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RESEARCH ARTICLE

Potential of *Pometia pinnata* Leaf Nano Powder in Stunting Prevention

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Abstract Matoa leaf (*Pometia pinnata* J.R. Forst & G. Forst.) is one of Indonesia's endemic plants originating from Papua. Matoa leaves can be utilized as a preventive measure against stunting. Increasing the absorption of active ingredients in a preparation can be achieved by creating nanoparticles. The purpose of this study was to test the potential of matoa leaf nano-powder as an additional ingredient to prevent stunting in children. The stages of this study include the preparation of nanoparticles using the Planetary Ball Mill (PBM) method, capsule preparation formulation, determination of total phenol and flavonoid content, antioxidant activity test, antibacterial test against *Escherichia coli* and *Staphylococcus aureus* bacteria, determination of protein content, determination of carbohydrate content, determination of fat content, and determination of caloric value. Based on the results of this study, the antioxidant activity of matoa leaf nano-powder is very strong, with an IC50 value of 38.2078 ± 0.26 µg/mL, corresponding to total phenol and flavonoid levels of 232.3319 ± 14.66 mg GAE/g sample and 4.5288 ± 0.99 mg QE/g sample, respectively. Matoa leaf nanoparticle capsules exhibit strong antibacterial activity against *Escherichia coli* bacteria at a dose of 150 mg (12.61 ± 2.07 mm), a dose of 200 mg (13.4 ± 2.00 mm), and a dose of 250 mg (13.4 ± 2.00 mm). For *Staphylococcus aureus*, the inhibition diameters obtained with doses of 150 mg, 200 mg, and 250 mg are 13.25 ± 0.78 mm, 13.81 ± 1.10 mm, and 14.13 ± 1.23 mm, respectively. The results of determining the total protein content of matoa leaf nano powder in the spectrophotometric method of 0.8571% and the Kjeldahl method of 11.2%, total carbohydrate content of 0.4617%, and crude fat content of 2.6916%, with a caloric value of 294.96 kcal / kg and 708.71 kcal / kg. It is concluded that matoa leaf nano powder has the potential to prevent stunting.

Keywords: *Pometia pinnata*, nano powder, antioxidant, antibacterial, calorific value.

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Introduction

Indonesia has a very high level of biodiversity. One plant species with potential medicinal properties is the matoa plant. Matoa (*Pometia pinnata* J.R. & G. Forst) is a plant from the Sapindaceae family that is distributed in Southeast Asia. This plant is an Indonesian endemic species native to Papua and has spread to almost all areas of the province, particularly in lowland regions. Matoa plants have also quickly spread across Indonesia, including Sumatra, Java, Sulawesi, Sumbawa Island (NTB), and Maluku [1, 2].

Matoa leaves contain various secondary metabolites that have multiple properties for the body. Based on a phytochemical screening study, secondary metabolites have been identified in the ethanol extract of matoa leaves, including flavonoids, tannins, terpenoids, saponins, and alkaloids. The properties of matoa leaves have been known to be antihyperglycemic, antibacterial, antioxidant, antidiabetic, anticancer, diuretic, antihyperuricemic, and antimicrobial. Matoa leaves also have potential as natural antioxidants and antibacterial agents [3, 4].

Stunting is a multifactorial condition that is not solely caused by inadequate nutrient intake but is also closely associated with recurrent infections, impaired intestinal function, reduced nutrient absorption, and chronic inflammation. In children, poor nutritional status and repeated infections may interact and contribute to growth failure, as infection can decrease appetite, impair nutrient utilisation, and aggravate malabsorption. Therefore, natural materials that provide nutritional value and also exhibit antibacterial and antioxidant activities may be relevant as supportive candidates for addressing biological pathways associated with stunting risk. In this context, the nutritional, antibacterial, and antioxidant properties of matoa leaves may be of interest, though they should not be interpreted as direct clinical evidence for stunting prevention [5, 6].

In addition, matoa leaves contain primary metabolites and have a high caloric value. Based on research, matoa leaf shoots have a calorie content of 4643 kcal/kg, crude protein of 7.33%, crude fat of 7.46%, and crude fiber of 7.46% [6]. To maximize nutrient uptake from matoa leaves, it is necessary to formulate preparations as powders with nano-sized particles. Nanotechnology has been widely applied in the treatment, diagnosis, monitoring, and control of biological systems. The development of nano dosage forms in phytoformulation research offers several advantages, including the potential to enhance activity and overcome issues associated with medicinal plants. Nanoparticles are considered a good drug carrier system because they can manipulate particle size and can modify basic properties such as solubility, diffusivity, and absorption. Particle shape and size are among the factors that influence a drug's effectiveness. Nanoparticles are particles that have a nanometer scale size with a diameter of 1-1000 nm. Nanoparticles have low energy and surface tension, making it easier for particles to enter cell membranes. The development of nano dosage forms in phyto formulation research can be achieved by encapsulating them in capsule dosage forms [7, 8].

Capsules are solid preparations containing drugs wrapped in a soluble shell, typically made of gelatin. Capsule preparations are considered to offer many advantages over other pharmaceutical dosage forms. The benefits of capsule preparations include eliminating contact between the drug and the patient’s mouth, as they can mask unpleasant tastes and odors, thereby increasing patient compliance. For patients who have difficulty swallowing, capsules can be opened and their contents sprinkled on food, which is particularly beneficial for pediatric and geriatric patients [9].

Based on the description above, researchers are interested in conducting research on the manufacture of capsules, testing the antioxidant and antibacterial activities, and analyzing the protein, carbohydrate, fat, and caloric content of matoa leaf nanoparticles. These evaluations were intended to provide preliminary evidence of the physicochemical, biological, and nutritional properties of matoa leaf nano powder that may be relevant to stunting-associated pathways.

Materials and Methods

Materials

The materials used in this research include glassware such as beaker glass, Erlenmeyer flasks, Soxhlet equipment, titration tools, and petri dishes. The instruments used include a particle size analyzer (Malvern), an incubator, an analytical balance, a scanning electron microscope (Fisitech), a UV-Vis spectrophotometer (Shimadzu-1800), an oven (Memmert UN55), and a Fourier Transform Infrared Spectrophotometer (Shimadzu-IRPrestige21).

