Antidiabetic activity from cinnamaldydhe encapsulated by nanochitosan

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Abstract. Diabetes mellitus (DM) is a disease characterized by chronic hyperglycemia and metabolic disorders of carbohydrates, proteins, and fats due to reduced function of insulin. Treatment of diabetes can be done by insulin therapy or hypoglycemic drugs. Hypoglycemic drugs usually contain compounds that can inhibit the action of α-glucosidase enzymes that play a role in breaking carbohydrates into blood sugar. Cinnamaldehyde has α-glucosidase inhibitory activity because it has a functional group of alkene that is conjugated with a benzene ring and a carbonyl group. 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%. Inhibition test result showed that cinnamaldehyde-chitosan nanoparticles at 100 ppm could inhibit α-glucosidase activity in 23.9% with 134,13 in IC₅₀. So it can be concluded that cinnamaldehyde can be encapsulated in nanoparticles of chitosan and proved that it could inhibit α-glucosidase.

1. Introduction

The worldwide estimation of diabetic patients around 2030 will be more than double from that of 2005, and most of these will be dominated by those suffering from type 2 diabetes [1]. So diabetes has become the third largest health killer, which is only second to cancer and cardiovascular diseases. At present, the drugs for diabetes include sulfonylureas and biguanides. Both of them have some beneficial effects on hyperglycemia and also have some adverse effects. So it was an urgent need to have some new kinds of drugs to solve this problem until α-glucosidase inhibitors turned up. The α-glucosidase inhibitors which inhibit the enzyme at the brush border of small intestine are effective for delaying glucose absorption and preventing postprandial blood glucose level elevation, therefore they play a significant role in the therapy as chemotherapeutic agents for Noninsulin-dependent diabetes mellitus (NIDDM) [2]. Such inhibitors, including acarbose and voglibose, are currently used clinically in combination with either diet or other anti-diabetic agents to control blood glucose levels of patients [3]. Natural resources provide a huge and highly diversified chemical bank from which we can explore for potential therapeutic agents by bioactivity-targeted screenings [2]. Recently, some of the natural phenolic compounds and polysaccharides (such as tea, grapefruit, strawberries) have been shown to inhibit the α-glucosidase enzyme and potential as the natural therapeutic agent for the treatment of diabetes mellitus [4].
Chitosan is prepared by the alkaline deacetylation of chitin [5]. Chitosan uses one as a means of delivering drugs and genes and biomaterials for immobilization. Chitosan is a natural biopolymer of interest because of the presence of reactive amino groups and hydroxyl functional groups. Chitosan has the desired biocompatibility characteristics and its ability to increase membrane permeability so that chitosan is very promising as it has the ability to form membranes [6]. Physical modification of chitosan involves changing the particle size or chitosan granules to be smaller for wider use. Utilization of physical modification produces nanoparticle size [7]. Biological activities of chitosan, such as its antitumor effect [8], cholesterol-lowering effects [9] and antibacterial effects [10] are well known. However, few reports are available on the antidiabetic effects of chitosan.

Cinnamaldehyde has an alkene functional group which is conjugated with a benzene ring and a carbonyl group. Research Subash et al [11] mentioned that sinamaldehyde has an antidiabetic effect in diabetic rats induced streptozotocin with a decrease in glucose levels of 63.29%. Sinamaldehyde contained in cinnamon oil is useful as an antidiabetic with IC50 value of 27.96 ppm [12], because sinamaldehyde has the most functional group similar to caffeine and cinnamic compounds compared to other cinnamon oil components. This advantage causes sinamaldehyde to have potential as an antidiabetic

This research investigated the characterization of chitosan structure, and anti-diabetic potential of encapsulation cinnamaldehyde-chitosan nanoparticles. Firstly, synthesized chitosan nanoparticles, nanoemulsion cinnamaldehyde, and encapsulated cinnamaldehyde in chitosan nanoparticles. To determine the encapsulation of cinnamaldehyde-chitosan nanoparticles as anti-diabetic was test by inhibition α-glucosidase enzyme.

2. Methods
2.1 Materials and Instrumentation

Materials; Chitosan, Cinnamaldehyde (Sigma aldrich), Aquades, Na-TPP (Sigma Aldrich), 1% glacial acetic acid, Ethanol 70%, Tween 80, ethanol, ether, bovine serum albumin, ρ-nitrophenyl α-D-glucopyranoside.

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 (LL1500), PSA (Microtrac-Particle Size Analyzer), FTIR (Shimadzu-Fourier Transform Infrared) and Spectrophotometer Ultraviolet-Visible (Spectroquant).

