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Nanoparticle formulation of ethanolic extract of *Syzygium polyanthum* leaf using chitosan and cross-linking method

Evi Nurul Hidayati[1*], Annisa Maulidia Rahayyu[2], Eni Masruriati[3]
[1]Pharmacy Department, Universitas Kusuma Husada Surakarta,
Jl. Jaya Wijaya No.11 Kadipiro, Surakarta, Indonesia
[2]Pharmacy Department, Institut Teknologi Sumatera,
Jl. Terusan Ryacudu – Way Huwi, Lampung, Indonesia
[3]Pharmacy Department, Sekolah Tinggi Ilmu Kesehatan Kendal,
Jl. Laut No.31, Ngilir, Kendal, Indonesia

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ABSTRACT

Syzygium polyanthum (bay leaves) is widely used in Indonesia and has been shown to have pharmacological activity, such as antihyperlipidemia. The nanoparticle is a delivery system that enhances therapy effectiveness, minimizes side effects, and ensures safety. Therefore, this study aimed to improve the antihyperlipidemic efficacy of *Syzygium polyanthum* extract by formulating it into nanoparticles. The polymer that is used in this nanoparticle formulation is chitosan, while the cross-linking agent that is used is sodium tripolyphosphate. Three formulations have been developed, each with different stirring times after crosslinking: F1 (20 minutes), F2 (90 minutes), and F3 (150 minutes). At the same time, nanoparticles produced were examined for particle size, ζ potential, polydispersity index, entrapment efficiency, and release study. *Syzygium polyanthum* extract is abundant in secondary metabolites, including alkaloids, flavonoids, saponins, triterpenoids, tannins, and quinones. The particle size data for F1, F2, and F3 were 257±6.68 nm, 232±2.54 nm, and 303±1.3 nm respectively, while the polydispersity index ranged from 0.242 to 0.383. The entrapment efficiency represented by quercetin, used to assess the extracted content of the nanoparticles, yielded results between 39.59% and 67.48%. A release study of nanoparticle *Syzygium polyanthum* (nanoparticle SP) showed that the extract represented by quercetin can be released from the system is 64-82% in 120 min. The ζ potential measurement in F2 indicated a value of 30.9±0.416 mV, suggesting that the nanoparticle SP formed possesses excellent stability. Among the formulas studied, F2 emerged as the most promising due to its combination of factors such as the smallest size, favorable polydispersity index, high entrapment efficiency, and desirable release profile values. All of the formula has the potential to provide a good therapeutic effect, such as antihyperlipidemia but it needs to be proven by further studies.

Keywords: *Syzygium polyanthum*, chitosan, nanoparticles, cross-linking method, stirring time

Corresponding author:
Evi Nurul Hidayati
Pharmacy Department, Universitas Kusuma Husada Surakarta
Jl. Jaya Wijaya No.11 Kadipiro, Surakarta, Indonesia
Email : evi.nurul03@gmail.com

INTRODUCTION

Indonesia is plentiful in natural resources, found both on land and in its oceans. Using plants in Indonesia to improve health has also begun to increase (Badan Pusat Statistik Indonesia, 2020). In 2022, there was an increase in exports of Indonesian medicinal plants by 5.55% from the previous year (BPS 2020). According to the World Health Organization (WHO), 80% of people in developing countries are expected to depend on plant-based medicines because they are considered safe and have little or no side effects (World Health Organization, 2010).

The salam plant (*Syzygium polyanthum*) is well-known throughout Indonesia, with its leaves commonly utilized as a culinary seasoning or flavor enhancer thanks to its unique aroma. In addition to its benefits as a food ingredient, bay leaves are used as an alternative medicine, due to their content of secondary metabolites with numerous pharmacological properties, such as alkaloids, saponins, steroids, phenolics, flavonoids, and essential oils (Widyawati et al., 2021; Qi et al., 2004).

One of the benefits of bay leaves is reducing dyslipidemia, especially in cases of hypertriglyceridemia, and lowering levels of low-density lipoprotein (LDL) (Harismah, 2017). Previous studies have shown that bay leaf extract effectively reduces cholesterol levels by inhibiting the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase. In experiments conducted on Wistar rats, administering *Syzygium polyanthum* extract for just 15 days resulted in significant reductions in triglyceride, LDL, and overall cholesterol levels. Saponins, alkaloids, flavonoids, quinones, and tannins are secondary metabolites known to potentially exhibit antidyslipidemic effects in bay leaf extract (Kusuma et al., 2011: Zargar et al, 2015; Widyawati et al., 2021; Widjajakusuma et al., 2019; Wu et al., 2017).

