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# Physical Stability Test and Total Phenolic Content Determination of Spray Gel Formulation with Ethanol 70% Melinjo Leaf (*Gnetum gnemon* L.) Extract with Combination of Carbopol 940 and HPMC as Gelling Agent

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**Abstract:** The stability of a pharmaceutical preparation is very important in formulation to ensure product efficacy and safety. This research aims to test the physical stability and total phenolic content of a spray gel preparation made from 70% ethanol extract of melinjo leaves (*Gnetum gnemon* L.) with a combination of Carbopol 940 and HPMC as gelling agents. Antioxidant activity was tested using the DPPH method, while total phenolic content was measured using the Folin-Ciocalteu reagent and expressed in gallic acid equivalents (mgGAE/g). The results analysis of total phenolic content in the 70% ethanol extract of melinjo leaves showed a value of 46.237 mg GAE/g. Meanwhile, the total phenolic content in the spray gel formulation of the 70% ethanol extract of melinjo leaves was recorded at 0.88 mg GAE/g."Based on evaluation, the best formula is a combination of Carbopol and Hidroxypropyl methylCellulosa, HPMC (0.4%:0.4%), but the stability test results show physical instability. Further modifications are required to produce a physically stable formula.

**Keywords** carbopol, HPMC, leaves, melinjo, spray gel, stability.

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## 1. INTRODUCTION

The background of this research is the utilization of natural ingredients from melinjo leaves (Gnetum gnemon L.), which possess antioxidant properties, developed into a spray gel formulation using a combination of Carbopol 940 and HPMC as gelling agents.This research aims to develop a spray gel formulation based on melinjo leaf extract gnemon (Gnetum L.) as a natural antioxidant active ingredient, using a combination of gelling agents HPMC a nd Carbopol 940. The spray gel was chosen because of its practicality of use, safety against microbial contamination, and protection against oxidation. By measuring the total phenolic content and evaluating the physical stability of the formulation, this research seeks to produce high quality and stable pharmaceutical preparations. HPMC and Carbopol were chosen because of their properties which support the formation of stable gels with optimal viscosity and spreadability. This development is relevant to the trend of using natural products, while utilizing the high antioxidant potential of melinjo leaves as a safe alternative for beauty and pharmaceutical products.

## 2. MATERIAL AND METHODS

### 2.1 Materials

Fresh melinjo leaves obtained from TP Sungkai Farm, Bogor, West Java, 70% ethanol, Mayer's reagent, Dragendorff's reagent, hydrochloric acid (HCl), 95% ethanol, amyl alcohol, magnesium powder, ferric chloride (FeCl3), distilled water, sulfuric acid, and anhydrous acetic acid. Carbopol 940, HPMC, DPPH

solution, and Folin-Ciocalteu reagent, propylene glycol, Carbopol 940, HPMC, TEA, menthol, methanol p.a., vitamin C, DPPH (2,2-difenil-1-pikrilhidrazil), Folin-Ciocalteu reagent, gallic acid, sodium bicarbonate.

### 2.2 Tools

Sartorius analytical balance, a set of rotary vacuum vaporizers (EYELA N-1000), blender (chopper), aluminum foil, maceration bottles, refrigerator (Sanyo), micropipette (DLAB), Sartorius pH meter, LV viscometer Brookfield, oven (Memmert), filter paper, desiccator (vakuumfest), furnace (Thermolyne), overhead stirrer (digital IKA® RW 20), IKA® C-MAG HS 7, other laboratory glassware, UV-Vis spectrophotometer (Hitachi).

### 2.3 Research Procedures

#### 2.3.1 Melinjo Leaf Extraction

The collected melinjo leaves (*Gnetum gnemon* L.) were washed under running water and then drained. To accelerate the drying process, the cleaned leaves were coarsely chopped. The leaves were subsequently sun-dried for five to seven days. The dried leaves were then ground using a blender to obtain uniformly sized simplicia powder, which was used as the raw material for extraction. Melinjo leaves were extracted using the maceration method with 70% ethanol. The filtrate is evaporated using a rotary evaporator to obtain a thick extract.