2.2 Eksperimental
2.2.1. Preparation of chitosan nanoparticles.

Chitosan of 0.2 g dissolved in 100ml glacial acetic acid 1% (v / v). Na-TPP 0.5 g dissolved in 100ml aquades. The Na-TPP solution was added dropwise into the chitosan 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 cynamaldehyde 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 Alpha-glucosidase inhibition test.

An enzyme solution was prepared by dissolving 1.0 mg α-glucosidase in 100 ml of phosphate buffer (pH 7.0) containing 200 mg bovine serum albumin. Before use as much as 250μL 20 mM p-nitrophenyl α-D-glucopyranoside as substrate, then added 490μL 100mM phosphate buffer (pH 7.0) and 10 μL solution of the synthesis compound in DMSO. The reaction mixture was incubated at the optimum temperature for 5 min, after which 250 μL of enzyme solution was added and incubated. The enzymatic reaction was discontinued by the addition of 1000 μl of 200 mM sodium carbonate. The reaction product is a p-nitrophenol compound which can be measured using a UV-Visible spectrophotometer at a 400nm wavelength. The sample used in the α-glucosidase inhibition test was sinamaldehyde with various concentrations of 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm with DMS solvent. Percent of inhibition can be calculated by the equation:

\[
\% = \frac{C - S}{C} \times 100\%
\]

with \( S \) = absorbance of the sample (obtained from the \( S_1 - S_0 \), where \( S_1 \) = absorbance of the sample with enzyme addition and \( S_0 \) = the absorbance of the sample without enzyme addition) and \( C \) = absorbance of control (DMSO), without sample (control-blank). IC50 values was obtained from: a linear regression equation \( Y = a + bx \).

\[
IC50 \rightarrow Y = 50, \text{ then it was substituted into the linear regression equation becomes:} \\
IC50 = (50-a)/b
\]

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 [13]. 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 allows chitosan to be a conductor of functional compounds or drugs to be more effective. The use of nanoparticles in the coating process can increase the absorption of active substances. The nanoencapsulation process means that various nutrients can be absorbed directly into the bloodstream where the nutrients are needed, it will be much 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 [17].
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 sinamaldehid emulsion has a particle size of 336.3 nm while nanokitosan has size 2249.7 nm. The value of encapsulation efficiency (EE) of cinnamaldehyde which encapsulated within chitosan nanoparticles is about 74%. This shows that the coated sinamaldehid in chitosan nanoparticles is 74%

3.2 Inhibition α-glukosidase test

The antidiabetic activity test of cinnamaldehyde-encapsulated with nanochitosan was performed by inhibition of glucosidase enzyme activity with glucobay tablet as standard. On this test, the alpha-glucosidase enzyme will hydrolyze p-nitrophenyl-α-D-glukopyranoside substrate into the yellow p-nitrophenol and glucose with the following the reaction:

\[
\alpha - \text{glukosidase} \rightarrow \text{p-nitrophenol} + \text{glucose}
\]

![Enzymatic reaction of α-glucosidase and p-nitrophenyl-α-D-glucopiranoside](image)

**Fig 1. An enzymatic reaction of α-glucosidase and p-nitrophenyl-α-D-glucopiranoside**

**Fig 2. Percent inhibition of α-glucosidase enzyme from cinnamaldehyde-encapsulated**

Enzyme activity was measured based on the absorbance of the p-nitrophenol which has yellow color. In the presence of Gallic acid-chitosan nanoparticles which acts as an alpha-glucosidase inhibitor so the p-nitrophenol produced will reduce which was characterized by a lowering in the intensity of yellow color. The p-nitrophenol is a product of the enzymatic reaction of alpha-glucosidase with p-nitrophenyl-α-
D-glucopyranose substrate. Gallic acid-chitosan nanoparticles can inhibit the action of the alpha-glucosidase enzyme which was characterized by a reduced concentration of p-nitrophenol.

In Figure 2 shows a percentage of inhibition of $\alpha$-glucosidase enzyme from sinamaldehyde-encapsulated at a concentration of 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm. From Figure 2 it can be seen that cinnamaldehyde-encapsulated at 100 ppm can inhibit $\alpha$-glucosidase enzyme by 23.916% with IC$_{50}$ value were 134,13 ppm. IC$_{50}$ value is a number indicating the concentration of sample (ppm) capable of inhibiting 50% of $\alpha$-glucosidase enzyme activity.

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