Release Profile of *Andrographis paniculata* Leaf Extract Nanocapsule as α-Glucosidase Inhibitors

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Abstract. *Andrographis paniculata* is one of 13 leading commodities Indonesian medicinal plants through the Ditjen POM. Andrographolide as main active compound has been shown to have many pharmacological activities, one of which is as α-glucosidase enzyme inhibitors which has clinical potential as an antitumor, antiviral, antidiabetic, and immunoregulator agents. This study aims to do nanoencapsulation of *Andrographis paniculata* leaf extract to increase its active compound bioavailability and get a release profile through synthetic fluids media simulation. Nanoencapsulation with ionic gelation method result the encapsulation efficiency and loading capacity values of 73.47% and 46.29% at 2%: 1% of chitosan:STPP ratio. The maximum α-glucosidase inhibition of 37.17% was obtained at 16% concentration. Burst release at gastric pH conditions indicate that most of the drug (in this study is an *Andrographis paniculata* leaf extract) adsorbed on the surface of the nanoparticles an indicates that the kind of nanoparticle formed is nanosphere.

1. Introduction

*Andrographis paniculata* is one of 13 leading commodities of Indonesian medicinal plants by the government [1]. The active ingredient contained in Andrographis paniculata is diterpene lactones and flavonoids [2]. Diterpene lactone found in Andrographis paniculata is andrographolide, 14-deoxy-11,12-didehydro andrographolide, neo andrographolide, and 14-deoksi andrographolide, while the two flavonoids were identified 5,7,2', 3'-tetrametoksiflavanon and 5-hydroxy-7, 2', 3'-trimetoksiflavon [3]. Andrographolide is the most active compounds and are most commonly found in *Andrographis paniculata*. Andrographolide have the ability as an antidiabetic, hepatoprotective, cardioprotective, anti-inflammatory, antiangiogenic, antithrombotic, and antiviral agents [4]. Diterpene compounds have the ability as an inhibitor of α-glucosidase enzyme, wherein α-glucosidase enzyme inhibitors has clinical potential that can serve among other things as an antitumor, antiviral, antidiabetic, and agents immunoregulator [5][6]. Various pharmacological activity of *Andrographis paniculata* makes this plant has a very good potential to be used to cure various diseases, including diabetes and hepatitis B. However, andrographolide as an active compound primarily on *Andrographis paniculata* is unstable in acidic conditions and alkaline digestive extreme, and also has a very short biological half-life (2 hours), so it need a method to deliver an active compound into the body. The application of nanotechnology is one method that can help this problem. Nanotechnology can increase the surface area of active compound,
increasing solubility, improving bioavailability in oral administration, and protect drug from fast degradation so it can sustain the release of active compounds in the long term. The polymer can be selected to facilitate the preparation of nanoparticles are in the form of a water-soluble polymer. One of the water-soluble polymer is chitosan. Chitosan has the properties of biocompatible, biodegradable, non-toxic and not expensive [7]. In addition, chitosan is a polysaccharide second in terms of its availability in nature and included as polyelectrolyte cationic [8]. The usage of chitosan as encapsulator has been widely used before, including the encapsulation of vitamin C [9] and encapsulation of tea [10]. However, using chitosan as encapsulator also has the disadvantage of mechanical properties of chitosan are fragile and should be stabilized by polyanion cross-linker. Polyanion cross-linker that most widely used is sodium tripolyphosphate (TPP) because not toxic and has multivalent. In addition, the use of tripolyphosphate for the formation of Chitosan gel can improve the mechanical properties of the particles that are formed due to the negative charge density tripolyphosphate has a high interaction with polycation Chitosan will be greater [11]. The existence of cross-linker to make chitosan formed is still not decayed so that the active substances contained cannot be separated before reaching the target when passing through the highly acidic pH. Based on this background, the study aims to do nanoencapsulation of Andrographis paniculata extract with chitosan-STPP and see its benefits as an inhibitor of α-glucosidase enzyme. Inhibition tes of α-glucosidase enzyme performed in in-vitro test with p-nitrophenyl-α-D-glucopiranoside substrate. In this case it is important to review the release profile of Andrographis paniculata leaf extract that has nanoencapsulated with a coating of chitosan in order to determine the performance of the drug in the body. In this study, the release profile test performed by in-vitro using synthetic fluids media of Simulated Gastric Fluid (SGF) pH 1.2 which shows the condition of the gastric and Simulated Intestinal Fluid (SIF) pH 7.4 which shows the condition of the intestine.

