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Research Article

Formulation and Characterization of Gelatin Nanoparticles from Manis Rejo Leaf Extract (*Vaccinium variangiaefolium* (Bl.) Miq.) with Glutaraldehyde Concentrations Variations as Crosslinker

Formulasi dan Karakterisasi Nanopartikel Gelatin dari Ekstrak Daun Manis Rejo (Vaccinium variangiaefolium (Bl.) Miq.) dengan Variasi Konsentrasi Glutaraldehid sebagai Crosslinker

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ABSTRACT

Manis Rejo *(Vaccinium variangiaefolium (Bl.) Miq*.) is a plant that dominates at the Tangkuban Parahu area and grows in mountainous regions. Its leaves contain flavonoids with various benefits. The ethyl acetate extract of these leaves has demonstrated cytotoxic activity, making it a potential active compound in nanoparticle-based drug delivery systems. This study aimed to investigate the effect of variations in the glutaraldehyde concentration on the characteristics of the gelatination nanoparticles encapsulating the Manis Rejo leaf extract. The organoleptic properties, moisture content, and solubility of the extract were evaluated and then developed into nanoparticles (NPs) using the desolvation method with glutaraldehyde concentrations of 100 µl, 200 µl, and 300 µl. The characterization included the nanoparticle stability, particle size, polydispersity index (PDI), zeta potential, and morphology. The results showed that the extract was green, had a distinctive odor, a dry texture, a moisture content of 4.31%, and was soluble in ethyl acetate (1:10). The nanoparticles remained physically stable for 20 days at room temperature (25 ºC), with particle sizes of 343.4±4.4 nm, 226.3±23.1 nm, and 199.7±1.6 nm, and PDIs of 0.381±0.0234, 0.446±0.0895, and 0.177±0.0195, respectively. The zeta potential values ranged from 15.8±1.1 to 17.8±1.1 mV. Transmission Electron Microscopy (TEM) analysis of formula III revealed a spherical

morphology supporting the nanoparticle stability. Glutaraldehyde variation significantly influenced the particle size and PDI.

Keywords: Desolvation, Stability, Morphology, Optimization, Biodegradable

ABSTRAK

Manis Rejo (Vaccinium variangiaefolium (Bl.) Miq.) adalah tanaman yang mendominasi kawasan Tangkuban Parahu dan tumbuh di daerah pegunungan. Daunnya mengandung flavonoid dengan berbagai manfaat. Ekstrak etil asetat dari daun ini telah menunjukkan aktivitas sitotoksik, sehingga berpotensi sebagai senyawa aktif dalam sistem penghantaran obat berbasis nanopartikel. Penelitian ini bertujuan untuk mengkaji pengaruh variasi konsentrasi glutaraldehida terhadap karakteristik nanopartikel gelatin yang mengenkapsulasi ekstrak daun Manis Rejo. Evaluasi dilakukan terhadap sifat organoleptik, kadar air, dan kelarutan ekstrak, kemudian dikembangkan menjadi nanopartikel menggunakan metode desolvasi dengan variasi konsentrasi glutaraldehida 100 µl, 200 µl, dan 300 µl. Karakterisasi meliputi stabilitas nanopartikel, ukuran partikel, indeks polidispersitas (PDI), potensial zeta, dan morfologi. Hasil penelitian menunjukkan bahwa ekstrak berwarna hijau, memiliki bau khas, tekstur kering, kadar air 4,31%, serta larut dalam etil asetat (1:10). Nanopartikel tetap stabil secara fisik selama 20 hari pada suhu kamar, dengan ukuran partikel masing-masing 343,4±4,4 nm, 226,3±23,1 nm, dan 199,7±1,6 nm, serta nilai PDI 0,381±0,0234; 0,446±0,0895; dan 0,177±0,0195. Nilai potensial zeta berkisar antara 15,8±1,1 hingga 17,8±1,1 mV. Analisis TEM pada formula III menunjukkan morfologi sferis yang mendukung kestabilan nanopartikel. Variasi konsentrasi glutaraldehida terbukti berpengaruh signifikan terhadap ukuran partikel dan nilai PDI.

