

Antidiabetic Activity from Gallic Acid Encapsulated Nanochitosan

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Abstract. Diabetes mellitus (DM) has become a health problem in the world because it causes death. One of the phenolic compounds that have antidiabetic activity is gallic acid. 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 result of chitosan nanoparticle's Scanning Electron Microscopy (SEM) showed that chitosan nanoparticle's size is uniform and it is smaller than chitosan. The value of encapsulation efficiency (EE) of gallic acid which encapsulated within chitosan nanoparticles is about 50.76%. Inhibition test result showed that gallic acid-chitosan nanoparticles at 50 ppm could inhibit α -glucosidase activity in 28.87% with 54.94 in IC₅₀. So it can be concluded that gallic acid can be encapsulated in nanoparticles of chitosan and proved that it could inhibit α -glucosidase.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder caused by dysfunction of insulin that causes hyperglycemia [1]. It caused long-term damage, dysfunction, and failure of various organs. The World Health estimates that in 2030 about 366 million people will suffer *diabetes mellitus* [2]. One of therapeutic to reduce hyperglycemia after a meal is prevented adsorption of glucose by inhibiting the enzyme hydrolysis on carbohydrate, such as α -glucosidase enzyme. The denial of α - glucosidase enzyme, glucose in the blood can be returned within normal limits [3]. 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 a polysaccharide derived from deacetylation of chitin which is found in the shells of crabs, shrimp, and squid. Chitosan has been shown afford to biodegradable, biocompatible, non-toxic, non-



antigenic, and antimicrobial, so chitosan could be applied in biomedical. Chitosan nanoparticles have activity as anti-diabetic [5].

Gallic acid is known as a phenolic compound that has drug activity for some disease, one of them as anti-diabetic. The use of gallic acid as *diabetes mellitus* (DM) results unsatisfactory because it is not stable at extreme temperatures or presence of oxygen or light [6] and is suspected because less of absorption by the human body the affected by particle's size is large, poor solubility during the absorption process. It is encouraging to modify for optimized the properties of the active compound by performing encapsulation in nanoparticles that serve to improve the solubility and *bioavailability*. *Encapsulation method is done to protect the bioactive compounds packaged in a secondary ingredient* (polysaccharide, protein, or lipid) to protect them from the effects of oxygen, heat and light that can affect the stability of compound [7].

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

2. Procedure

2.1 Materials and Instrumentation

Materials; Chitosan with 80-85% deacetylation degree that obtains from deacetylation of chitin, acetic acid p.a (Merck), sodium tripolyphosphate (Na-TPP) p.a (Sigma-Aldrich), water de-ionization, gallic acid (Sigma Aldrich), 4-nitrophenol, phosphate buffer, α -glucosidase enzyme, bovine albumine serum, dimethyl sulfoxide (DMSO), *p*-nitrophenyl, α -D-glucopyranoside as a substrate.

Research tools used are glass that commonly used in the laboratory, Blender, Oven, Hot Plate Stirrer (IKA C-MAG HS 7), Centrifuge (Corning LSE Compact), Freeze drying (LLI500), 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 nanoparticles were prepared based on the ionic gelation method: Chitosan was synthesized by chitin deacetylation isolated from shrimp shells. Chitosan was dissolved with acetic acid, then stirred until homogeneous. Hereafter, it added by solution of sodium tripolyphosphate slowly. Gel suspension was centrifuged and dried using freeze dryer, then characterized by FT-IR, and SEM.

2.2.2. Encapsulation of gallic acid in chitosan nanoparticles. Encapsulation of gallic acid in chitosan nanoparticles were prepared based on inclusion complexation method. First, gallic acid 0,1% was added chitosan nanoparticles. Gel suspension was centrifuged and dried using freeze dryer, to be followed by determination of encapsulation efficiency.

