Optimizing antioxidant properties of salam (Syzygium polyanthum) leaf extract through nanoencapsulation technology

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Abstract. Nanoencapsulation preparation was carried out to optimize the antioxidant properties of salam leaf extract (Syzygium polyanthum). The ionic gelation method was used in the process of making nanoencapsulation with chitosan and Sodium Trypolypospat (STPP) as a coating material. The parameters observed included the antioxidant activity of salam leaf extract and the characteristics of the nanoencapsulation of salam leaf extract. NSLE characterization was measured, such as particle size, zeta potential (using Particle Size Analyzer), and nanoparticle morphology (using Transmission Electron Microscopy). Descriptive analysis was used to discuss the results obtained which are equipped with tables and figures. The results showed that the optimum formulation of NSLE was the ratio of extract nanoencapsulation: chitosan: STPP was 1: 4: 1/90. The antioxidant activity of the salam leaf extract was 68.55%, then the measured NSLE particle size was 478.3 nm with a Polydispersity Index (PI) of 0.52 and a measured zeta potential value of + 42.3 mV. NSLE particle morphology looks round with a rough surface. Characterization of NSLE has the potential to protect bioactive compounds so that they can optimize the performance of antioxidant activity.

1. Introduction

Indonesia is known for its high biodiversity, such as the diversity of herbal plants. One of the plants that are often used for herbal medicine is salam leaf [1]. Salam leaf contains various secondary metabolite compounds that have pharmacological activity in overcoming various diseases [2]. The synergistic effect between secondary metabolite compounds causes pharmacological effects [3]. The secondary metabolite compounds found in salam leaves include phenolic, saponin, flavonoid, and alkaloid [4]. Active compounds found in salam leaves have a variety of activities, one of which is as an antioxidant. Salam leaves extract has a weakness such as low bioavailability that causes the active compound activity cannot work optimally, so the right strategy is needed to make preparations that can protect the active compound by using the nanoencapsulation method.

Nanoencapsulation is a technology used to protect bioactive compounds on the nanoparticle scale [5]. Nanoparticles can penetrate spaces between cells that can be penetrated by colloidal particles. The application of nanotechnology can improve thermal stability, oral bioavailability, and its solubility in water [6]. Nanoencapsulation allows the active ingredient to release periodically through the encapsulant layer so that it can increase the efficiency of using active ingredients [7]. Nanoencapsulation can be implemented as protection and control of the release of bioactive compounds at the right time and in the right place [8]. Nanoencapsulation can be made by various methods. One method which often used is the ionic gelation method. The basis of this method is the nature of chitosan that undergoes a liquid-gel
transition due to ionic interactions with a polyanion. This interaction occurs between the positively charged ammonium chitosan group and a crosslinker, for example, Sodium tripolyphosphate (STPP) [9].

The characteristics of nanoencapsulation can be observed by particle size, zeta potential, and morphology. Particle size and zeta potential are measured using Particle Size Analyzer (PSA) while the morphology of nanoparticles is observed using Transmission Electron Microscopy (TEM). Particle size is determined by the average diameter of a particle. A particle is explained to have a nano-size if its diameter ranges from 1-1000 nm [10]. Nanoparticles play an important role in increasing interactions between particles which causes an increase in viscosity where nanoparticles have a large surface area with a small volume [11]. Zeta potential is used to characterize the surface charge properties of particles associated with electrostatic interactions of nanoparticles [12]. Zeta potential that indicates the level of stability of nanoparticles is more than +30 mV or -30 mV [13]. Surface morphology affects the ability of nanoparticles to penetrate the target cell membrane. The shape and surface state of nanoparticles can provide information about the nature of drug release [14].

2. Material and Methods

2.1. Material
Salam leaves obtained from the Yogyakarta area, chitosan (Chitosan FG Powder, Chimultiguna, Indramayu, Indonesia), Sodium tripolyphosphate (Brataco, Yogyakarta), citric acid, and aquades.

2.2. Methods

2.2.1. Preparation of plant materials (salam leaves). Salam leaves were selected with the same color qualification (medium green) and then cleaned. The salam leaves were chopped and dried in an oven drying at 55°C for 24 hours. Salam leaves that have dried and then smoothed using a Willey mill to 1 mm in diameter.

2.2.2. Extraction of plant materials (salam leaves). 100 grams of salam leaf powder macerated with 70% alcohol as much as 500 ml. Maceration was carried out for 72 hours and stirred every 24 hours. After 3 days the filter was filtered and the filter was evaporated using a water bath until it was thick.