Kata Kunci: Desolvasi, Stabilitas, Morfologi, Optimasi, Biodegradable

I. INTRODUCTION

Indonesia is recognized as a country with abundant and diverse natural resources, particularly in its rich plant biodiversity. Numerous plant species have long been used traditionally as herbal medicines and are now increasingly being explored as valuable raw materials for the development of modern phytopharmaceutical-based drugs (Roziaty & Agustina, 2023). Empirical knowledge passed down through generations demonstrates that plants contain bioactive compounds beneficial to health, making them worthy of investigation and development into safe, effective, and economically valuable pharmaceutical preparations (Roziaty, 2022).

One of the plants with great potential is Cantigi, also known as Manis Rejo (*Vaccinium variangiaefolium* (Bl.) Miq), which has traditionally been used by communities in various regions of Indonesia. The young leaves of Manis Rejo, characterized by their reddish color, are known to contain higher levels of

flavonoids compared to mature leaves, thereby exhibiting stronger antioxidant activity (Yulyana et al., 2016; Soleha et al., 2020). Flavonoids are polyphenolic compounds that exhibit various pharmacological activities, including anti-inflammatory, anticancer, antimicrobial, and cardioprotective effects (Rahmawati et al., 2024). Previous studies have also demonstrated that the ethyl acetate extract of Manis Rejo leaves possesses potential cytotoxic activity against several cancer cell lines, making it an attractive candidate for the development of natural product–based therapeutic agents (Yulyana et al, 2016).

However, the direct use of plant extracts often encounters several limitations, such as poor aqueous solubility, low stability during storage, and restricted bioavailability. To overcome these challenges, nanoparticle technology has emerged as a significant innovation in drug delivery systems. Nanoparticles can enhance the solubility of poorly water-soluble active compounds, improve stability, increase absorption in the body, modify the release profile of active agents, and enable lower yet effective dosing (Dipahayu & Kusumo, 2021; Kosasih et al., 2024).

One of the most widely used techniques for producing nanoparticles is the desolvation method, which enables the formation of nanosized particles with high stability and low tendency to aggregate. In this study, gelatin nanoparticles were employed because gelatin is a natural polymer that is biodegradable, biocompatible, non-toxic, and readily available. Type-B gelatin was selected because of its superior stability under biological conditions, allowing for the sustained and controlled release of active compounds. Type-B gelatin, which is derived from alkaline hydrolysis of collagen and contains a higher proportion of anionic groups, was selected because of its superior stability under biological conditions, allowing for sustained and controlled drug release. (Indriana & Fitriana, 2021).

To further enhance the stability of the gelatin nanoparticles, glutaraldehyde was used as a crosslinker, forming covalent bonds with the polymerizer chains, thereby strengthening the particle structure and increasing its resistance to degradation. In addition, Pluronic F-68 was incorporated as a stabilizer to prevent particle aggregation during preparation and storage (Kosasih et al., 2022b).

The novelty of this study lies in the development of gelatin nanoparticle formulations encapsulating the ethyl acetate extract of *V. variangiaefolium* leaves, using various concentrations of glutaraldehyde as a crosslinker. In contrast

to the study by Kosasih et al. (2021), which employed ethanol extracts and a single crosslinker concentration, this research systematically investigates how different glutaraldehyde concentrations influence key nanoparticle characteristics. Furthermore, previous studies primarily focused on basic parameters such as particle size and antioxidant activity, whereas this study provides a more comprehensive physicochemical evaluation—including particle size, polydispersity index, zeta potential, morphology, solubility, and 20-day storage stability. Additionally, the use of *Manis Rejo* leaf extract in nanoparticle-based delivery systems, particularly using ethyl acetate as the extraction solvent, has been rarely reported. Therefore, this research offers a deeper understanding of formulation behavior while promoting the potential of local Indonesian plants for advanced pharmaceutical applications.