Sample Preparation

Collected as much as 3 kg of fresh, young matoa leaves, then cut them into small pieces and removed the leaf blade. The leaves were then dried in a drying cabinet at approximately 40 °C until dry (as indicated by breaking), and weighed as the dry weight. Dried matoa leaves were blended into a powder and then sieved through a No. 40 mesh. The matoa leaf simplisia was then pulverized into nanoparticles using a Planetary Ball mill. Furthermore, the characterization of Matoa leaf Simplicia nanoparticles was carried out using a Particle Size Analyzer (PSA) and a Scanning Electron Microscope (SEM). The

procedure was carried out at PT Nanotech Herbal Indonesia (Gd. Nanoplex, Batan Lama, No. A12, Setu, South Tangerang). Characterization was continued using a Fourier-transform infrared (FTIR) spectrometer [10–12].

Analysis of Total Phenol and Flavonoid Content

A total of 25 mg of matoa leaf nano powder was accurately weighed and dissolved in 25 mL of methanol. The solution was sonicated for 60 minutes, then left to stand at room temperature for 24 hours before being filtered to obtain a stock solution at 1000 µg/mL. For the determination of total phenol content, 1 mL of the stock solution was pipetted into a 5 mL volumetric flask and diluted with distilled water to a final concentration of 200 µg/mL. Subsequently, 0.5 mL of this diluted solution was transferred to a test tube, followed by the addition of 0.5 mL of 10% Folin–Ciocalteu reagent. The mixture was vortexed for 1 minute, allowed to stand for 5 minutes, and then 1 mL of 10% Na_2CO_3 was added. The solution was incubated at room temperature (27 °C) for 35 minutes, and its absorbance was measured at 742 nm using a UV-Vis spectrophotometer [13].

The total flavonoid content was determined by pipetting 0.5 mL of the same stock solution, followed by the addition of 0.1 mL of $AlCl_3$, 0.1 mL of CH_3COONa, and 2.8 mL of distilled water. The mixture was incubated at room temperature (27 °C) for 25 minutes. The absorbance was then measured at the maximum wavelength of 431 nm using a UV-Vis spectrophotometer [14].

Antioxidant Testing

Weighed as much as 25 mg of matoa leaf nano powder and dissolved in 25 mL of methanol, sonicated for 60 minutes, allowed to stand for 24 hours, then filtered. obtained a sample standard mother solution with a concentration of 1000 µg/ml. Pipetted sample parent solution as much as 0.05 mL; 0.1 mL; 0.15 mL; 0.2 mL; 0.25 mL to obtain concentrations of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL, then added 0.8 mL of 200 µg/mL DPPH standard mother liquor into a 5 mL volumetric flask and sufficed with methanol until the mark line and homogenized, allowed to stand for 30 minutes and then measured the absorption using a UV- Visible spectrophotometer at the maximum absorption wavelength obtained which is 516 nm [15, 16].

Capsule Preparation

The preparation begins with the preparation of 16-mesh lactose granules. Then the nanoparticle active ingredients and other additives, such as Avicel PH 102, talcum, and magnesium stearate, are weighed to the required amount into the mortar. All ingredients were stirred homogeneously using a stirrer. The granule preformulation test was conducted before the granules were placed into the capsule shell. The capsule formula, used in 3 doses, is adjusted based on the activity test of matoa leaf nanoparticles against Escherichia coli and Staphylococcus aureus, which meet the bacterial inhibition test. The doses of matoa leaf nanoparticles used in this study were 150, 200, and 250 mg (Table 1). Preformulation tests carried out on capsule preparations include tap index, flow time, and angle of repose tests [17].

Table 1. Capsule formula of matoa leaf nanoparticles

Material	Weight (mg)		
	F1	F2	F3
NPDM (active ingredient)	150 mg	200 mg	250 mg
Avicel PH 102 (developer material)	19 mg	17.5 mg	16 mg
Talcum (lubricating agent)	3.8 mg	3.5 mg	3.2 mg
Mg Stearate (lubricating agent)	3.8 mg	3.5 mg	3.2 mg
Lactose (filler)	Ad 530	Ad 550	Ad 570

Capsule evaluation tests included weight uniformity and destruction time tests. The test procedure for weight uniformity was to weigh 20 capsules simultaneously, then weigh each capsule individually, then remove the contents of all capsules, and weigh all empty shells. Then the weight of the capsule contents and the average weight of each capsule content were calculated. The percent difference in the weight of the capsule contents from the average weight of the contents of each capsule should not be more than that specified in column A (7.5%), and 2 capsules should not be more than that specified in column B (15%). The procedure for the disintegration time test was to place six capsules in each basket in the disintegrator device, insert a disk into each basket tube, set the temperature to 36°C-38°C, and turn on the device. Let the basket move until the capsule is destroyed. The capsule was declared destroyed if no part of it remained on the gauze. The test requirement for the destruction time is no more than 15 minutes [17].

Antibacterial Activity Test

The antibacterial activity test was initiated by preparing the test solution from the powder obtained from each capsule dose. Briefly, 1 g of powder was transferred into a centrifuge tube, mixed with 2 mL of distilled water, and centrifuged at 10,000 rpm for 10 min. The supernatant was then collected as the test solution. For the assay, 15 mL of molten Mueller–Hinton Agar (MHA) was poured into a sterile Petri dish, and 0.1 mL of bacterial inoculum suspension of *Escherichia coli* or *Staphylococcus aureus* was added, mixed, and allowed to solidify. Sterile paper discs impregnated with the test solution were then placed on the agar surface. An amoxicillin disc (30 µg) was used as the positive control, and sterile distilled water as the negative control. The plates were left at room temperature for 10–15 min to allow initial diffusion and then incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured in millimetres. All tests were performed in triplicate for each bacterial strain, and the results are presented as mean ± standard deviation [18, 19].