Dyslipidemia treatment conventionally falls into several classes based on mechanism, including HMG CoA reductase inhibitors, nicotinic acid, fibrates, and others. However, there's been significant progress in enhancing these conventional treatments by utilizing nanoparticle delivery systems. This approach offers numerous advantages and has been extensively adopted to enhance the potency of dyslipidemia drugs in therapy. Research findings indicate that Atorvastatin nanoparticles can enhance both safety and effectiveness as antihyperlipidemic agents (Mahmood et al., 2019). Nanoparticles employed in drug delivery offer numerous benefits. Their particle dimensions and surface properties can be carefully modified, enabling targeted drug delivery to specific areas through both passive and active mechanisms after parenteral administration. Additionally, nanoparticles allow for the controlled and sustained release of active pharmaceutical ingredients at the targeted location, enhancing therapeutic efficacy, also minimizing side effects. Moreover, this versatile system can be utilized for drug delivery through various paths, such as oral and parenteral administration (Mohanraj and Chen, 2006). However, the advancement of nanoparticle-based drug delivery systems for treating dyslipidemia like this has been developed for chemical synthesis drugs only, such as Atorvastatin nanoparticles, Lovastatin, Simvastatin, and Estradiol (Mahmood et al., 2019). There isn't research on nanoparticle drug delivery systems for antihyperlipidemia from natural ingredients yet.

Due to the large biological natural resources that Indonesia has, as well as advances in the development of current pharmaceutical technology, it is necessary to formulate bay leaf extract nanoparticles (*Syzygium polyanthum*) to obtain a formula with optimal physical characteristics and release profile and is proven to be able to provide better effectiveness compared to only bay leaf extract so that it can increase the use value of plants of Indonesian herbal medicines.

MATERIAL DAN METODE

Material

The tools used in this study were a magnetic stirrer, centrifuge (DLAB), water bath sonicator, scales, Zetasizer Nano ZS ZEN3600 (Malvern, United Kingdom), and UV-Vis spectrophotometer Genesys-50 (Thermo). The materials applied in this study were chitosan (CV. Bio Chitosan Indonesia), sodium tripolyphosphate (STPP) (Xilong Scientific), ethanol extract of *Syzygium polyanthum* (PT. Borobudur Industri Jamu), ethanol (Merck), syringe filter (Allopure), sodium acetate (Merck), dialysis membrane MWCO 14000 Da (Wards Science, USA), aluminum chloride, Phosphate Buffered Saline (PBS) tablet (Oxoid, Thermo), and quercetin (Merck).

Method

Phytochemical screening

In the ethanol extract from *Syzygium polyanthum*, a phytochemical screening was conducted, encompassing tests for alkaloids, flavonoids, quinone, saponin, tannin, and triterpenoids.

Alkaloid

The extract was weighed as much as 0.4 grams, then combined with ammonia 25% and 0,02 L of chloroform, it was agitated and filtered. Subsequently, 2 N HCl was added until two distinct phases emerge: an aqueous phase and a chloroform phase, facilitating liquid-liquid extraction. The aqueous phase was transferred into two reaction tubes, with Dragendorff reagent added to the first tube. The appearance of a red deposit confirmed the presence of alkaloids in the extract. In the second tube, the presence of alkaloids was confirmed by the formation of a white precipitate upon adding Meyer's reagent (Silva et al., 2017).

Flavonoid

The extract was weighed as much as 1 gram, then, it was heated in 50 mL of solvent for 0,25 hour, and filtered. The filtrate was utilized to detect flavonoids, tannins, saponins, and quinones. 5 mL of filtrate, 1 mL of HCl, 5 mL of amyl alcohol, and magnesium powder were added. The mixture was thoroughly shaken and allowed to separate. The appearance of a red, brick red, or purplish red color in the amyl alcohol layer confirmed the presence of flavonoids in the extract (Jagessar, 2017).

Quinone

A drop of the filtrate previously prepared was added to a spot plate, afterward by the addition of a drop of 1 N NaOH. The development of a red color denoted a positive result (Silva et al., 2017; Singh & Kumar, 2017).

Saponins

5 mL of filtrate was vigorously shaken for 10 minutes until consistent foam forms, reaching a height of 1-10 cm and persisting for 10 minutes. The consistent foam formed after adding a few drops of 2 N HCl demonstrated that the extract contains saponin (Singh & Kumar, 2017; Tiwari et al., 2011).

