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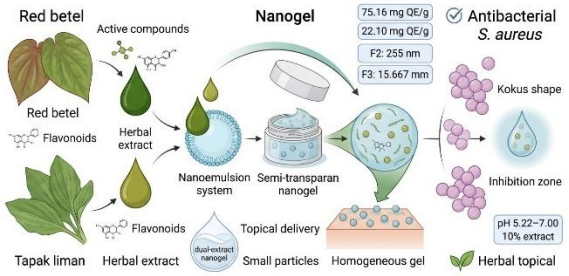
# Determination of Total Flavonoid Content and Antibacterial Activity of Nanogel Combination of Red Betel Leaf and Tapak Liman

**Hanun Aishy Marwa[1], Nadine Ayu Syaima Gustaf Prins[1], Alliva Ro'ain Abbiyu Imanita[2], Fania Putri Wiba Maharani[1], Rahmawati Salsa Dinurrosifa[1*]**

[1] *Pharmacy Study Program, Faculty of Medicine, Universitas Negeri Semarang, Semarang, Indonesia*
[2] *Public Health Study Program, Faculty of Medicine, Universitas Negeri Semarang, Semarang, Indonesia*

## ABSTRACT




Flavonoids are secondary metabolites known for their antioxidant, antibacterial, and anti-inflammatory activities, positioning them as strong candidates for modern topical formulations; however, nanogels incorporating combined extracts of red betel (Piper crocatum) and tapak liman (Elephantopus scaber L.) remain scarcely reported. This study quantified the total flavonoid content of both extracts and developed a combined nanogel formulation using maceration with 70% ethanol followed by UV-Vis spectrophotometric analysis with quercetin as the reference standard. The red betel extract exhibited a flavonoid level of 75.15 ± 0.55 mg QE/g, while tapak liman contained 22.10 ± 0.25 mg QE/g, and the resulting nanogels incorporated 39.66–40.30 mg QE/g while meeting physicochemical standards for topical preparations. Among the formulations, Formula 2 demonstrated the most favorable physical characteristics, including a particle size of approximately 255 nm, physiological pH, and uniform homogeneity, whereas Formula 3 exhibited the strongest antibacterial activity against Staphylococcus aureus. Overall, the findings indicate that the combination of red betel and tapak liman extracts can be effectively formulated into stable antibacterial nanogels, with the innovation of this dual-extract system offering potential as a modern herbal topical preparation.

**Keywords:** *antibacterial activity; flavonoids; nanogel formulation; piper crocatum; elephantopus scaber; topical herbal preparation.*

*Corresponding Author: salsadinurrosifa@mail.unnes.ac.id

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## INTRODUCTION

The use of herbal medicinal plants is widely recognized as an alternative approach to addressing public health problems for both prevention and treatment purposes. This approach also supports the conservation and sustainable use of plant biodiversity [1]. Modern development of herbal medicine has increasingly focused on dosage forms that can improve the effectiveness of natural compounds. Nanotechnology-based systems, such as nanogels, have gained attention because they can enhance the stability, skin penetration, and bioavailability of active phytochemicals, thereby improving their therapeutic performance [2].

Indonesia has a rich diversity of medicinal plants with potential

pharmacological properties, including red betel leaf (Piper crocatum) and tapak liman (Elephantopus scaber L.). Red betel leaf contains alkaloids, flavonoids, tannins, and essential oils that are associated with antibacterial and anti-inflammatory activities [3]. Tapak liman also contains flavonoids, tannins, saponins, and triterpenoids/steroids, which are reported to contribute to similar biological activities [4]. The overlapping phytochemical profiles of these two plants, particularly their flavonoid constituents, indicate their potential to be developed as a combined herbal preparation.

Flavonoids are of particular scientific interest because they represent one of the most abundant groups of secondary metabolites in plants and contribute significantly to antioxidant, antibacterial, and anti-inflammatory activities [5]. These compounds are often used as chemical markers to support the pharmacological standardization of herbal preparations. In topical antibacterial formulations, flavonoid-containing extracts are relevant because they may support bacterial growth inhibition while also providing additional biological activities that are beneficial for skin application.

