Karakterisasi Fisik Nanopartikel Kitosan Ekstrak Daun Salam (*Syzygium polyanthum*)

Physical Characterization of Chitosan-based *Syzygium polyanthum* Leaves Extract Nanoparticles

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Abstrak

Indonesia merupakan salah satu negara dengan kekayaan alam yang melimpah. Daun salam merupakan salah satu tanaman yang sering digunakan oleh masyarakat dan memiliki beberapa manfaat seperti antidislipidemia. Saat ini, penggunaan teknologi nanopartikel cukup popular dalam pengobatan dislipidemia. Penggunaan teknologi nanopartikel memiliki kelebihan salah satunya peningkatan potensi dari obat antidislipidemia. Penelitian ini bertujuan untuk mengembangkan nanopartikel kitosan dengan ekstrak daun salam (*Syzygium polyanthum*) menggunakan metode tautan silang. Pada penelitian ini, digunakan kitosan sebagai polimer dan STPP sebagai *crosslinker* dengan rasio 5:1. Formula 1 (F1) merupakan nanopartikel kitosan yang mengandung ekstrak, sedangkan Formula 2 (F2) merupakan nanopartikel blanko. Berdasarkan hasil skrining metabolit sekunder, *Syzygium polyanthum* mengandung alkaloid, flavonoid, saponin, kuinon dan tanin. Kandungan flavonoid total dihitung sebagai kuersetin sebanyak 0,04%. Karakteristik fisik nanopartikel seperti ukuran, indeks polidispersitas dan zeta potensial juga dievaluasi. Ukuran nanopartikel dari F1 dan F2 adalah 180,1 ± 0,5 nm dan 464,3 ± 25,98 nm dengan nilai PI masing-masing formula adalah 0,220 ± 0,02 dan 0,563 ± 0,112. Nilai zeta potensial dari F1 dan F2 yaitu 21,8 ± 1,74 mV and F2 is 17,8 ± 0,21 mV. Pengujian efisiensi penjebakan (EE) dilakukan untuk menentukan jumlah ekstrak dalam nanopartikel dan didapatkan hasil EE sebesar 47,13%. Berdasarkan hasil tersebut, nanopartikel kitosan dengan kandungan ekstrak daun salam memiliki karakteristik fisik yang baik. Penelitian lebih lanjut diperlukan untuk mengetahui potensi nanopartikel kitosan ekstrak daun salam sebagai antidislipidemia.

Kata Kunci: nanopartikel, kitosan, *Syzygium polyanthum*, gelasi ionik

Abstract

Indonesia is one of the most abundant in natural resources countries in the world. The bay leaves are well-known among the locals and have various pharmacological properties, including anti dyslipidemia. Currently, the use of nanoparticle technologies in dyslipidemia treatment is quite popular. These treatments have numerous advantages such as enhancing the potency of dyslipidemia agents. The purpose of this study is to develop chitosan-based nanoparticles made from Syzygium polyanthum leaves extract using crosslink method. Chitosan is used as the polymer and STPP as the crosslinker with the ratio of chitosan:STPP is 5:1. Two formula is generated, F1 is chitosan-based nanoparticle extract and F2 is chitosan-base nanoparticle blank. Syzygium polyanthum extract contains alkaloid, flavonoid, saponin, quinone, and tannin, as determined by secondary metabolites screening. The total flavonoid content of Syzygium polyanthum leaves extract determined as quercetin is 0.04%. Physical properties of generated nanoparticles such as size, polydispersity index (PI) and zeta potential are investigated. Particle sizes of nanoparticles F1 and F2 are 180.1 ± 0.5 nm and 464.3 ± 25.98 nm with the PI values of F1 and F2 are 0.220 ± 0.02 and 0.563 ± 0.112 respectively. Zeta potential value for F1 is 21.8 ± 1.74 mV and F2 is 17.8 ± 0.21 mV. The entrapment efficiency is evaluated to determine the extract content of the nanoparticle, and the result is 47.13%. From this research, the chitosan-based nanoparticles containing Syzygium polyanthum formula have good physical properties. Further investigation is needed to evaluate its potential as anti-dyslipidemia.