Tannin

5 mL of the filtrate that was prepared earlier was poured into three test tubes. To the first tube, 5% $FeCl_3$ was added, and the presence of tannins was indicated by the development of a green. dark blue, blue, or greenish-blue color. In the second test tube, 1% gelatin was introduced, and a white precipitate signals the presence of tannins. In the third test tube, 2 mL of 40% formaldehyde and 1 mL of concentrated HCl were added, and the mixture was then heated to boiling. The appearance of red precipitation indicated tannin (Pandey & Tripathi, 2014; Singh & Kumar, 2017).

Triterpenoid

0.2 grams of extract was combined with 20 mL of ether, then filtered and evaporated using an evaporator cup. The residue left in the cup was treated with 2 drops of acetic anhydride. Then, a drop of concentrated sulfuric acid was added. Positive triterpenoid resulted if a red, pink, or purple color was formed (Singh & Kumar, 2017).

Total flavonoid content determination

The total flavonoid content was represented as quercetin. 200 mg of the extract dissolved in ethanol and let it rested for 60 minutes, then filtered and added ethanol to the final volume of 25 mL. A series of quercetin dilutions (10 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm) was prepared as a reference. From each dilution, 500 µL of the extract and quercetin samples were pipetted into separate tubes. To each tube, 100 µL of 1 M sodium acetate, 1500 µL of ethanol, 100 µL of 10% aluminum chloride, and demineralized water was added to adjust the total volume of the mixture to 5 mL. Then incubated for

30 minutes. The wavelength and instrument that was used were 436 nm and spectrophotometer UV-Vis. A standard curve was constructed using the quercetin standard solution. The total flavonoid content was determined in terms of milligrams of quercetin equivalents per gram of dry extract. Equation 1 was used to calculate the total flavonoid content (Departemen Kesehatan Republik Indonesia, 2017).

$$\% \text{ flavonoid} = \frac{C \times V \times f}{W} \times 100\% \quad \text{.....(1)}$$

C: Concentration of sample solution (mg/mL)
V: Volume of test solution before dilution (mL)
f: Dilution factor
W: Weight of sample test (mg)

Formulation of nanoparticle *Syzygium polyanthum*

In the formulation process of nanoparticles *Syzygium polyanthum* (nanoparticle SP), a cross-linking method was used in which chitosan polymer and sodium tripolyphosphate (STPP) were used as cross-linkers. The nanoparticle formula was prepared by modifying the previously described method (Hidayati et al, 2023; Rahayyu et al, 2024). The first step in nanoparticle formulation involved preparing chitosan at a concentration of 1.4 mg/mL by dissolving it in a 1% acetic acid glacial solution. Then pH adjusted to 7.4 using 5 M NaOH, resulting in the formation of cationic chitosan. *Syzygium polyanthum* extract stock solution were prepared in demineralized water at concentrations of 5,000 ppm and 1.4 mg/mL STPP was prepared with demineralized water. The chitosan: STPP ratio was 3:1. The extract solution was combined with the chitosan solution to obtain a final concentration of 2,500 ppm, and the mixture was allowed to incubate for 0.5 hours in the dark. Next, the solution was mixed at 800 rpm for 3 minutes. Then, STPP was slowly added using a micropipette, stirring the mixture for 20 minutes (F1), 90 minutes (F2), and 120 minutes (F3) to produce nanoparticles. Then centrifuged at 14,000 rpm for a duration of 10 minutes. It was used to collect the nanoparticles, then rinsed with demineralized water, and subjected to sonication for 60 minutes. The *Syzygium polyanthum* nanoparticle formula was presented in Table 1.

Table 1. Nanoparticle *Syzygium polyanthum* extract formulation

Formula Name	Stirring time (minutes)
F1	20
F2	90
F3	150

Characterization of nanoparticle *Syzygium polyanthum*

The *Syzygium polyanthum* nanoparticles were then characterized by assessing their particle size, polydispersity index, zeta potential (ζ potential), entrapment efficiency, and conducting a release study.

Particle size and polydispersity index

Zetasizer Nano ZS ZEN3600 was used to analyze particle size and polydispersity index of the resulting nanoparticle *Syzygium polyanthum*. The test was carried out in triplicate (Hidayati et al., 2023; Rahayyu et al., 2024).

ζ potential

Zetasizer® Nano ZS was used to characterize the ζ potential value of the resulting nanoparticles *Syzygium polyanthum*. The test was carried out in triplicate (Hidayati et al., 2023; Rahayyu et al., 2024).

