

**Cinnamomum casia** Extract Encapsulated Nanochitosan as Antihypercholesterol

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**Abstract.** Atherosclerosis vascular disease with clinical manifestations such as cardiovascular disease and stroke are the leading cause of death in Indonesia. One solution to these problems is a natural antihypercholesterol medicine by utilizing *Cinnamomum casia* extract. However, the use of natural extracts to lower blood cholesterol levels do not provide optimal results because it is possible that the active components of extract have been degraded /damaged during the absorption process. So that, we need to do the research to get a combination of chitosan nanoparticles-*Cinnamomum casia* extract as a compound which has an antihypercholesterol activity through the in vitro study. Modification of natural extracts encapsulated nanochitosan be a freshness in this study, which were conducted using the method of inclusion. The combination of both has the dual function of protecting the natural extracts from degradation and deliver the natural extracts to the target site. Analysis of nanochitosan using the Particle Size Analyzer (PSA) shows the particle size of synthesis product that is equal to 64.9 nm. Encapsulation efficiency of *Cinnamomum casia* extract-Chitosan Nanoparticles known through UV-VIS spectrophotometry test and obtained the efficiency encapsulation percentage of 84.93%. Zeta Potential at 193,3 mv that chitosan appropriate for a delivery drug. Antihypercholesterol activity tested in vitro assay that showed the extract-nanoparticle chitosan in concentration 150 ppm gave the highest cholesterol decreasing level in the amount of 49.66% w/v. So it can be concluded that *Cinnamomum casia* extract can be encapsulated in nanoparticles of chitosan and proved that it has a cholesterol-lowering effect through the in vitro study.

1. **Introduction**

According to data from the Ministry of Health in 2013 the number of patients with cardiovascular disease, stroke and heart attack showed an upward trend and that the disease has become one of the major causes of death in Indonesia. Cardiovascular disease caused by hypercholesterolemia, one of them is a medical condition characterized by blood cholesterol levels more than normal. Many factors cause hypercholesterolemia among others caused by genetic factors such as familial hypercholesterolemia and polygenichypercholesterolemia, that habit, obesity and lack exercise [1]. The main purpose of treatment of hypercholesterolemia is to maintain normal range of cholesterol levels. Treatment of hypercholesterolemia may include weight control, exercise and diet. But it does not help much to lower blood cholesterol that usually needs to be given cholesterol-lowering drug therapy. Anti-cholesterol drugs have been widely available in the market, but almost all is synthetic compounds would pose a risk to use in a long time so it needs to be developed natural anti-cholesterol drugs by utilizing several Indonesia’s plant extracts.
Cinnamomum casia is a popular plant Indonesian and most widely used as a traditional medicine. Cinnamomum is believed to have very large benefits including increased appetite, anti-cholesterol, anti-inflammatory, anemia, antioxidant, cancer prevention, and anti-microbial. Cinnamomum bark is composed of three fractions, namely fraction of cinnamic acid, cinnamaldehyde and essential oil [2]. The use of natural extracts to reduce blood cholesterol levels still give unsatisfactory results when compared with the use of synthetic drugs [3]. The efficiency of absorption of the body is less because of the relatively large particle size, low solubility and possible active components of the extract has been degraded/damaged during the absorption process. This research will be a combination of Cinnamomum extract with nanochitosan. The cinnamomum will be encapsulated in nanoparticles of chitosan. The purpose of this are protecting the natural extracts from degradation and it delivering to the target side [4]. Starting from the above problems, it appeared an innovation to improve and explore types of antihypercholesterol compounds with high activity so as to reduce, and even be able to cope with demand antihypercholesterol synthetic drugs, and replace them with cheap raw materials and with a simple method. Overall these activities are divided into three groups: the extraction of bioactive compound from cinnamomum and synthesize of nanochitosan, a modified nanoparticle of chitosan-cinnamomum extract as antihypercholesterol drug and in vitro assay of antihypercholesterol. The advantages from cinnamomum extract encapsulated in nanoparticles of chitosan are natural extract protected from degradation and it can be released slowly and continuously over a certain period, so the delivery toward the target cells occurs precisely as well as the use of natural extracts to be optimal. Nano chitosan - cinnamomum extract is an innovation in the field of nano herb that has a synergistic effect. That is mutually reinforcing in order to produce high nutritious as an antihypercholesterol drug.

2. Experimental
2.1 Materials and procedure
Cinnamomum casia were collected in the surroundings of Semarang, Central Java province (Indonesia), in February 2016, shrimp shells were collected from Traditional Market at Semarang, Central Java province (Indonesia), Cholesterol was purchased from Sigma Chemicals Company, St. Louis, Mo. USA. All other and reagents were of analytical grade and purchased from Organic Laboratories, Diponegoro University.
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 Active Components Extraction of Cinnamomum casia
The active components of plants extracted with ethanol for 24 hours. The next stage is filtered to collect the active component. The solvent is evaporated by using rotary evaporator.

2.3 Phytochemical screening Extract
Phytochemical screening was conducted to determine the active component from The obtained extract. Screening/qualitative tests were performed such as alkaloids, saponin, flavonoids, phenolics, tannins, steroids and terpenoids tests.

2.4 Nanochitosan Synthesis of Shrimp Shell
Demineralization, shell shrimp or crab shell has been mashed into a powder which then it was added by HCl. The mixture was heated at 80 °C for 4 hours while stirred with a stirrer of 50 rpm and filtered. The solids washed by distilled water to remove HCl solution. Floured solids dried in the oven at 70 °C for 24 hours. The shrimp shell powder or dried crab shell from demineralization stage added NaOH and it stirred at 90 °C for 1 hour. Furthermore the result obtained is filtered then washed with distilled water until neutral pH. The result is a chitin then added it with 70% ethanol. The precipitate washed in hot distilled water and acetone to remove the color. It is a chitosan. Chitosan nanoparticles
were prepared based on the ionic gelation method. Chitosan was dissolved in acetic acid. Then stirred it until homogeneous. Forward it added by sodium tripolyphosphate slowly. Gel suspension centrifuged and dried it with freeze dryer. Fathemore nanochitosan charachterized by FT-IR and SEM.