Keywords: nanoparticles, chitosan, Syzygium polyanthum, ionotropic gelation

INTRODUCTION

Dyslipidemia is a metabolic disorder characterized by an increase in Low-Density Lipoprotein (LDL) and Triglyceride (TG), as well as a decrease in High-Density Lipoprotein (HDL) (Agusmansyah, 2021). One of the risk factors for cardiovascular disease is dyslipidemia (Kamso et al., 2002), according to Riskesdas data from 2018, the prevalence of cardiovascular disease at every age is 1.5%. In general, the treatment for dyslipidemia is a change in lifestyle and the taking of lipid-influencing medications such as statins, fibrates, bile-acid binding, and niacin (Gotto, 2002).

Statins are the most frequently used anti-dyslipidemia medicines, however, their application is limited due to side effects such as rhabdomyolysis, musculoskeletal problems, and hepatotoxicity (Golomb and Evans, 2012). Thus, the exploration of natural ingredients for alternative medicine with low side effects such as bay leaf extract (Agusmansyah, 2021; Damanik et al., 2019; Prianwari et al., 2019), is currently undertaken.

Syzygium polyanthum is widely accessible in Indonesia and the leaves are commonly used as a spice in culinary. Other than as a spice, bay leaves have numerous health benefits due to their flavonoid, tannin, alkaloid, steroid, triterpenoid, and saponin content. These secondary metabolites have antibacterial, antioxidant, antidiabetic, and antidyslipidemia properties (Kusuma et al., 2011; Agusmansyah, 2021). The administration of 400 mg bay leaf extract for 30 days in dyslipidemia patients resulted in a decrease in lipoprotein (a) levels (Prinawari et al., 2018).

Flavonoid content may inhibit the HMG-CoA reductase enzyme, decreasing cholesterol production and hence its level (Chen et al, 2001). *Syzygium polyanthum* leaves have potency as antidyslipidemia. However, the research that has been carried out is only as an extract (Damanik et al., 2019;

Prianwari et al., 2019). Furthermore, some active compounds, such as flavonoids, have limited solubility, resulting in poor bioavailability (Smith et al., 2011). Thus, developing therapeutic delivery systems to improve the potency of bay leaf extract is critical; one example is formulation as polymer-based nanoparticles.

Nanoparticles are solid particles with sizes ranging from 10 to 1000 nm. In general, drugs delivered as nanoparticles are dissolved, entrapped, adsorbed or encapsulated in matrix. The advantages of this technique over the conventional methods are it may be modified for a specific tissue or cell, improves drug bioavailability, allows for controlled release, increases drug intake in cells, and facilitates drug accumulation, all of which improve therapeutic effectiveness (Singh and Liliard, 2009). For example, liver-targeted nanoparticles made from *Clerondendrum infortunatum* L extract had a particle size of 608 nm, a zeta potential of -30mV, a drug efficiency of 32.8%, and an entrapment efficiency of 98.40% when utilized as hypercholesterol treatments (Suman et al., 2013).

Ionotropic gelation, spray drying, high pressure homogenization, ultrasonication, and crystallization are some methods for nanoparticle formulation. Because the ionotropic gelation approach is straightforward and does not require a specialized reaction, it is the favored method in the formulation of nanoparticles (Shrimal et al., 2019; Yang et al., 2009). Chitosan, gelatin, and albumin are natural polymers utilized in the creation of nanoparticles, meanwhile methacrylate is an example of a synthetic polymer used in the formulation (Armendáriz-Barragán et al., 2016).

Chitosan is a natural polymer formed from positively charged deacetylated chitin that may adhere to cells and penetrate cells with negatively charged membranes (Qi et al., 2004). The crosslinker is another chemical required for the formulation. One of the most

common crosslinkers is sodium tripolyphosphate (STPP). The chitosan:STPP ratio is a significant characteristic that influences the quality of the produced nanoparticles, including particle size, polydispersity index, and zeta potential. These physical properties would have an impact on the stability and effectiveness of nanoparticles (Anthoniou et al., 2014).