Determination of Total Protein Content

a) UV-Vis Spectrophotometric Method

Concentration solutions of 1000 µg/ml, 2000 µg/ml, 3000 µg/ml, 4000 µg/ml, and 500 µg/ml were prepared. Pipetted 0.6 ml, 1.2 ml, 1.8 ml, 2.4 ml, and 3 ml of each solution. The solution was treated with 2 ml of biuret reagent, and the volume was brought to 6 ml with distilled water. The solution was homogenized and incubated for 28-30 minutes at room temperature. The absorbance of each solution was measured with a UV-Vis spectrophotometer at the maximum wavelength obtained. A calibration curve was created by plotting concentration versus absorbance. The regression equation $y = ax + b$ was then calculated. 5 grams of sample, add 5 ml of 1 M NaOH, and distilled water to 25 ml. Then heated at 90 °C for 10 minutes. After that, the solution was cooled and centrifuged at 2000 rpm for 10 minutes. Then, 5 mL of supernatant was collected, and 5 mL of biuret reagent was added [20, 21].

Furthermore, dilution was performed at a 1:10 ratio, with 0.5 ml of the solution added to 10 ml of biuret reagent. The sample solution was then homogenized and incubated for 28-30 minutes at room temperature. Then the absorbance of the sample was measured with a UV- Vis spectrophotometer at the maximum wavelength obtained [20, 21].

b) Kjeldahl Method

Weigh the sample to 0.5 grams, then place it in a 100 ml Kjeldahl flask. Added 2 grams of selen mixture and 25 ml of H2SO4 (p). Heated on an electric heater or burner until it boils and the solution becomes clear greenish (about 2 hours). Then allowed the solution to cool, diluted it, and transferred it to a 100 ml volumetric flask. Sufficient to the line mark. Piped 5 ml of solution and put into a distiller, then added 5 ml of 30% NaOH and a few drops of PP indicator. Distilled for 10 minutes. As a reservoir, 10 ml of a 2% boric acid solution mixed with the indicator was used. Rinsed the cooling tip with distilled water, and titrated with 0.01N HCl solution. Titrated until a constant pink color is reached. The determination of the blank was also carried out [22].

Determination of Total Carbohydrate Content

5 grams of the sample were placed in a round-bottom flask. Added 200 ml of 3% HCl solution and simmered for 3 hours with upright cooling. Then cool the sample solution and neutralize it with 30% NaOH. The solution was filtered, and the filtrate was collected. Sufficient with distilled water until the total volume becomes 250 ml. Pipetted 25 ml of distilled water solution and 25 ml of Luff Schoorl solution and then put them in an Erlenmeyer. Heated the solution for 10 minutes. Added 15 ml of 20% KI and 25 ml of H_2SO_4 slowly. Titrated with $Na_2S_2O_3$ until the solution is pale yellow. Added 1% starch solution up to 2 ml and titrated again until the exact blue color disappeared. Recorded the volume of $Na_2S_2O_3$ used [23, 24].

Determination of Crude Fat Content

Fat content was determined using the Soxhlet method. First, the sample was weighed up to 5 grams, wrapped in filter paper, tied, and then dried in an oven at 80 °C for 1 hour. Then, prepared a round-bottom flask and dried it in an oven at 105 °C for 30 minutes. Cooled in a desiccator and then weighed the weight of the empty flask. Connect the lower end of the soklet micro tube with the dried soklet flask. Place the sample wrapped in filter paper into the soklet tube, then pour n-hexane solvent up to 250 ml. The condenser is then installed, and the heater is set to boil n-hexane. The extraction process is carried out until the solvent that wets the sample is clear or colorless. After the extraction process is complete, the remaining solvent is evaporated, and the fat is dried in a round-bottom flask in an oven at 105 °C. Cooled the flask in a desiccator and then weighed the flask containing the fat [25, 26].

Determination of Caloric Value

Caloric value is obtained from the levels of protein, total carbohydrates, and fat using the formula: P x 4 Kcal + G x 4 Kcal + L x 9 Kcal = X Kcal/100 g (P = protein content, G = carbohydrate content, L = fat content, X = energy value) [24].

Results and Discussion

Characterization of Matoa Leaf Nanoparticles

Particle size analysis (PSA) showed that the matoa leaf powder had an average particle size of 750.6 nm. Although this value is at the upper end of the nanoscale range, it remains within the 1–1000 nm interval commonly used for nanoparticulate materials. Therefore, the term “nano-powder” is used in this study to describe matoa leaf powder that has been reduced to the submicron/nano-sized range, while acknowledging that the obtained particles are relatively large compared with many engineered nanoparticle systems. SEM analysis was performed to evaluate particle morphology and particle aggregation tendency. As shown in Figure 1, SEM observation at 5000× magnification revealed irregular particles with apparent sizes of approximately 5 µm. This apparent increase in size is likely due to particle aggregation, as cellulose-rich particles tend to form agglomerates, leading to a surface morphology that appears larger than the average size measured by PSA. Overall, the results indicate that the prepared material tended to aggregate and exhibited a large, irregular, and relatively smooth surface morphology. From a formulation perspective, particle-size reduction to the submicron/nanometer range may still increase surface area and improve dispersion of bioactive constituents; however, the present material should be considered a relatively large nano-powder rather than an optimised nanodelivery system [17, 27].

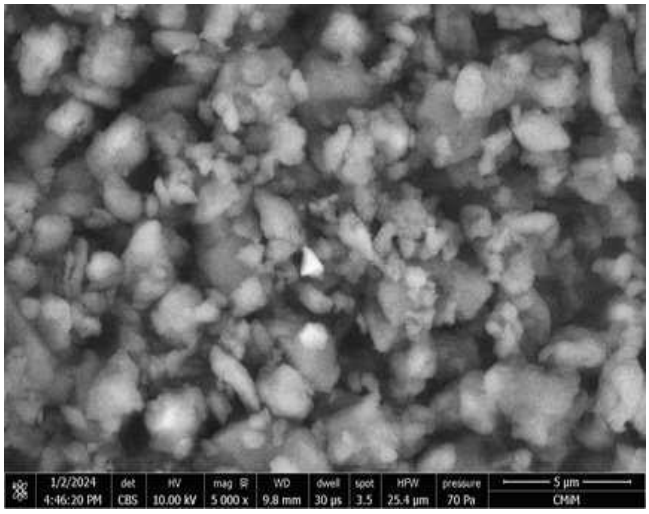

Figure 1. Morphological results of Matoa Leaf Nanoparticles with SEM

Characterization of functional groups was performed using Fourier-transform infrared (FTIR) spectroscopy (Figure 2). The wavelengths observed at 3734, 3400, 2929, 2322, 1631, 1512, 1444, 1072, 881, 823, and 773 are associated with several functional groups. Precisely at 3716 is the stretching frequency of the -OH group, and at 3394 is the stretching frequency of the -NH group. The identification of functional groups indicates the presence of alcohol and amine compounds in nanoparticles on *Pometia pinnata* J.R. Forst & G. Forst [17, 27].