Previous studies have reported the antibacterial potential of red betel leaf and tapak liman extracts, as well as the usefulness of flavonoids as bioactive markers in herbal preparations [3]–[5]. Nanogel systems have also been increasingly explored to improve the delivery of plant-derived compounds because they can enhance dispersion, skin contact, and penetration of active ingredients [2]. Most available studies, however, still focus on single-extract formulations or conventional gel preparations, while dual-extract nanogel systems combining plants with complementary phytochemical profiles remain limited. This limitation indicates the need to develop a combined herbal nanogel that contains measurable flavonoid content and demonstrates acceptable physicochemical characteristics and antibacterial activity.

The pharmacological benefits of red betel leaf and tapak liman have been reported separately, but studies examining their combined use in a nanogel formulation remain limited. This study therefore aims to determine the total flavonoid content of each extract, formulate a combined red betel–tapak liman nanogel, and evaluate its physicochemical characteristics and antibacterial activity. The novelty of this study lies in the development and evaluation of a dual-extract nanogel from red betel leaf and tapak liman as a potential herbal-based topical antibacterial preparation.

## METHODS

### 1. Types of Research

This research is an experimental laboratory research with a quantitative approach, which includes determining the total flavonoid content of red betel leaf (*Piper crocatum*) and tapak liman leaf (*Elephantopus scaber L.*) extracts, as well as the formulation of nanogel preparations.

### 2. Material

The materials used in this study were powdered red betel leaf (*Piper crocatum*) and tapak liman leaf (*Elephantopus scaber L.*) simplicia, extracted with 70% analytical-

grade ethanol. The flavonoid analysis required quercetin standard (≥98% purity), concentrated hydrochloric acid, magnesium powder, aluminum chloride hexahydrate, acetic acid solution (5%), ethanol (70%), and distilled water. Preparing the nanoemulsion required the ethanol extracts, olive oil for the oil phase, Tween 80 as the surfactant, PEG 400 as the cosurfactant, and distilled water. The nanogel formulation used hydroxypropyl methylcellulose (HPMC, pharmaceutical grade) as the gelling agent; methyl paraben; propylene glycol; 96% ethanol; distilled water; and the combined extracts as the active ingredients. Information on the chemical grade and purity is included to support reproducibility, and instrument calibration follows standard laboratory procedures.

Flavonoid content was calculated from a quercetin calibration curve. The analyzer's software was used to process the particle size data, and the nanogel characteristics (spreadability, adhesion, drying time, and viscosity) were measured in triplicate.

**3. Instruments**

The instruments used in this study included a closed glass jar for maceration, a rotary evaporator for solvent evaporation, and a vortex mixer and magnetic stirrer with a temperature controller for homogenization. Flavonoid analysis was conducted using a UV-Vis spectrophotometer with the aid of a measuring pipette, measuring cylinder, volumetric flask, and analytical balance. The preparation of nanoemulsions and nanogels was assisted by a sonicator. Particle size testing was conducted using a Malvern ZEN169 Particle Size Analyzer (PSA). Evaluation of the physical properties of the gel used various tools, namely glass slides and spherical glass for homogeneity and spreadability tests, an adhesive strength tester with a 250-gram weight, a pH meter for pH measurement, and a viscometer and glass beaker for viscosity testing.

**4. Extraction**

A total of 200 grams of red betel leaf powder and 200 grams of tapak liman leaf powder were each macerated using 2000 mL of 70% ethanol in a closed glass jar at room temperature for 24 hours. The maceration process was carried out for three days (3 × 24 hours) with two remacerations using new solvents to ensure optimal extraction of active compounds. After that, the maceration solution was filtered, then the filtrate obtained was evaporated with a rotary evaporator to obtain a thick ethanol extract

**5. Qualitative Analysis of Flavonoid Content**

Qualitative flavonoid screening was carried out using the magnesium-HCl test. A total of 2 mL of extract solution was heated for 5 minutes, followed by the addition of concentrated HCl and 0.2 g of magnesium powder. The mixture was shaken, and the formation of red, yellow, or orange coloration was interpreted as a positive indication of flavonoid compounds [6].

**6. Thin Layer Chromatography (TLC) Analysis**

Thin Layer Chromatography (TLC) was used to confirm the presence of flavonoid compounds in the extract. The TLC plate was placed in a chamber saturated with the mobile phase consisting of n-hexane and ethyl acetate in a 1:9 ratio. Quercetin was used as the reference standard for comparison [7], [8]. The extract solution and quercetin standard solution were spotted on the TLC plate at an appropriate distance from the lower edge. The plate was then developed in the saturated chamber until the mobile phase reached approximately three-quarters of the plate length. The developed plate was dried and sprayed with 5% $AlCl_3$ reagent. Observations were conducted under UV light at 254 nm and 366 nm. Similarity in spot position and color between the extract and quercetin standard was used as an indication of flavonoid compounds.