Considering the potency of bay leaf extract as an antidyslipidemic, there has been little research into the formulation of extract for antidyslipidemia and the possibility of increasing its effectivity; thus, in this study, a nanoparticles formulation using chitosan as a polymer and STPP as a crosslinker is carried out. Following formulation, the physical characteristics of nanoparticles such as particle size, polydispersity index, zeta potential, and entrapment efficiency (EE) are evaluated.

RESEARCH METHODOLOGY
Instruments
Magnetic stirrer (Nesco, China), micropipette (Dragon Lab, China), centrifuge (DLAB, China), water bath sonicator (GT Sonic, China), Zetasizer® Nano ZS (Malvern, United Kingdom), Spectrophotometer UV-Vis (Thermo Scientific, USA).

Materials
Syzigium polyanthum leaves extract (PT. Borobudur Extraction Center, Indonesia), Methanol p.a (Sigma Aldrich, USA), water, chitosan (Biochitosan, Indonesia), Sodium Tripolyphosphate (Xylong Scientific, China), Acetic acid glacial (Sigma Aldrich, USA), $AlCl_3$ (Sigma Aldrich, USA), Sodium acetic (Sigma Aldrich, USA), Quercetin (Sigma Aldrich, USA).

Screening of Secondary Metabolites
Flavonoid
1 gram of extract is boiled in 50 mL for 15 minutes then filtered. The filtrate can be used to identify flavonoid, tannin, saponin, and quinone. Mg powder,1 mL HCl and 5 mL amyl alcohol are added to 5 mL of filtrate. The mixture is shaken vigorously and allowed to separate. The appearance of red, brick red, or purplish red at the amyl alcohol layer indicates the presence of flavonoid in the extract (Fransworth, 1966).

Tannins
5 mL filtrate that previously prepared is pour into three test tubes. $FeCl_3$ 5% is added into the first tube and positive tannin content is shown by the formation of blue, dark blue, green, greenish blue. The second tube, 1% gelatin is added. The presence of white precipitation indicates the presence of tannins. The third tube, 2 mL of formaldehyde 40% and 1 mL of concentrated HCl are added, then boiled. The red precipitation indicates the tannin content (Fransworth, 1996).

Saponin
5 mL filtrate is shaken vigorously for 10 minutes until stable foam is formed as high as 1-10 cm for 10 minutes. The steady foam formed after adding a few drops of HCl 2N showed that the extract includes saponin (DepKes RI, 1995).

Alkaloid
0.4 grams extract is mixed with 25% ammoniac and 20 mL of chloroform, then shaken and filtered. The liquid-liquid extraction is then carried out by adding HCl 2N until two phases are created, namely the water phase and the chloroform phase. Two reaction tubes are filled with the water phase. The Dragendorff reagent is introduced to the first tube, and the formation of red precipitate reveals the alkaloid content of the extract. The second tube is added with Meyer reagent, a white precipitate that produced shows the alkaloid's content (Cordell, 1981).

Quinone
The previously prepared filtrate is dripped onto a spot plate and a drop of NaOH 1 N is added. The appearance of red indicates a positive result (Harborne, 1996).

Determination Total Flavonoid Content
The total flavonoid content is expressed as quercetin. After macerating 0.2 gram extract in ethanol for one hour, the extract solution is filtered and added ethanol up to 25 mL. The dilution series of quercetin, as

reference, are prepared for 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. Pipette up to 0.5 mL of each previously sampled extract and quercetin dilution series. 1.5 mL ethanol, 0.1 mL AlCl$_3$ 10%, 0.1 mL sodium acetate 1 M and 2.8 mL water are added into each tube. Incubate for 30 minutes. The absorbances are measured using spectrophotometer UV-Vis. Maximum wavelength screening are conducted in 300 - 500 nm. The maximum wavelength that is used in this study is 436 nm.