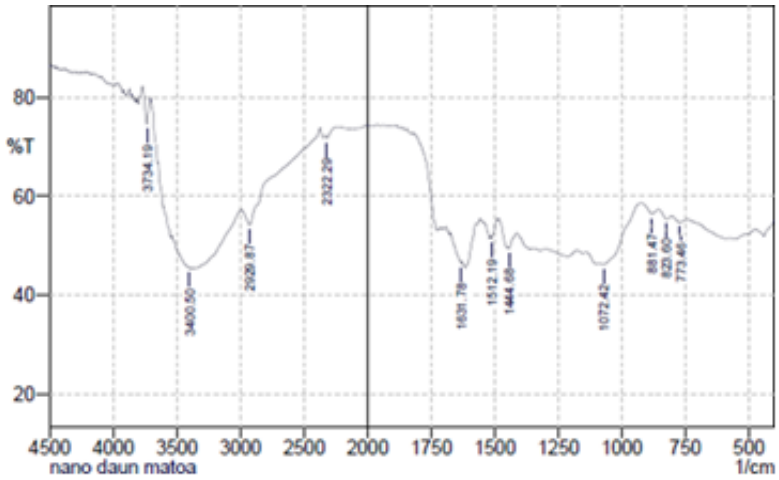

Figure 2. FTIR Spectrum of Matoa Leaf Nanoparticle

Analysis of Total Phenol and Flavonoid Content of Matoa Leaf Nano Powder

The determination of total phenol content was carried out by the colorimetric method using the Folin-Ciocalteu reagent and a gallic acid standard. Phenol compounds form a blackish-green molybdenum complex when reacted with Follin-Ciocalteau. Molybdenum complexes form faster in an alkaline atmosphere.

In the determination of total phenol content, measurements were taken at 738 nm after 54 minutes of incubation. The average total phenol content of matoa leaf nano powder obtained was 232.3319 ± 14.66 mg GAE/g sample. This states that each gram of Matoa Leaf Nano Powder contains phenol compounds equivalent to 232.3319 mg gallic acid. Based on the research, the total phenol content of the ethanol extract from matoa fruit peel was 201.450 ± 0.017 mg GAE/g sample. This is not much different from the results of determining the total phenol content obtained for Matoa Leaf Nano Powder [11].

The total flavonoid content was determined by a colorimetric method using AlCl3 and CH3COONa as reagents, with quercetin as a standard. Quinine can form a colored complex with $AlCl_3$. To determine the total flavonoid content, measurements were taken at 430 nm after 22 minutes of incubation. The results of determining the flavonoid content of matoa leaf nano powder were obtained at 4.5288 ± 0.99 mg QE/g sample. This indicates that each gram of matoa leaf nano powder contains flavonoid compounds equivalent to 4.5288 mg of quinine. In a study, the total flavonoid content of the ethanol extract of matoa fruit peel was determined to be 3.092 ± 0.005 mg QE/g sample. This is not much different from the results of determining the total phenol content obtained for matoa leaf nano powder [13].

Based on the results of this study, it is known that matoa leaf nano-powder samples contain phenolic and flavonoid compounds. The phenol compounds contained in the sample are greater than the flavonoid compounds because not all the significant levels of phenols are flavonoid compounds. Polyphenolic compounds are the largest group of secondary metabolites in plants, comprising phenolic acids, melanin, coumarin, flavonoids, lignin, and tannins [28].

Antioxidant Activity Test of Matoa Leaf Nano Powder

The antioxidant activity of phenol compounds is directly related to their chemical structure, including the degree of glycosylation and the number and positions of hydroxyl groups associated with carboxyl groups. These phenol compounds make a significant contribution to antioxidant activity due to their radical-binding properties. Free radicals have harmful effects on biological systems. Phenolic compounds can donate hydrogen atoms or electrons to free radicals to form stable intermediates. These compounds bind free radicals, decompose oxidation products, and chelate metal ions [15, 16].

The absorbance of DPPH was measured at 516 nm. The average IC50 value of the standard comparator quercetin was 3.2961 ± 0.079 µg/mL, while the average IC50 value of matoa leaf nano powder was 38.2078 ± 0.26 µg/mL. The antioxidant activity of matoa leaf nano powder is weaker than that of the

standard comparator, quercetin. However, matoa leaf nano powder has very strong antioxidant activity, making it a source of natural antioxidants.

DPPH can be reduced, and the violet color changes to yellow when exposed to substances that can provide hydrogen. Antioxidant activity is expressed in Inhibitor Concentration (IC50), which is the concentration needed to produce a 50% decrease in DPPH activity. The smaller the IC50, the stronger the antioxidant activity.

Formulation Test and Capsule Preformulation

The results of the preformulation test of matoa leaf nanoparticle capsule powder (NPDM) can be seen in Table 2.

Table 2. Preformulation test results of NPDM capsule powder

PreformulationTest	Formula			Terms
	F1	F2	F3	
Flow Time	6 g/sec	7.1 g/sec	7.49 g/sec	<10 g/sec
Angle of Repose	22,7º	25,33º	25,07º	20º ≤ x < 40º
Tap Index	15,33%	17,33%	18%	≤ 20%

Based on Table 2 of the flow time test, the results obtained for each formula are 6 g/sec for F1, 7.1 g/sec for F2, and 7.49 g/sec for F3. The average flow time results from the three formulations obtained meet the requirements, with values less than 10 g/second. The binder concentration influences the difference in flow time between granules. In testing the stationary angle, the average results of the stationary angle in each formula were obtained, namely in formula 1 of 22.7º, F2 of 25.33º, and F3 of 25.07º. The Angle of Repose obtained from the three formulas ranged from 20º to 40º, so the three formulations are declared to meet the requirements for Angle of Repose. The resulting angle of repose can be influenced by the number of granules, granule size, humidity, funnel diameter, pouring method, and vibration. It shows that the smaller the Angle of Repose, the better the granule's flow properties. Tap index testing yielded results of 15.33%, 17.33%, and 18% for formulas 1, F2, and F3, respectively. The results obtained have met the requirements where a good compressibility value is less than 20%. The wetting factor influences the tap index in the granule as well as the shape and texture of the particles [17].

