



ACTIVITY OF NANOPARTICLE GEL FROM MELINJO BARK EXTRACT (GNETUM GNEMON L.) AGAINST PROPIONIBACTERIUM ACNES

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ABSTRACT

Acne is a common skin problem that often occurs in teenagers and adults, and it can reduce confidence and quality of life. One of the main causes of acne is the bacteria *Propionibacterium acnes*, which lives in hair follicles and triggers inflammation in the skin. Conventional treatment usually involves topical or oral antibiotics, but long-term use may lead to resistance and reduced effectiveness. Therefore, natural alternatives are needed, including plant extracts with antibacterial activity. This study aimed to formulate a nanoparticle gel from melinjo (*Gnetum gnemon* L.) peel extract and evaluate its physical quality and antibacterial activity. The extract was obtained through maceration using 96% ethanol, then prepared into nanoparticles by ionic gelation with chitosan and sodium tripolyphosphate. The nanoparticles had an average size of 44.01 nm, monodisperse distribution, and a zeta potential of +42.81 mV, indicating good stability. The gel formulations were tested for organoleptic properties, viscosity, spreadability, and pH. All formulas met the standard criteria for topical gels. Antibacterial testing showed that the nanoparticle gel (F2) produced a larger inhibition zone against *P. acnes* (6.6 mm) compared to the extract gel (F1) (5.7 mm). These results demonstrate that melinjo peel extract formulated in nanoparticle gel has good potential as a natural topical antibacterial agent for acne treatment.

Keywords: antibacterial; flavonoids; melinjo peel; nanoparticles; *propionibacterium acnes*

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INTRODUCTION

Acne is one of the most common skin disorders, especially among teenagers and adults. This condition not only causes problems for skin health but also affects self-confidence and the overall quality of life. One of the key factors contributing to acne development is the presence of *Propionibacterium acnes*, a bacterium that lives in hair follicles and plays an important role in triggering inflammation that worsens the skin condition (Vilar et al., 2015). Treatment of acne is generally carried out using antibiotics in both topical and oral forms. Although effective, long-term use of antibiotics can lead to resistance, which reduces the effectiveness of therapy. Therefore, it is necessary to develop alternative treatments based on natural ingredients that are safer but still have significant antibacterial potential. One approach that has gained much attention is the use of plant extracts containing bioactive compounds (Leung et al., 2021).

Melinjo (*Gnetum gnemon* L.) peel is one of the natural materials with promising potential as an antibacterial agent. It contains active compounds such as flavonoids, tannins, and stilbenoids, which are known for their antimicrobial and anti-inflammatory properties. However, the application of these compounds is often limited because of their poor solubility and relatively large particle size, which restricts their penetration into the skin (Lokanata et al., 2025). One strategy to overcome these limitations is nanoparticle-based formulation. The application of this technology allows better solubility and penetration of active compounds into the skin layers. A nanoparticle gel preparation is expected to increase the effectiveness of flavonoids and other bioactive compounds in inhibiting

the growth of *Propionibacterium acnes*. With enhanced absorption of active substances, the antibacterial effect can be optimized, making it a potential alternative solution for acne treatment.

METHOD

Tools and Materials

The instruments used in this study included a Particle Size Analyzer (PSA), Field Emission Scanning Electron Microscope (FESEM), analytical balance, pH meter, oven, and porcelain dishes. The materials used in this research were melinjo peel (*Gnetum gnemon L.*), Carbopol ULTREZ 10, triethanolamine, propylene glycol, methylparaben, sodium metabisulfite, distilled water (aquadest), Mueller Hinton Agar (MHA) media, chitosan, sodium tripolyphosphate, *Propionibacterium acnes* bacteria, FeCl 1%, concentrated HCl 2N, Dragendorff reagent, magnesium powder, amyl alcohol, 70% ethanol, clindamycin, and DMSO.

Preparation of Simplicia

The initial process of preparing melinjo peel simplicia began with wet sorting to separate dirt or unwanted parts. The next step was washing to remove dust and impurities, followed by slicing to reduce the size of the material and increase the surface area. The sliced melinjo peel was then dried in an oven at 65 °C for 24 hours or until completely dry. After drying, dry sorting was carried out to ensure no foreign materials remained. The dried material was then ground using a blender to obtain melinjo peel powder simplicia.

Extraction of Melinjo Peel

The extraction process was carried out using the maceration method with 96% ethanol as the solvent. The simplicia powder was soaked for three days (3×24 hours) with occasional stirring to optimize the diffusion of active compounds. After soaking, the mixture was filtered to obtain a liquid extract. The filtrate was then evaporated using a rotary evaporator to produce a concentrated extract. The final step was the calculation of yield percentage to determine the efficiency of the extraction process (Nofita et al., 2022).