The flavonoid content is calculated using the following formula (Geissman, 1962):

$$\% \text{ flavonoid} = \frac{C \; x \; V \; x \; f}{W} \; x \; 100\%$$

C : Concentration of sample solution
V : Volume of test solution before dilution
f : Dilution factor
W : Weight of sample test

Chitosan-based Extract Nanoparticle Preparation

As shown in **Table 1**, two formulas are prepared using ionotropic gelation method: chitosan-based nanoparticle containing *Syzigium polyanthum* leaves extract (F1) and chitosan-based nanoparticle blank (F2). The nanoparticles are prepared using previously method with modification (Hidayati, 2021).

Table 1. Formulation of *Syzygium polyanthum* Nanoparticles

| Formula | Concentration | | |
	Extract (ppm)	Chitosan (mg/ml)	STPP (mg/ml)
F1	2500	1	1,4
F2	-	1	1,4

The ratio chitosan: STPP is 5:1. 1 mg/mL chitosan is dissolved in 1% acetic acid glacial, then 5M NaOH is added until the pH is 4.7. STTP and *Syzygium polyanthum* leaf extract solutions are made in demineralized water at concentrations of 1.4 mg/mL and 5000 ppm, respectively. The extract solution is added to the chitosan solution until it reaches a concentration of 2500 ppm and incubate for 30

minutes in dark condition. After incubation, the solution is stirred for 3 minutes at 800 rpm, and STPP solution is gently dropped using a micropipette, followed by 90 minutes of stirring to form nanoparticles. The nanoparticles are harvested by centrifugation at 14.000 rpm for 10 minutes. The formed nanoparticles are rinsed using demineralized water and then sonicated for 60 minutes.

Characterization of Nanoparticles

The nanoparticles are dispersed in demineralized water. The particle size, polydispersity index and zeta potential of nanoparticles are measured using Zetasizer® Nano ZS.

Entrapment Efficiency (EE) of Nanoparticles

The EE of *Syzygium polyanthum* leaves extract in nanoparticle is determined by measuring the amount of free extract using colorimetry method. The AlCl$_3$ is used as complex to produce color and the quercetin is used as reference. The method is modified using spike. The calibration curve is generated by using various concentration of quercetin at 30 - 100 ppm. As much as 0.5 mL of each quercetin dilution is added with 0.1 mL AlCl$_3$ 10%, 0.1 mL sodium acetate 1M, 1.5 mL methanol and 2.5 mL demineralized water, followed by 30 minutes of incubation in dark condition. After incubation, the absorptions are measured using spectrophotometer UV-Vis. Maximum wavelength screening are conducted in 300 - 500 nm. The maximum wavelength that is used in this study is 436 nm.

The supernatant that produced at nanoparticle purification is used as sample. The mixture of 0.5 mL sample, 0.5 mL quercetin 50 ppm as spike, 0.1 mL AlCl$_3$ 10%, 0.1 mL sodium acetate 1M, 1.5 mL methanol and 2.3 mL demineralized water is incubated in dark condition for 30 minutes. The sample absorbance is measured at the maximum wavelength of quercetin.

## RESULT AND DISCUSSION
Secondary Metabolites Screening

The extract used in this study is a dry extract of *Syzygium polyanthum* leaves with a maltodextrin filler proportion of 10:1 purchased from PT. Borobudur Extraction Center. The aim of this screening is to identify and confirm the secondary metabolite content of *Syzygium polyanthum* leaf extracts qualitatively. The result test was presented in **Table 2**. The result is consistent with other studies (Nasyanka et al., 2020; Kusuma et al., 2011; Widjajakusuma et al., 2018).

Table 2. Secondary Metabolites Screening of *Syzygium polyanthum* Extract

No	Secondary Metabolite	Result
1	Alkaloid	+
2	Saponin	+
3	Kuinon	+
4	Flavonoid	+
5	Tanin	+