2.2.3. Preparation of nanoencapsulation salam leaves extract. Nanoencapsulation of salam leaf extract was prepared using ionic gelation method by mixing salam leaf extract: chitosan: STPP (1: 4: 1/90) w/v. Two (2) grams of chitosan was added to 100 ml of acetic acid (1% w/v) and dissolved 0.04 mg of STPP into distilled water and stirred for 1 hour. The salam leaf extract is added to the mixture (chitosan is dissolved in a pH 4 acetic acid solution) and stirred using a magnetic stirrer for 30 minutes. Then the STPP is added and stirred for another 30 minutes. Then the solution and sediment are separated.

2.2.4. Determination of Antioxidant Activity. Analysis of antioxidant activity using the DPPH method [15]. 2, 2-diphenyl-1-picryl hydroxyl (DPPH) is a stable free radical compound that will react with antioxidants. A total of 10 mg of sample was dissolved in 30 mL methanol and centrifuged (Sartorius Sigma 3–18 K) for 10 minutes. The supernatant was added to 3 mL of 0.025 g / l DPPH in methanol. Absorbance was measured after 40 minutes at room temperature using a UV-Vis spectrophotometer with methanol as a comparison. Antioxidant activity is calculated by the formula:

\[
\% \text{ inhibition} = \frac{\text{sample blank} - \text{sample uptake}}{\text{sample uptake}} \times 100\% \tag{1}
\]

2.2.5. Characteristication of nanoencapsulation salam leaves extract. Particle size. The particle size of NSLE was measured using Zetasizer Nano ZS (Horiba Scientific SZ-100, Horiba, Kyoto, Japan)
following the Balakumar [16] and Hussain-Sahudin [17] methods. The sample was diluted at a ratio of 1: 0.01 (v/v) using distilled water at 24.9° C with a detection angle of 90 °.

**Zeta potential analysis.** Zeta potential of NSLE was measured by dynamic light scattering techniques using particle size analyzers (Horiba Scientific SZ-100, Horiba, Kyoto, Japan) with the Balakumar [16] and Hussain-Sahudin [17] samples. Diluted samples with a ratio of 1: 100 (v/v) with distilled water.

**Nanoencapsulation morphology.** The morphology of NSLE was examined using high-performance digital imaging Electron Transmission Microscopy (Joel JEM-1400 CX, Hitachi High-Technologies Corp., Tokyo, Japan). The analysis was carried out by diluting nanoencapsulation of salam leaf extract with distilled water, a drop of the solution was placed into a micro copper box that was previously stained with phosphotungstic acid and allowed to evaporate to dry at room temperature (25± 2 ° C). Dry microgrids are then seen at various resolutions under TEM [17].

2.2.6. **Data Analysis.** The results obtained were analyzed using descriptive methods that are displayed in the form of tables and figures.

3. **Results and Discussion**

3.1. **Antioxidant activity**

The results of the analysis of the antioxidant activity of salam leaves extract using the DPPH method were 68.55%. This result is lower than Perumal [18] with antioxidant activity values of 91.43± 2,52%. It might be caused by differences in plant origin and the methods used. The antioxidant activity of salam leaf extract shows the number of compounds that play a role in the antioxidant process in counteracting free radical compounds in this case in the form of compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radical compounds. Polyphenol compounds and flavonoids are natural antioxidants that are found in many plants. Flavonoids which have antioxidant activity include flavones, flavonoids, isoflavones, catechins, and chalcones [19].

3.2. **Optimum formulation of nanocaptulation salam leaves extract**

The ratio of extract, chitosan, and STPP in the formulation is one of the parameters to control particle size. Comparison of formulations from 1: 1: 1/10 to 1: 4: 1/90 salam leaf extract: chitosan: STPP is evaluated one by one to get the optimal formulation. The results showed that a ratio of 1: 4: 1/90 (Table 1) was chosen to be the optimal formulation. An ionic crosslinking occurs between the tripolyphosphate (polyanion) STPP ion and thiamine-NH3+ (cation) group in chitosan [20]. The more ionic cross-linking occurs between chitosan and STPP, the more nanoparticle molecules of the active substance are formed.

| Table 1. Formulation ratio of salam leaves extract: chitosan: STPP |
|-----------------|-----------------|
| Ratio of extract: chitosan: STPP | Visual observation |
| 1:1:1/10 | Cloudy |
| 1:2:1/20 | Cloudy |
| 1:3:1/30 | Cloudy |
| 1:4:1/40 | Cloudy |
| 1:4:1/90 | Clearly |
3.3. Characterization of nanoencapsulation salam leaves extract