**7. Quantitative Analysis of Total Flavonoid Content**

Total flavonoid content was determined using the aluminum chloride colorimetric method with quercetin as the reference standard. A quercetin stock solution was prepared by weighing 10 mg of pure quercetin and dissolving it in 70% ethanol to a final volume of 10 mL to obtain a concentration of 1000 ppm. The stock solution was then diluted to obtain a 100 ppm standard solution for calibration curve preparation.

The maximum wavelength of quercetin was determined by scanning the 100 ppm quercetin standard solution using a UV-Vis spectrophotometer at a wavelength range of 370–450 nm. The selected wavelength was then used for sample measurement. Operating time was determined by mixing 1 mL of 100 ppm quercetin solution with 0.1 mL of 10% $AlCl_3$ reagent and 0.1 mL of 5% acetic acid. Absorbance was measured every two minutes for 30 minutes until a stable absorbance value was obtained.

A series of quercetin standard solutions at concentrations of 5, 10, 15, 20, 25, 30, 40, and 50 ppm was prepared from the 100 ppm standard solution. Each standard solution was reacted with 10% $AlCl_3$ and 5% acetic acid, then incubated according to the determined operating time before absorbance measurement.

Extract solutions were prepared by dissolving 10 mg of thick red betel leaf extract or tapak liman leaf extract in 70% ethanol to a final volume of 10 mL, followed by homogenization using a vortex mixer. Total flavonoid content was determined by taking 1 mL of extract solution, adding 10% $AlCl_3$ and 5% acetic acid, and homogenizing the mixture. The solution was incubated according to the determined operating time, and absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of quercetin. Total flavonoid content was calculated using the linear regression equation obtained from the quercetin calibration curve and expressed as mg quercetin equivalent per gram sample.

**8. Nanoemulsion Preparation**

The composition of the nanoemulsion formulations is presented in Table 1. Three nanoemulsion formulas were prepared with different ratios of red betel leaf extract and tapak liman leaf extract, while the

concentrations of olive oil, Tween 80, PEG 400, and distilled water were kept constant.

**Table 1.** Composition of Nanoemulsion Formulations Containing Red Betel Leaf and Tapak Liman Extracts

| Material | Function | F1 | F2 | F3 |
|---|---|---|---|---|
| Red betel leaf extract | Active ingredient | 15% | 10% | 5% |
| Tapak liman leaf extract | Active ingredient | 5% | 10% | 15% |
| Olive oil | Oil phase | 8% | 8% | 8% |
| Tween 80 | Surfactant | 17% | 17% | 17% |
| PEG 400 | Co-surfactant | 5% | 5% | 5% |
| Distilled water | Solvent | q.s. to 50 mL | q.s. to 50 mL | q.s. to 50 mL |

Nanoemulsion preparation was carried out by mixing Tween 80, PEG 400, and olive oil, followed by the addition of the extract combination according to each formula (F1, F2, and F3). The mixture was homogenized using a magnetic stirrer at 50°C for 15 minutes. Distilled water was then added gradually under constant stirring. The homogeneous mixture was sonicated at 35°C for 60 minutes to reduce droplet size and stabilize the nanoemulsion.

### 9. Particle Size Analysis

Particle size analysis was conducted using a Particle Size Analyzer (Malvern ZEN169) based on the Dynamic Light Scattering principle [9]. Each nanoemulsion sample was analyzed to determine the particle size distribution and to evaluate whether the formulation had reached the nanometer range.

### 10. Nanogel Formulation

The composition of the nanogel formulations is presented in Table 2. Each nanoemulsion formula was incorporated into a gel base containing Carbopol, propylene glycol, triethanolamine, methyl paraben, and distilled water.