Evaluation tests carried out include the weight uniformity test and the disintegration time test. The results of the evaluation test for matoa leaf nanoparticle capsules (NPDM) are presented in Table 3.

Table 3. Evaluation Test of Matoa Leaf Nanoparticle Capsules

Formula	Evaluation test	Parameters	Results
F1	Weight Uniformity	A1 (%)	3,23%
		A2 (%)	1,33%
		B1 (%)	3,23%
	Crush time	Time (minutes)	9.48 minutes
F2	Weight Uniformity	A1 (%)	4,19%
		A2 (%)	2,37%
		B1 (%)	4,19%
	Crush time	Time (minutes)	10.66 minutes
F3	Weight Uniformity	A1 (%)	2,73%
		A2 (%)	0,97%
		B1 (%)	2,73%
	Crush time	Time (minutes)	11.42 minutes

Based on Table 3, the results of the weight uniformity test showed that there were no dose deviations that were too far away where based on Pharmacopoeia Edition III, the requirements for capsule weight uniformity are that there are no 2 capsules that are greater than the price set in column A (7.5%) and there is no single capsule that is greater than the price of column B (15%). From the uniformity test on all formulas, it was found that no capsules deviated from the requirements. Weight uniformity can be influenced by pharmacists' accuracy in formulating drugs and by the duration of mixing or grinding, which can lead to deviations and non-uniform doses [17].

Based on the destruction time test results obtained in Table 3, the times are 9.48 minutes for F1, 10.66 minutes for F2, and 11.42 minutes for F3. The destruction time obtained meets the requirements of the Farmakope Indonesia [29], which stipulate a destruction time of less than 15 minutes. The results show that the greater the dose of capsules, the longer the destruction time obtained. The destruction time test is influenced by the impact force resulting from the contact between the capsule and the ballast.

Activity Test Results of Matoa Leaf Nanoparticles

The antibacterial activity of matoa leaf nanoparticles (NPDM) was determined using the agar diffusion method on paper plates. The results of measuring the average diameter of the growth-inhibition zones of Escherichia coli and Staphylococcus aureus are shown in Table 4.

Table 4. Antibacterial activity of matoa leaf nanoparticles against Escherichia coli and Staphylococcus aureus

Concentration(mg/mL)	Average Diameter of Obstacle Area (mm)*	
	Escherichia coli	***Staphylococcus aureus***
Chloramphenicol	22,46 ± 1,95	25,46 ± 2,96
400	13,3±1,54	13,6±0,91
300	10,65±0,32	11,35±1,03
250	10,52±0,30	11,15±1,65
200	10,21±0,56	10,53±0,44
150	10,0±0,26	10,23±0,07
100	8,76±0,27	9,6±0,39
50	8,93±0,45	9,2±0,70
Negative control	-	-

Description:
(*) The average diameter of the bacterial growth inhibition area is three times the repetition (-). There is no area of bacterial growth inhibition.

Classification of bacterial growth inhibition, there are 4 categories of bacterial inhibition zones, namely the weak inhibition zone category has an inhibition zone diameter of less than 5 mm, a medium inhibition zone with a diameter of 5-10 mm, a strong inhibition zone with a diameter of 10-20 mm, and a very strong inhibition zone with a diameter of more than 20 mm. According to Pakpahan, D., and Sutrianingsih (2020), the effective concentration of an active substance can serve as a benchmark for determining the minimum value in the antibacterial test when the inhibition zone diameter is greater than 10 mm. Based on this, concentrations of 150 mg/mL, 200 mg/mL, 250 mg/mL showed inhibition zones of 10 mm, 10, 21 mm, 10.52 mm and 10.23 mm, 10.53 mm, and 11.15 mm respectively on *Escherichia coli* bacteria so that they are included in the strong category and can be used as active ingredients in making capsule preparations. According to the research by Wulandari, L., and Umam, K. (2023), the higher the concentration, the larger the inhibition zone. This is because the more active compound content will have a greater ability to kill or inhibit bacterial growth[12, 17].

Nanoparticles are known to exhibit favourable chemical properties in antibacterial testing, including a large surface area and high reactivity. This is because the small particle size can increase interactions between bacterial surface charges, causing instability in the cell membrane and leakage of intracellular substances, ultimately leading to cell death [12, 17].

Antibacterial activity test of capsule-filled formulations Matoa Leaf Nanoparticles

The results of measuring the average diameter of the growth-inhibition area for Escherichia coli and Staphylococcus aureus are presented in Table 5 and Figure 3.

Table 5. Measurement of antibacterial activity test of matoa leaf nanoparticle capsule filler against *Escherichia coli* and *Staphylococcus aureus* bacteria

Concentration (mg/mL)	Average Diameter of Obstacle Area (mm)*	
	Escherichia coli	*Staphylococcus aureus*
F1	12,61 ± 2,07	13,25 ± 0,78
F2	13,40 ± 2,00	13,81 ± 1,10
F3	13,91 ± 1,69	14,13 ± 1,23

Table 5 shows that the capsule formulas F1, F2, and F3 yielded inhibition zone diameters against Staphylococcus aureus that were greater than those against Escherichia coli. According to [31], the diameter of the inhibition zone is influenced by the concentration of the test solution, which affects the compound's ability to inhibit bacteria. In addition to concentration, there are differences in the sensitivity of each bacterium to antibacterial substances, which vary in composition and structure. *Staphylococcus aureus* is a gram-positive bacterium with a thick peptidoglycan layer and a simpler cell wall than those of gram-negative bacteria such as Escherichia coli, making itore susceptible to antibacterial compounds [12, 17].

The results of the activity test on Escherichia coli and Staphylococcus aureus show that the larger the volume of bacteria inoculated into the test media, the smaller the inhibition zone. Conversely, the fewer volumes inoculated into the test media, the greater the zone of inhibition. This is because the large number of bacteria used increases their ability to withstand compounds in the test solution [12, 17].