2.2.3. Alpha-glucosidase inhibition test. The enzyme inhibition assay was done based on the methods from Lee and Lee, 2001; and Prashanth et al., 2001. Enzyme solution was prepared by dissolving 1.0 mg of α -glucosidase in 100 mL of phosphate buffer (pH 7.0) containing 200 mg of bovine albumin serum. Prior to use, as much as 1 mL of that solution was diluted 25 times using phosphate buffer (pH 7.0) which then referred as the enzyme solution. A total of 250 μ L 20 mM *p*-nitrophenyl α -D-glucopyranoside as a substrate, then added 490 μ L 100 mM phosphate buffer (pH 7.0) and 10 μ L solution of synthesized compounds in DMSO. The reaction mixture was incubated at the optimum

temperature for 5 minutes, then 250 μL of enzyme solution was added and then it was incubated. Enzymatic reaction was stopped by the addition of 1000 μL of 200 mM sodium carbonate. The product of the enzymatic reaction was *p*-nitrophenol which can be measured by using UV-visible spectrophotometer at 400 nm. The concentration of sample were 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm in DMSO solvent. Percent inhibition can be calculated from the equation: $[(C - S) / C] \times 100$; 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). IC_{50} values was obtained from: a linear regression equation $Y = a + bx$.

$\text{IC}_{50} \rightarrow Y = 50$, then it was substituted into the linear regression equation becomes:

$$\text{IC}_{50} = (50 - a)/b$$

3. Results and Discussion

3.1 Characterization

Analysis of FTIR spectra provided information about the functional groups of products. The spectra showed that the compound has a functional group as expected compound. FTIR spectra of chitin (**Fig.1**) showed some vibration patterns, which are in 3490 cm^{-1} from NH, 3310 cm^{-1} from-OH, 1650 cm^{-1} from stretching vibration of C=O, and 1560 cm^{-1} from stretching vibration of NH. The stretching vibration of NH is characteristic for chitin due to an NH group in -NH-CO- (acetylated amine group).

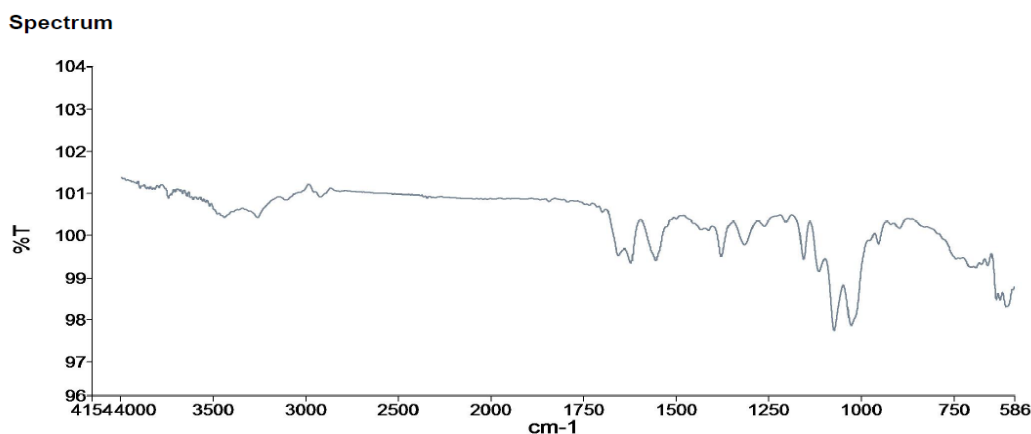


Fig. 1. FTIR Spectra of Chitin

Based on the Fig. 1 showed that vibration of CH_3 from chitin at 1310 cm^{-1} coincides with C-N amide vibration at 1400 cm^{-1} . The vibration of the amine group of chitin in the 3490 cm^{-1} coincided with OH vibration because hydrogen bond in amine is weak and less polar. It makes N-H bond vibration becomes less intense than OH. Other vibration at 2970 cm^{-1} is a stretching from aliphatic C-H. This absorption is weak because chitin structure is dominated by $\text{R}_3\text{C-H}$ (methine) which has a weak absorption (Sastrohamidjojo 1992). The presence of an absorption band at $1010\text{--}1150\text{ cm}^{-1}$ showed a vibration of C-O. Absorption pattern appears to indicate that the residue is a chitin as expected.