Total Flavonoid Content

The colorimetry method is used because the keto group at C-4 and the hydroxy group at C-3 or C-5 from quercetin could form a complex with $AlCl_3$. The process is stabilized by the addition of sodium acetate. The maximum wavelength of quercetin is 436 nm, which is consistent with previous research that found quercetin maximum wavelength to be between 380 - 480 nm (Das el al., 2013). The total flavonoid content of extract is calculated based on calibration curve of quercetin. **Figure 1** shows the calibration curve of quercetin with R2 value of 0.9945.
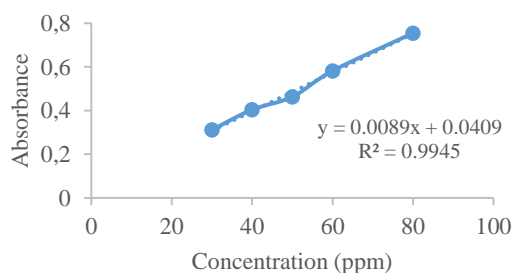
Figure 1. Calibration curve of quercetin for Total Flavonoid Contents

Quercetin is one of the flavonoid compounds. From the calculation, the total flavonoid content of extract is 0.04%. According to the Indonesian Herbal Pharmacopoeia standard, the total flavonoid concentration of *Syzygium polyanthum* leaves thick extract should be not less than 1.14%, it is measured as quercetin.

Total flavonoid content of the dry extract in this study is lower than the thick extract as stated in Indonesian Herbal Pharmacopoeia standard. The dry extract used in this research contains maltodextrin, whereas maltodextrin is commonly used in the production of extract powders. According to other studies, increasing the maltodextrin concentration reduces the overall flavonoid content of the extract (Widyaningsih et el., 2021).

### Particle Size, Polydispersity Index and Zeta Potential

Nanoparticles are classified into two types: nanospheres and nanocapsules (Qi et al., 2004). The nanosphere is generated in this study using the ionotropic gelation process. Chitosan is a biodegradable polymer that is soluble in acid as a result of protonation of the amino group from glucosamine and positively charge in acid. The protonation group can be crosslinked with multivalent polyanions such as STPP via inter and intramolecular bonds.

Table 3. Particle size, polydispersity index and zeta potential

| Formula	Size (nm)	PDI	Zeta Potential (mV)
F1	180.1 ± 0.5	0.22 ± 0.02	21.8 ± 1.74
F2	464.3 ± 26.0	0.56 ± 0.11	17.8 ± 0.21

The mass ratio of chitosan and STPP in critical for controlling the particle size and distribution of nanoparticle, the higher the chitosan content, the more ionotropic gelation occurs between chitosan and STPP (Antoniou et al., 2014). The optimal chitosan: STPP ratio found in this study is 5:1. Physical

characteristic of nanoparticles are presented in **Table 3**, **Figure 2**, and **Figure 3**.

(a)   (b)
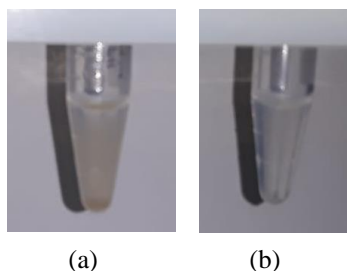
Figure 2. Synthesis of Nanoparticles F1: *Syzygium polyanthum* (a) and F2: blank (b)

The particle size of the nanoparticle containing extract (F1) $\leqslant$ 200 nm, indicate that the nanoparticle has beneficial properties. That size is sufficient to circumvent the Reticuloendothelial System (RES), overcome the biological barrier, and effectively treat hyperlipidemic patients (Umair et al., 2016). F1 has particle size < 200 nm, indicate that this nanoparticle has potential to give good theraphy effect on hyperlipidemic patients.
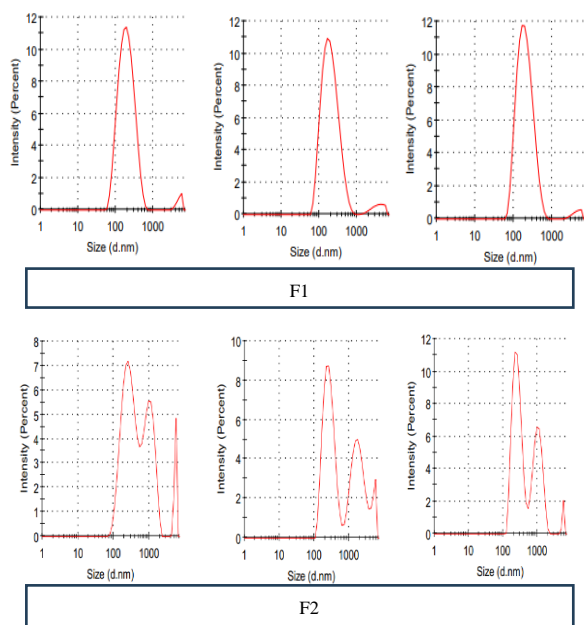
Figure 3. Nanoparticle Distribution