Qualitative Phytochemical Screening

Alkaloids

The alkaloid test was carried out by weighing 0.5 grams of extract, followed by the addition of 1 mL of 2 N hydrochloric acid and 9 mL of distilled water. The mixture was heated in a water bath for two minutes, then cooled and filtered. The filtrate was divided into three test tubes. Each tube was filled with 0.5 mL of filtrate and then treated with Mayer, Wagner, and Dragendorff reagents. A positive reaction was indicated by the formation of a white precipitate with Mayer reagent, a brown precipitate with Wagner reagent, and an orange precipitate with Dragendorff reagent, confirming the presence of alkaloids (Zhang et al., 2022).

Flavonoids

A total of 1 mL of the extract solution was transferred into a clean test tube; then 3–5 fragments of metallic magnesium (Mg) and 2 drops of concentrated HCl (1–2 M) were gradually added. The mixture was gently shaken for 1–2 minutes and the color change was observed. The appearance of pink, red, or orange color indicated the presence of flavonoids, as the reduction of flavonoids by magnesium produces colored complexes (Edo et al., 2023).

Tannins

The tannin test was carried out by weighing 5 grams of melinjo peel extract, which was then macerated with 10 mL of distilled water. The mixture was filtered, and the obtained filtrate was diluted with distilled water until it became colorless. A total of 2 mL of the diluted solution was added with 2 drops of ferric chloride (FeCl₃) reagent. A positive result was shown by the appearance of a greenish-black color, indicating the presence of tannin compounds in the extract

(Liman et al., 2021).

Saponins

The saponin test was performed by weighing 0.5 grams of extract, which was then mixed with 10 mL of hot water. After cooling, the solution was shaken vigorously for about 10 seconds until foam appeared. Next, 1 drop of 2 N HCl was added to observe foam stability. The persistence of stable foam after acid addition confirmed the presence of saponins in the extract (Rai et al., 2023).

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a qualitative analytical method widely used to identify groups of secondary metabolites, including flavonoids. The separation principle in TLC is based on differences in polarity that lead to different interactions between the stationary phase (silica gel) and the mobile phase (eluent) (Carolina et al., 2022). In this study, melinjo peel extract was analyzed using TLC to detect the presence of alkaloids, flavonoids, tannins, and saponins.

Quantitative Analysis of Flavonoid Content

Determination of Maximum Wavelength (λ_{max}) of Quercetin

The maximum wavelength of quercetin was determined by scanning the standard solution in the range of 400–450 nm using a UV-Vis spectrophotometer. The scan results showed that quercetin had maximum absorption at 435 nm. This value was then set as the optimum wavelength for measuring the absorbance of the melinjo peel extract samples (Shanko et al., 2024). The selection of the maximum wavelength aimed to achieve better sensitivity and accuracy, because at this point quercetin molecules show the highest light absorption, providing more reliable and representative quantification results.

Preparation of Quercetin Standard Curve

An accurate weight of 25 mg of quercetin standard was dissolved in 25 mL of ethanol to prepare a stock solution. From this solution, 1 mL was pipetted and diluted with ethanol to a final volume of 10 mL, resulting in a concentration of 100 ppm. From the 100 ppm standard solution, a series of concentrations were prepared at 3, 4, 5, 6, 7, 8, and 9 ppm. Each standard solution was pipetted at 1 mL, then mixed with 1 mL of 2% AlCl_3 solution and 1 mL of 120 mM potassium acetate solution. The mixtures were incubated at room temperature for 60 minutes to allow the formation of flavonoid complexes. Absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of 435 nm (Shraim et al., 2021).

Determination of Total Flavonoid Content in Melinjo Peel Extract

A total of 15 mg of extract was carefully weighed and dissolved in 10 mL of ethanol to obtain a solution with a concentration of 1500 ppm. From this stock solution, 1 mL was taken for further analysis. Absorbance was then measured using the UV-Vis spectrophotometer at the maximum wavelength of 435 nm, which had previously been determined as the optimum condition. To ensure data reliability, each sample was analyzed in three replications. The absorbance values obtained were then averaged to produce more accurate results that represent the actual condition (Sim et al., 2024).

Nanosuspension of Melinjo Peel Extract

A total of 1 gram of chitosan was dissolved in 100 mL of 1% glacial acetic acid solution using a magnetic stirrer until a 1% (b/v) chitosan solution was obtained. Then, 500 mg of ethanol extract of melinjo peel was dissolved in a solvent mixture consisting of 20 mL propylene glycol, 20 mL of 70% ethanol, 20 mL of 10% DMSO, and 100 mL of distilled water. After that, 40 mL of 1% chitosan solution was added to achieve a final concentration of 0.2%. The mixture was stirred with a magnetic stirrer for 10 minutes. The next step was the addition of 20 mL of 0.4% sodium tripolyphosphate (TPP) solution, which was introduced slowly using a burette at a rate of one drop

every three seconds, while stirring at 300 rpm with a magnetic stirrer until a nanoparticle suspension was formed, indicated by uniform turbidity. Stirring was continued for 15 minutes to obtain a stable nanosuspension of melinjo peel extract. The stability of the suspension was evaluated for five days by observing turbidity, color, clarity, and the presence of sediment. Afterward, the suspension was dried in an oven at 50 °C for 24 hours to obtain dry nanoparticles of melinjo peel extract (Indriastuti et al., 2024).