#### 2.3.2 Spray Gel Formulation

Table 1. HPMC Carbopol Combination Spray Gel Base Formula with Modifications (Ramdha & Azizah, 2021)

| Material | Function | Concentration (%) | | |
|---|---|---|---|---|
| | | F1 | F2 | F3 |
| Carbopol 940 | *Gelling agent* | 0.4 | 0.6 | 0.4 |
| HPMC | *Gelling agent* | 0.4 | 0.4 | 0.6 |
| Propylen glycol | Humectant | 15 | 15 | 15 |
| TEA | *Alkalizing agent* | 0.25 | 0.25 | 0.25 |
| Methyl paraben | Antimicrobial preservative | 0.18 | 0.18 | 0.18 |
| Prophyl paraben | Antimicrobial preservative | 0.02 | 0.02 | 0.02 |
| Menthol | *Enhancher* | 0.05 | 0.05 | 0.05 |
| Ethanol 95% | Menthol solvent | 5 | 5 | 5 |
| Aquadest ad | Solvent | ad 100 | ad 100 | ad 100 |

This study involved the formulation of a spray gel using hydroxypropyl methylcellulose (HPMC) and Carbopol 940 as gelling agents in three concentration variants. Carbopol 940 was initially dispersed in distilled water and stirred until uniformly distributed, followed by the addition of triethanolamine (TEA) to obtain a transparent gel mass (M1). HPMC was dispersed in hot distilled water, stirred, and subsequently combined with M1 to form a transparent gel (M3). Menthol dissolved in ethanol, along with methyl paraben and propyl paraben dissolved in propylene glycol, was added to M3. The mixture was stirred until homogeneous, and the remaining distilled water was added to obtain the desired final weight. The resulting spray gel base was transferred into a closed vial for physical evaluation. After a base formulation that met the required criteria was obtained, 2% melinjo leaf (*Gnetum gnemon* L.) extract was incorporated. The final spray gel formulation was then subjected to physical stability and antioxidant activity evaluations.

#### 2.3.3 Physical Stability Test

**a. Organoleptic:**

Observation of color, smell and texture (Cendana *et al*., 2021).

**b. Homogenity**

Homogeneity testing is carried out using a transparent glass substrate to detect particles or substances that have not formed completely in the spray gel formulation.(Cendana *et al*., 2021).

**c. pH**

pH testing was carried out with a pH meter calibrated using a pH 4 buffer. A total of 0.1 gram of spray gel was dissolved in 100 ml of distilled water, and the pH was measured after the electrode was soaked. The formula should have a pH of 4.5–7.0, corresponding to the skin's pH (Akbar *et al*., 2021).

**d. Viscosity**

Viscosity was measured with a Brookfield viscometer using spindle number 64 on 100 grams of spray gel. Once the reading stabilizes, the viscosity is measured with an optimal range of 500-5000 cps (Kresnawati *et al*., 2022).

**e. Flow Properties**

Flow quality was assessed using a Brookfield Viscometer type LVT, specifically with spindle number 64. The speed of the device was first set at 0.3; 0.6; 1.5; 3; 6; 12; 30; 60 rpm and then reversed to 60; 30; 12; 6; 3; 1.5; 0.6; 0.3 rpm. The scale is determined by monitoring the red needle after it reaches a stable position (Febriani *et al*., 2020).

**f. Spray Pattern**

The spray pattern was evaluated by spraying the preparation on a plastic sheet at a distance of 3, 5, 10, and 15 cm, then measuring the spray weight and diameter. The experiment was carried out three times to observe the pattern, diameter and weight of the preparation. A longer spray distance produces a larger diameter, with the final weight expected to be consistent (Kresnawati *et al*., 2022).

**g. Sticky Spreadability**

The adhesive spreadability was tested by spraying the spray gel on a clean and dry upper arm, then measuring whether the preparation adhered or dripped within 10 seconds (Cendana *et al*., 2021).

**h. Cycling Test**

Chemical stability was evaluated through a cycling test procedure conducted over 6 cycles, each consisting of 24 hours of cooling at a temperature of 4 ± 2°C stored in a freezer and 24 hours of heating at a temperature of 40 ± 2°C stored in an oven. at a temperature of 4 ± 2°C and 24 hours of heating at a temperature of 40 ± 2°C. Physical stability was tested through organoleptic measurements, viscosity, pH, uniformity, spray pattern and adhesive spreadability before and after the 6th cycle (Suryani et al., 2017).

**2.3.4 Antioxidant Activity of Extracts Melinjo Leaves and Preparation of Spray Gel**

**a. Preparation of 0.1 mM DPPH Stock Solution**

A 0.1 mM DPPH solution was obtained by dissolving 3.9432 mg DPPH in methanol p.a. up to 100 ml (Hasanah *et al*., 2017).