2.5 Encapsulation of cinnamomum in chitosan nanoparticles
Encapsulation cinnmomum in chitosan nanoparticles were prepared based on inclusion complexation method. First, 0.1% of cinnamomum was added chitosan nanoparticles. Gel suspension was centrifuged and dried using freeze dryer, then determined of encapsulation efficiency.

2.6 Chemical and physical properties tests
Morphology of natural extracts encapsulated nano chitosan characterized using Particle Size Analyzer (PSA) to analyzing nanoparticles of chitosan size. The efficiency of the extract encapsulation process in nano chitosan was evaluated by calculating the value of encapsulation efficiency (Encapsulation Efficiency, EE), and loading capacity (Loading Capacity, LC) extracts in nanoparticles of chitosan. Levels of natural extracts in the supernatant was analyzed by ultraviolet-visible spectrophotometry. EE value and LC is calculated using the formula below:

\[
\text{Where, } \text{EE} (\%) = \frac{m_0 - m_s}{m_0} \times 100 \\
\text{LC} (\%) = \frac{m_0 - m_s}{w_{np}} \times 100
\]

\[m_0 = \text{initial mass of natural extracts}\\
M_s = \text{mass of natural extracts in the supernatant}\\
w_{np} = \text{total weight of the naturally obtained extract of nanoparticles [5]}
\]

2.7 Anticholesterol assay
Anticholesterol assay conducted by cholesterol Lieberman-Burchard reaction. It determines the amount of free cholesterol in the sample which is reacted in green. Furthermore, its absorbance measured by UV-Vis spectrophotometer. If the concentration of free cholesterol in the sample increased then the solution color become lighter. [6]

3. Results and Discussion
3.1 Phytochemical study
The purpose of the phytochemical assay was determine the secondary metabolite that content in cinnamomum extract. The result of phytochemical of cinnamomum extract is presented in table 3.1.

| No | Group              | Result |
|----|--------------------|--------|
| 1  | Flavonoids         | (+)    |
| 2  | Saponins           | (+)    |
| 3  | Alkaloid           | (-)    |
| 4  | Steroids/Triterpenids | (+)  |
| 5  | Tannin             | (+)    |
| 6  | Phenolic           | (+)    |
Cinnamomum extract is dark brown that was successfully obtained from cinnamomum maceration in ethanol. Based on the phytochemical screening which are flavonoids, triterpenoids and phenolic compounds.

3.2 FTIR spectra analysis of Chitosan.
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.

![Figure 3.1. FTIR Spectra of Chitosan](image1)

FTIR spectra of chitosan residues showed a broad absorption band at 3490 cm\(^{-1}\) indicating hydrogen bond of OH stretching vibration. The absorption band at 2950 cm\(^{-1}\) and 1050 cm\(^{-1}\) are responsible for stretching vibration of C-H methylene group and C-O group, respectively. Chitosan absorption band are similar to that of chitin. The differences occur after deacetylation step, in which there are changes in the absorption spectrum at 1690 cm\(^{-1}\) from stretching of C=O. The spectra (Fig 3.3) showed a shift of C=O absorption from 1650 cm\(^{-1}\) to 1690 cm\(^{-1}\) and a decrease of N-H absorption band in CONH group at 1560 cm\(^{-1}\) in chitosan. It also showed a new appearance of a weak absorption at 1555 cm\(^{-1}\).

3.3 Data analysis of PSA
Particle size analyzer phase aims to determine the size of particles produces from chitosan become nanoparticles-chitosan.

![Figure 3.2 Analysis PSA of Nano chitosan](image2)
Chitosan nanoparticles of the PSA test is known particles size of nano chitosan synthesized is 64.9 nm. Based on the result of PSA test that chitosan nanoparticles synthesis has been successful.

3.4 Percentage of Encapsulation efficiency from cinnamomum Extract-Chitosan Nanoparticles

Encapsulation of cinnamomum extract in chitosan was added crosslink agent Na-TPP. The interaction between chitosan and Na-TPP will optimal cause the distance between chitosan chains stretched so that the amine group is protonated form -\(\text{NH}_3^+\) that will interact with cinnamomum. Percentage of encapsulation efficiency suggests how much cinnamomum coated in nanoparticles of chitosan.

![Absorbance Vs. Concentration](image)

**Figure 3.3** Calibration curve of Standard cinnamomum Extract

The percentage of encapsulation efficiency is 84.93%. This showed that as much as 84.93% of cinnamomum are successfully encapsulated by chitosan nanoparticles.

3.5 Anticholesterol assay

Anticholesterol assay aims to determine encapsulation nanoparticles-cinnamomum extract as in vitro anticholesterol activity.

![Average value of cholesterol lowering](image)

**Figure 3.4** Reduction of cholesterol concentration

Based on the percent reduction obtained that ethanol extract at 150 ppm decreased by 49.666% w/v. It showed that *cinnamomum* extract encapsulated by nanochitosan have hypercholesterol activity. On the Figure 3.4 also showed that the larger sample concentration of ethanol extract of *cinnamomum* encapsulated by nanochitosan provides anti-cholesterol activity.

4. Conclusion

*Cinnamomum casia* extract can be encapsulated by nanoparticles of chitosan and proved that it has a cholesterol-lowering effect through the invitro study.
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