Release Profile and Inhibition Test of The Nanoparticles A. Paniculata Extract as Inhibitor of α-Glucosidase in The Process of Carbohydrates Breakdown Into Glucose Diabetes Mellitus

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Abstract. Andrographis paniculata (A.paniculata) contain the main active substances Andrographolide which helps lower glucose levels in diabetics by inhibiting the enzyme α-glucosidase. The ability of the extract A.paniculata in lowering glucose levels will increase with the technique encapsulation with a coating of composition Chitosan-STPP as a drug delivery to the target organ. This study aimed to get an overview of A.paniculata release profile of nanoparticles in a synthetic fluid media with various concentrations of coating and inhibition testing nasty shard extract in inhibiting the enzyme α-glucosidase. This research resulted in nanoparticles by coating efficiency and loading capacity of chitosan greatest variation of 2% and 1% STPP 60% and 46.29%. The ability of A.paniculata extracts as α-glucosidase enzyme inhibitors has been demonstrated in this study, the percent inhibition of 33.17%.

1. Introduction
Diabetes mellitus (DM) is a disease that cannot be cured but the quality of life of patients can be maintained with a good metabolic control. DM is a major cause of morbidity and mortality in the world. Mortality in DM increased two times higher due to the complications of diabetes which include cardiovascular disease, retinopathy, nephropathy, and neuropathy DM. Indonesia is a country with the number of people with diabetes mellitus fourth largest in the world. That number is expected to increase from 8.4 million in 2000 to around 21.3 million by 2030 in future [1]. The DM generally divided into two types namely, DM type 1 or insulin-dependent diabetes mellitus (TYPE 1) and type 2 DM or noninsulin-dependent diabetes mellitus (NIDDM). Type 1 diabetes is a chronic metabolic disease or disorder with multiple etiologies characterized by high blood sugar levels followed by impaired metabolism of carbohydrates, lipids, and proteins as a result of insufficiency of insulin function. Insufficiency of insulin function may be caused by impaired or deficient production of insulin by the β Langerhans cells of the pancreas that caused reaction autoimmune [1]. Type 2 diabetes
occurs due to interference with the binding of glucose by the production of insulin receptor but still within normal limits so that patients are not dependent on insulin. In development, business pharmacological treatment for diabetics is already widely used, such as therapy with oral hypoglycemic drugs such as sulfonylureas and acarbose, insulin therapy that is useful to stabilize blood sugar concentration, or a combination of both. Therapy insulin is one the requirement for patients dm type 1. Antidiabetic drugs used often have a side effects like therapy insulin in DM type 1 [2]. A side effect is a decrease in blood glucose levels below normal and obese.

Herbal plant extract of *sambiloto* (*Andrographis paniculata*) contain the main active substances i.e. andrographolide responsible for pharmacological activity and efficacious as an antidiabetic [3]. Andrographolide can stimulate the release of insulin and inhibiting the absorption of glucose by a-glucosidase and α-amylase [4]. Sambiloto has less soluble in water, limiting the distribution of the bio, unstable in alkaline and acid on digestion which is extreme, and have very short biological half-life (2 hours). The application of nanotechnology can help the problems of *A. paniculata* in delivering the active compound into the body. Advantages of the application of nanotechnology include increasing the surface area, increasing solubility, improving bioavailability in oral administration, drug protection from degradation, and maintain release in the long term. Polymers that facilitates the preparation of nanoparticles can be selected in the form of water-soluble polymers, one of which is chitosan. Chitosan has the ideal properties, i.e., biocompatible, biodegradable, non-toxic, and not expensive [5]. Use of Chitosan as an encapsulator has been widely used previously, such as the coating of vitamin C [6], the coating of tea catechins [7], and recent studies shows that absorption of chitosan nanoparticles can improve insulin and has the ability to interact with the intestinal mucosa [8]. Meanwhile, chitosan as encapsulator has disadvantage that the mechanical properties of chitosan fragile. The addition of a crosslinking agent sodium tripolyphosphat (STPP) can improve mechanical properties chitosan, so when drug passing a very acidic condition, the matrix of chitosan formed is not degraded until the active substances that released reaching the target. Profile release from *A. paniculata* extract which has been nanoencapsulated with the chitosan coating is important for review in order to know the performance of the drug in the body to reduce blood glucose levels for patients with diabetes, as well as α-glucosidase inhibitory activity of nanoencapsulation extracts *A. paniculata* by chitosan in vitro.