**b. Determination of Maximum Wavelength and Absorbance of DPPH Blanks**

A solution containing 2.7 mL of 0.1 mM DPPH and 1.3 mL of methanol was incubated for 30 minutes. Absorbance was then measured using a UV–Visible spectrophotometer by scanning wavelengths between 515 and 520 nm, with the reading taken at the wavelength showing the maximum absorbance. The procedure was conducted in triplicate (Hasanah et al., 2017).

**c. Preparation and Measurement of Vitamin C Comparator Solutions**

A 1000 ppm vitamin C solution was prepared, and test solutions with concentrations of 2, 4, 6, 8, and 10 ppm were prepared. For each concentration, 2.7 mL of a 0.1 mM DPPH solution was added, followed by a 30-minute incubation period, after which the absorbance was determined using a UV–Visible spectrophotometer. (Hasanah *et al*., 2017).

**d. Preparation and Measurement of Test Solutions for Spray Gel Preparations**

A standard solution of 2000 ppm was made with 200 mg of spray gel and methanol. Solutions with a concentration of 250-1250 ppm are prepared by mixing the stock solutions. After 30 minutes of incubation, absorbance was measured using a UV-Vis spectrophotometer. The test was repeated three times (Hasanah *et al*., 2017).

**e. Determination of IC50 Value**

The IC50 value was measured to determine the antioxidant activity of melinjo leaf extract, and it was obtained by calculating the percentage of inhibition at various sample concentrations, followed by constructing a concentration response curve to determine the concentration required to inhibit 50% of the DPPH radical activity.

**f. Determination of AAI Value**

Antioxidant Activity Index (AAI) was calculated to evaluate the antioxidant activity of melinjo leaf extract. The AAI value can be calculated using the following formula:

$$\text{AAI Value} = \frac{\text{DPPH Concentration (ppm)}}{\text{IC}_{50}\text{ Value (ppm)}}$$

If the AAI value is < 0.5, the antioxidant activity is considered weak. An AAI value > 0.5 indicates moderate antioxidant activity. An AAI value between 1 and 2 indicates strong antioxidant activity, while an AAI value > 2 indicates very strong antioxidant activity (Paraeng et al., 2016).

**2.3.5 Determination of Total Phenolic Content of Spray Gel Preparations**

**a. Preparation of Gallic Acid Mother Solution**

A 500 µg/mL gallic acid solution was obtained by dissolving 50 mg gallic acid in 100 mL ethanol p.a. (Alfian & Susanti, 2012).

**b. Preparation Na2CO3 Solution**

The Na2CO3 solution is made by dissolving 7.5 grams of Na2CO3 in 80 mL of distilled water, then boiling and filtering until the volume reaches 100 mL (Alfian & Susanti, 2012).

**c. Determination of Maximum Absorbance Wavelength of Gallic Acid**

Gallic acid solution (5 µg/mL) was homogenized with Folin-Ciocalteu reagent and Na2CO3 solution. After incubation, absorbance was measured at a wavelength of 600-850 nm (Alfian & Susanti, 2012).

**d. Determining Operating Time**

The gallic acid solution was reacted with Folin–Ciocalteu reagent followed by the addition of a sodium carbonate ($Na_2CO_3$) solution, after which the absorbance was monitored at the maximum wavelength until a stable reading was achieved. (Alfian & Susanti, 2012).

**e. Determination of Gallic Acid Standard Curve**

Gallic acid solutions with various concentrations (1-5 µg/mL) were homogenized with Folin-Ciocalteu reagent and Na2CO3, then the absorbance was measured to create a calibration curve (Alfian & Susanti, 2012).

**f. Determination of Phenol Content in Spray Gel Preparations**

The spray gel preparation was dissolved in methanol-water, then homogenized with Folin-Ciocalteu reagent and Na2CO3. Absorbance was measured using a UV-Vis spectrophotometer to determine phenolic content (Alfian & Susanti, 2012).

### 2.3.6 Data Analysis

The SPSS 29 statistical data processing tool which contains the One Way ANOVA test for testing at room temperature storage and the Paired T-Test for cycling tests is used to analyze data from physical evaluation test results of preparations.