3.3.1. Particle Size. The average particle size and polydispersity index (PI) are calculated from the volume, intensity, and bimodal distribution assuming spherical particles. Particle size and distribution are the most important characteristics in nanoparticle systems. It is used to estimate the in vivo distribution, biological, toxicity, and aiming ability of the nanoparticle system [21]. The particle size distribution of the selected formulation was found 478.3 nm. Particle size is an important factor in formulations because it determines the rate of drug release as well as absorption and increases bioavailability [22]. The size of the NSLE is considered sufficient to carry phytochemical compounds salam leaves. The graph of NSLE size and particle index value can be seen in Figure 1.

![Graph of NSLE nanoparticle size and Particle Index](image)

Figure 1. Graph of NSLE nanoparticle size and Particle Index

Polydispersity index values indicate uniformity of particle size. Based on observations of NSLE particles it has a polydispersity index of 0.521 which means that the size of the NSLE particles is homogeneous. According to [23], the smaller the polydispersity index value the more homogeneous particle size. The numerical values range of PI is from 0.0 (for perfectly uniform samples with particle size) to 1.0 (for highly polydisperse samples with multi-particle size populations) [24].

3.3.2. Potential Zeta. The zeta potential value is positive for the selected formulation. Zeta potential indicates a positively charged surface. The zeta potential value of the selected formulation was found +42.3 mV. This shows that NSLE particles have a fairly high level of stability so that no aggregation occurs in each particle. The combination of chitosan and STPP in the ionic gelation method produces positive potential zeta. On the other hand, the ionization process in the amino group in chitosan causes a cationic process that produces a potentially positive zeta [25]. Zeta potential that indicates the level of stability of nanoparticles is more than +30 mV or -30 mV [13]. Therefore, the zeta potential measurement results show a stable formulation. Zeta potential is used to characterize the surface charge properties of particles associated with electrostatic interactions of nanoparticles [12]. The suspension which has a high potential zeta value will prevent particles from experiencing flocculation and aggregation.

![Graph of NSLE Potential Zeta](image)

Figure 2. Graph of NSLE Potential Zeta
The main characteristic of nanoparticles with chitosan: STPP is the value of zeta potential positively charged due to the presence of amine groups in chitosan [26]. Mucus consists of a glycoprotein called mucin, which contains a negative charge because it has residual sialic acid. The mucoadhesive properties of chitosan can extend retention time and increase the time of the release of nanocapsules in the digestive tract [27]. In the digestive tract, chitosan is positively charged because of the acidic environment and, therefore, can interact with mucin by electrostatic forces.

Zeta potential is commonly used to characterize the surface charge properties of nanoparticles, related to electrostatic interactions of nanoparticles. Electrostatic interactions will determine the tendency of aggregation and rejection. Zeta potential is a measure of the surface charge of particles scattered in a dispersing medium. Ideally, the zeta potential charge of a particle must be higher than the dispersing medium to prevent aggregation [14].

3.3.3. Morphology of nanoparticles. The morphology of NSLE are small dots or circles on a dark background with a homogeneous round shape (Figure 3). The morphology of the nanoencapsulation form of salam leaf extract is spherical with a rough surface. Self-aggregation and different nanoparticle droplets indicated homogeneously distributed in size and stable [28]. Surface morphology affects the ability of nanoparticles to penetrate the target cell membrane. Spherical nanoparticle surfaces more easily enter cells. The shape and surface state of nanoparticles can provide information about the nature of drug release [14]. The less spherical a particle is, the more angles there are of the particle, which can cause agglomeration to form.

![Figure 3. Morphology of salam leaf extract nanoparticles with TEM](image)

Morphology of NSLE was shown in figure 3 with a white circle appears and a black outline. The black outline indicates that salam leaf extract compounds which are nonpolar (white) have been successfully encapsulated using chitosan and STPP [29]. Nanoencapsulation of salam leaf extract using chitosan and STPP produces an oval-shaped capsule. This form occurs because of the interaction of opposing ions between the active ingredient which is negatively charged, chitosan which is positively charged, and STPP which is negatively charged, so that the ionic gelation process forms rounded particles [30].

4. Conclusion
Characterization of NSLE has the potential to protect bioactive compounds so that they can optimize the performance of antioxidant activity.

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