F1 has a polydispersity index (PDI) less than 0.5, indicating that the particle distribution is monodisperse with low variability and no aggregation. Meanwhile, the nanoparticle blank (F2) has a high PDI value, indicating that it is highly variable and prone to aggregate (Mahmood et al., 2019). The objective of measuring zeta potential is to obtain information about the charge characteristics of nanoparticles based on electrostatic interactions. Potentially positive zeta created by cationic reaction from the ionization of amino group in chitosan (Afifah et al., 2021).

The zeta potential is used to estimate colloidal stability in solution. Particles with high stability typically have zeta potential values more than (+) 30 mV or less than (-) 30 mV. Because of the interparticle interaction, nanoparticles having zeta potential values less than (+) 25 mV or greater than (-) 25 mV would eventually agglomerate (Kumar & Dixit, 2017).

The interaction of nanoparticles *in vivo* condition with the cell membrane can be predicted by the value of zeta potential because the cell membrane usually negative charged (Agarwal et al., 2018).

The zeta potential values of F1 and F2 are (+) 21,8 ± 1,74 mV and (+) 17,8 ± 0,21 mV respectively. Positive charged of F1 and F2 showed that the nanoparticles have tendency to bind with the cell membrane to form ionic bonds. Zeta potential value of F1 and F2 show that the nanoparticles have fair stability and have the possibility to form agglomerate. Increasing chitosan concentration can increase zeta potential value of nanoparticles (Yien et al., 2012).

The concentration of chitosan that used in this research is 1 mg/ml. The value of zeta potential can increase when the concentration of chitosan larger than 1 mg/ml.

**Entrapment Efficiency (EE)**

Entrapment efficiency is defined as the ratio of trapped active compounds in nanoparticles to total active compounds added in the formula (Vrignaud et al., 2011). The EE of nanoparticles varies from 22-100%, depend on the formulation condition (Mattu et al., 2013; Ghadiri et al., 2012). The average EE from F1 is 47.13% as seen in **Table 4**

indicate that the concentration of *Syzygium polyanthum* extract that entrapped in nanoparticles is 1178 ppm.

Tabel 4 Entrapment Efficiency (EE) of NP SP

Formula	EE (%)	Mean EE (%)
F1	47.13	47.13 ± 0.58
	43.03	
	47.95	

Colorimetry (Chang Method) is used to determine the free extract. The free extract is found in the supernatant of the nanoparticle purification process by centrifugation. The calibration curve for determining the quercetin content in the supernatant shows in **Figure 4**.
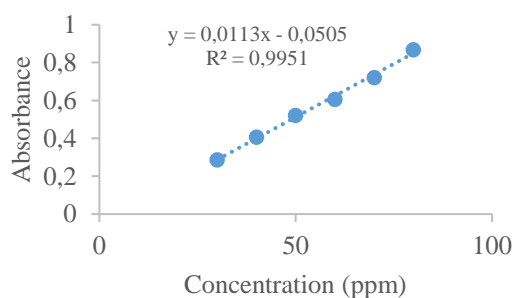
Figure 4. Calibration curve of quercetin for EE

Drug solubility in matrix or polymer impacts EE value, which is determined by polymer composition, molecular weight, drug-polymer interactions, and functional group in the drug (Wu et al., 2017).

CONCLUSIONS

The chitosan-based nanoparticles with *Syzygium polyanthum* leaves extract have an excellent size and polydispersity index, as well as a fair zeta potential and entrapment efficiency value. Further study is needed to evaluate the safety and efficacy of the *Syzygium polyanthum* nanoparticles.

ACKNOWLEDGMENTS

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