Formulation of Nanoparticle Gel of Melinjo Peel Extract

Carbopol Ultrez 10 was dispersed in 60 mL of distilled water and neutralized gradually with triethanolamine while being homogenized until a gel base was formed. Methyl paraben and propyl paraben were dissolved in part of the propylene glycol, while EDTA and sodium metabisulfite were separately dissolved in distilled water. These solutions were then incorporated into the carbopol mixture. Finally, melinjo peel extract or its nanoparticles were added to the mixture, and all ingredients were homogenized to form the gel.

Physical Quality Evaluation

Organoleptic Test

The organoleptic test was carried out to evaluate the odor, color, and texture of the samples. Each parameter was observed systematically to describe the physical properties that represent product quality. The test was conducted with three replications for each formula, ensuring reliable results and allowing comparisons between formulas. Replication aimed to minimize data variation and increase observation validity, so the generated organoleptic information was accurate and consistent (Agustina et al., 2021).

Spreadability Test

An amount of 0.5 grams of gel was weighed and placed in the center of a Petri dish in an inverted position. The sample was left for one minute before being loaded with weights of 25 g, 50 g, and 100 g, each for one minute. This test measured the spreadability of the gel, with an acceptable standard ranging from 5 cm to 7 cm (Agustina et al., 2021).

Viscosity Test

Viscosity was measured using a viscometer equipped with spindle number 4 at a rotation speed of 12 rpm. This condition was applied to obtain consistent rheological data in accordance with gel formulation standards. The required viscosity range for gel preparations is between 2000 and 4000 cps. Thus, the measurement results served as a physical quality parameter and an indicator of formulation suitability for stable and easily applicable gels.

pH Measurement

The pH of the gel preparation was measured using a pH meter stick, which was dipped directly into the sample. The obtained pH value was compared with the normal skin pH range. The acceptable and safe range is between 4.5 and 6.5, as it does not cause skin irritation. This standard serves as a reference for ensuring the safety and quality of the gel preparation.

RESULT

Extraction

Table 1.
Extraction Results

Weight of simple Ingredients	Extract Weight	% Extract Yield
560 g	85 g	15,17%

Phytochemical Screening Results

Phytochemical screening tests are an important step in determining the potential compounds present in plant materials, particularly their secondary metabolites (Astuti et al., 2013).

Table 2.
Phytochemical Screening Results

No	Active Substance	Reagents	Test Results	Conclusion
1	Alkaloid	Dragendroff	Orange Sediment	+
		Mayer	White Sediment	+
		Wagner	Brown Sediment	+
2	Flavonoid	Mg Stearat	Change Color to Brick Red	+
3	Saponin	shake the distilled water vigorously	Formation of foam which is stable and the foam will disappear with the addition of HCL	+
4	Tanin	FeCl ₃	Blackish green	+

Thin Layer Chromatography of Extracts

Tabel 3.
Thin Layer Chromatography of Extracts

Compound	Stain Distance	Eluent Distance	Rf
Ekstrak Kulit Melinjo	5,3	6	0,88
Quersetin	5,0	6	0,83

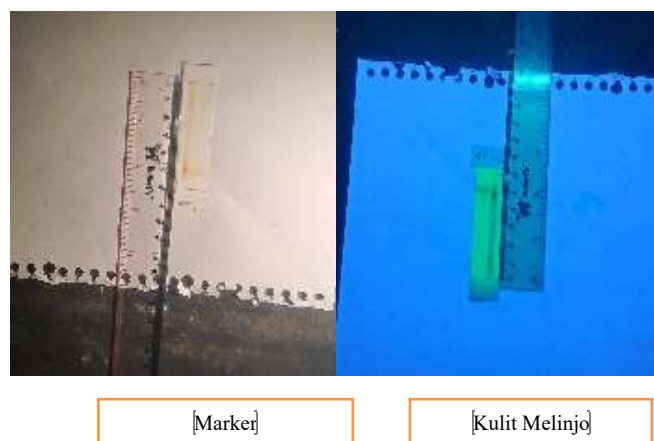


Figure 1. TLC results of *Melinjo* (*Gnetum gnemon*) peel extract under UV light at a wavelength of 254 nm

Calculation of Flavonoid Concentration Levels in Melinjo Skin Extract (*Gnetum gnemon* L.)