II.METHOD

A. Tools

This study employed several laboratory instruments, including a drying oven (Memmert, Germany), Whatman No. 1 filter paper (Cytiva, UK), a rotary evaporator (Buchi Rotavapor R-210, Switzerland), a magnetic stirrer (IKA C-MAG HS7, Germany), a refrigerated centrifuge (Eppendorf 5804R, Germany), a moisture analyzer (AND MX-50, Japan), a particle size analyzer (Horiba SZ-100, Japan), a Zetasizer Nano ZS (Malvern Instruments, UK), and a transmission electron microscope (TEM) (JEOL JEM-1400, Japan).

B. Materials

The materials used in this study included young leaves of Vaccinium variangiaefolium (Bl.) Miq. (Manis Rejo), technical n-hexane (Merck, Germany), analytical grade ethyl acetate (Merck, Germany), dimethyl sulfoxide/DMSO (Merck, Germany), analytical grade acetone (Merck, Germany), type-B gelatin (Sigma-Aldrich, USA), glutaraldehyde (Merck, Germany), Pluronic F-68 (Sigma-Aldrich, USA), 0.1 N hydrochloric acid (Merck, Germany), and distilled water.

C. Preparation of Plant Material

Young reddish leaves of *V. variangiaefolium* were collected from the Tangkuban Perahu crater area, North Bandung, West Java, Indonesia. Botanical identification was performed at the Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong. The leaves were washed with running water and dried at 40 °C until the moisture content was less than 10%.

D. Preparation of Simplicia

A total of 5 kg of fresh leaves were thoroughly washed, dried at 40 °C using a drying oven, and sorted to remove impurities. The dried leaves were grounded and sieved using a No. 4/18 mesh sieve. The resulting simplicia powder was stored in a clean, dry container protected from light (Kosasih & Alifiya, 2024).

E. Extraction

A total of 374 g of simplicia powder was subjected to maceration using n-hexane at room temperature (25–27 °C) for 24 hours to remove non-polar compounds such as chlorophyll and waxes. The mixture was stirred manually every 6 hours. After filtration, the plant residue was air-dried and further extracted with ethyl acetate for 3 consecutive days (3 × 24 hours) with intermittent stirring (approximately every 8 hours) to enhance solvent penetration and metabolite diffusion (Yulyana et al., 2016; Kosasih & Alifiya, 2024).

F. Preparation of gelatin nanoparticles

The formulation of gelatin nanoparticles containing *V. variangiaefolium* leaf extract was prepared with the following composition: 100 mg of gelatin, 40 mg of leaf extract, 15 ml of ethyl acetate, 10 ml of dimethyl sulfoxide (DMSO), 300 mg of Pluronic F-68, 30 ml of acetone, and 25 ml of distilled water. The variations were applied to the concentration of glutaraldehyde used as the crosslinker, namely 100 µl for Formula I, 200 µl for Formula II, and 300 µl for Formula III.

The nanoparticles were prepared using the desolvation method. Type-B gelatin was dissolved in distilled water (adjusted to pH 3 using 0.1 N hydrochloric acid) to a concentration of 1% w/v, then heated at 40 °C and stirred with a magnetic stirrer at 250 rpm until completely dissolved. The ethyl acetate extract of Manis Rejo leaves was dissolved in a mixture of DMSO and ethyl acetate until homogeneous and subsequently added to the gelatin solution under constant stirring. Pluronic F-68 was added as a stabilizer, followed by the dropwise addition of glutaraldehyde according to the respective formula while stirring at 1000 rpm until a nanoparticle suspension was formed (Kosasih et al., 2021). The resulting suspension was centrifuged at 12,000 rpm for 30 min at 4 °C to separate the nanoparticles from the supernatant. The nanoparticle pellets were washed twice with distilled water to remove the residual solvents and unbound compounds.

G. Characterization of Extract and Nanoparticles

1. Organoleptic test

A visual observation was carried out to assess the color, odor, and physical form of the obtained ethyl acetate extract.

2. Moisture content

The moisture content was determined using the Karl Fischer titration method with a standardized Karl Fischer reagent (Christian et al., 2022).

3. Solubility Test

The solubility of the extract was tested in distilled water, dimethyl sulfoxide (DMSO), ethyl acetate, and acetone at ratios ranging from 1:10 to 1:100. The mixtures were stirred, and the solubility was visually observed (Christian et al., 2022).