2. Equipment and Materials

2.1 Equipment.
Sonicator from *Elmasonic*, Blendor, Beaker glasss, Spectrophotometry, digital incubator from *Boeke*, rotary evaporator from *IKA*, magnetic stirrer, Freeze dryer from *Eyela Word*, syringe, centrifuge from Hanil MF 50, FESEM by *Zeiss*.

2.2 Material.
*Andrographis paniculata*, Ethanol 70%, Aquade, NaOH, HCl, Chitosan (*low*), STPP, Tween 80, Acetat acid, enzim α-glucosidase from sigma aldrich, p-Nitrophenyl α-D-glucopyranoside from sigma aldrich, KH2PO4, dan KCl.

2.3 Simplesia Preparation.
At this stage carried out preparation of plant leaves from sambiloto simplicia (*Andrographis paniculata*). Preparation of simplicia consists of sorting the leaves dried with rods downsizing, and sieving to the size of the resulting homogeneous ± 1.15 mm

2.4 A.paniculata Extraction.
Extraction leaves A. panniculata is done using ethanol 70% by sonication method. Simplicia that have been prepared and weighed as much as 50 grams of ethanol, and then mixed with 70% with a ratio of 1:20 (w / v) in the glass beaker and covered with plastic wrap and aluminum foil. Extraction with a sonicator lasted for 60 minutes with the operating conditions on the frequency of 40 kHz and a
temperature of 600°C. The filtrate is obtained subsequently separated using a vacuum rotary evaporator until it formed a thick extract.

2.5 Phytochemicals Qualitative Test.
Qualitative test aims to identify the presence of phytochemicals andrographolide class. Silica gel 60 F254 plates prepared with a size 2 × 75 cm with a mobile phase (eluent) used chloroform: methanol (9: 1). Then the eluent was taken by 4 ml and poured in chamber which is then allowed to stand for 30 minutes. Next, as much as 5μL extract spotted on spotted point that is within 1 cm of the bottom line using a capillary tube. After 30 minutes, the plate was spotted eluted by inserting a plate into the chamber and the elution process that occurs until the eluent reaches the upper limit. Then the silica plate is taken to stop the elution process. The stains formed were examined under UV light at a wavelength of 254 nm to measure the value of Rf.

2.6 Nanoparticles Process.
Making nanoparticles A. paniculata leaf extract in this study using ionic gelation method with a coating composition concentration ratio of chitosan and STPP. This study will be conducted for the release profile variation of chitosan-STPP (1%: 1%; 1% to 1.5% and 2%: 1%). Making starts with 1% acetic acid as the solvent chitosan. Chitosan is dissolved into a solution of acetic acid 1% (w / v) with the desired ratio (1% and 2%) using a magnetic stirrer. Furthermore, A. paniculata extract as much as 0.15 grams of prepared and then diluted with an ethanol 70% about 5 drops. A. paniculata leaf extract mixed into a solution of chitosan. Preparing STPP solution in aquades to the desired concentration (1% and 1.5%), dripping a solution of chitosan extract using a syringe into a solution of STPP followed by magnetic stirrer. The process of size reduction is done by sonicator at a frequency of 20 kHz for 60 minutes. Centrifugation carried out on the mixed solution to separate the nanoparticles (sediment) from the supernatant with a speed of 10,000 rpm for 15 minutes. Nanoparticles have been obtained used as solids (powders) using freeze drying.