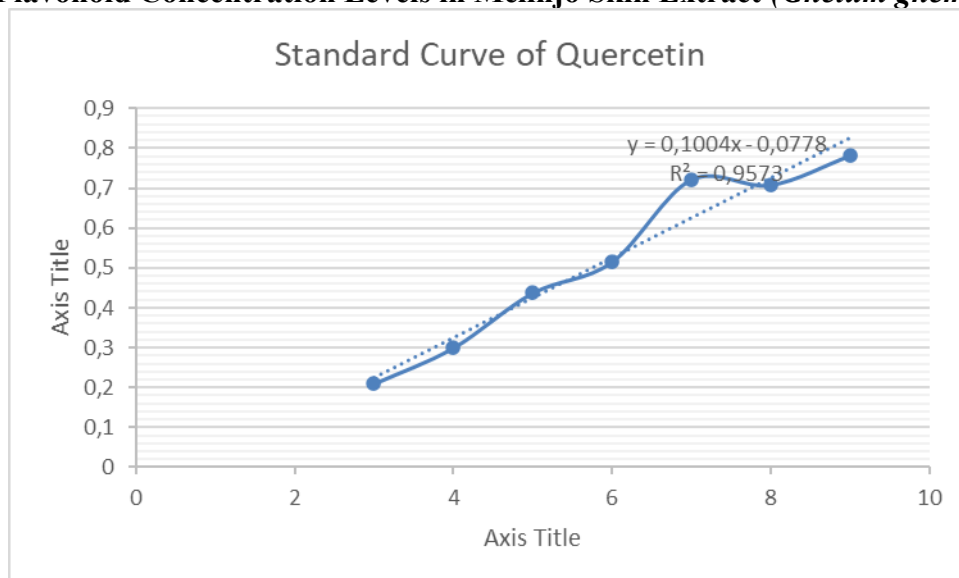


Figure 3. Quercetin Standard Curve

Table 4.
Absorbance Results of Gnetum gnemon peel extract preparations

Replication	Absorbance
1	5,1725
2	5,0558
3	5,1622

$$\text{Average absorbance} = \frac{5,1725 + 5,0558 + 5,1622}{3} = 5,1302$$

$$\begin{aligned} \text{Quercetin equivalent concentration} &= \frac{y+0,778}{0,1004} \\ &= \frac{5,1302+0,778}{0,1004} = \frac{5,208}{0,1004} = 51,86 \mu\text{g/ml} \end{aligned}$$

Analysis of the flavonoid content in melinjo peel extract showed an average absorbance of 5.1302, which is equivalent to a quercetin concentration of 51.86 $\mu\text{g/mL}$. These results indicate that melinjo peel extract is rich in flavonoids, secondary metabolites that play an important role as antioxidants, anti-inflammatory agents, and antibacterial agents. The presence of flavonoids at this concentration supports the extract's pharmacological effectiveness as a topical preparation.

Characterization of Nanosuspension and Nanoparticle Gel Formulations

PSA (Particle Size Analyzer) Characterization

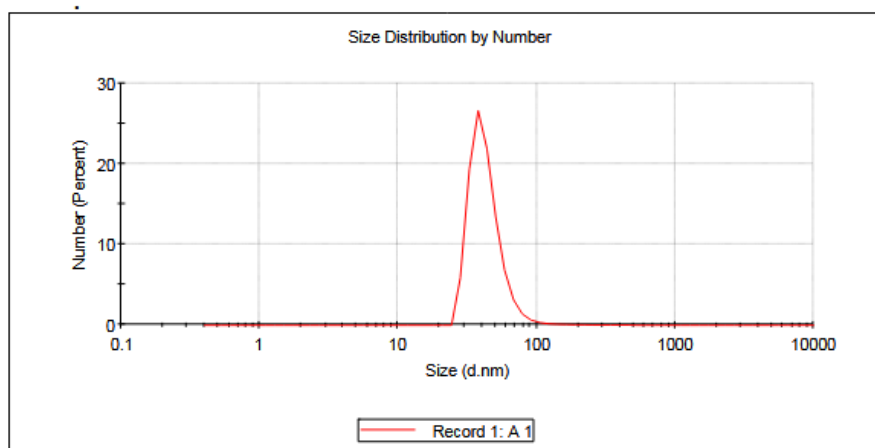
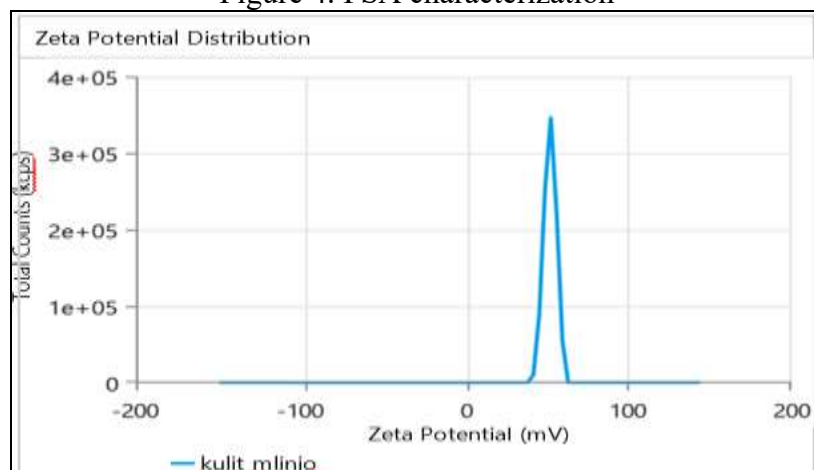


