RESEARCH ARTICLE

Characterization and Evaluation of Anti-inflammatory Melinjo Seed Extract Nanoparticles

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ABSTRACT

Melinjo seed extract is rich in various active compounds, including trans-resveratrol, commonly known as "melinjo resveratrol," with antioxidant and antimicrobial pharmacological functions. However, a comprehensive exploration of the anti-inflammatory potential of melinjo seed extract has not been conducted. Converting melinjo seed extract into nanoparticles offers distinct advantages, including the ability to permeate intercellular spaces and higher cell walls through diffusion or opsonification. This flexibility allows for synergies with various technologies, presenting extensive potential for diverse applications and targets. Therefore, this experimental research aimed to obtain an extract from melinjo seed through an extraction process and apply it in nanoparticle dosage forms. Characterization was performed by evaluating the particle size, zeta potential, surface morphology, and anti-inflammatory activity. The results showed that the nanoparticles were successfully prepared using the ionic gelation method, yielding a particle size of 267.5 nm, a stable potential zeta, and well-defined spherical morphology. Additionally, the nanoparticles produced the highest anti-inflammatory activity compared to treatments with conventional melinjo seed extract and sodium diclofenac.

Keywords: Melinjo seed extract, Nanoparticles, Anti-inflammatory.

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INTRODUCTION

Melinjo (*Gnetum gnemon* L.) is a popular plant native to the Indo-Malaya[1] region, with its flowers, fruits, and leaves being used as food, while the stems serve as raw material for tools. In Indonesia, 'emping' melinjo, one of the well-known processed products, is often consumed as a snack or alongside various traditional culinary items. The seed of this plant possesses high purine content, leading to increased uric acid production and potential chronic joint inflammation.[2] Which discourages people with gout from consuming these parts. However, the skin and seed extract contains various health-beneficial active ingredients. Research has shown that melinjo flour comprises nutraceutical (nutritional-pharmaceutical) content with potential application in the development of food products.[3]

Melinjo seed extract contains the active compound trans-resveratrol, commonly referred to as "melinjo resveratrol", which has been previously examined for pharmacological activities including antioxidant and antimicrobial properties.[4] Moreover, some research showed that consuming 50 to 100 mg of melinjo seeds daily[5] generates antitumor activity by triggering apoptosis in breast, colon, prostate, and pancreatic cancer cells.[5] Six stilbenoid compounds present in melinjo seed extract, namely trans-resveratrol (3,5,4'-tryhydroxy-trans-stilbene), gnetin C, gnetin L, gnemonoside A, gnemonoside C, and gnemonoside D.[6] often exert chemopreventive and anticancer activity. Particularly, resveratrol enhances the apoptosis of MCF-7/ASPP1 cells through p53 stimulation.[7]

Another serious health condition is inflammation, characterized by swelling, pain, redness, and heat, which is generally treated using expensive modern drugs commonly associated with side effects (Santoso, 1998). The application of currently available non-steroidal anti-inflammatory drugs (NSAIDs) with analgesic, antipyretic, and anti-inflammatory properties as a remedy for inflammatory diseases may lead to

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peptic ulcers (Robbins *et al*., 2007). Based on these concerns, the exploration of traditional medicines with anti-inflammatory potential becomes crucial.[5]

Various advanced drug delivery systems, including liposomes, microspheres, phytosomes, nanoparticles, etc., have been reported as effective carriers for herbal medicines. The use of nanoparticles as a modern drug delivery system shows great potential for cancer therapy. Furthermore, all these delivery systems enhance solubility, stability, protection against toxicity, pharmacological activity, gradual delivery, as well as physical and chemical stability of active ingredients.[8]

Based on the explained background, this research aimed to provide more information on the anti-inflammatory potential of melinjo seed. Additionally, the experimental process comprises melinjo seed extraction, preparation of extract nanoparticles, characterization of particle size, zeta potential, and surface morphology, as well as evaluation of anti-inflammatory properties of the nanoparticles in Wistar rats.

MATERIAL AND METHOD

The tools used in this research were analytical balance (Sartorius, Germany), magnetic stirrer (IKA C-MAC HS 7), pH meter (Eutech 510, Singapore), Particle Size Analyzer (Malvern), UV-1800 Spectrophotometer (Shimadzu, Japan), freeze dryer (Scanvac), desiccator, glassware, macerator, rotary evaporator, and centrifuge.

Materials for extraction and isolation included 70% ethanol (Merck), ethyl acetate p.a (Merck), chloroform p.a (Merck), and n-hexane p.a (Merck) used as solvents for the maceration extraction stage. Furthermore, melinjo seed was sourced from plantations in Karanganyar district, Central Java, and Wistar rats were obtained from the Pharmacology Laboratory of the National College of Health Sciences with ethical approval from the College Ethics Committee.