Entrapment efficiency (% EE)

The method for determining the entrapment efficiency (% EE) of the resulting nanoparticles SP was an indirect method using supernatant samples obtained from centrifugation. Quercetin was the compound observed in the supernatant sample, and it was analyzed using the Chang method (Hidayati, 2021; Morales-Olan et al, 2021). The value of % EE from the nanoparticle was calculated using the Equation 2.

$$\% EE = \frac{weight\ of\ loaded\ extract}{weight\ of\ initial\ extract}\ x\ 100 \quad \text{.....(2)}$$

Release study

The procedure for conducting an in vitro release study of *Syzygum polyanthum* nanoparticles was the dialysis bag method, with simulated blood circulation (phosphate-buffered saline / PBS at pH 7.4) as the release medium. The SP nanoparticle formulation dispersed in water, then placed inside a dialysis membrane. The membrane was immersed in 5 mL of phosphate-buffered saline (pH 7.4) kept at 37±0.5°C and continuously stirred at 100 rpm. At predetermined time intervals (5, 15, 30, 45, 60, 90, and 120 minutes), 1,000 µL samples were taken from the release medium, and the medium was replaced with 1,000 µL of fresh phosphate-buffered saline (pH 7.4). The collected sample was analyzed using a spectrophotometer UV-Vis with the Chang method using quercetin as a marker (Hidayati, 2021).

Data Analysis

The differences in this study were analyzed using a one-way ANOVA in SPSS version 20, with a significance level set at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening test

The phytochemical screening test aimed to qualitatively identify and ascertain the content of secondary metabolites in the extract of *Syzygium polyanthum*. The result of the phytochemical screening test is presented in Table 2. These findings align with previous studies that analyzed the phytochemicals in *Syzygium polyanthum* extract (Kusuma et al., 2011; Widyawati et al., 2021; Widjajakusuma et al, 2019).

Table 2. Phytochemical screening test of *Syzygium polyanthum* extract

Secondary Metabolite	Result
Alkaloid	+
Saponin	+
Kuinon	+
Flavonoid	+
Tanin	+
Triterpenoid	+

Determination of total flavonoid content

The colorimetric method is utilized as the keto group at C-4 and the hydroxy group at either C-3 or C-5 in quercetin are capable of forming a complex with $AlCl_3$. The addition of sodium acetate then stabilizes this complex. λ_{max} of quercetin is 436 nm, aligning with earlier research showing its peak wavelength between 380 and 480 nm (Das et al., 2013). The calibration curve of quercetin is shown in Figure 1 with an R^2 value of 0.9967.

Quercetin is a flavonoid compound, and the calculation reveals that the total flavonoid content in the *Syzygium polyanthum* extract is 0.04%. The Indonesian Herbal Pharmacopoeia standard requires that the total flavonoid content in the concentrated extract of Syzygium polyanthum leaves be at least 1.14%, expressed as quercetin. The total flavonoid of the dry extract content in this study is smaller

than the standard for concentrated extracts outlined in the Indonesian Herbal Pharmacopoeia. Maltodextrin, a common additive in extract powder production, is used in this extract. Other studies suggest that the total flavonoid content of the extract decreases as the concentration of maltodextrin increases (Widyawati et al., 2021).

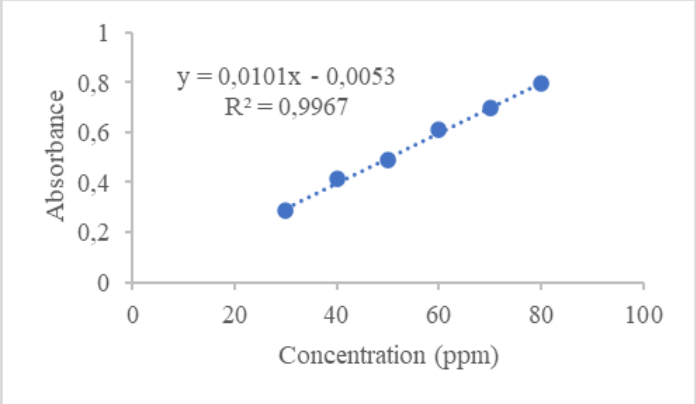

Figure 1. Quercetin calibration curve

Characterization of *Syzygium polyanthum* nanoparticles

Depending on the preparation method, there are two types of nanoparticles, namely nanospheres and nanocapsules (Qi et al., 2004). This study produces nanosphere. Chitosan undergoes an ionic gelation process due to the formation of STPP and cationic chitosan polyanion complexes through electrostatic energy and the formation of spherical particles (Zargar et al., 2015; Wu et al., 2017). Some parameters influence the size and stability of chitosan nanoparticles, such as stirring time, chitosan: STPP mass ratio, chitosan concentration, initial chitosan pH, and NaCl addition in formula (Antoniou et al., 2015). The variable that is used in this study is stirring time which is F1 (20 min), F2 (90 min), and F3 (150 min). The nanoparticle SP formula is presented in Table 2.

