. Ionic gelation is often followed by polyelectrolyte complexation with polyelectrolyte opposite. The formation of the cross connecting bond will strengthen the mechanical strength of the particles formed. Polymer nanoparticles are usually made using biodegradable polymers and hydrophilic such as chitosan, gelatin and sodium alginate. Chitosan is a natural polysaccharide [β (1→4) glucosamine (2-amino-2-deoxy-d-glucose)-N-acetyl-d-glucosamine (2-acetamido-2-deoxy-d-glukosa)], and widely applied in the pharmaceutical industry, food and health. Chitosan has several beneficial properties that are anti-microbial, wound healing, non-toxic, inexpensive, biocompatible, biodegradable and water soluble. The form of micro or nanoparticles of chitosan has many advantages that are not toxic, unstable during in use, high surface area,

and it can be used as matrices for different types of medications and plant extracts¹³. Chitosan has further demonstrated capacity to enhance macromolecules epithelial permeation through transient opening of epithelial tight junctions. In addition chitosan is known to be biocompatible and to exhibits very low toxicity. Compared to many other natural polymers, chitosan has a positive charge and mucoadhesive¹⁴.

The principle of the method is that the existence of ionic interactions between the amino groups of positively charged chitosan with negatively charged poly-anion compounds forms a three-dimensional network structure. Cross linker poly-anion which is most widely used is sodium tripolyphosphate, because it is non-toxic and a multivalent. The process is not only the physical cross avoiding the use of organic solvents, but also preventing the possibility of damage to the active ingredient to be packaged as a product of chitosan nanoparticles. Making product of nanoparticles using chitosan as drug delivery materials can be performed by the method of Wu⁸.

MATERIAL AND METHOD

Apparatus and reagent

Glassware, analytical balance, evaporator Buchi Rotavapor R-114, magnetic stirrer, sentrifuge, scanning electron microscopy (SEM, Jeol T-300), particles size analysis (PAS, Horiba 550), Zeta potential, refrigerator, and Spectronic 20 (Genesys) were commonly used in this work.

Ethanol, aquabidest, chitosan (low molecular weight, Sigma), Sodium Tripoliphosphat (Na-TPP, Sigma-Aldrich), Acetic acid (p.a. Sigma), chloroform (p.a. Sigma) Rhizome of *K. rotunda*, 1,19-diphenyl-2-picrylhydrazyl (DPPH, Aldrich), and ascorbat acid (Aldrich) were purchased and used without further purification.

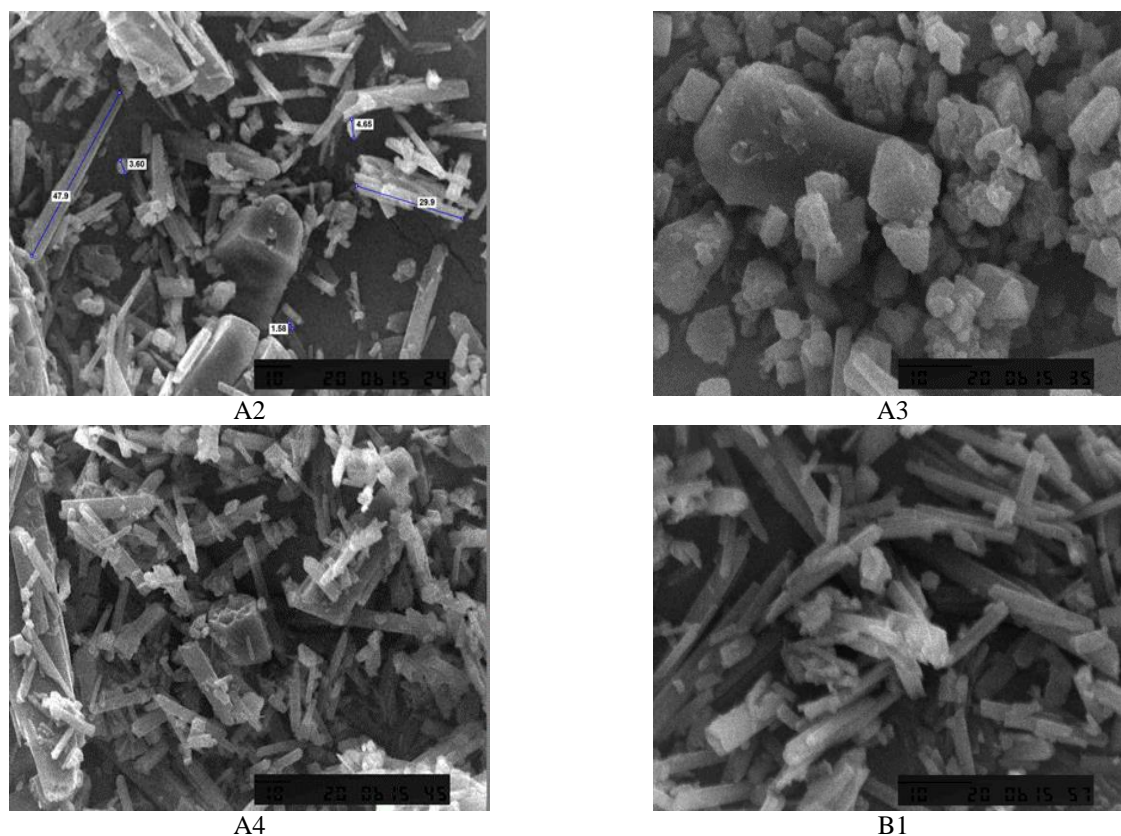


Figure 1: Scanning electron microscopy data of the nanoparticle products

Preparation of chloroform fraction of *K. rotunda*

The milled dried rhizoma of *K. rotunda* (3 kg) was extracted exhaustively with ethanol. The ethanol extract was partitioned three times with *n*-hexane, chloroform, and ethyl acetate respectively. The chloroform fraction was evaporated using vacuum evaporator to dry to yield brown residue of about 230 g.

