Antioxidant activity from encapsulated Cinnamaldehyde-Chitosan

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Abstract. Cinnamaldehyde compound is a powerful antioxidant agent that can effectively combat the free radicals referred to superoxide anions and hydroxy radicals, as well as other free radicals in vitro testing. An antioxidant is an electron donor or reductant. Antioxidants are also compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. As a result, cell damage will be inhibited. However, the use of this compound still provides unsatisfactory results due to its degradation during the absorption process. The solution offered to solve the problem is by encapsulated it within chitosan nanoparticles that serve to protect the bioactive compound from degradation, increases of solubility and delivery of a bioactive compound to the target site by using freeze-drying technique. The value of encapsulation efficiency (EE) of cinnamaldehyde which encapsulated within chitosan nanoparticles is about 74,389%. Also antioxidant activity test showed that cinnamaldehyde encapsulated by nanochitosan could inhibit free radicals of 223.44 in IC_{50}.

Keyword: Cinnamaldehyde, Encapsulated, antioxidant activity

1. Introduction
Antioxidants are inhibitors that can be used to inhibit auto-oxidation of carbohydrates, nucleic acids, and lipids by free radicals in the body [1]. The existence of oxidation reaction due to free radicals in the body can cause disease [2]. The body does not have an excessive oxidative defense system, so if there is exposure to excessive radical body requires exogenous antioxidants [3]. Cinnamaldehyde is a well-known natural antimicrobial compound that has been shown to be effective against a broad spectrum of food-borne pathogens [4]. The aldehyde group in cinnamaldehyde can react with the amino group in chitosan through the Schiff base reaction. Studies have shown that cinnamaldehyde cross-linked chitosan nanoparticles can be prepared using water-in-oil emulsions as templates [5]. According to Hu et al. 2003 [6] one of the safe materials used as chitosan coating which is the result of extraction of animal skin waste class of crustaceans. Chitosan has been widely used as a drug coating for the purpose of optimizing drug absorption on cell targets. Gallic acid encapsulated-chitosan has been experimented with an encapsulation efficiency value of 50.76% [7] and Cinnamomum casia extract encapsulated-chitosan has been experimented with an encapsulation efficiency value of 84.93%. [8]. The mechanical properties of the brittle chitosan must be stabilized with sodium tripolyphosphate (STPP) as a crosslinking. Desai & Park [9] have proven that crosslinked chitosan microspheres with STPP can be used as a coating of drugs by spray drying method. The methods that can be used for the manufacture of nanoparticles are ultrasonication, homogenization, and magnetic scavengers.
2. Procedure
2.1 Materials and Instrumentation
Materials; Chitosan, Cinnamaldehyde (Sigma aldrich), Aquades, Na-TPP (Sigma Aldrich), 1% glacial acetic acid (Merck), Ethanol 70% (Merck), Tween 80 (Merck), DPPH (Merck) test solution, ascorbic acid (Merck) and methanol p.a (Merck) 
Research tools used are glass that commonly used in the laboratory, Hot Plate Stirrer (IKA C-MAG HS 7), Centrifuge (Corning LSE Compact), Freeze drying (LLI500), PSA (Microtrac-Particle Size Analyzer), and Spectrophotometer Ultraviolet-Visible (Spectroquant).

2.2 Eksperimental

2.2.1. Preparation of chitosan. Chitosan of 0.2 g dissolved in 100ml glacial acetic acid 1% (v / v). Na-TPP 0.5 g solution, stirred using a magnetic stirrer for 1 hour. Subsequently the chitosan nanoparticle suspension was generated at centrifugation at 6000 rpm for 10 min. The centrifugation results were then dried using freeze drying and PSA characterization was performed to detect the nano size formed.

2.2.2 Preparation of cinnamaldehyde emulsion. Preparation of nanoemulsion using spontaneous emulsification technique. The emulsion system consists of organic phases (sinamaldehyde and ethanol 70%) and water phases (aquades and Tween 80). The organic phase was prepared by mixing the cyclamaldehyde and 70% ethanol solvent to reach the total dissolved solids of 20° brix. The spontaneous emulsification technique is done by adding the organic phase into the water phase through drip (dropwise). At the time of dripping the organic phase into the water phase, the water phase is stirred using a magnetic stirrer, the surfactant used is Tween 80. The best emulsion formula is determined by the best characteristics (particle size, polydispersity index value, zeta potential) using light-scattering Particle Size Analyzer DelsaTM Nano C (Beckman Coulter, France).