Particle size is an essential parameter that influences the biocompatibility and bioactivity of nanoparticles. Parameters to indicate nanoparticle homogeneity is polydispersity index, while ζ potential shows colloidal stability (Mahmood et al., 2019; Departemen Kesehatan Republik Indonesia, 2017). From the formula that has been made, the size and polydispersity index results of nanoparticles SP are obtained as shown in Table 3.

In the formulation of *Syzygium polyanthum* nanoparticle, there are three formulas were used, namely F1, F2, and F3 where the variable used was the stirring time after cross-linking processes. The cross-linking process occurs when a mixture of chitosan solution and extract is added drop by drop with sodium tripolyphosphate (STPP) accompanied by stirring at a speed of 800 rpm. The figure of *Syzygium polyanthum* nanoparticle is presented in Figure 2 shows that the formula formed has a clear colloidal form and a yellowish color.

Table 3. Size and polydispersity index of nanoparticle of *Syzygium polyanthum extract*

Formulas	Size (nm)	Polydispersity Index
F1	257 ± 6.68	0.383 ± 0.04
F2	232 ± 2.54	0.333 ± 0.02
F3	303 ± 1.30	0.242 ± 0.01

Based on Table 3, the result of the size is all of the formulas have a size <300 nm. The statistical result of the size of SP nanoparticle is no significant difference between the three formulas. The best formula in this study regarding size is F2 because it has the smallest size. F2 is statistically significantly different from F1 and F3 ($p<0.05$). In the stirring process, the variables that can affect the results of the nanoparticle size obtained are the stirring speed and stirring time. In this study, the

variable used was the length of the stirring process, where the duration of stirring F1, F2, and F3 was 20 minutes, 90 minutes, and 150 minutes respectively. The stirring speed and sonication time were kept constant, namely 800 rpm and 60 minutes respectively. Chitosan nanoparticles with a size < 1000 nm have been proven to have therapeutic effects, such as antihyperlipidemic effect (Zhang et al., 2011: Tao et al., 2011). F1, F2, and F3 have the potential to give a good therapeutic effect, especially the antihyperlipidemia effect because its size is less than 1000 nm.

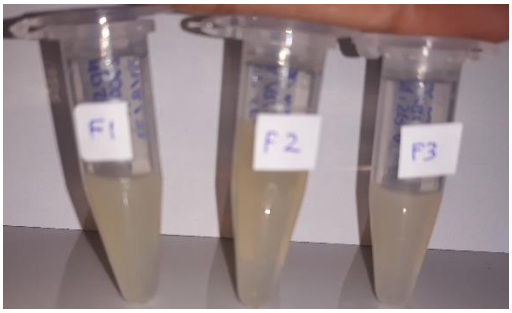

Figure 2. Nanoparticle *Syzygium polyanthum*

The polydispersity index values obtained in F1, F2, and F3 are presented in Table 3. This value indicates that this formulation has good homogeneity because it has a value of less than 0.5. The polydispersity index of F2 and F3 is significantly different. In addition, the polydispersity index value indicates that the distribution of nanoparticles is monodispersed with little variation and no aggregate formed (Mahmood et al., 2019; Kementrian Kesehatan Republik Indonesia, 2019).

The next characterization performed is the measurement of the potential. The sample selected for the ζ potential test is the best formulation, which has the smallest particle size, a favorable polydispersity index, and optimal manufacturing efficiency in terms of mixing time. This sample is sample F2 because it has the smallest particle size among the three formulas and has a polydispersity index because it has a value of less than 0.5, and the stirring time is shorter than F3. It should be written in one paragraph with the previous sentencein Table 4. These results suggest that the particle dispersion is stable, as it has a ζ potential value exceeds +30 mV or below than -30 mV, which is attributed to the electrostatic forces among the particles. The presence of a positive charge originating from chitosan causes a positive value in the ζ potential analysis of nanoparticles (Mahmood et al., 2019).