In this experimental research, melinjo seed extract obtained through the extraction process was applied in nanoparticle dosage forms. Characterization was conducted by evaluating the size, zeta potential, surface morphology, and anti-inflammatory activity of the nanoparticles in Wistar rats. Additionally, sodium diclofenac and melinjo seed extract were examined in the treatment groups.

Preparation of Melinjo Seed Extract Nanoparticles (*Gnetum Gnemon* L.)

Melinjo seed was dried with sunlight or an oven at 400°C, then ground using a grinder, and the resulting powder was macerated with 70% ethanol solvent for five days, accompanied by stirring. The macerate was filtered and the filtrate collected was concentrated using a rotary evaporator to obtain an extract. Subsequently, nanoparticles were prepared through the modified method of Wu *et al*. by weighing 2 g of thick melinjo seed extract in a 100 mL beaker. The material was dissolved in 50 mL of ethanol: water at a ratio of 70:30, then mixed with 100 mL of 2% chitosan solution and diluted with distilled water to 1000 mL. Approximately 700 mL of 0.1% Na-TPP solution was gradually added to the mixture while stirring at 12,500 rpm. The produced melinjo seed extract nanoparticles were separated by centrifugation, and the powder form was prepared using the freeze-drying method.[9]

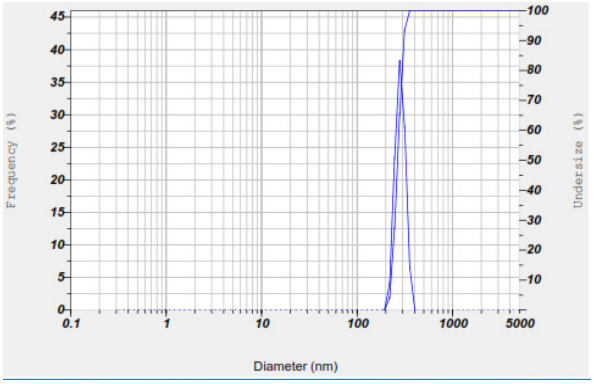

Figure 1: The particle size of 267.5 nm

Characterization of Nanoparticles

Particle diameter size

The particle diameter size was estimated through the light scattering method using a particle size analyzer (PSA) at 25°C. During this process, melinjo seed extract nanoparticle powder was dispersed in phosphate buffer with pH 6.8, then ± 1-mL of the mixture was collected and placed in a cuvette to be measured.

Zeta potential

Zeta potential of nanoparticles formed was determined using PSA Horiba Scientific-100 by filling 1-mL of the sample in a zeta potential cuvette and inserting it into the PSA tool holder to measure the value of this parameter (Zulfa, 2019).

Morphology

Morphological characterization of melinjo seed extract nanoparticles was conducted using scanning electron microscopy (SEM) (Haskell, 2006). Prepared samples were placed on carbon tape attached to an SEM plate, which was subsequently inserted into the microscope and bombarded with electrons for imaging at 1500X magnification (Dian *et al*., 2018).

Anti-inflammatory test

A total of 20 male white Wistar rats were fasted for 18 to 24 hours and divided into four groups, each consisting of 5 randomly selected rats. The left foot of these test animals was labeled at the ankle with a sharp-tipped marker, and the initial foot volume (Vo) was measured with a plethysmometer. The rats were treated with sodium diclofenac, melinjo seed extract, and melinjo seed extract nanoparticles 1-hour before injecting 0.1 mL of 1% carrageenin into the subplantar part of the foot to induce edema. Subsequently, the marked foot volume was measured with a plethysmometer every 0.5 hours until the volume of the foot edema decreased (Vt).

The data obtained from the anti-inflammatory effect test included the foot volume of the treated rats. The edema volume was calculated as the difference between the foot volume

before and after inflammation with 1% carrageenin using the following formula:

$$Vu = Vt - Vo$$

Information:
Vu: Volume of foot edema at time t
Vt: Volume of rat foot after being inflamed with 1% carrageenin at a certain time.
Vo: Initial volume of rat foot before inflammation

The quantitative data of this research were in the form of area under the curve (AUC) and percentage anti-inflammatory effect. The AUC value is the average area under the curve, depicting the relationship between the average edema volume per unit of time with the following formula:

$$AUC^{t_n}_{t_{n-1}} = \frac{Vt_{n-1}+Vt_n}{2}(t_n - t_{n-1})$$

Information:

Vt_{n-1} : Average edema volume at t_{n-1}

Vt_n : Average edema volume at t_n

The percentage anti-inflammatory power (inhibition of edema volume) was calculated based on the percentage reduction in edema using the following formula:

%anti-inflammatory power =

$$\frac{AUC_k - AUC_p}{AUC_k} \times 100\%$$

Data analysis

The characterization results of ethanol extract nanoparticles of melinjo seed (*G. gnemon* L.) manufactured through the ionic gelation method were analyzed descriptively. Furthermore, the data obtained from the characterization of particle size, zeta potential value, morphology, and anti-inflammatory power were analyzed using SPSS software version 26.0 with the one way ANOVA test. This was followed by the Post Hoc Duncan test to identify meaningful differences in each treatment group.