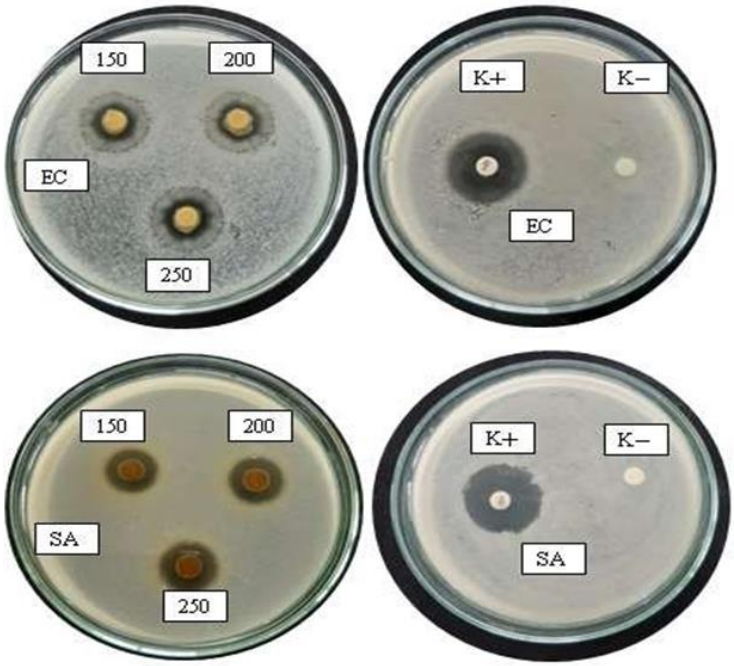

Figure 3. Results of Antibacterial Inhibition Zone Measurement of Matoa Leaf Nanoparticle Capsules

Capsule preparations are formulated with additional ingredients, such as microcrystalline cellulose (Avicel). Based on research, microcrystalline cellulose can exhibit antibacterial activity against Escherichia coli and Staphylococcus aureus. It is known that microcrystalline cellulose can enhance the adsorption properties of cellulose and its antimicrobial activity.

Determination of Total Protein Content

The protein content of matoa leaf nano powder was determined by the spectrophotometric and Kjeldahl methods. The calibration curve for the spectrophotometric method yielded a regression equation, y = 0.0001x + 0.011, with a correlation coefficient (r) = 0.9955. The obtained correlation coefficient meets the acceptable criterion of 0.995. Based on these results, there is a positive correlation between

concentration and absorbance: the greater the BSA solution concentration, the greater the absorbance [30, 31]. The results of the determination of total protein content in matoa leaf nano powder are shown in Table 6.

Table 6. Total Protein Content of Matoa Leaf Nano Powder

Sample	Spectrophotometric Method Protein Content (%)	Protein Content of Kjeldahl Method (%)
Matoa Leaf Nano Powder	0,8571	11,2

Protein content in leaves can be influenced by chlorophyll content, where old leaves contain more chlorophyll than young leaves. Chlorophyll consists of carbon, nitrogen, hydrogen, and oxygen. Nitrogen atoms in the sample will bind to Cu^{2+} ions from biuret, forming a purple complex that indicates the presence of protein.

In addition, protein content can also be affected by sample processing. During nanoparticle manufacturing, a grinding process can generate heat from collisions between the grinding ball and the sample. Heating can denature proteins, reducing protein content in a sample. However, the presence of heat energy only breaks non-covalent bonds (such as hydrogen bonds and hydrophobic interactions) but does not break covalent bonds, namely peptide bonds, in the sample.

Determination of Total Carbohydrate Content

Determination of carbohydrate content in matoa leaf nano powder was carried out by the Luff Schoorl method. This method was chosen because it is the best for measuring carbohydrate content, with an error rate of 10%, and is more practical and low-cost [39]. The principle is based on determining the amount of cuprous oxide (Cu_2O) in the solution before reacting with reducing sugar (blank titration) and after reacting with the reducing sugar sample (sample titration). Determination is done by titration using sodium thiosulfate ($Na_2S_2O_3$). The difference between the blank titration and the sample titration is equivalent to the amount of cuprooxide (Cu2O) formed and also to the amount of reducing sugar in the sample [30, 31].

The result of determining the carbohydrate content of matoa leaf nano powder is 0.4617%. Carbohydrate content is obtained by subtracting the titration volume of the blank from that of the sample, using the available table that describes the relationship between the amount of reducing sugar and multiplying by 0.9, which is the weight of starch in the material. The results of the carbohydrate content determination are shown in Table 7.

Table 7. Carbohydrate Content of Matoa Leaf Nano Powder

Sample	Carbohydrate Content (%)	Average carbohydrate (%)
Matoa Leaf Nano Powder	0,4389	0,4617
	0,4954	
	0,4508	

Determination of Crude Fat Content

The fat content of matoa leaf nano powder was determined to be 2.6916%. The determination of fat content in matoa leaf nano powder was carried out using the Soxhlet method with n-hexane solvent. Soxhlet extraction is a continuous process of extracting samples using a pure solvent. The process is to heat the solvent to its boiling point so it evaporates and falls, wetting the sample. The solvent will soak the sample, and the substances it contains will be extracted. When the solution reaches the height of the siphon pipe, it will flow down the flask, where the extracted substance will accumulate. The collected extract is then heated again to evaporate the solvent, leaving the fat in the flask. The results of the fat content determination are shown in Table 8.

Table 8. Fat Content of Matoa Leaf Nano Powder

Sample	Crude Fat Content (%)	Average Crude Fat Content (%)
Matoa Leaf Nano Powder	2,6334	2,6916
	2,7425	
	2,6991	

Calorific Value Determination

Caloric value is obtained from the levels of protein, total carbohydrates, and fat using the formula: P x 4 Kcal + G x 4 Kcal + L x 9 Kcal = X Kcal/100 g. The results of the caloric value determination are shown in Table 9.

Table 9. Caloric Value of Matoa Leaf Nano Powder

Sample	Total Carbohydrate Content (%)	Crude Fat Content (%)	Total Protein Content (%)	Caloric Value (kcal/kg)
Matoa Leaf Nano Powder	0,4617	2,6916	0,8571	294,96
			11,2	708,71

In the manufacture of nanoparticles, the grinding process involves high-energy milling, which generates heat. The heating process can affect the content of a material, such as minerals, proteins, fats, and carbohydrates, decreasing their levels. Additionally, plant nutrient content can be influenced by environmental factors, particularly rainfall, sunlight, and air temperature.