Table 4. ζ potential nanoparticle *Syzygium polyanthum*

Formula	ζ potential (mV)	Average of ζ potential (mV)
F2	31.0	30.9 ± 0.416
	31.2	
	30.4	

Entrapment efficiency

The entrapment efficiency test is one of the characterizations that needs to be done to find out how much concentration of the extract is entangled in the nanoparticle system. The entrapment efficiency is greatly affected by the solubility of the drug within the matrix or polymer used, which is influenced by the molecular weight, interactions between the drug and the polymer, polymer composition, and the presence of functional groups in the drug (ester or carboxyl group) (Wu et al., 2017).

Entrapment efficiency of nanoparticle *Syzygium polyanthum* was determined by measuring the amount of *Syzygium polyanthum* extract that was not entangled by nanoparticles by calorimetry (Chang Method) using AlCl3 modified by the spike method and using quercetin as a standard comparison and the method for determining entrapment efficiency used was the determination of total flavonoids referring to the provisions of the Pharmacopoeia Indonesian Herbs.

The stages of carrying out this entrapment efficiency test are the preparation of a quercetin standard curve followed by an analysis of the test sample which is the supernatant from the centrifugation results in the nanoparticle manufacturing process. The quercetin calibration curve in this study is described in Figure 1 while the entrapment efficiency values for each formula are described in Table 5. The standard quercetin curve obtained in this study is included in the good category because the value of the differentiation coefficient is close to 1.

Table 5. Entrapment efficiency of nanoparticle *Syzygium polyanthum*

Formula	Entrapment efficiency (%)	Concentration of trapped extract (ppm)
F1	48.18	1204
F2	64.19	1605
F3	67.48	1687

Entrapment efficiency is defined as the ratio of the active compounds trapped within the nanoparticle to the total active compounds incorporated into the formula (Vrignaud et al., 2011). The value of entrapment efficiency of nanoparticles range between 22% and 100% (Mattu et al., 2013). Drug solubility in a matrix or polymer affects the entrapment efficiency, which is influenced by molecular weight, polymer concentration. functional group in the drug, and drug-polymer interaction (Wu et al., 2017). Entrapment efficiency obtained in this research was in the range of 48.18 - 67.48%. The initial extract concentration used in this nanoparticle formulation is 2,500 ppm. *Syzygium polyanthum* concentration that is enclosed in the nanoparticles is 1,204 ppm (F1), 1,605 ppm (F2), and 1,687 ppm (F3). Colorimetry is utilized to quantify the free extract, which is present in the supernatant after the nanoparticles are purified through centrifugation.

Release study of nanoparticle *Syzygium polyanthum*

The release study of nanoparticle SP is one of characterization to ensure the extract that is entrapped in the nanoparticle can be released from the system and produce therapeutic effects. The in vitro release behavior of nanoparticle SP uses phosphate-buffered saline (PBS) pH 7.4 as the release medium at body temperature (37ºC) with stirring at 100 rpm (Rizvi et al., 2019). Figure 3 explains the release profile of nanoparticle *Syzygium polyanthum* for 5-120 min.

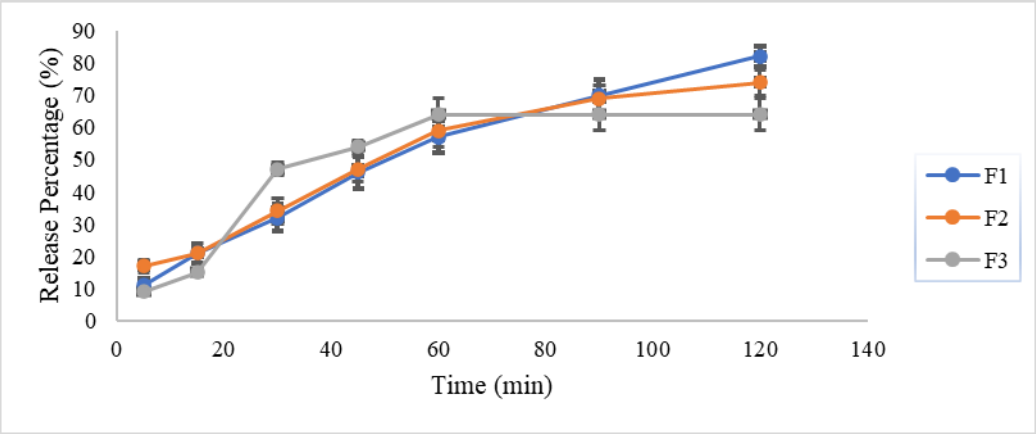

Figure 3. Release profile of *Syzygium polyanthum* nanoparticle

All of the formulas have good release profiles indicating that 64-82% of extract concentration can be released from the system in 120 min which release profiles of F1, F2, and F3 are 82 ± 3%, 74 ± 4%, and 64 ± 5% respectively. The formula that shows the best release profile is F1 which has a higher release percentage of extract. After release from the system, the extracts can start to play their role in

producing therapeutic effects. Therapeutic effects that potential can be produced by this formula are antihyperlipidemia and antihypertension, but this effect need to be proven with further study.