**Table 2.** Composition of Nanogel Formulations

| Material | Function | F1 | F2 | F3 |
|---|---|---|---|---|
| Carbopol | Gelling agent | 1% | 1% | 1% |
| Propylene glycol | Humectant | 5% | 5% | 5% |
| Triethanolamine | pH adjuster | 2% | 5% | 5% |
| Methyl paraben | Preservative | 0.1 % | 0.1 % | 0.1 % |
| Distilled water | Solvent | q.s. to 50 mL | q.s. to 50 mL | q.s. to 50 mL |

Nanogel preparation was started by dispersing Carbopol in distilled water at 50°C until a gel base was formed. Propylene glycol and methyl paraben were then added and homogenized using a magnetic stirrer. Triethanolamine was added gradually while stirring to adjust pH and support gel formation. Each nanoemulsion formula was incorporated into the gel base and homogenized until a uniform nanogel preparation was obtained [10].

### 11. Evaluation of Nanogel Characteristics

Nanogel characteristics were evaluated through organoleptic,

homogeneity, spreadability, adhesion, drying time, pH, and viscosity tests. Organoleptic evaluation was conducted by observing the color, odor, and texture of each formula. Homogeneity testing was performed by placing a small amount of nanogel between two glass slides and observing the presence or absence of coarse particles or clumps. A homogeneous preparation was indicated by the absence of visible aggregates and uniform distribution of the active ingredients.

Spreadability testing was conducted by placing 0.5 g of nanogel on a glass plate, followed by the application of a load for one minute. The spread diameter was measured, and the average value was calculated. Topical gel preparations generally require an appropriate spreadability range to support ease of application and contact with the skin surface [26].

Adhesion testing was conducted using an adhesive strength tester. A 0.5 g sample was placed between two glass slides, loaded with a 250 g weight for 5 minutes, and the time required for the two slides to separate was recorded. A longer adhesion time indicates a longer contact duration between the preparation and the skin surface [27].

Drying time was evaluated by applying the nanogel to the skin surface and recording the time required for the preparation to dry. The pH test was conducted using a calibrated pH meter to determine whether the preparation was within the acceptable skin pH range. Viscosity was measured using a viscometer to evaluate the flow properties and consistency of the nanogel preparation [29], [30].

**12. Antibacterial Activity Test**

Antibacterial activity was evaluated against Staphylococcus aureus using the inhibition zone method. Each nanogel formulation, single extract, positive control, and negative control was tested to compare antibacterial effectiveness. The inhibition zone diameter was measured in millimeters. A larger inhibition zone indicated stronger antibacterial activity against the tested bacteria.

## RESULTS AND DISCUSSION

This study used plant materials that had passed the determination test at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. Plant determination was conducted to confirm the authenticity of the samples and to avoid errors in plant collection or possible mixing with other plant species [12].

The confirmed plant materials were then processed into simplicia. Simplicia refers to natural materials used as herbal or traditional medicine that have not undergone advanced processing [13]. The dried and powdered simplicia were extracted to separate soluble active compounds from the plant matrix using a suitable solvent [14].

**1. Extract Yield**

The extraction process was conducted using the remaceration method with 70% ethanol as the solvent. Ethanol 70% was selected because flavonoid compounds are generally present as polar glycosides and are more effectively dissolved in polar solvents. Ethanol 70% has higher polarity than 96%

ethanol, making it suitable for extracting polar phytochemical constituents [15]. The maceration method was selected because it is simple and allows the extraction of many compounds, although some compounds may have limited solubility at room temperature [16].

**Table 3.** Percentage of Extract Yield

| Extract Name | Yeild (%) |
|---|---|
| **Red Betel Leaf** | 17,6 |
| **Tapak Liman leaf** | 9,9 |

The extraction of 200 g of red betel leaf and tapak liman leaf simplicia produced yields of 17.6% and 9.9%, respectively, as shown in Table 3. These results met the Indonesian Herbal Pharmacopoeia standards, which require ethanol extract yields of not less than 17.0% for red betel leaf and not less than 5.5% for tapak liman leaf [17]. This indicates that the extracts obtained in this study fulfilled the standard quality requirements for herbal extracts.

## 2. Qualitative Analysis of Flavonoid Content

The qualitative flavonoid test showed that both red betel leaf extract and tapak liman leaf extract contained flavonoids, as indicated by color changes to brick red and orange. The flavonoid reaction mechanism is shown in Figure 1. The formation of red, orange, or purplish-red coloration indicates the presence of flavonoids, while orange coloration may indicate flavones, chalcones, or aurones. The color change occurs due to a reduction reaction involving $Mg^{2+}$ under acidic conditions after the addition of concentrated HCl, producing yellowish-red, orange, or purplish-red coloration [19].