## 3. RESULTS AND DISCUSSION

### 3.1 Test The Antioxidant Activity of Extracts and Preparation

Tabel 2. Results of Antioxidant Activity Test of Vitamin C

| Concentration (ppm) | Absorbantion average | % inhibition | $IC_{50}$ (ppm) | AAI |
|---|---|---|---|---|
| 2 | 0.375 ± 0.01 | 27.448 | | |
| 4 | 0.302 ± 0.008 | 41.623 | | |
| 6 | 0.233 ± 0.01 | 55.026 | 4.96 | 7.96 |
| 8 | 0.133 ± 0.01 | 74.227 | | |
| 10 | 0.021 ± 0.01 | 96.005 | | |

Table 3. Results of Antioxidant Activity Test of Melinjo Leaf Extrac

| Concentration (ppm) | Absorbantion average | % Inhibition | $IC_{50}$ (ppm) | AAI |
|---|---|---|---|---|
| 250 | 0.623±0.01 | 20.239 | | |
| 500 | 0.496±0.007 | 36.507 | | |
| 750 | 0.390±0.01 | 50.085 | 771.80 | 0.051 |
| 1000 | 0.287±0.01 | 63.194 | | |
| 1250 | 0.213±0.01 | 72.715 | | |

Table 4. Results of Antioxidant Activity Test of Melinjo Leaf Extract Gel Spra

| Concentration (ppm) | Absorbantion average | % Inhibition | $IC_{50}$ (ppm) | AAI |
|---|---|---|---|---|
| 250 | 0.749 | 4.01 | | |
| 500 | 0.720 | 7.73 | | |
| 750 | 0.691 | 11.53 | 3501.89 | 0.011 |
| 1000 | 0.668 | 14.47 | | |
| 1250 | 0.638 | 18.27 | | |

This research was conducted to assess the antioxidant activity of melinjo leaf extract employing the DPPH method. Antioxidant activity is measured by the IC50 value, which indicates a substance's ability to inhibit 50% oxidation. DPPH undergoes a color transition from purple to yellow, with its maximum

absorbance recorded at a wavelength of 515 nm. Vitamin C shows a calculated $IC_{50}$ value of 4.96 ppm, which reflects very strong antioxidant activity, whereas the melinjo leaf extract exhibits an $IC_{50}$ value of 771.80 ppm, indicating very weak activity. This comparison shows that the antioxidant activity of melinjo leaf extract is only 0.64% of vitamin C, because the raw extract contains disturbing substances that can inhibit the antioxidant process. Environmental factors, such as temperature and CO2 levels, also influence the production of secondary metabolites that play a role in plant antioxidant activity. Melinjo leaf extract contains phenolic and flavonoid compounds which have antioxidant properties with the ability to provide hydrogen atoms to neutralize free radicals.

The antioxidant assay of the spray gel formulation produced a linear regression equation of $y = 0.0141x + 0.6234$ with an $R^2$ value of 0.9985, and the $IC_{50}$ was calculated to be 3501.89 ppm, classifying it as having very weak antioxidant activity. The calculated AAI value of 0.011 is also included in the weak category. The IC50 value of melinjo leaf extract spray gel is only 21.57% compared to melinjo leaf extract, this is because the extract content is only 2% of the total formula. Even though the extract has a lower IC50, the spray gel preparation has met the minimum required IC50 threshold, this shows that the antioxidant activity of the extract remains stable during the formulation and storage process.

### 3.2 Determination of Total Phenolic Content of Extracts and Preparations

Table 5. Results of Determination of Total Phenol Content of Melinjo Leaf Extract

| Concentration | Maximum Wavelength | Absorbantion |
|---|---|---|
| 100 ppm | 750.5 | 0.620<br>0.586<br>0.605 |
| **Level (mgGAE/g)** | **Mean levels ± SD** | |
| **47.77**<br>**44.58**<br>**46.36** | 46.237 ± 1.599 | |

Table 6. Results of Determination of Total Phenol Content of Melinjo Leaf Extract Gel Spray Preparation

| Concentration | Maximum Wavelength | Absorbantion |
|---|---|---|
| 100 ppm | 750.5 | 0.120<br>0.120<br>0.119 |
| **Level (mgGAE/g)** | **Mean levels ± SD** | |
| 0.91<br>0.91<br>0.82 | 0.88 ± 1.599 | |

To measure the correlation between antioxidant activity and total phenolic content, the phenolic content was determined using gallic acid as a standard with the Folin-Ciocalteu reagent. Phenol, with its hydroxyl group, is able to neutralize free radicals by donating hydrogen atoms. The maximum wavelength of gallic acid was identified at 750.5 nm with an absorbance of 0.691. The gallic acid calibration curve yielded the equation $y = 0.1067x + 0.1103$ with an $R^2$ value of 0.9959. Analysis of the total phenolic content of the 70% ethanol extract of melinjo leaves resulted in a value of 46.237 mg GAE/g.