CONCLUSION

The formulas F1, F2, and F3 nanoparticle *Syzygium polyanthum* have good characteristics which is the best formula was F2 because it has the smallest particle size among the three formulas, good polydispersity index (less than 0.5), more efficient stirring time, good entrapment efficiency and has good release pattern. The safety and effectiveness of *Syzygium polyanthum* nanoparticles need to be further evaluated in the next study.

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REFERENCES

Antoniou, J., Liu, F., Majeed, H., Qi, J., Yokoyama, W., & Zhong, F. (2015). Physicochemical and morphological properties of size-controlled chitosan–tripolyphosphate nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *465*, 137–146. https://doi.org/10.1016/j.colsurfa.2014.10.040

Badan Pusat Statistik Indonesia. (2020). *Produksi tanaman biofarmaka (Obat) 2018-2020*. *Bps*. https://www. bps. go. id/indicator/55/63/1/produksi-tanaman-biofarmaka-obat-. html.

Das, A. (2013). Characterization of antioxidants and antioxidative properties of various unifloral Honeys procured from West Bengal, India. *IOSR Journal Of Environmental Science, Toxicology And Food Technology*, *7*(3), 56–63. https://doi.org/10.9790/2402-0735663

Departemen Kesehatan Republik Indonesia. (2017). *Farmakope Herbal Indonesia Edisi II*. Kementerian Kesehatan Republik Indonesia.

Harborne, J. B., Padmawinata, K., & Soediro, I. (2006). *Metode fitokimia, penuntun dan cara modern menganalisis tumbuhan*. ITB Bandung.

Harismah, K. (2017). Pemanfaatan daun salam (*Eugenia polyantha*) sebagai obat herbal dan rempah penyedap makanan. *Warta LPM*, *19*(2), 110–118. https://doi.org/10.23917/warta.v19i2.2742

Hidayati, E. (2021). *Studi inhibisi Spermatogenesis Nanopartikel Phyllanthus niruri melalui jalur persinyalan testosteron non klasik secara in vitro pada Lini Sel TM4. (Tesis)*. Institut Teknologi Bandung.

Hidayati, E. N., Rahayyu, A. M., & Azzahra, F. (2023). Physical characterization of chitosan-based syzygium polyanthum leaves extract nanoparticles. *Pharmacon: Jurnal Farmasi Indonesia*, *20*(2), 120-128.

Jagessar, R. (2017). Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of Passiflora edulis and Vicia faba L. (Fabaceae). *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 1714–1721.

Kementrian Kesehatan Republik Indonesia. (2019). *Infodatin. Beban Kanker di Indonesia*. Jakarta: Pusdating Kemenkes RI.

Kusuma, I. W., Kuspradini, H., Arung, E. T., Aryani, F., Min, Y.-H., Kim, J.-S., & Kim, Y. (2011). Biological activity and Phytochemical analysis of three Indonesian medicinal plants, Murraya koenigii, *Syzygium polyanthum* and *Zingiber purpurea*. *Journal of Acupuncture and Meridian Studies*, *4*(1), 75–79. https://doi.org/10.1016/S2005-2901(11)60010-1

Mahmood, M. A., Madni, A., Rehman, M., Rahim, M. A., & Jabar, A. (2019). Ionically cross-linked Chitosan Nanoparticles for sustained delivery of docetaxel: fabrication, post-formulation and acute oral toxicity evaluation. *International Journal of Nanomedicine*, *Volume 14*, 10035–10046. https://doi.org/10.2147/IJN.S232350

Mattu, C., Li, R., & Ciardelli, G. (2013). Chitosan nanoparticles as therapeutic protein nanocarriers: The effect of ph on particle formation and encapsulation efficiency. *Polymer Composites*, *34*(9), 1538–1545. https://doi.org/10.1002/pc.22415

Mohanraj, V. J., & Chen, Y. (2007). Nanoparticles - a review. *Tropical Journal of Pharmaceutical Research*, *5*(1). https://doi.org/10.4314/tjpr.v5i1.14634

Morales-Olán, G., Luna-Suárez, S., Figueroa-Cárdenas, J. D. D., Corea, M., & Rojas-López, M. (2021). Synthesis and characterization of chitosan particles loaded with antioxidants extracted from Chia (*Salvia hispanica* L.) seeds. *International Journal of Analytical Chemistry*, *2021*, 1–12. https://doi.org/10.1155/2021/5540543

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*, *2*(5), 115–119.