2. Methods

2.1. Instruments

Main instruments used consist of a beaker glass (Pyrex Iwaki), analytical massbalance (Kern, 870), magnetic stirrer (ColeParmer), syringe (Terumo), sentrifuge (Hanil), digital incubator (Boekel133730Model), sonicator (Ultrasonic Cleaner ElmaS30H), and UV Vis Spectrophotometry (Labomed,Inc.).

2.2. Materials

Materials used consist of Andrographis paniculata dry leaf, chitosan, sodium tripolyphosphate (STPP), tween80, acetic acid (Merck), α-glucosidase enzymes (Sigma Aldrich), the p-nitrophenyl-α-D-glucopiranoside substrate (SigmaAldrich), bovine serum albumin (Merck), andrographolide standard (Sigma Aldrich), ethanol, methanol, chloroform (Merck), KH$_2$PO$_4$ (Merck), NaOH (Merck), Na$_2$CO$_3$ (Merck), dimethyl sulfokside (DMSO) (Merck), and aquades.

2.3. Andrographis paniculata Extract

Extraction using ethanol 70% as solvent with sonication method. 50 g of Andrographis paniculata dry leaves is dissolved with a ratio of 1:10 (w/v) (do two circulation). Extraction with sonicator lasted for 60 minutes at a frequency of 40 kHz and a temperature of 30°C. Filtrate obtained was concentrated using a vacuum rotary evaporator to form a thick extract.

2.4. Nanoencapsulation

One gram of chitosan dissolved in 50 mL of acetic acid 1% (b/v), then 0.15 extract added and stirred with magentic stirer until homogene. In other beaker glass, 0.25 g of STPP dissolved in 25 mL of aquades, and 200 μL Tween 80 0.1% (v/v) was added to the solution of STPP and stirred. Solution of chitosan-extract then added with syringe into STPP solution and stirred for 30 minutes. Then, centrifuge at 10,000 rpm speed for 15 minutes. Sediment formed then dried with freeze dryer.
Calculation of loading capacity is done by dry nanocapsule formed, and calculation of encapsulation efficiency is done by the andrographolide level on nanocapsule using High Performance Liquid Chromatography (HPLC). Particle size and morphology are identified using Field Emission Scanning Electron Microscopy (FE-SEM).

2.5. Solution Prepare
Buffer phosphate with pH 6.8 made by KH$_2$PO$_4$ 0.1 M solution and NaOH 0.1 N solution. Substrate solution made by 0.0315 g of p-nitrophenyl-α-D-glucopyranoside dissolved in 20 mL buffer phosphate. Enzyme carrier solution made by 6 mg of BSA dissolved in buffer phosphate until 6 mL, then enzyme solution made by dissolve α-glucosidase in BSA solution until get 0.3 u/mL, 0.15 u/mL, 0.075 u/mL, and 0.0375 u/mL concentration. Na$_2$CO$_3$ solution to end enzymatic reaction made by dissolve 1.06 g of Na$_2$CO$_3$ in 100 mL buffer phosphate. Sample of crude extract, nanocapsule of *Andrographis paniculata* leaf extract, and andrographolide standard dissolved by DMSO then buffer phosphate until get concentration of 0.5%, 1%, 2%, 4%, 8%, 12%, 16%, and 18%.

2.6. α-Glucosidase Inhibition [12]
Sample testing of crude extract, nanocapsule, and andrographolide standard done by adding 10 μL of sample with various concentrations into 490 μL of buffer phosphate and 250 μL of substrate solution. Solution then incubated for 5 minutes at 37°C. 250 μL of enzyme solution 0.0375 u/mL was added, then the solution was incubated for 15 minutes. Reaction end by adding 2000 μL of Na$_2$CO$_3$. Absorbance measured at wavelength 400 nm. Sample control testing done by adding 10 μL of sample with various concentrations into 490 μL of buffer phosphate and 250 μL of substrate solution. Solution then incubated for 5 minutes at 37°C. 2000 μL Na$_2$CO$_3$ was added, then the solution was incubated for 15 minutes. Then 250 μL of enzyme solution 0.0375 u/mL was added. Absorbance measured at wavelength 400 nm.

2.7. Inhibition Percentage Calculation
Inhibiton calculated with the equation of:

\[
\% \text{Inhibition} = \frac{A_B - A_S}{A_B} \quad (1)\]

where $A_B$ is blank absorbance minus blank control absorbance, and $A_S$ is sample absorbance minus sample control absorbance.