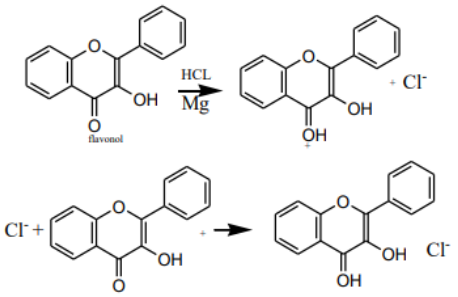



**Figure 1.** Flavonoid reaction equation [18]

## 3. Thin Layer Chromatography (TLC) Analysis

The Thin Layer Chromatography (TLC) method was used to confirm the presence of flavonoid compounds in the extract after phytochemical screening [20]. TLC analysis aimed to identify flavonoids in the red betel leaf extract by comparing its chromatographic profile with the quercetin reference standard. Table 4 shows that red betel leaf extract produced two spots, Rf 1 and Rf 3, both with an Rf value of 0.70, while quercetin showed an Rf value of 0.71. Rf values within the range of 0.20–0.75 are generally associated with flavonoid compounds [21]. The similarity between the Rf values of the extract and quercetin suggests that the extract contains compounds with chromatographic characteristics similar to the reference standard [22].

Under UV 254 nm, both the red betel extract and quercetin exhibited chocolate-colored spots. Under UV 366 nm, the extract exhibited fluorescent magenta coloration, while quercetin showed glowing green fluorescence. These visual differences arise

from the interaction between the UV light and the fluorescence indicator on the TLC plate [23]. The brownish coloration under 254 nm and the fluorescent colors under 366 nm are consistent with the typical behavior of flavonoids, particularly flavones and glycoflavones [21].

**Table 4.** Results of Rf values on 254 nm and 366 nm UV lamps

| Sample | Code | Rf value | Color | |
|---|---|---|---|---|
| | | | UV 254 nm | UV 366 nm |
| Red Betel Leaf Extract | Rf 1 | 0.7 | Chocolate | Fluorescent Magenta |
| Red Betel Leaf Extract | Rf 3 | 0.7 | - | Fluorescent Magenta |
| Quercetin | Rf 2 | 0.71 | Chocolate | Glowing Green |

## 4. Quantitative Analysis of Flavonoid Content

Total flavonoid content was determined using $AlCl_3$ reagent, in which flavonoids form a colored complex with $Al^{3+}$. The absorbance of the complex was measured using a UV-Vis spectrophotometer at the maximum wavelength of quercetin, and the total flavonoid content was calculated using the regression equation of the quercetin standard curve. The reaction between quercetin and $AlCl_3$ is shown in Figure 2 [24].

The quercetin calibration curve is presented in Figure 3. The relationship between quercetin standard concentration and absorbance showed a strong linear correlation, with the regression equation y = 0.0274x + 0.0283 and an $R^2$ value of 0.9906. An $R^2$ value approaching 1 indicates a strong relationship between the standard concentration and absorbance value, while an $R^2$ value close to 0 indicates weak explanatory ability [22]. The high $R^2$ value obtained in this study indicates that the calibration curve had good linearity and was suitable for determining total flavonoid content in the samples.

The linearity of the calibration curve was confirmed before the regression equation was used to calculate flavonoid content from the absorbance values of each extract and formulation sample. The results were expressed as mg QE/g and are presented in Table 5.