2.7 The Analytical Determination of Nanoparticles.
Analytical determination nanoparticles were obtained by FESEM.

2.8 Test In-Vitro Release Profile.
Test in vitro release profile of nanoparticles A. paniculata done using synthetic fluid media. Media synthetic fluids used were simulated gastric fluid (pH 1.2) and Simulated Intestinal Fluid (pH 7.4). The testing process starts by mixing 0.1 g matrix of any variation with 50 ml of synthetic fluid pH 1.2 and save in an incubator with a temperature of 37°C. Sampling was done by taking 10 ml solution from a beaker glass with syringes and moved into the tube centrifugation, centrifugation done at a speed of 2000 rpm for 10 minutes. A total of 4 ml samples were taken from the centrifuge tube and inserted into the bottle as a sample vial, then the liquid in the tube centrifugation returned into a beaker glass and put in 4 ml of synthetic fluid new media to keep synthetic fluid volume constant. The succession of buffer is done on hour-3 with pH 7.4 and additional enzymes α-amilase 0,003% (w/w). The data taken until the hour to the 7 th. Sample absorbance measured using UV spectrophotometry with wavelength 208 nm.

3. Result and Discussion
3.1 A. paniculata Extraction.
The value of the average yield obtained on extracting the leaf A. paniculata is 15.96% where percent yield has values that are almost the same with the research done by [9] using the extraction method and the same solvent, of 15.88%.The results of different research done by [10], where the use of solvent methanol produces yield value is higher than the solvent ethanol of 39.8%. This is due to the solvent with low viscosity, more easily to channeled. Methanol has a lower viscosity values (0.597 mPa.s) compared with ethanol merely 1.200 mPa.s. The viscosity is reduced (cohesion are also reduced) resulting in decreased surface tension. The surface tension decreases can help solvent get into
the solid matrix, thereby increasing the speed of the reaction. This causes the percentage of yield of methanol.

3.2 Phytochemicals *A.paniculata* results.
Based on the results of these observation, Rf value obtained on the same andrographolide standards such as the Rf values obtained on the leaf extract of *A.paniculata* 0.61. This may indicated that in the samples of *A.paniculata* extract contains a bioactive compound andrographolide. In this case the selection of solvent affects the existence of active compounds in the extract. This indicates that the use of ethanol solvents to extract andrographolide active compounds contained in leaf *A.paniculata* can produce the extraction process of active compounds target properly.

3.3 *A.paniculata* Nanoparticles.
Tables 1 shows the data encapsulation efficiency and loading capacity gained from the leaf extract *A.paniculata* nanoparticles

| Table 1. The results of the encapsulation efficiency and loading capacity leaf extract of *A.paniculata* nanoparticles |
|-------------------------------------------------|
| **Variation**            | **Encapsulation efficiency** | **Loading Capacity** |
|-------------------------|-----------------------------|---------------------|
| Chitosan 1% : STPP 1%   | 55.89%                      | 30.62%              |
| Chitosan 1% : STPP 1,5% | 28.79%                      | 11.09%              |
| Chitosan 2% : STPP 1%   | 60%                         | 46.29%              |

Based on table 1 of the greatest efficiency values obtained on the variations of Chitosan: STPP (2:1) by 60%, where the higher the concentration of Chitosan then the percentage encapsulation efficiency also higher. This is due to the larger number of nanoparticles of chitosan, then the amount of the coated andrographolide also increasingly due to the surface area of the particles increases so that efficiency may be even greater. While the higher concentration of TPP then the less andrographolide that absorbed because the function of the TPP as a substance that strengthens cross-matrix binding nanoparticles. Nanoparticle become more compact and dense due to the concentration of TPP the larger, so that the rate of diffusion out ingredients (andrographolide) declining, which is in accordance with the theory that the more TPP will further strengthen the ties of Ionic revolt on nanoparticle so that the drugs will be difficult to remove the back [11]. The largest percentage of loading capacity in line with the largest percentage encapsulation efficiency. This is due to the more TPP used the more ions triplyphosphat that interact with NH3 which cause the bond between the two ions stronger so that the structure of the solid-matrix more compact because mass ratio increased to chitosan : TPP, which lead to an increase in the amount of nanoparticle formed, resulting in increased efficiency encapsulation and loading capacity in nanoparticle. The results of this study are consistent conducted by [12].