4. Stability Test

Stability testing was conducted by storing the nanoparticle formulations in sealed glass vials within a stability chamber maintained at 25 ± 2 °C for 20 days. Observations included changes in color and precipitation formation.

5. Particle size and polydispersity index (PDI)

The particle size and polydispersity index (PDI) of the nanoparticles were measured using a particle size analyzer at 25 °C. The PDI value was used to evaluate the uniformity of the particle size distribution (Christian & Rahmat, 2022).

6. Zeta Potential

The zeta potential of the nanoparticles was determined using a Zetasizer to measure the surface charge, which serves as an indicator of the colloidal dispersion stability (Christian & Rahmat, 2022)

7. Transmission Electron Microscopy (TEM)

Samples were prepared by placing a drop of the nanoparticle suspension onto a carbon-coated copper grid. The sample was then air-dried at room temperature and examined using Transmission Electron Microscopy (TEM) at 80 kV to assess the particle shape (spherical) and size distribution. (Christian & Rahmat, 2022).

H. Statistical Analysis

All numerical data were processed using Microsoft Excel. The results are presented as mean ± standard deviation (SD) from three replicates. A one-way ANOVA test was applied to assess the significance of differences between the three nanoparticle formulations. A significance level of $p < 0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

The plant material used in this study was the young, reddish leaves of *V. variangiaefolium* known locally as cantiga or manis rejo. This selection was based on

evidence that red pigmentation in young Ericaceae leaves correlates with elevated anthocyanin and flavonoid levels Figure 1, which are known for their antioxidant properties.

Figure 1. The reddish young leaves, indicated by the upper shoots, are rich in anthocyanins and flavonoids, which contribute to their antioxidant potential

Yulyana et al. (2023) reported that anthocyanin content in young leaves can be two to three times higher than in mature ones, justifying their selection to enhance extract potency.

The leaves were collected from the Tangkuban Perahu area (±1,800 m altitude), where the cool climate and high humidity are known to favor the biosynthesis of secondary metabolites in Ericaceae species. While environmental factors like altitude and temperature can modulate phytochemical levels, this study did not directly compare samples from different altitudes. Drying was performed using a controlled oven at 40 °C, which is considered a safe temperature to retain heat-sensitive active compounds such as flavonoids and anthocyanins. Previous studies have shown that drying temperatures below 50 °C are effective in minimizing the degradation of polyphenolic compounds. However, prior studies support that such ecological conditions promote higher polyphenol accumulation (Yulyana et al., 2023a).

Unlike previous studies that used bulk leaf samples without age selection, this study specifically utilized young, reddish leaves to ensure optimal bioactive compound content for nanoparticle formulation.

A. Plant Determination

The identity of the plant material used in this study was taxonomically confirmed as *Vaccinium variangiaefolium* (Bl.) Miq., which belongs to the Ericaceae family. The identification was based on distinct morphological characteristics, including elliptical leaf shape, entire leaf margins, smooth surface texture, and age-dependent leaf coloration that ranges from green in mature leaves to reddish in younger ones. When available, additional diagnostic features such as stem morphology, floral structure, and fruit characteristics were also examined to ensure accurate classification and avoid

misidentification with other *Vaccinium* species. Accurate species identification was essential to ensure the validity and consistency of the study, as variations in plant species can result in significant differences in phytochemical content and pharmacological activity. (Noviyanti, 2023) (Yulyana et al., 2023a).

B. Preparation of Simplicia

Young reddish leaves of *V. variangiaefolium* were collected based on pre-established selection criteria to ensure high bioactive compound content. Upon harvesting, a foreign organic matter (FOM) assessment was conducted to remove non-target materials such as mature leaves, twigs, debris, and other plant parts. The separation process was performed manually by grouping materials on a white paper divided into quadrants, allowing for detailed inspection of each group to ensure sample purity (Kosasih & Alifiya, 2024).

Two consecutive evaluations revealed FOM contents of 154.8 g and 197.9 g, corresponding to 0.54% of the total raw material weight. These values were well below the maximum acceptable limit of 2% as specified by the Indonesian Herbal Pharmacopoeia, thereby confirming that the raw material met the established quality requirements.