Preparation of chitosan–chloroform fraction of *K. rotunda* nanoparticles

Nanoparticles of chloroform fraction of *K. rotunda* loaded chitosan were synthesized by ionic gelation. Firstly, the chloroform fraction of *K. rotunda* was dissolved in 35 mL of ethanol and 35 mL of aquadest. After homogen it was added 100 ml of chitosan in various concentration (0.1 – 0.4 g of chitosan in 100 ml of acetic acid 1%), and was mixed by magnetic stirring until homogen. The resulting solution was further added with 350 mL of Na-TPP (in various concentration (0.01-0.02% in aquadest), and was kept for complete dissolution by magnetic stirring for 2 hours. The mixture was stabilized overnight in refrigerator. The nanoparticles were collected by centrifugation at 12.000 rpm for 15 minutes, dried and then stored in refrigerator till further used. Yield of the nanoparticles synthesis were calculated by the formula:

$$\% \text{ yield} = \frac{[\text{weight of nanoparticles obtained}]}{[\text{weight of sample fraction} + \text{weight of chitosan used for synthesis}]} \times 100\%$$

The product was analyzed in particle size, zeta potential, and SEM (Scanning Electron Microscopy).

Antioxidant activity of the nanoparticles of chloroform fraction of *K. rotunda* loaded Chitosan

To analyze the effect of the nanoparticle of chloroform fraction of *K. rotunda* loaded chitosan we tested the free-radical scavenging activity. In this work, 1,19-diphenyl-2-picrylhydrazyl (DPPH) was used as the source of free-radicals. The nanoparticle suspended in ethanol was used for the analysis. About 5 ml of the nanoparticle was mixed with 5 ml of methanolic solution of DPPH (0.12 mM) and kept in dark at room temperature for 30 minutes. The DPPH scavenging activity was determined using spectronic 20 (Genesys) at 516 nm against DPPH solution as control. The samples were tested in triplicates. The antioxidant activity was calculated as percentage of DPPH that was decreased in comparison with the control, and was calculated with the formula:

$$\% \text{ Antioxidant activity} = \frac{[\text{A control} - \text{A sample}]}{[\text{A control}]} \times 100\%$$

and thus the inhibition activity could be calculated to determine IC₅₀.

Statistical analysis

The data of all experiments were represented as Mean ± SD and were analyzed with SPSS 13.0 statistic software. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

In this study, the main data were obtained through 7 designed experiment on various concentrations of chitosan and Na-TPP (Table 1). Data in Table 1 show particle size, zeta potential, yield, and physical properties of each product. The syntheses of nanoparticles of chloroform fraction of *K. rotunda* loaded chitosan are carried out at

ambient temperature. The preparations are simple, rapid, and reliable. The corresponding nanoparticles products are obtained spontaneously under very mild conditions. These data show that nanoparticle products obtained at a concentration ratio of chitosan/Na-TPP, 10: 1, is as much as 100%, and the products obtained in comparison of chitosan/Na-TPP 15: 1 and 20: 1 are to be 64.2 % and 65%, respectively. When the concentrations of chitosan are high, the particles formed from the reaction electrostatic between chitosan and Na-TPP are very much and dense, so that these are clustered to form aggregates into micro-sized particles. Nanoparticles products obtained are relatively less compared with the product microparticles. This may lead to suggestion that the nanoparticle products are more stable and more difficult to be separated by centrifugations.

From these results it can be seen that the particle size is strongly affected by the use of concentration ratio of chitosan and Na-TPP, where the particle size increases with increasing concentration ratio of chitosan and Na-TPP. Previous research was adjusted to get a chitosan/Na-TPP ratio of 6:1. Chitosan nanoparticles thus were obtained in the size range of 300–400 nm with a positive surface charge ranging from +54 to +25 mV¹³. However, the nanoparticle product is highly dependent on the deacetylation of chitosan used, because it involves gelation of the protonated amino group of chitosan.

Zeta potential of these products showed in the range of +28 to +38 mV, that was indicated a stable colloidal system. In general, nanoparticles showed zeta potential of about +30 mV. If the value of the particle zeta potential is large, the colloidal system will be stable. Conversely, if the particle zeta potential is relatively small, the colloidal system will agglomerate.

The morphology of nanoparticle products of chloroform fraction of *K. rotunda* was evaluated using optical microscope by scanning electron microscope. For SEM analysis, the working distance was 10 mm, beam energy was 20.0 kV, spot size was 5.0, and magnification was 2000x. The nanoparticle products were loaded on a double sided carbon tape put on studs before being examined by SEM. Figure 1 shows that the SEM micrographs revealed cylinders in shape with smooth surfaces.

The DPPH assay was used to study the free-radical scavenging capacity of these products. The results are shown in Table 2. The antioxidant activity of nanoparticles of chloroform fraction of *K. rotunda* loaded chitosan shows less activity compared with the previous fractions before modified. This is consistent with previous studies¹⁵, which showed that the encapsulated products requires time to leach out into the DPPH solution for the scavenging activity to take place.

CONCLUSION

The nanoparticle products of chloroform fraction of *K. rotunda* loaded chitosan were successfully obtained by ionic gelation method. The products can be synthesized at a ratio of 10:1 in concentration of chitosan/Na-TPP. The size of the nanoparticles were in the range of 172 to 877 nm, with a zeta potential of +28.06 to +38.03 mV. The

particles were cylinders in shape with smooth in surface. The antioxidant activity of chitosan nanoparticle of chloroform fraction of *K. rotunda* showed less activity compared with the previous fraction before modified.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest in this article.

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