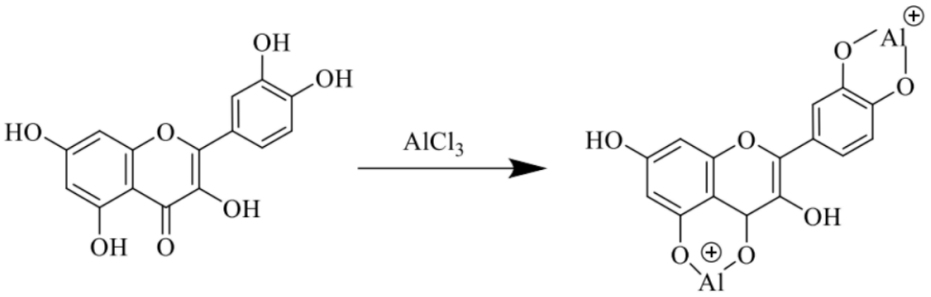



**Figure 2.** Reaction of Quercetin with $AlCl_3$[24]

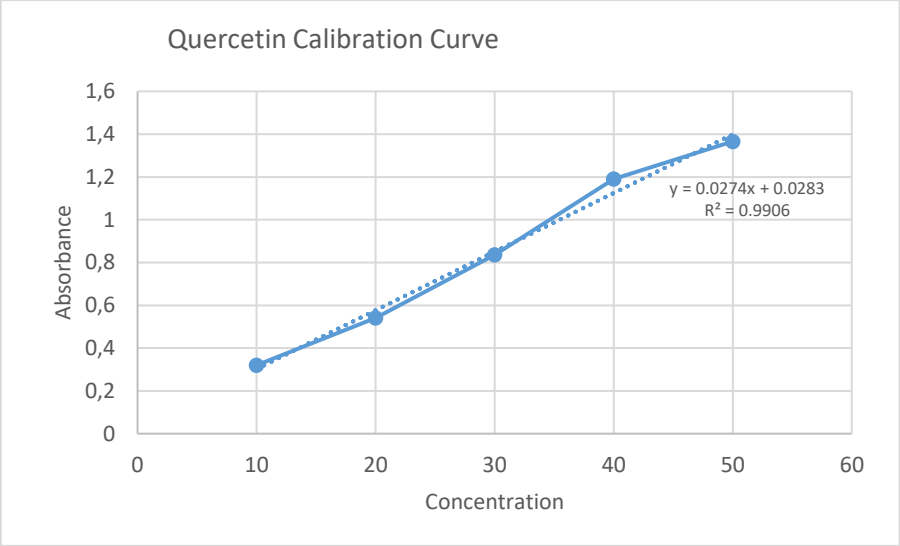



**Figure 3**. Quercetin Calibration Curve at Maximum Wavelength of 422 nm

**Table 5**. Results of Determination of Total Flavonoid

| Sample | Absorbance | Concentration (ppm) | Concentration (mg QE/g) | Mean ± SD |
|---|---|---|---|---|
| Tapak Liman Extract | 0.637 | 22,10 | 22,10 | 22,10 ± 0,25 |
| Red Betel Extract | 2,112 | 75,16 | 75,16 | 75,16 ± 0,55 |
| Formulation 1 | 1,382 | 44,59 | 44,59 | 44,59 ± 0,41 |
| Formulation 2 | 1,424 | 40,30 | 40,30 | 40,30 ± 0,32 |
| Formulation 3 | 1,424 | 39,66 | 39,66 | 39,66 ± 0,27 |

The results in Table 5 show that red betel leaf extract had the highest flavonoid content at 75.16 mg QE/g, while tapak liman leaf extract contained 22.10 mg QE/g. These results indicate a clear difference in flavonoid content between the two extracts. The combination formulas F1, F2, and F3 produced flavonoid contents ranging from 39.66 to 44.59 mg QE/g. Although these values were lower than that of the single red betel leaf extract, the combined formulations still showed measurable and relatively stable flavonoid levels.

Formula 1 showed the highest flavonoid content among the three formulations, at 44.59 mg QE/g. Formula 2 and Formula 3 showed relatively similar flavonoid levels, at 40.30 mg QE/g and 39.66 mg QE/g, respectively. The relatively small differences among the formulations indicate that variation in the extract ratio did not drastically reduce the total flavonoid content in the combined preparations. This finding suggests that the extract combination can be further considered for nanogel development, particularly in relation to its physical characteristics and antibacterial activity.

### 5. Particle Size Test of Preparations

Nanogel preparations consist of nanoemulsion and gel components. Nanoemulsions are delivery systems that can increase drug permeability on membrane surfaces [11]. Nanoparticle technology has been widely developed in drug delivery

systems, with particle sizes generally ranging from 10 to 1000 nm [9].

**Table 6**. Particle Size Analyzer (PSA) Test Results

| Sample Name | Particle Size (nm) | Notes |
|---|---|---|
| Formula 1 | 649,133 | Largest and most inconsistent |
| Formula 2 | 255,433 | Smallest and most uniform |
| Formula 3 | 472,733 | Largest with poor uniformity |

The particle size results are presented in Table 6. Each formula showed different particle sizes, which may be influenced by differences in extract composition and nanoemulsion formation. Formula 2 was considered the most optimal formulation in terms of particle size because it produced the smallest particle size and showed a more uniform distribution than the other formulas.