2.8. Making of Synthetic Fluid Media
Buffer media with pH 1.2 was prepared by dissolving 4.0824 g KH$_2$PO$_4$ with aquades to 300 mL, then 0.8 g NaOH dissolved with aquades to 200 mL. Buffer media with pH 7.4 was prepared by dissolving 1.7 mL of 37% HCl to 100 ml with aquades, then as much as 6 g KCl dissolved with aquades to 400 mL.

2.9. Release Profile
A total of 1 g nanocapsule dissolved in 50 mL of buffer pH 1.2, and then incubated at 37°C. The data was undertaken every 1 hour by taking 10 ml solution and centrifuge at speeds 2000 rpm for 10 minutes. Then, supernatant was taken to measure its absorbance. Replacing the media buffer from pH 1.2 to pH 7.4 is done on the 3rd hours by filter media solution pH 1.2 with paper Whatmann paper No. 42 by vacuum pump. Nanocapsule solids were filtered and diluted with media buffer pH 7.4. The data taken were previously as procedure.
3. Result and Discussion

3.1. Andrographis paniculata Extraction

Andrographis paniculata extract is performed three times to produce an average yield of 16% which is almost the same as Yonanda study [13] that is equal to 15.88% and vary by study Mathew, et al. [14] using methanol and generate value yield of 39.8%. This is because the solvent with low viscosity, easier to pour. The use of percolation methods by Dewi [15] produces a yield of 17.09%.

3.2. Nanoencapsulation

Dry nanocapsule leaf extract produced is as much 1.88 g. Calculation result the loading capacity and the encapsulation efficiency that high enough of 46.29% and 73.47%. This is due to the magnitude of the chitosan concentration that cause chitosan molecules will be increasingly bound to each other which leads into the increasing of intermolecular crosslinking bonds [16]. The bonds formed at these conditions are strong and stable so produce particles that hard and not easily broken. The resulting particle size ranged from 557.9 nm to 892.6 nm. This particle size meets the definition of nanoparticles between 10-1000 nm [14], but not yet to meet the range of nanoparticles for drug of <100 nm [11]. The morphology of the particles shown in Figure 1 are identified by FE-SEM 1,000x zoom in and 2,000x zoom in, result particles that not spherical perfect with a smooth surface and agglomerated each other but still visible form of a circle. This is due to the use of high concentration of chitosan that cause increased viscosity solution that produces a strong form of the particle surface with the interaction by TPP, so the particles result will form the more spherical [17].

![Figure 1. SEM result of Andrographis paniculata-Chitosan nanoparticle in 1,000x zoom in (a) and 2,000x zoom in (b).](image)

3.3. α-Glucosidase Inhibition

α-glucosidase enzyme inhibition of sample shows in the Figure 2. The maximum percent inhibition and optimum concentration needed from each sample are different each other. Crude extract result maximum inhibition of 33.17% at 12% concentration. Nanocapsule result higher maximum inhibiton of 37.17%, however the concentration required to get that value is 16%.
3.4. Release Profile

In Figure 3 shows the percent cumulative release of 10.45% on hour-7. This shows that the concentration ratio of chitosan: STPP produce a strong cross-linking between them and a strong matrix so difficult split and make the release (release) drug lasts longer. Characteristics of chitosan unstable under acidic conditions led to the electrostatic repulsion between molecules on the surface of chitosan nanoparticles that produces the drug adsorbed diffusion, thereby making high levels of drug release from the matrix [18][19]. Burst release indicates that some drugs (in this study is an extract of *Andrographis paniculata*) adsorbed on the surface of the nanoparticles [20] and it also indicates that the kind of nanoparticle formed is nanosphere [21]. Sustained release profile caused by the properties of chitosan as a main ingredient matrix properties insoluble at pH below 6.0, but has resistance at pH above that value, and the hydrogen bonds between the molecules of chitosan nanoparticles create stronger which causes diffusion into the molecule becomes more difficult, followed by release of the drug lasts longer [18].
4. Conclusion
Nanoencapsulation of *Andrographis paniculata* can take the problem of andrographolide short half-life by maintain the drug release into the organ target by percent cumulative release of 10.45% on hour-7 in order to inhibits α-Glucosidase activity. Inhibition test of α-glucosidase activity by *Andrographis paniculata* nanocapsule result the maximum inhibition of 37.17% with 16% concentration.

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