3.4 The Analytical Determination of Nanoparticles Using FESEM.
The results of measurement with zoom FESEM 1,000 – 10,000 times can be seen in Figure 1.
Figure 1. FESEM test results (a) variation 1 (Chitosan1%: TPP1%) (b) the variation 2 (Chitosan1%: TPP 1.5%) and (c) the variation 3 (Chitosan 2%: TPP 1%).

Table 2. The Diameter of the particle size on the variation 1, variation 2, variation 3

| Variations | Diameter of Particles (nm) | Min   | Max   | Average | Deviation Standard |
|------------|---------------------------|-------|-------|---------|--------------------|
| 1          |                           | 837.6 | 604   | 728.1   | 480.4              | 672.4              | 480.4              | 664.5              | 133.81             |
| 2          |                           | 301.5 | 234.7 | 264.7   | 284.7              | 268.2              | 234.7              | 301.5              | 270.76             | 24.91              |
| 3          |                           | 669.4 | 557.9 | 557.4   | 892.6              | 669.4              | 892.6              | 669.3              | 136.74             |

From the figure 2 can be known that the size of the smallest particles found on the second variation with a diameter 234.7 nm, while the size of the largest particles found on the third variation with a diameter 892.6 nm. The distribution of the particle size approaches the uniform is occupied by the second variation as seen from the above data with the value of the standard deviation variation 2 variation is much smaller than the other, which indicates the diameter of the particle size distribution variation of 2 is homogeneous. It can be concluded that the size of the particles obtained from this research have reached the size of the nano, but to be an effective drug delivery required particle size <100 nm. The form is obtained from the variation of 1 to 3 variations show that less spheric form and the size of its particles relatively large. According to Wahyono [11] the addition number of number of Chitosan with a fixed number of TPP led to coagulation (agglomeration) which causes the shape of
nanoparticles is not spheric. The particle size is not uniform is also expected because of *A. paniculata* leaf extracts not only entered into the matrix of chitosan nanoparticles but also attached to the nanoparticle surface.

3.5 α-Glucosidase Inhibition.

Extraction of *A. paniculata* leaf positively can inhibit α-glucosidase enzymes are shown in Figure 2.

![Figure 2](image)

**Figure 2.** Comparison of inhibition test all samples.

The figure above shows that the standard andropholide have percent highest enzyme inhibition while extracting the leaf *A. paniculata* have percent enzyme inhibition low against the enzymes α-glucosidase. The percentage increase enzyme inhibition andrographolide occurs along with the addition of the concentration of andropholide, where the maximum absorbance peak is at a concentration of 16% with the percent inhibition of 99.56% and at a concentration of 18% inhibition andrographolide power has decreased. This means that the concentration of andrographolide 16 percent is the maximum concentration to inhibit troubleshooting enzymatic reactions pati or carbohydrates into glucose in the small intestine.

Percent inhibition crude extract of leaf *A. paniculata* is much smaller than the comparison substance andrographolide under the same conditions, where the percent inhibition highest crude extract of *A. paniculata* leaf is 33.17% which in konsentrasi12%. The percent difference significant inhibition is the possibility of other chemical compounds. Such compounds may be no synergistic in inhibiting the enzyme α-glucosidase. In nanoencapsulation *A. paniculata* leaf extract, the highest percent inhibition obtained, the same as the percent inhibition of crude extract of 33.17% but this occurred at a concentration of 16%. Percent enzyme inhibition is still smaller than the substance of comparison such as acarbose and diabetano. This is due to the fact that there are still other chemical compounds in the leaf extract *A. paniculata* nanoencapsulation. In addition, the percent inhibition nanoencapsulation little *A. paniculata* leaf extract also caused quite a small andrographolide content in the sample, of 2.5 mg / ml, whereas according to the research that has been done Subramanian et al, [4] required 62.5 mg / ml andrographolide to reach a maximum of 89% percent inhibition. Inhibition of the action of the enzyme can effectively reduce complex carbohydrate digestion and absorption, thereby reducing post-prandial glucose levels in diabetics.