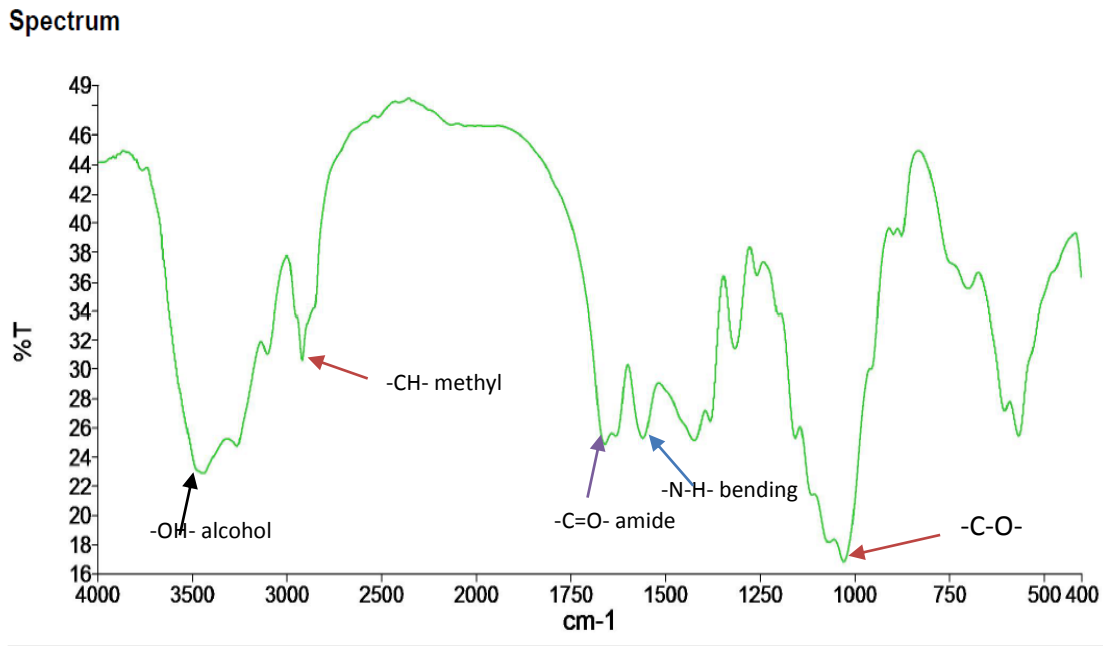


Fig. 2. FTIR Spectra of Chitosan

Based on Fig.2 that the changing of FTIR spectra of chitin and chitosan can be confirmed that deacetylation was successfully transformed chitin into chitosan. However, the carbonyl group absorption band at 1690 cm^{-1} showed that chitosan is not perfectly deacetylated. Due to that reason, it is a need to calculate the degree of deacetylation (DD) based on the baseline method, according to the following equation:

$$\% DD = 1 - \left[\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \right] \times 100\%$$

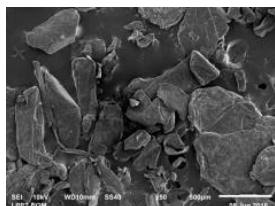
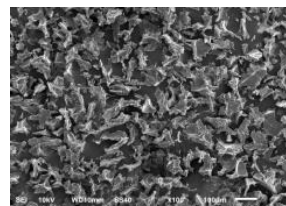
A_{1655} = absorbance at 1655 cm^{-1} wavelength number (amide absorption)

A_{3450} = absorbance at 3450 cm^{-1} wavelength number (hydroxyl absorption)

1.33 = A_{1655} for chitin which is fully deacetylated (100%).

Based on chitosan spectra (**Fig.2**), it is resulted in a degree of deacetylation of 87.78%. It means only about 89.98% residual chitin which is deacetylated into chitosan. The degree of acetylation of commercial chitosan is between 20-25%. In other words, the degree of deacetylation is between 70-90%. It can be concluded that deacetylation done in this research is in a good range.

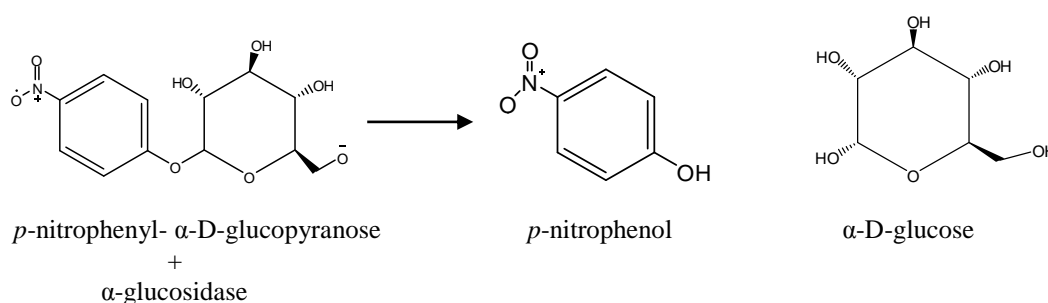
The next stage is characterisation by *Scanning Electron Microscope* (SEM): the SEM data showed the morphological differences between chitosan and nanochitosan.