The caloric value of matoa leaf nano powder was estimated from the measured protein, carbohydrate, and crude fat contents using standard conversion factors. In the present study, two caloric values were obtained: 294.96 kcal/kg and 708.71 kcal/kg, as the calculation used two different protein results obtained by two analytical methods, i.e., the spectrophotometric and Kjeldahl methods. The lower caloric value (294.96 kcal/kg) was calculated using the spectrophotometric protein result (0.8571%), whereas the higher value (708.71 kcal/kg) was calculated using the Kjeldahl protein result (11.2%). Because the Kjeldahl method is more widely used for crude protein determination in proximate nutritional analysis, the caloric value based on the Kjeldahl result is presented as the primary result in this study. In contrast, the spectrophotometric-based value is retained for comparison and methodological transparency. Therefore, the difference between the two caloric values reflects the use of different protein-determination methods rather than inconsistencies in the carbohydrate or fat data [30, 31].

The relevance of the present study to stunting lies in the multifactorial nature of growth failure. Stunting is closely linked to inadequate nutrition, recurrent infection, intestinal dysfunction, and impaired nutrient absorption. Therefore, the nutritional composition of matoa leaf nano powder, together with its antibacterial and antioxidant activities, may support its potential as a supplementary natural product relevant to stunting-related pathways. However, the current study does not provide direct clinical evidence of stunting prevention; rather, it offers preliminary in vitro and compositional data that justify further investigation [30, 31].

Conclusions

The determination of total phenol content of matoa leaf nano powder obtained was 232.3319 ± 14.66 mg GAE/g sample, and the flavonoid content of matoa leaf nano powder obtained was 4.5288 ± 0.99 mg QE/g sample. The antioxidant activity test result of matoa leaf nano powder showed very strong activity, with an IC50 value of 38.2078 ± 0.26 µg/mL. Matoa leaf nanoparticle capsules showed strong antibacterial activity against *Escherichia coli* bacteria at a dose of 150 mg, which was 12.61 ± 2.07 mm, a dose of 200 mg, which was 13.4 ± 2.00 mm, and a dose of 250 mg, which was 13.4 ± 2.00 mm. Against *Staphylococcus aureus*, the inhibition diameters obtained with doses of 150 mg, 200 mg, and 250 mg were 13.25 ± 0.78 mm, 13.81 ± 1.10 mm, and 14.13 ± 1.23 mm, respectively. The results of determining the total protein content of matoa leaf nano powder were 0.8571% by the spectrophotometric method and 11.2% by the Kjeldahl method, with a total carbohydrate content of 0.4617% and crude fat content of 2.6916%, yielding calculated caloric values of 294.96 kcal/kg and 708.71 kcal/kg. Overall, these findings indicate that matoa leaf nano-powder has antioxidant, antibacterial, and nutritional potential and

may serve as a supportive functional ingredient that could contribute to stunting prevention. However, this study provides only preliminary in vitro and compositional evidence; therefore, further in vivo and clinical studies are required to confirm this potential.

Conflicts of Interest

The authors have no conflicts of interest regarding this investigation.

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References

[1] Styani, E., Irawan, C., Putri, I., & Sukiman, M. (2021). Phytochemical and antidiabetic activity studies of n-hexane, ethyl acetate and methanol extracts of matoa (*Pometia pinnata*) fruit peel using alpha-glucosidase enzyme. In *Proceedings of the International Conference on Innovation in Science and Technology (ICIST 2020), 208*.

[2] Sarima, S., Pistanty, M. A., Fathia, M., & Winanda, W. (2025). The potential of various matoa plant (*Pometia pinnata*) extracts as antibacterial agents: Systematic literature review. *Jurnal Pembelajaran dan Biologi Nukleus, 11*.

[3] Utoro, P. A. R., Witoyo, J. E., & Alwi, M. (2022). A short literature review on bioactivity of Indonesian matoa (*Pometia pinnata*) leaves extracts and its application on food products. *Journal of Tropical AgriFood, 4*.

[4] Sulastri, L., Sugihartini, N., & Efiana, N. (2025). *Pometia pinnata* in pharmaceutical research: Bioactivity, mechanisms, and formulation prospects. *Sciences of Pharmacy, 4*. https://doi.org/10.58920/sciphar0403331.

[5] Mulyani, A. T., Khairinisa, M. A., Khatib, A., & Chaerunisaa, A. Y. (2025). Understanding stunting: Impact, causes, and strategy to accelerate stunting reduction—A narrative review. *Nutrients, 17*.

[6] Kayadoe, M., Rochana, A., Tanuwiria, U. H., & Sinaga, S. (2019). Short communication: Effect of different feed combination on the growth development of spotted cuscus (*Spilocuscus maculatus*) in captivity. *Biodiversitas, 20*. https://doi.org/10.13057/biodiv/d200231.

[7] Masfria, M., Sumaiyah, S., Syahputra, H., & Jenifer, J. (2024). Anti-inflammatory and anti-aging activity of hydrogel with active ingredient *Phyllanthus emblica* L. fruit nanosimplicia. *Journal of Medicinal Plants, 22*, 49–64.

[8] Masfria, M., Suci, N., Syahputra, H., & Dalimunthe, A. (2026). Exploring the safety and phytochemical composition of nanoherbal formulations from *Phyllanthus emblica* L. fruit. *International Journal of Medical Toxicology and Forensic Medicine, 16*. https://doi.org/10.22037/ijmtfm.v16.50297.

[9] Naharros-Molinero, A., Caballo-González, M. Á., de la Mata, F. J., & García-Gallego, S. (2024). Shell formulation in soft gelatin capsules: Design and characterization. *Advanced Healthcare Materials, 13*.

[10] Masfria, M., Syahputra, H., Celina, A., *et al*. (2025). Standardisation and toxicity assessment of *Dioscorea hispida* (Dennst.) tubers: Acute and subchronic dosage studies. *Plant Science Today, 12*. https://doi.org/10.14719/pst.4277.