The determination of powder fineness was conducted to evaluate particle size uniformity. Based on the sieving results, all samples passed through mesh No. 4 (4.75 mm), indicating the absence of coarse particles. Meanwhile, 24.36% to 24.48% of the powder passed through mesh No. 18 (1.00 mm), suggesting that a portion of the material had a fine particle size suitable for enhancing surface area and extraction efficiency. These values provide an estimate of the particle size distribution prior to further processing, which is essential for optimizing the subsequent formulation and nano-sizing steps. Particles that are too fine may clog the filter medium and complicate solvent separation, while particles that are too coarse may limit surface area and reduce solvent penetration efficiency (Kosasih & Alifiya, 2024).

Uniform particle size plays a vital role in optimizing solvent diffusion into plant tissues, directly influencing extraction yield and the concentration of extracted bioactive compounds. The simplicia preparation protocol in this study successfully ensured not only the cleanliness and purity of the raw material but also achieved the desired particle size distribution, which supports more efficient and consistent extraction outcomes (Kosasih et al., 2022a).

C. Results of Extract

A two-step maceration process was used to extract secondary metabolites from

Vaccinium variangiaefolium (Manis Rejo) leaves. In the first stage, 374 grams of powdered simplicia were macerated with n-hexane to remove non-polar constituents such as chlorophyll, essential oils, and certain terpenoids (Kosasih et al., 2022a). The filtrate was then concentrated under reduced pressure at 40 °C to preserve heat-sensitive compounds.

The remaining plant residue underwent a second extraction using ethyl acetate, a semi-polar solvent capable of dissolving flavonoids, tannins, and alkaloids not captured by n-hexane. The resulting extract was again evaporated under the same controlled conditions to obtain a thick, concentrated ethyl acetate extract (Yulyana et al., 2023a).

This process yielded 68.33 grams of extract, equivalent to 18.27% of the initial raw material weight (Table I). The calculated Drug Extract Ratio (DER-native) was 5.39, meaning that 5.39 grams of dried leaf powder were required to produce 1 gram of ethyl acetate extract. These results suggest that the optimized extraction method, combining appropriate solvent polarity with proper temperature control, successfully isolated a semi-polar extract rich in bioactive compounds (Christian et al., 2022). The extract obtained through this method is considered suitable for further formulation into gelatin nanoparticles due to its high yield, stability, and targeted chemical profile (Salsabillah et al., 2025).

Table I. Results of extract

Extract Evaluation	Result
Leaf weight (g)	374
Extract yield (g)	68,33
Yield (%)	18,27
DER-Native	5,39

Figure 2. The extract

Based on the extraction process Figure 2, a thick ethyl acetate extract of 68.33 g was obtained. The extract yield was calculated based on the ratio between the obtained extract weight and the initial weight of the simplicia, resulting in a value of 18.27%. This yield indicates a fairly efficient extraction process, considering the content of the secondary metabolites in the Manis Rejo leaves.

In addition, the DER-native was also calculated to determine the amount of simplicia required to obtain 1 g of extract. The DER-native value was 5.39, meaning that approximately 5.39-g Manis Rejo simplicia is required to produce 1 g of ethyl acetate extract. This value provides a

quantitative description of the concentration of the active compounds in the raw material (Christian et al., 2022).

Overall, the extraction process of Manis Rejo leaves in this study successfully isolated a semi-polar fraction rich in bioactive compounds, which can subsequently be formulated into gelatin nanoparticles to enhance stability, bioavailability, and pharmacological effectiveness.

D. Organoleptic Evaluation

The organoleptic assessment of the ethyl acetate extract of *V. variangiaefolium* revealed a dark greenish-black color, a distinctive aromatic profile, and a dry, powder-like consistency. The coloration is likely due to residual chlorophyll, partially removed during the initial n-hexane extraction, and the presence of polyphenolic compounds such as flavonoids and tannins, both of which are known to contribute to dark pigmentation and antioxidant activity (Mokhamad & Shelly , 2022).