Figure 4. PSA characterization



Statistics Table					
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	42.81	-	-	42.81	42.81
Zeta Peak 1 Mean (mV)	42.81	-	-	42.81	42.81

Figure 5. Characterization ZETA

Table 5.
. Organoleptic Test Results

Formula	Week To	Consistency	smell	Color	Documentation
F0	1	Gel	Odorless	Clear	
	2	Gel	Odorless	Clear	
	3	Gel	Odorless	Clear	
F1	1	Gel	Special extract	Dark brown	
	2	Gel	Special extract	Dark brown	
	3	Gel	Special extract	Dark brown	
F2	1	Gel	Parfum	Orange	
	2	Gel	Parfum	Orange	
	3	Gel	Parfum	Orange	

Table 6.
Results of Spreadability Test

Formula	Week To	Spread Power			Average	Documentation
		20g	50g	100g		
F0 (0%)	Week 1	4,5 cm	4,8 cm	5 cm	4,7	
	Week 2	5,2 cm	5,5 cm	5,8 cm	5,5	
	Week 3	5,4 cm	5,9 cm	6 cm	5,7	
F1 (80% extract gel)	Week 1	4,2 cm	4,5 cm	4,9 cm	4,5	
	Week 2	5,6 cm	6,1 cm	6,6 cm	6,1	
	Week 3	6,5 cm	6,8 cm	7,3 cm	6,8	
F2 (80% nanoparticle gel)	Week 1	4,7 cm	4,8 cm	5,1 cm	4,8	
	Week 2	5,6 cm	6 cm	6,4 cm	6,0	
	Week 3	6,2 cm	6,5 cm	6,8 cm	6,5	

Table 7.
Homogeneity Test Results

Replication	F0	F1	F3	Documentation
Week 1	Homogeneous	Homogeneous	Homogeneous	
Week 2	Homogeneous	Homogeneous	Homogeneous	
Week 3	Homogeneous	Homogeneous	Homogeneous	

Table 8.
Results of pH Measurement Test

Formula	pH results			Average
	Week 1	Week 2	Week 3	
F0 (0%)	5,41	5,55	5,71	5,55
F1 (80% extract gel)	5,29	5,80	5,74	5,61
F2 (80% nanoparticle gel)	5,31	5,47	5,67	5,48

Table 9.
Viscosity Test

Formulasi	Week to	Spindle/Rotor	Speed	Viscosity
F0	Week 1	4	12	2380
	Week 2	4	12	2397
	Week 3	4	12	2421
F1	Week 1	4	12	2413
	Week 2	4	12	2427
	Week 3	4	12	2412
F2	Week 1	4	12	2373
	Week 2	4	12	2381
	Week 3	4	12	2385

Phytochemical Screening of Nanoparticle Gel Preparations

Table 10.

Phytochemical Screening of Nanoparticle Gel Preparations

No	Active Substance	Reagents	Test Results	Conclusion
1	Alkaloid	Dragendroff	Orange Sediment	+
		Mayer	White Sediment	+
		Wagner	Brown Sediment	+
2	Flavonoid	Mg Stearat	Change Color to Brick Red	+
3	Saponin	shake the distilled water vigorously	Formation of foam which is stable and the foam will disappear with the addition of HCL	+
4	Tanin	FeCl ₃	Blackish green	+

Identification of Flavonoid Compounds TLC Gel Nanoparticles

Table 11.