Qi, L., Xu, Z., Jiang, X., Hu, C., & Zou, X. (2004). Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydrate Research*, *339*(16), 2693–2700. https://doi.org/10.1016/j.carres.2004.09.007

Rahayyu, A. M., Hidayati, E. N., & Masruriwati, E. (2024). Effect of Chitosan concentration on physical characteristic of extract ethanolic of Bay Leaf (*Syzygium polyanthum*) Nanoparticle prepared by Cross-Linking Methods. *Jurnal Farmasi Sains Dan Praktis*, 150–155. https://doi.org/10.31603/pharmacy.v10i2.9233

Rizvi, S. Z. H., Shah, F. A., Khan, N., Muhammad, I., Ali, K. H., Ansari, M. M., Din, F. ud, Qureshi, O. S., Kim, K.-W., Choe, Y.-H., Kim, J.-K., & Zeb, A. (2019). Simvastatin-loaded solid lipid nanoparticles for enhanced anti-hyperlipidemic activity in hyperlipidemia animal model. *International Journal of Pharmaceutics*, *560*, 136–143. https://doi.org/10.1016/j.ijpharm.2019.02.002

Silva, G. O. De, Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*, *5*(2), 29–32.

Singh, V., & Kumar, R. (2017). Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand region. *International Journal of Life-Sciences Scientific Research*, *3*(6), 1451–1458.

Tao, Y., Zhang, H., Gao, B., Guo, J., Hu, Y., & Su, Z. (2011). Water-soluble chitosan Nanoparticles inhibit Hypercholesterolemia induced by feeding a high-fat diet in Male Sprague-Dawley Rats. *Journal of Nanomaterials*, *2011*, 1–5. https://doi.org/10.1155/2011/814606

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *International Pharmaceutica Sciencia*, 98-106.

Vrignaud, S., Anton, N., Gayet, P., Benoit, J.-P., & Saulnier, P. (2011). Reverse micelle-loaded lipid nanocarriers: a novel drug delivery system for the sustained release of doxorubicin hydrochloride. *European Journal of Pharmaceutics and Biopharmaceutics*, *79*(1), 197–204. https://doi.org/10.1016/j.ejpb.2011.02.015

Widjajakusuma, E. C., Jonosewojo, A., Hendriati, L., Wijaya, S., Surjadhana, A., Sastrowardoyo, W., Monita, N., Muna, N. M., Fajarwati, R. P., & Ervina, M. (2019). Phytochemical screening and preliminary clinical trials of the aqueous extract mixture of *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Syzygium polyanthum* (Wight.) Walp leaves in metformin treated patients with type 2 diabetes. *Phytomedicine*, *55*, 137-147

Widyawati, T., Pase, A., Daulay, M., & Sumantri, I. B. (2021). *Syzygium polyanthum* (Wight.) Walp ethanol extract Decreased Malondialdehyde level in type 2 diabetic patients. *Pharmacognosy Journal*, *13*(6s), 1557–1561. https://doi.org/10.5530/pj.2021.13.198

World Health Organization. (2010). *Traditional herbal remedies for primary health care*.

Wu, J., Wang, Y., Yang, H., Liu, X., & Lu, Z. (2017). Preparation and biological activity studies of resveratrol loaded ionically cross-linked chitosan-TPP nanoparticles. *Carbohydrate Polymers*, *175*, 170–177. https://doi.org/10.1016/j.carbpol.2017.07.058

Zargar, V., Asghari, M., & Dashti, A. (2015). A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. *ChemBioEng Reviews*, *2*(3), 204–226.

https://doi.org/10.1002/cben.201400025
Zhang, H., Tao, Y., Guo, J., Hu, Y., & Su, Z. (2011). Hypolipidemic effects of chitosan nanoparticles in hyperlipidemia rats induced by high fat diet. *International Immunopharmacology*, *11*(4), 457–461. https://doi.org/10.1016/j.intimp.2010.12.015