2.2.3 Encapsulation of cinnamaldehyde nanoemulsion. After the manufacture of nanoemulsion, a coating material of nanocitosan was added with a ratio of 1: 1 (v / v). Then stirring for 1 hour using a magnetic stirrer. A slightly thicker solution is produced, then centrifuged at 6000 rpm for 30 minutes to produce gel suspension and supernatant. The gel suspension is dried using a freeze dryer to produce a cinnamaldehyde encapsulation powder. The sinamaldehyde encapsulation powder was then calculated by encapsulation efficiency (% EE) using a UV-Visible spectrophotometer.

2.2.4 Antioxidant Activity Test of DPPH Damping Method
2.2.4.1 DPPH Parent Solution. The DPPH parent solution was made by diluting 2 mg of DPPH powder in a 50 mL volume flask with p.a methanol and stored in a dark bottle.

2.2.4.2 Blenko and Control. The control used was DPPH solution and the blank used was methanol p.a. The maximum wavelength of the control is determined by measuring the maximum absorbance at a wavelength of 400-600 nm.

2.2.4.3 Antioxidant Test. Cinnamaldehyde encapsulation-chitosan sample was dissolved in p.a methanol with various concentration variations. A total of 1 mL of metabolite samples from each concentration variation added 3 mL DPPH 0.1 mM incubated for 30 min in darkness. The next step, measured absorbance at a maximum wavelength of 517 nm [10] and performed the same on ascorbic acid as antioxidant comparator. Absorbance control and absorbance of test samples were used to calculate % inhibition using the following formula:

\[
\% \text{ inhibition} = \frac{(Abs \ control - Abs \ solution \ test)}{abs \ control} \times 100\%
\]
3. Results and Discussion

3.1 Characterization

Nanoparticles have very specific properties and surface area that multiply, usually can increase the chances of the occurrence of more chemical reactions. A substance can be absorbed directly into the bloodstream where it is needed so that it is more effective than broken down during the digestive system [11]. Factors that affect the work of substances such as repeated doses and income [12]. The application of nanotechnology to chitosan that is in the formation of nanoparticles or microparticles allows chitosan to be a conductor of functional compounds or drugs to be more effective. The use of nanoparticles or microparticles in the coating process can increase the absorption of active substances. Encapsulation process means that various nutrients can be absorbed directly into the bloodstream where the nutrients are needed, it will be more effective than broken down during processing or breakdown with enzymes in the digestive system. New mechanisms can be developed with nanotechnology in drug delivery issues. The foundation used in the drug delivery system is to stimulate the effectiveness of the drug, through specific targeting and specific cells, the acceleration of delivery time, and the prevention of digestive enzymes in breaking down the drugs being consumed [11].

Modern particle calculations generally use image analysis or some kind of particle counting such as Particle Size Analyzer (PSA) analysis. The average particle size distribution obtained by the size of the sinamaldehyde emulsion has a particle size of 336.3 nm while microchitosan has size 2249.7 nm. The value of encapsulation efficiency (EE) of cinnamaldehyde which encapsulated within chitosan nanoparticles is about 74.389%. This shows that the coated sinamaldehyde in chitosan microparticles is 74.389%.

3.2 Antioxidant Activity

Test of antioxidant activity in this study was conducted with the aim of knowing the profile of radical damping capacity by encapsulation cinnamaldehyde-chitosan. Antioxidant capacity was measured by DPPH Scavenging Activity method using DPPH reagent (1,1-diphenyl-2-picrilhidrazil). The result of antioxidant activity test by DPPH damping method is shown in Figure 3.1.

![Figure 3.1](image_url)

**Figure 3.1** % Graph of sample inhibition against DPPH

Ghasemi et al. [13] states that DPPH is a free radical that is stable at room temperature and is often used to evaluate the antioxidant activity of some compounds or extracts of natural materials. The principle of antioxidant activity test method is quantitative measurement of antioxidant activity by measuring DPPH radical capture by a compound having antioxidant activity using UV-Vis spectrophotometry so that known value of free radical damping activity is expressed by IC50 (Inhibitory Concentration fifty) value [14]. In Figure 3.1 shows the value of IC50 in the antioxidant test in the encapsulation cinnamaldehyde-chitosan sample with a value 223.44 ppm.
4. Conclusion
Encapsulation Cinnamaldehyde-chitosan has moderate antioxidant capability. Encapsulation cinnamaldehyde-chitosan can be an alternative antioxidant.

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