Flavonoid Compounds TLC Nanoparticle Gel

Compound	Stain Distance	Eluent Distance	Rf
Preparation of Melinjo Peel Nanoparticles	4,9	8	0,61
Marker (Quercetin)	5	8	062

Calculation of Flavonoid Concentration Levels in Nanoparticle Formulations

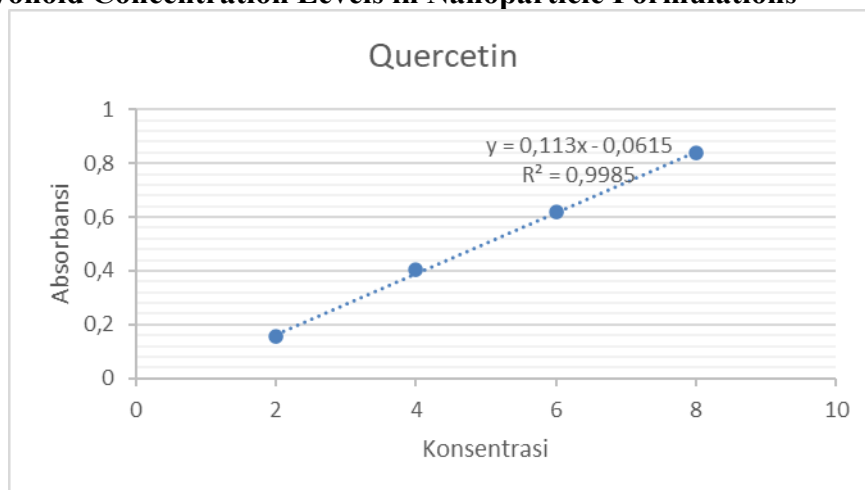


Figure 3. Quercetin Standard Curve

Table 12.

Absorbance Results of Gnetum gnemon peel nanoparticle preparations

Replication	Absorbance
1	2,1641
2	2,1375
3	2,2084

$$\begin{aligned} \text{Average absorbance} &= \frac{2,1641+2,1375+2,2084}{3} = 2,17 \\ \text{Quercetin equivalent concentration} &= \frac{y+0,0615}{0,113} \\ &= \frac{2,17+0,778}{0,113} = \frac{2,948}{0,113} = 26,08 \mu\text{g/ml} \end{aligned}$$

Antibacterial Activity Test

Table 13.

Antibacterial test results

replication to	Inhibition zone diameter			
	Klindamisin	F0 (Base)	F1 (Extract gel)	F2 (Nanoparticle gel)
1	8,5	0	5,7	6,6
2	8,3	0	6	6,5
3	8,7	0	5,4	6,7
St Deviasi	0,2	0	0,3	0,1

DISCUSSION

Extraction

Extraction was carried out using the maceration method by soaking 560 g of melinjo peel simplicia powder in 5.6 L of 95% ethanol. The process produced a thick extract with a total weight of 85 g, resulting in a yield percentage of 15.7%.

Phytochemical Screening Results

Phytochemical screening tests are an important step in determining the potential compounds present in plant materials, particularly their secondary metabolites (Astuti et al., 2013). The results of phytochemical screening of melinjo peel extract showed the presence of secondary metabolites, including alkaloids, flavonoids, saponins, and tannins. This test was carried out to ensure the consistency of active compounds in the extract so that it can maintain the expected pharmacological effects. In the alkaloid test, these compounds are known as organic substances with a heterocyclic ring containing nitrogen atoms as their main component. Testing with Dragendorff, Mayer, and Wagner reagents produced positive results, indicated by the formation of colored precipitates, which confirmed the presence of nitrogen bonds in the complex structure of the extract.

The flavonoid test showed the formation of a brick-red precipitate. Flavonoids are polyphenolic compounds with a basic C6-C3-C6 framework, consisting of two aromatic rings connected by a heterocyclic ring. The hydroxyl groups in flavonoid structures act as electron donors, allowing them to neutralize free radicals, which explains the antioxidant potential of the extract. The results also showed the presence of saponins, which are glycoside compounds with a lipophilic aglycone and a hydrophilic sugar group. This combination makes saponins act as natural surfactants, as demonstrated by the formation of stable foam when the solution was shaken. These findings support the biological role of saponins, including their antimicrobial effects. Furthermore, the tannin test gave positive results with FeCl_3 reagent, producing a dark green color. Tannins are polyphenolic compounds with chemical structures in the form of gallic acid polymers (hydrolyzed tannins) or flavan-3-ol (condensed tannins). The phenolic hydroxyl groups in tannins can form complexes with proteins and metal ions, which supports their potential as bioactive compounds.

Thin Layer Chromatography of Extracts

Based on the observations, the formed spot showed a movement distance of 5.3 cm with an eluent distance of 6 cm. The retention factor (Rf) value obtained was 0.88. This relatively high value indicates that the flavonoid compounds in the melinjo peel extract have lower polarity compared to the stationary phase, allowing them to move further along with the mobile phase. This result is consistent with the properties of some flavonoid aglycones, which are generally more nonpolar than their glycoside forms. As a comparison, the quercetin marker showed a movement distance of 5 cm. The similarity of Rf values between the sample and the marker supports the possibility that the compounds in the melinjo peel extract belong to the flavonoid group. Visual documentation under visible light and UV light further confirmed the presence of characteristic flavonoid spots, which usually fluoresce at certain wavelengths.