**Fig 3.** SEM of chitosan**Fig 4** SEM of Nanochitosan

To determine the size of synthesized chitosan, it is necessary to measure the chitosan particles using SEM. Based on fig. 3 showed that chitosan has a particle size of 64.9 nm. Based on the fig.4 showed that the chitosan has a nano-sized particles and the nano chitosan has a uniform shape. The value of encapsulation efficiency (EE) of gallic acid which encapsulated within chitosan nanoparticles is about 50.76%.

3.2 Inhibition of Alpha-glucosidase activity test

Inhibition test of the alpha-glucosidase enzyme was conducted to determine the antihyperglycemic activity of each extract. On this test, the alpha-glucosidase enzyme will hydrolyze *p*-nitrophenyl- α -D-glucopyranoside substrate into the yellow *p*-nitrophenol and glucose with the following the reaction:

**Fig. 5.** An enzymatic reaction of α -glucosidase and *p*-nitrophenyl- α -D-glucopyranoside

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-glucopyranoside substrate. Gallic acid-chitosan nanoparticles can inhibit the action of the alpha-glucosidase enzyme which was characterized by a reduced concentration of *p*-nitrophenol.

It clearly is seen showed on **fig. 5** that 50 ppm of cinnamon oil could inhibit the enzyme alpha-glucosidase by 28.87 % and IC_{50} values were 54.94 ppm. IC_{50} values are numbers that indicate the concentration of the sample (in ppm) which are able to inhibit 50% of α -glucosidase enzyme activity. The results indicate that cinnamon oil could potentially inhibit α -glucosidase enzyme.

The alpha-glucosidase enzyme is an enzyme that plays a role in the termination of 1-4 glycoside bonds of carbohydrates to produce blood sugar (glucose). Inhibiting the alpha-glucosidase enzyme work will lead to prevent increasing concentration of glucose levels in blood after eating.

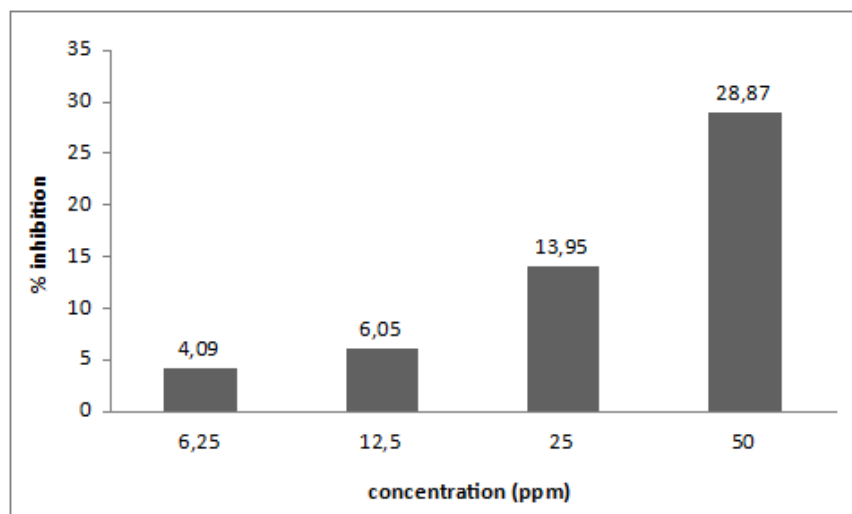


Fig.6 Percent inhibition of alpha-glucosidase enzyme from gallic acid-chitosan nanoparticles in varying concentration

4. Conclusions

Gallic acid can be encapsulated in nanoparticles of chitosan and proved that it has inhibited α -glucosidase. It showed that gallic acid encapsulated nanochitosan can inhibit 28.87 % at 50 ppm with $IC_{50} = 54.94$.

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