The extract exhibited a specific aromatic scent characteristic of Manis Rejo leaves, attributable to naturally occurring volatile compounds including essential oils and aromatic metabolites. This sensory profile may serve as a qualitative identity marker to distinguish Manis Rejo from other herbal extracts (Mahroji & Kurnia, 2024).

The dry form of the extract indicates an effective solvent removal process, with minimal residual moisture. This physical state is favorable for downstream formulation into nanoparticles, as it reduces the risk of degradation or clumping due to excess water.

These organoleptic properties, color, aroma, and texture, are consistent with the chemical characteristics of the extract and the method of preparation. Together, they serve as preliminary quality indicators prior to further physicochemical analyses such as moisture content and solubility testing.

In summary, the organoleptic evaluation confirmed that the ethyl acetate extract of *V. variangiaefolium* possesses physical characteristics consistent with its chemical properties and extraction process. The dark greenish-black color, distinctive odor, and dry form represent quality markers that can serve as preliminary standards before further physicochemical testing, such as moisture content determination and solubility.

E. Moisture Content of Extract

Moisture content is a critical indicator of extract stability, as excess water can lead to microbial contamination and degradation of active compounds

through hydrolytic reactions. In this study, the ethyl acetate extract of *Vaccinium variangiaefolium* leaves exhibited a moisture content of 4.31%, which is below the maximum acceptable limit of 5% as defined by the Indonesian Herbal Pharmacopoeia for dried extracts (Christian et al., 2022).

This low moisture level reflects the efficiency of the solvent evaporation process using a rotary evaporator and indicates that the extract is relatively stable during storage. A moisture content below 5% minimizes the risk of microbial growth and chemical instability, while also preserving the integrity of heat- and moisture-sensitive bioactive compounds.

From a formulation perspective, reduced water content supports the physical and chemical stability of nanoparticle systems by preventing agglomeration during nanosizing and enabling better control over dosing. Additionally, dry extracts tend to maintain consistent color, odor, and texture over time, which are important quality attributes in pharmaceutical applications.

These findings confirm that the extract meets standard moisture requirements, making it suitable for further development into gelatin-based nanoparticle formulations with improved shelf life and performance.

Figure 3. Solubility of manis rejo extract in ethyl acetate

F. Solubility of Ethyl Acetate Extract

Solubility plays a key role in determining the appropriate solvent system during formulation, especially for nanoparticle development. In this study (Figure 3), the ethyl acetate extract of *V. variangiaefolium* exhibited its highest solubility in ethyl acetate, with a solubility ratio of 1:10, classified as freely soluble. This is consistent with the extract's phytochemical profile, which is dominated by semi-polar compounds such as flavonoids and tannins that dissolve well in semi-polar solvents (Nasution et al., 2024).

Conversely, the extract showed limited solubility in distilled water, DMSO, and acetone, with solubility ratios of 1:100 or lower, classified as slightly soluble. The poor solubility in water is attributed to the low polarity compatibility between water and the predominantly semi-polar to non-polar constituents of the extract. Similarly, the limited solubility in acetone may be due to its higher non-polarity, which restricts its

ability to dissolve all fractions of the extract effectively (Nasution et al., 2024).

This solubility profile has direct implications for formulation design. A combination of ethyl acetate and DMSO was selected as the solvent system for dissolving the extract prior to nanoparticle formation via the desolvation method. This approach facilitates the homogeneous dispersion of bioactive compounds within the gelatin matrix, supporting the physical stability of the nanoparticles and maximizing the delivery potential of key phytochemicals (Kosasih et al., 2022b; Yulyana et al., 2023b).

Overall, the favorable solubility in ethyl acetate confirms the compatibility of the extract with the selected formulation solvents, reinforcing its suitability for nanoparticle-based delivery systems.

G. Stability Test of Gelatin Nanoparticles Containing Manis Rejo Leaf Extract

The physical stability of gelatin nanoparticles containing *V. variangiaefolium* extract was evaluated by visual observation over a 20-day storage period at room temperature (±25 °C). Three formulations were tested, each incorporating different glutaraldehyde concentrations (100 µL, 200 µL, and 300 µL) and stabilized with Pluronic F-68.