RESULT AND DISCUSSION

Particle Size

The characterization results of melinjo seed extract nanoparticle preparations showed that the size ranged from 20 to 400 nm as shown in Figure 1.

Through the achievement of the nanoparticle size, the melinjo seed extract has the potential to be developed into pharmaceutical preparations with better drug content percentage and loading capacity.[10]

Zeta Potential

Zeta potential is the charge of particles in a dispersing medium, which shows the magnitude of repulsive force between the same and adjacent charges. A high positive or negative zeta potential value (minimum of ± 30 mV or close to 30 mV) provides good stability because it can prevent the aggregation of particles. The presence of a high zeta potential creates a repulsive force and electrical stabilization of the nanoparticle dispersion. When the value is small, an attractive force greater than the repulsive force is generated, thereby triggering coagulation and flocculation.[9]

The surface charge properties of nanoparticles are characterized by zeta potential in relation to the electrostatic interactions of the material component. Electrostatic interactions determine the tendencies of aggregation and repulsion which have an impact on particle size stability of the medicinal preparations. Increased electrostatic charges between the agglomerates prevent the particles from coalescing in the emulsion, but a reduction in the charges can cause phase separation. Therefore, electrostatic charges must be controlled by monitoring the zeta potential value[9] of melinjo seed extract nanoparticles, as shown in Figure 2. The zeta potential of melinjo seed extract nanoparticles showed that this composition was quite stable, characterized by values ranging from -25 to -50 mV, and test samples with values below -25 mV or above +25 mV tended to be stable.[11]

Characterization of the Surface Morphology of the Nanoparticles Using SEM

Figure 3 shows the morphology of melinjo seed extract nanoparticle preparations observed using SEM. The results of SEM photos with 500X, 1000X, 3000X, and 5000X magnifications presented a fairly good morphology in the nanoparticles based on the round or spherical shape with a soft surface.

Anti-Inflammatory Test

This test investigated the presence or absence of anti-inflammatory power in melinjo seed extract and melinjo seed extract nanoparticles, as well as determined the level of power possessed. Furthermore, the time after 1% carrageenin injection and average weight of the edematous foot of rats in the groups treated with melinjo seed extract and melinjo seed extract nanoparticles are shown in Figure 4.

The results showed that the 1% carrageenin control group had the highest average weight of edematous foot compared to the treatment group. Additionally, the group given melinjo seed extract nanoparticles had the smallest edematous foot weight and greater ability to reduce edema compared to those treated using marketed x products and melinjo seed extract. Based on these observations, melinjo seed extract has anti-inflammatory power which is effectively enhanced through conversion into the nanoparticle dosage forms.

According to Figure 5, the melinjo seed extract nanoparticle treatment exerted an anti-inflammatory effect on foot edema in the Wistar rats. The dosage forms of the nanoparticles produced the highest percentage of anti-inflammatory power. Moreover, the analysis of variance (ANOVA) test conducted using SPSS showed a significant result of 0.001, surpassing the predefined significance level ($\alpha = 0.05$). This result presented a substantial anti-inflammatory effect of the nanoparticles on foot edema, which exceeded the impact observed in groups given finished products obtained from the market and conventional melinjo seed extract.

The AUC value is the area under the curve representing the relationship between the average volume of edema *versus*

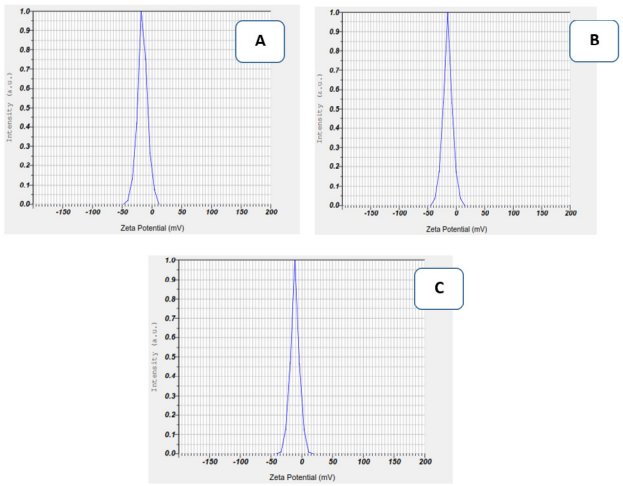

Figure 2: Zeta Potential of melinjo seed extract nanoparticles (A) -16.0 mV, (B) -15.1 mV, (C) -11.3 mV