## 6. Gel Characteristics

### Test Organoleptic Test

Organoleptic testing was conducted to evaluate the physical appearance of the gel preparation, including color, odor, and texture [25]. The organoleptic results are presented in Table 7. All nanogel formulas showed relatively similar characteristics, including deep green color, typical leaf simplicia odor, and thick liquid texture. The deep green color corresponded to the presence of red betel leaf and tapak liman leaf extracts, indicating that the extracts were dispersed in the nanogel preparation. The typical leaf simplicia odor also indicated that the formulation process did not cause major odor changes.

**Table 7.** Organoleptic Test Results

| Parameter | Formula 1 | Formula 2 | Formula 3 |
|---|---|---|---|
| **Color** | Deep green | Deep green | Deep green |
| **Odor** | Typical leaf simplicia | Typical leaf simplicia | Typical leaf simplicia |
| **Texture** | Thick liquid | Thick liquid | Thick liquid |

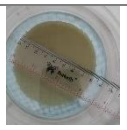
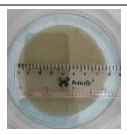
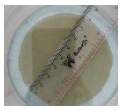
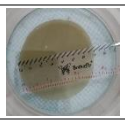
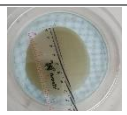
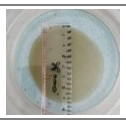
### Homogeneity Test

The homogeneity test showed that all nanogel formulas were homogeneous. This was indicated by the absence of coarse particles or clumps and by the even distribution of active ingredients and additives within the gel base.

### Spread Power Test

The spreadability test was conducted to determine the ability of the gel preparation to spread on the skin surface, which may affect drug absorption and the release rate of active substances at the application site. The required spreadability range for topical preparations is generally 5–7 cm [26]. The spreadability results are presented in Table 8. The results in Table 8 show that Formula 1 had an average spreadability diameter of 7.93 cm, Formula 2 had 7.86 cm, and Formula 3 had 7.23 cm. These values exceeded the standard range for topical preparations. Higher spreadability indicates that the preparation is easier to spread, although it may also reflect relatively low viscosity. This characteristic can improve user comfort and allow wider skin coverage with a relatively small amount of preparation.

**Table 8.** Result of Spread Test

| Sample Name | Diameter 1 (cm) | | Diameter 2 (cm) | | Diameter 3 (cm) | | Average (cm) |
|---|---|---|---|---|---|---|---|
| **Formula 1** | 8 |  | 7,9 |  | 7,9 |  | 7,93 |
| **Formula 2** | 7,8 |  | 8 |  | 7,8 |  | 7,86 |
| **Formula 3** | 7 |  | 7,3 |  | 7,4 |  | 7,23 |

**Adhesion Test**

Adhesion testing was conducted to determine the ability of the gel preparation to adhere to the skin after application. Strong adhesion can support drug delivery because longer contact between the preparation and the skin may improve the expected therapeutic effect. A good gel preparation should have an adhesion time of more than 1 second [27]. The adhesion results are presented in Table 9.

**Table 9.** Adhesion Test Results

| Sample Name | Time |
|---|---|
| Formula 1 | 1 minute 45 seconds |
| Formula 2 | 1 minute 9 seconds |
| Formula 3 | 1 minute 26 seconds |

The results in Table 9 show that all formulas had adhesion times of more than 1 second, indicating that they met the requirement for good gel adhesion. Formula 1 showed the longest adhesion time, followed by Formula 3 and Formula 2. These findings indicate that formulation composition influenced the adhesion ability of the nanogel, where longer adhesion may increase skin contact time and potentially improve therapeutic effectiveness.

**Dry Time Test**

The drying time test was conducted to measure the time required for the preparation to dry and form a film layer on the skin [28]. The drying time results are presented in Table 10. Formula 1 had the fastest drying time at 2 minutes 52 seconds, followed by Formula 2 at 3 minutes 42 seconds and Formula 3 at 4 minutes 58 seconds.