Throughout the observation period, no visible changes in color or clarity were detected. All formulations consistently exhibited a light green dispersion without sedimentation, indicating that the nanoparticles remained stable in suspension. These results suggest that the formulations maintained their thermodynamic and kinetic stability during storage (Iskandar, 2025).

The presence of Pluronic F-68, a non-ionic surfactant, contributed significantly to stability through steric stabilization mechanisms. This polymer forms a protective layer around each particle, preventing aggregation and preserving particle size distribution. Additionally, the desolvation technique used in nanoparticle preparation promoted the formation of uniformly sized and smooth-surfaced particles, reducing the likelihood of coalescence. The increasing glutaraldehyde concentration across the formulations also enhanced the structural integrity of the gelatin matrix by forming stable crosslinks. This crosslinking further minimized degradation and deformation during storage, especially when combined with steric stabilization from Pluronic F-68.

In conclusion, the combination of controlled crosslinking and steric stabilization successfully maintained the physical stability of the nanoparticles for at least 20 days under ambient conditions.

These findings demonstrate the effectiveness of the formulation strategy in preserving the visual and colloidal stability of protein-based nanoparticles during short-term storage.

Table II. Particle size and polydispersity index (pdi) of gelatin nanoparticles in three formulations

Formulation	Particle Size (nm)	Polydispersity Index (PDI)
1	343,4 ± 4,4	0,381±0,023
2	226,3±23,1	0,446±0,090
3	199,7±1,6	0,177±0,020

The measurement results of the particle size and distribution of the gelatination nanoparticles loaded with the ethyl acetate extract of Manis Rejo leaves revealed that formulation 1 had a particle size of 343.4 ± 4.4 nm, formulation 2 measured 226.3 ± 23.1 nm, and formulation 3 exhibited the smallest size of 199.7 ± 1.6 nm. All three formulations fall within the nanoparticle size range of 1–1000 nm (Table II), confirming their classification as suitable nanoparticles for pharmaceutical applications. A clear trend was observed, where higher concentrations of glutaraldehyde used as the crosslinker produced smaller particle sizes. This finding is consistent with the theory that increasing the crosslinker concentration strengthens the intermolecular bonding gelatination chains, leading to a denser particle matrix and smaller size.

The polydispersity index (PDI) values also reflected trends relevant to the nanoparticle stability. Formulation 1 exhibited a PDI of 0.381 ± 0.023, formulation 2 recorded 0.446 ± 0and while formulation 3 demonstrated the lowest PDI of 0.177 ± 0.020. PDI values closer to zero indicate a narrower size distribution, implying that the dispersion system is more homogeneous and less prone to aggregation during storage. Among the three formulations, formulation 3, prepared with the highest glutaraldehyde concentration, showed the lowest PDI value, suggesting superior long-term physical stability (Saputri et al., 2024; Hidayati & Sari, 2025).

Statistical analysis using one-way ANOVA yielded a p-value of 0.000 (< 0.05), indicating a significant difference between the glutaraldehyde concentrations with respect to the particle size and PDI. These findings confirm that the glutaraldehyde concentration plays a crucial role in modulating the physicochemical characteristics of the gelatination nanoparticles containing the Manis Rejo extract, not only in terms of particle size but also in the stability of the size distribution. Thus, optimizing the crosslinker concentration is essential for enhancing the stability and controlling the particle size in gelatin-based nanoparticle drug delivery systems.

Table III. Zeta potential of the three gelatin nanoparticle formulations containing manis rejo leaf extract

Formulation	Zeta Potential (mV)
1	17,8 ± 1,1
2	16,6±0,2
3	15,8±1,1

The zeta potential is one of the key parameters in nanoparticle characterization, as it reflects the surface charge particle, which directly affects the stability of the dispersion. In general, nanoparticles with high positive or negative zeta potential values tend to repel each other, thereby preventing aggregation and extending the shelf life of the formulation (Christian & Rahmat, 2022; Amalia et al., 2024).