Calculation of Flavonoid Concentration Levels in Melinjo Skin Extract (*Gnetum gnemon* L.)

Analysis of the flavonoid content in melinjo peel extract showed an average absorbance of 5.1302, which is equivalent to a quercetin concentration of 51.86 $\mu\text{g/mL}$. These results indicate that melinjo peel extract is rich in flavonoids, secondary metabolites that play an important role as antioxidants, anti-inflammatory agents, and antibacterial agents. The presence of flavonoids at this concentration supports the extract's pharmacological effectiveness as a topical preparation.

Characterization of Nanosuspension and Nanoparticle Gel Formulations

PSA (Particle Size Analyzer) Characterization

Characterization of nanoparticle gel particle size of melinjo peel extract using the Particle Size Analyzer (PSA) is shown in Figure 1. The analysis results indicated that the formed particles had a single size distribution (monodisperse) with an average size of 44.01 nm and a range of less than 100 nm, which meets the nanoparticle criteria defined by the European Commission. The percentage of detected particles reached 100% with a standard deviation of 22.10. This small particle size offers advantages by increasing the surface area, supporting better interaction with the skin, and accelerating the release of active compounds from the preparation. The monodisperse

distribution also reflects the success of the nanoprecipitation method in producing homogeneous particles.

The organoleptic test aimed to evaluate the physical properties of the nanoparticle gel of melinjo peel extract, observed based on consistency, odor, and color during three weeks of storage. Formula F0, as the control, showed the best stability with uniform gel consistency, a perfume-like odor, and a clear color without significant changes until the third week. This indicates that the gel base has good physical stability. Formula F1 remained stable in terms of consistency but had a distinctive extract odor with a dark brownish color. This change was assumed to be influenced by the presence of flavonoids and polyphenols, which tend to undergo oxidation. In contrast, Formula F2 showed stable gel consistency, a perfume-like odor, and a consistent orange color, suggesting better stability compared to F1. Overall, the organoleptic evaluation confirmed that variations in odor and color were influenced by the concentration of extract in the formulation.

The spreadability test was carried out to evaluate the ability of the gel to spread on the surface, which serves as an indicator of comfort during topical use. A total of 0.5 grams of the sample was placed in the center of a Petri dish, then gradually loaded with 25 g, 50 g, and 100 g, each for one minute. The standard criteria for good spreadability of topical preparations are in the range of 5–7 cm. The observations showed that formula F0 only approached the criteria, with an average spread of 4.7–5.7 cm. Formula F1 showed an increase from 4.5 cm in the first week to 6.8 cm in the third week, which meets the standard. Formula F2 demonstrated more consistent values, ranging from 4.8–6.5 cm. Therefore, both formula F1 and F2 fulfilled the requirements for good spreadability.

In this study, a homogeneity test was conducted to ensure that the melinjo peel nanoparticle gel had an even distribution without any coarse particles or phase separation. The results above show that the melinjo peel nanoparticle gel met the criteria as a homogeneous preparation without any coarse particles, phase separation, and homogeneity is very important to ensure that each part of the gel contains active ingredients with equal concentrations so that it produces a consistent effect in the use of the topical preparation (Vera and Silalahi., 2022).

In formula F1 (extract gel), a slight increase in pH was observed from the first to the second week, followed by stabilization in the third week. This change is assumed to be influenced by the interaction of secondary metabolites with the gel base until stability was achieved. Meanwhile, formula F2 (nanoparticle extract gel) showed smaller pH fluctuations, indicating that the nanoparticle system played a role in maintaining the stability of the preparation. Overall, the pH values of all formulas remained within the safe range for topical use and were stable during storage. These findings confirm that the nanoparticle gel of melinjo peel extract is more stable compared to the conventional extract gel.

Viscosity testing of the nanoparticle gel formulation of melinjo peel extract was carried out using a viscometer with spindle number 4 at a speed of 12 rpm to obtain consistent rheological results. The measurement data showed that all formulas, including F0, F1, and F2, had viscosity values within the range of 2000–4000 cps, which meets the quality standards for gel preparations. Formula F0, used as the control, showed a gradual increase in viscosity from 2380 cps in the first week to 2421 cps in the third week, indicating good physical stability. Formula F1 maintained relatively constant viscosity values ranging from 2412 to 2427 cps, suggesting no significant changes during storage. Meanwhile, Formula F2 displayed slightly lower viscosity values of 2373–2385 cps, but still remained within the required range. These results confirm that all formulations possessed appropriate physical consistency, were easy to apply, and remained stable throughout the storage test.