**Table 10.** Drying Time Test Results

| Sample Name | Time |
|---|---|
| Formula 1 | 2 minute 52 seconds |
| Formula 2 | 3 minute 42 seconds |
| Formula 3 | 4 minute 58 seconds |

A shorter drying time may improve user comfort because the preparation does not leave a prolonged sticky sensation on the skin. Formula 1 can therefore be considered the most favorable formulation in terms of drying comfort, whereas Formula 3 required

the longest drying time and may be less practical for users.

**pH Test**

The pH test was conducted to determine the acidity level of each nanogel formulation. Preparations that are too alkaline may irritate the skin, while preparations that are too acidic may cause scaling and itching [29]. A suitable pH range for skin application is generally 4.5–8.0 [30]. The pH test results are presented in Table 11.

**Table 11.** Adhesion Testing Result

| Sample Name | Result |
|---|---|
| Formula 1 | 7,00 |
| Formula 2 | 5,50 |
| Formula 3 | 5,22 |

The results in Table 11 show that all formulas were within the acceptable pH range for skin application. Formula 2 and Formula 3 were closer to physiological skin pH, indicating their potential to provide better comfort and minimize irritation risk. Formula 1 had a pH close to neutral and remained within the safe range, although its value was relatively higher than the natural skin pH.

**Viscosity Test**

The viscosity test results are presented in Table 12. The results show differences in viscosity among the formulas, which may be influenced by variations in active ingredient composition. The required viscosity range for gel preparations is generally 500–10,000 mPa·s [29]. Formula 1 showed the highest viscosity at 40.466 mPa·s, followed by Formula 3 at 30.300 mPa·s and Formula 2 at 21.300 mPa·s.

**Table 12.** Viscosity Test Results

| Sample Name | Result (mPa.S) |
|---|---|
| Formula 1 | 40.466 |
| Formula 2 | 21.300 |
| Formula 3 | 30.300 |

The viscosity values obtained in this study were below the required standard for gel preparations. This indicates that the formulations had not achieved the structural integrity expected for a typical gel. The low viscosity values may be caused by insufficient gelling agent concentration, incomplete polymer hydration, or suboptimal neutralization during gel formation. These factors may limit gel network formation and produce a more fluid-like preparation. Further formulation optimization is therefore required to improve rheological properties and ensure acceptable viscosity performance.

**Antibacterial Test**

**Table 13.** Antibacterial Activity Test Results of Nanogel Formulations and Extracts

| Types of Formulation | % Extract | Inhibition Zone Diameter (mm) |
|---|---|---|
| Formulation 1 | 10% | 11,333 |
| Formulation 2 | 10% | 7,333 |
| Formulation 3 | 10% | 15,667 |
| Positive Control | - | 24,000 |
| Negative Control | - | 0,000 |
| Tapak Liman Leaf Extract | 100% | 9,000 |
| Red Betel Leaf Extract | 100% | 12,333 |

The antibacterial activity test results are presented in Table 13. All nanogel formulations at a concentration of 10% inhibited the growth of Staphylococcus

aureus with varying inhibition zone diameters. Formula 3 produced the largest inhibition zone at 15.667 mm, followed by Formula 1 at 11.333 mm and Formula 2 at 7.333 mm.

The antibacterial activity of single extracts showed that red betel leaf extract produced a larger inhibition zone of 12.333 mm than tapak liman leaf extract at 9.000 mm. The positive control showed the largest inhibition zone at 24.000 mm, while the negative control showed no antibacterial activity. These results indicate that the combined nanogel formulations had antibacterial potential, with Formula 3 showing the strongest activity among the tested formulas.

## CONCLUSION

Flavonoids were confirmed in both extracts through qualitative tests, TLC, and quantitative analysis, with red betel extract showing the highest content (75.16 mg QE/g) and tapak liman lower (22.10 mg QE/g). The combined nanogels contained 39.66–44.59 mg QE/g and met basic physical requirements, where Formula 2 produced the smallest particle size (±255 nm) and good homogeneity. All formulas showed antibacterial activity against *Staphylococcus aureus*, with Formula 3 producing the largest inhibition zone. Because this study only assessed flavonoid levels, physical characteristics, and simple antibacterial zones, the findings cannot yet be interpreted as evidence of broader pharmacological potential. Further studies, including advanced in vitro assays, stability testing, in vivo evaluation, and synergy-specific analysis, are required to determine clinical relevance and to confirm whether the increased antibacterial effect of the combined extracts is synergistic or merely additive

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