[11] Masfria, M., Dalimunthe, A., Syahputra, H., *et al*. (2023). Standardization study of simplicia nanoparticle from *Phyllanthus emblica* L. fruit. In A. A., B. W., & S. B. S. (Eds.), *AIP Conference Proceedings*. American Institute of Physics.

[12] Masfria, M., Sumaiyah, S., Syahputra, H., & Silvia. (2025). Antibacterial and anti-inflammatory activities of nanosimplicia *Phyllanthus emblica* L. fruit in suspension formulation. *International Journal of Applied Pharmaceutics, 17*, 376–383. https://doi.org/10.22159/ijap.2025v17i2.52608

[13] Masfria, M., Syahputra, H., Haro, G., & Nasution, L. R. (2024). Phenolic and flavonoids contributions to the antioxidant, antidiabetic, and anticholesterol activities of *Eriobotrya japonica* fruit extract: An in vitro analysis. *International Journal of Drug Delivery Technology, 14*, 1962–1970. https://doi.org/10.25258/ijddt.14.4.4.

[14] Masfria, M., Syahputra, H., Khairani, L., *et al*. (2024). Analysis of phytochemical, mineral, total phenol, flavonoids and antimicrobial from ethanol extracts and fractions of *Phyllanthus acidus* (L.) Skeels fruit. *Journal of Medicinal and Pharmaceutical Chemistry Research, 6*, 1368–1382. https://doi.org/10.48309/jmpcr.2024.440641.1100.

[15] Masfria, M., Syahputra, H., Wahyuni, H. S., *et al*. (2024). Analysis of total flavonoid and antioxidant activity of coconut shell liquid smoke (*Cocos nucifera* L.) as an antibacterial. *Pharmacy Education, 24*, 39–45. https://doi.org/10.46542/pe.2024.242.3945.

[16] Syahputra, H., Fadila, N., & Siringoringo, Y. L. (2025). Phytochemical screening, antioxidant, and anti-inflammatory activities of *Dioscorea hispida* (Dennst.) tuber extracts and fractions. *Research Journal of Pharmacy and Technology, 18*, 4689–4694. https://doi.org/10.52711/0974-360X.2025.00674.

[17] Masfria, M., Sumaiyah, S., Syahputra, H., & Witarman, M. (2023). Formulation and evaluation of antibacterial and anti-inflammatory capsules containing *Phyllanthus emblica* L. fruit nanoparticles. *Science and Technology Indonesia, 8*, 607–615. https://doi.org/10.26554/sti.2023.8.4.607-615.

[18] Syahputra, A., Ervina, I., Darwis, A. F., *et al*. (2025). In vitro antimicrobial activity of *Punica granatum* L. extract and anti-inflammatory activity of nano gel in vivo in Wistar rats gingiva. *International Journal of Applied Pharmaceutics, 17*, 189–199. https://doi.org/10.22159/ijap.2025v17i3.53619.
[19] Masfria, M., Syahputra, H., Samosir, V. C., & Manalu, E. (2024). Study of total phenols, flavonoids, antioxidant and antibacterial activities from *Dioscorea hispida* tubers. *International Journal of Drug Delivery Technology, 14*, 754–758. https://doi.org/10.25258/ijddt.14.2.24.
[20] Arunima, & Verulkar, S. (2022). Comparative analysis of different protein estimation methods. *The Pharma Innovation Journal, 11*.
[21] Hadju, Y., Salimi, Y. K., Bialangi, N., & Ischak, N. I. (2022). Analysis of protein and bioactivity of nike fish (*Awaous melanocephalus*) extract as antioxidant. *Jambura Fish Processing Journal, 4*. https://doi.org/10.37905/jfpj.v4i2.15254
[22] Vukmanović, S., Vitas, J., Kravić, S., *et al*. (2024). Influence of main production variables on nutritional characteristics of winery effluent kombucha. *Chemical Industry and Chemical Engineering Quarterly, 30*. https://doi.org/10.2298/CICEQ231002001V.
[23] Syam, A., Zainal, Wahiduddin, *et al*. (2023). Nutrient content and toxicity of pumpkin seed flour. *Food Research, 7*. https://doi.org/10.26656/fr.2017.7(6).069.
[24] Syahrullah, A. U. H., Sukendar, N. K., Djalal, M., & Wangdi, J. T. (2023). Physicochemical properties of fettuccine paste products made from tapioca flour and corn flour based resistant starch type 3. In *AIP Conference Proceedings*.
[25] Khan, A., Talpur, F. N., Bhanger, M. I., *et al*. (2021). Extraction of fat and fatty acid composition from slaughterhouse waste by evaluating conventional analytical methods. *American Journal of Analytical Chemistry, 12*. https://doi.org/10.4236/ajac.2021.125013.
[26] Liu, Z., Liu, Q., Zhang, D., *et al*. (2021). Comparison of the proximate composition and nutritional profile of byproducts and edible parts of five species of shrimp. *Foods, 10*. https://doi.org/10.3390/foods10112603.
[27] Masfria, M., Dalimunthe, A., Suci, N., & Syahputra, H. (2024). Phytochemical constituent analysis of *Phyllanthus emblica* L. fruit nanoherbals by LC-HRMS and their antimutagenic activity and teratogenic effects. *Molecules, 29*. https://doi.org/10.3390/molecules29071642.
[28] Sari, R. P., Rumanti, R. M., & Fathta, D. N. (2025). Identification of flavonoid compounds and antioxidant activity test of ethyl acetate extract of matoa leaves (*Pometia pinnata*) using the DPPH method. *International Journal of Health Engineering and Technology, 4*. https://doi.org/10.55227/ijhet.v4i4.434.
[29] Kementerian Kesehatan Republik Indonesia. (2020). *Farmakope Indonesia* (Edisi VI).
[30] Mandosir, Y. M., Prasetyo, Y., Suhartini, B., & Kristanto, B. (2025). Nutrients in matoa for health and exercise performance: A literature study. *Formosa Journal of Science and Technology, 4*. https://doi.org/10.55927/fjst.v4i1.13648.
[31] Hasan, H., Taupik, M., Andi Suryadi, A. M., & Ihsan, M. (2024). Toxicity effect test of matoa (*Pometia pinnata*) plant fractions as raw materials for anticancer drugs. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy), 10*. https://doi.org/10.22487/j24428744.2024.v10.i2.17016.