Phytochemical Screening of Nanoparticle Gel Preparations

The results of the phytochemical screening of the nanoparticle gel containing melinjo peel extract showed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and tannins. The positive results for alkaloids with Dragendorff, Mayer, and Wagner reagents were indicated by the formation of orange, white, and brown precipitates. Alkaloids are known to have antibacterial activity by disrupting bacterial cell wall components and inhibiting protein synthesis, thus supporting the potential of the nanoparticle gel as a topical preparation.

In addition, the presence of flavonoids was indicated by a brick-red color change in the magnesium stearate test. Flavonoids act as strong antioxidants that can neutralize free radicals and support anti-inflammatory activity in the skin. The saponin content, which produced stable foam in the shaking test, as well as tannins that showed a greenish-black color with FeCl_3 reagent, further reinforced the antimicrobial and astringent activity of the extract. The combination of these secondary metabolites has the potential to provide synergistic effects in enhancing the effectiveness of the nanoparticle gel as a topical antibacterial and anti-inflammatory agent.

Identification of Flavonoid Compounds TLC Gel Nanoparticles

The identification of flavonoid compounds using the thin layer chromatography (TLC) method showed that the nanoparticle gel of melinjo peel extract had an R_f value of 0.61, while the quercetin standard marker had an R_f value of 0.62. The closeness of the R_f values between the sample and the standard marker indicates that the flavonoid compounds contained in the preparation are consistent with quercetin. This confirms that the nanoparticle formulation process did not damage or reduce the stability of the flavonoid compounds, allowing them to remain detectable within the gel matrix.

Calculation of Flavonoid Concentration Levels in Nanoparticle Formulations

The analysis of flavonoid content in the nanoparticle gel of melinjo peel extract showed an average absorbance of 2.17, which is equivalent to a quercetin concentration of 26.08 $\mu\text{g/mL}$. This result indicates that melinjo peel extract is rich in flavonoids, secondary metabolites that play an important role as antioxidants, anti-inflammatory, and antibacterial agents. The presence of flavonoids at this concentration supports the pharmacological effectiveness of the nanoparticle gel as a topical preparation. A nanoparticle-based formulation offers advantages in maintaining flavonoid stability, while also improving their solubility and bioavailability. Flavonoid compounds generally have limited solubility in water, so incorporating them into a nanoparticle system can help optimize their biological activity. Furthermore, the consistent measurement of flavonoid levels demonstrates that the formulation method used is capable of preserving the integrity of the active compounds within the preparation.

Antibacterial Activity Test

The results of the antibacterial activity test showed that the nanoparticle gel of melinjo peel extract (F2) had an average inhibition zone of 6.6 mm, while the extract gel (F1) exhibited an average inhibition zone of 5.7 mm. The control formula (F0) did not show any antibacterial activity, whereas clindamycin as the positive control produced an average inhibition zone of 8.5 mm. These findings indicate that the nanoparticle formulation (F2) provided higher antibacterial effectiveness compared to the extract gel (F1). This difference may be related to the smaller particle size in the nanoparticle formulation, which increases the surface area of contact with the bacteria and facilitates the penetration of active compounds into bacterial cells. Nanoparticles are also known to enhance the release of bioactive compounds more efficiently, leading to stronger antibacterial activity.

CONCLUSION

The results of this study showed that melinjo peel (*Gnetum gnemon* L.) contains several secondary metabolites, including flavonoids, alkaloids, saponins, and tannins, which contribute to its

biological activity. Extraction using the maceration method produced a yield of 15.17%, and quantitative analysis of flavonoids showed a total content of 51.86 mg QE/g of dried sample. Identification using TLC confirmed the similarity of the extract's flavonoids with the quercetin marker, proving that the bioactive compounds were preserved during the formulation process.

Nanoparticle formulation using the nanoprecipitation method produced particles with an average size of 44.01 nm, monodisperse distribution, and a zeta potential of +42.81 mV, indicating high stability. Physical quality evaluation of the gel showed that all formulas (F0, F1, and F2) had viscosity, pH, spreadability, and organoleptic characteristics within the standard range. F1 (extract gel) showed a typical color change of the extract, while F2 (nanoparticle gel) had a stable orange color and better pH stability.

Antibacterial testing showed that the nanoparticle gel (F2) produced an average inhibition zone of 6.6 mm against *Propionibacterium acnes*, higher than the extract gel (F1) at 5.7 mm. The positive control (clindamycin) produced an inhibition zone of 8.5 mm, while the base control (F0) showed no activity. These results demonstrate that the nanoparticle system can improve the antibacterial effectiveness of melinjo peel extract by increasing the surface area and bioavailability of the active compounds. In conclusion, the nanoparticle gel of melinjo peel extract has great potential as a safe, stable, and effective topical antibacterial preparation, and it can be developed as a natural alternative therapy for acne treatment.

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