In this study, the zeta potential of three gelatin nanoparticle formulations encapsulating the ethyl acetate extract of Vaccinium varingifolium (Bl.) Miq was evaluated (Table III). The results showed that formulation 1 exhibited a zeta potential of 17.8 ± 1.1 mV, formulation 2 of 16.6 ± 0.2 mV, and formulation 3 of 15.8 ± 1.1 mV. These values fall within the positive range sufficient to provide dispersion stability, although not as high as systems with strong surface charges (≥ ±30 mV) (Christian & Rahmat, 2022). The stability observed in this study was further supported by the use of Pluronic F-68 in all formulations, which acted as a steric stabilizer by forming a protective layer around the particles, reducing interparticle attraction, and preventing sedimentation.

Moreover, the nanoparticle preparation was carried out at an acidic pH (pH 3) to optimize the cationic properties of the gelatination under such conditions. This facilitated effective electrostatic interactions between the gelatin and glutaraldehyde during particle formation. The acidic environment also contributed to the generation of positive zeta potential values, consistent with the results obtained in all three formulations.

Compared to previous studies, the zeta potential values obtained in this work are sufficient to maintain the short- to medium-term physical stability of the formulations. However, to ensure long-term stability, higher zeta potential values may be required, depending on the intended application and storage conditions (Saputra, 2024).

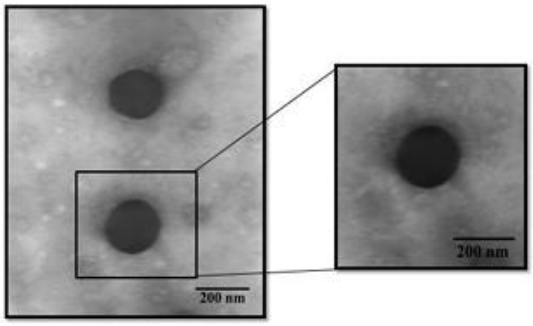

Figure 4. TEM image of formulation 3 of gelatin nanoparticles containing Vaccinium variangiaefolium leaf extract

Particle morphology is one of the essential parameters in nanoparticle characterization, as the shape and surface structure can influence their physical stability, dissolution behavior, and even biological interactions. In this study, morphological observations were carried out using transmission electron microscopy (TEM), which operates on principles similar to an optical microscope but employs electrons as the illumination source. The use of electrons enables imaging at a much higher resolution than visible light, allowing very small particle sizes to be observed in precise detail (Kosasih et al., 2022b).

The TEM results revealed that the gelatin nanoparticles encapsulating the ethyl acetate extract of *V. variangiaefolium* leaves exhibited a spherical morphology (Figure 4). Spherical morphology is highly desirable in nanoparticle formulations because it minimizes direct contact between particles that could otherwise lead to aggregation or clumping. With a symmetrical shape and relatively smooth surface, particle–particle interactions are better controlled, thereby reducing the risk of instability during storage.

The observations were conducted at a magnification of 50,000 with an accelerating voltage of 80 kV, producing clear images of the particle morphology. The consistency in the particle diameter demonstrated the success of the preparation method in the product uniform particle size and shape. Morphological uniformity is not only important for physical stability but also contributes to a more controlled and reproducible release of active compounds upon application (Kosasih et al., 2021).

Moreover, the spherical morphology combined with the small particle size increases the specific surface area, which directly improves the solubility and bioavailability of the active compounds in biological systems. Based on these findings, it can be concluded that the nanoparticle preparation method successfully produced particles with optimal morphology for pharmaceutical and biomedical applications (Hidanah et al., 2022).

IV.CONCLUSION

This study confirms that the ethyl acetate extract of *Vaccinium variangiaefolium* (Bl.) Miq. meets the quality standards of the Indonesian Herbal Pharmacopoeia, with favorable organoleptic properties, low moisture content, and good solubility. Gelatin nanoparticles formulated via the desolvation method exhibited optimal characteristics, including particle sizes of 199.7–343.4 nm, low PDI, spherical morphology, and positive zeta potential. The 20-day stability test supported the

short-term physical stability of the formulation, indicating its potential for further pharmaceutical development.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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