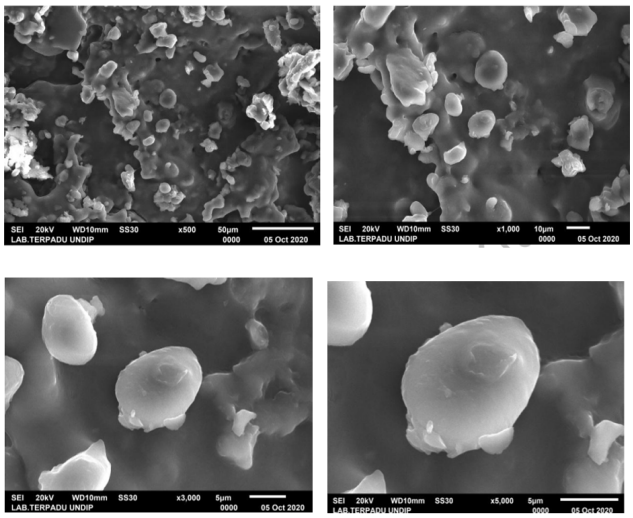

Figure 3: Surface morphology of melinjo seed extract nanoparticles

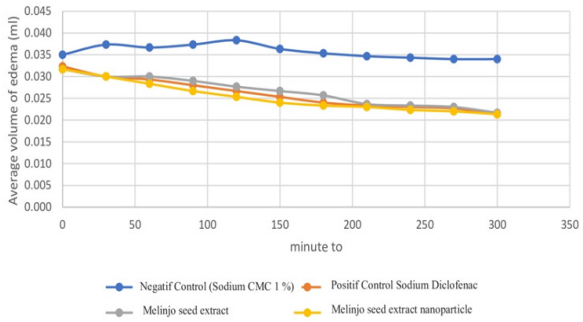

Figure 4: Graphical representation of the foot edema volume after being inflamed by carrageenin

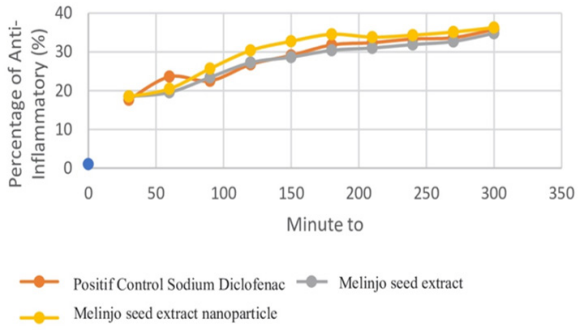

Figure 5: Percentage of anti-inflammatory power

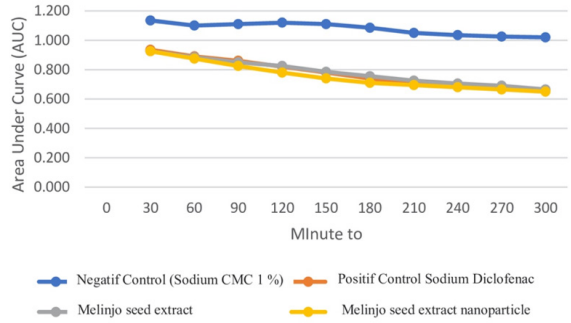

Figure 6: AUC value

time. Based on Figure 6, the AUC value of the nanoparticle treatment group was the lowest compared to the negative and the positive control treatment group given sodium diclofenac 6.75 mg/Kg BW and melinjo seed extract.

The percentage of anti-inflammatory power and the AUC value showed that melinjo seed extract has anti-inflammatory potential. The anti-inflammatory mechanism is attributed to the presence of stilbenoid compounds, which can inhibit prostaglandin production. This is mediated through the inhibition of cyclooxygenase enzyme activity and its ability to bind oxygen free radicals capable of inducing the inflammatory process. Besides, melinjo seed extract contains six types of stilbenoid compounds,[6] including resveratrol, a natural phytoalexin expressed in plants as a defensive response to fungal infections and other environmental stressors. Melinjo seed was found to possess antitumor potential when consumed at a dose of 50 to 100 mg per day,[5] as well as the ability to induce apoptosis in breast, colon, prostate, and pancreatic cancer cells.[12] In this research, the anti-inflammatory activity of melinjo seed extract was strengthened by application in nanoparticle dosage forms, which increased the diffusion of the compound components to produce a greater effect compared to the extract form.

CONCLUSION

In conclusion, this research used the ionic gelation method to successfully prepare melinjo seed extract nanoparticles with a size of 267.5 nm, a stable zeta potential, and good surface morphology, which showed a spherical shape. Furthermore, the nanoparticles produced the highest anti-inflammatory activity compared to both melinjo seed extract and sodium diclofenac.

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