Nanoencapsulation leaf *A. paniculata* extract also inhibit the enzymes α-amilase pancreas working in hydrolyzing polysaccharide in caused by small intestine. Nanoencapsulation leaf *A.paniculata* extract as inhibitor enzymes α-glucosidase also function in inhibits the expression of the glucose transporter SGLT1 and GLUT-4 translocation mRNA is increased by 3 times in diabetic patients who...
are permanently in the membrane apical resulting in an enzyme α-amylase and α-glucosidase also increased so that the taking of glucose in the intestine increased 3 times faster than the normal. This transporter functions to move glucose from one compartment to another compartment to pass through biological membranes so that glucose can be absorbed by the body [13].

Standard Andrographolide, diabetano, and acarbosa have the same concentration in inhibit the activity of the enzymes α-glucosidase of 16%. It is needed 3 times to extract the leaves A. paniculata to inhibit the enzymes α-glucosidase in accordance with drugs acarbose and diabetano.

3.6 Release Profile. These test results are shown in Figure 3.

![Figure 3](image_url)

**Figure 3.** Percent cumulative release variation 1, variation 2 and variation 3, where the clock to 1-3 indicates the condition of the stomach and the clock to 4-7 shows the small intestine.

Based on Figure 3 it can be seen that the variation 2 produces the largest cumulative release percent ie 74.83% at 7, followed by the first variation of 19.92% and 10.45% of the variation 3 at the same hour. The result of this release is inversely related to the results of encapsulation efficiency and loading capacity that produces optimum value in three variations. This shows that the concentration ratio of chitosan : STPP on third variation of 2%: 1% (g/ml) resulted in a strong cross linking between them and the characteristics of a strong matrix so that difficult broken and make the release of the drug lasts longer. The cumulative amount of drug release produced variations on the hour 3 to 7 is worth the least. This is in line with the results of research [14] which stated that the increased concentration of chitosan make the secretion of drugs requires a longer time.

The release profile of nanoencapsulation extract of A. paniculata leaf describes the events burst release on the condition of gastric and intestines sustained released on conditions. Events burst release is due to the dissociation of the drug on the surface of chitosan nanoparticles that cause diffusion of the drug entrapped in nanoparticles contained in the release of high level experience. 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 the drug release rate that is higher than the matrix [15] and [13]. Events burst release indicates that some drugs (in this study is an extract of A. paniculata leaf) entrapped on the surface of the nanoparticles [16].

Events sustained release can be caused by the nature of chitosan as the main material of the matrix has not dissolved at pH under 6.0, but have durability at pH above the value, and hydrogen bonds between the molecules chitosan make nanoparticles stronger that cause soft diffused into the molecules become more difficult and then followed by the secretion of the drug lasted longer [15].
4. Conclusion

The highest percent inhibition α-glucosidase obtained diabetano herbal medicine by 99.631% on the concentration of 16 percent and percent enzyme inhibition lowest obtained nanoencapsulation A.paniculata extract of 33.17 on should concentrate on 16%. To match the ability of inhibition diabetano it takes 3 times the dose nanoencapsulation of A. paniculata extract that can inhibit the enzyme α-glucosidase optimally. The application of nanotechnology is proven to help the problems of Andrographis paniculata is limited to the distribution of bio in delivering the active compound into the body by maintaining the release in the long term. This research proves the nanoencapsulation characteristics of chitosan 2% and STPP 1% is able to withstand burst release and bring A. paniculata extract to the target organ small intestine.

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