The test results for the total phenolic content in the spray gel formulation of 70% ethanol extract of melinjo leaves were 0.88 mgGAE/g. Although the total phenol content of the spray gel preparation decreased compared to melinjo leaf extract because the extract was only 2% of the formula, the total phenol content of the preparation approached the minimum theoretical value of 0.92 mgGAE/g. This shows that the active phenol in the extract remains stable after being formulated into a spray gel and during the storage process.

### 3.3 Physical Evaluation of The Spray Gel Base

Table 7. Comparison of Optimization Results for Spray Gel Base Optimization

| Parameter | Requirements | Referral | Room Temperature | | |
|---|---|---|---|---|---|
| | | | FB1 | FB2 | FB3 |
| Organoleptic | No change in shape, color, smell | Cendana *et al*. (2021) | √ | √ | √ |
| Homogenity | Homogent | Sayuti (2015) | √ | √ | √ |
| pH | 4,5-7 | Akbar (2021) | √ | √* | √* |
| Viskocity | 500 – 5000 cPs | Kresnawati *et al*. (2022) | √ | - | √* |
| Flow properties | Thixotropic | Indriyati (2019) | √ | √ | - |
| Diameter per spray | Distance increases, diameter increases | Kresnawati *et al*. (2022) | √* | √* | √* |
| Weight per spray | Uniform | Wicaksono (2019) | √ | √ | √* |
| Sticky spreadability | Stick | Kresnawati *et al*. (2022) | √ | √ | √ |
| | Spread | | - | - | - |
| **Parameter** | **Requirements** | **Referral** | ***Cycling Test*** | | |
| | | | **FB1** | **FB2** | **FB3** |
| Organoleptic | No change in shape, color, smell | Cendana *et al*. (2021) | √ | √ | √ |
| Homogenity | Homogent | Sayuti (2015) | √ | √ | √ |
| pH | 4,5-7 | Akbar (2021) | √ | √* | √* |
| Viskocity | 500 – 5000 cPs | Kresnawati *et al*. (2022) | √ | - | √* |
| Flow properties | Thixotropic | Indriyati (2019) | √ | √ | - |
| Diameter per spray | Distance increases, diameter increases | Kresnawati *et al*. (2022) | √* | √* | √* |
| Weight per spray | Uniform | Wicaksono (2019) | √ | √ | √ |
| Sticky spreadability | Stick | Kresnawati *et al*. (2022) | √ | √ | √ |
| | Spread | | - | - | - |

Note:
√ = meets the requirements
- = does not meets the requirements
* = significantly different p (<0,05)

Table 8. Evaluation Results of Melinjo Leaf Extract Gel Spray

| Parameter | Requirements | Referral | Room Temperature |
|---|---|---|---|
| Organoleptic | No change in shape, color, smell | Cendana *et al*. (2021) | √ |
| Homogenity | Homogent | Sayuti (2015) | - |
| pH | 4,5-7 | Akbar (2021) | √ |
| Viskocity | 500 – 5000 cPs | Kresnawati (2022) | - |
| Flow properties | Thixotropic | Indriyati (2019) | √ |
| Diameter per spray | Distance increases, diameter increases | Kresnawati (2022) | √ |
| Weight per spray | Uniform | Wicaksono (2019) | √ |
| Sticky spreadability | Stick | Kresnawati (2022) | - |
| | Spread | | - |
| **Parameter** | **Requirements** | **Referral** | ***Cycling Test*** |
| Organoleptic | No change in shape, color, smell | Cendana *et al*. (2021) | √ |
| Homogenity | Homogent | Sayuti (2015) | - |
| pH | 4,5-7 | Akbar (2021) | √ |
| Viskocity | 500 – 5000 cPs | Kresnawati (2022) | - |
| Flow properties | Thixotropic | Indriyati (2019) | √ |
| Diameter per spray | Distance increases, diameter increases | Kresnawati (2022) | √ |
| Weight per spray | Uniform | Wicaksono (2019) | √ |
| Sticky spreadability | Stick | Kresnawati (2022) | - |
| | Spread | | - |

Note :
√ = meets the requirements
- = does not meets the requirements
* = significantly different p (<0,05)

The physical stability of the spray gel formulation was assessed during 21 days of storage at room temperature as well as through a cycling test procedure. The formulation was assessed using several evaluation criteria, including organoleptic characteristics, homogeneity, pH value, viscosity, flow behavior, spray distribution, and adhesive spreading capacity. Based on the physical evaluation of the

base optimization formula, a formula was obtained that met the requirements, namely FB1 with a concentration ratio of Carbopol and HPMC (0.4%:0.4%). Physical evaluation of the melinjo leaf ethanol extract spray gel preparation carried out at room temperature and during the cycling test showed that melinjo leaf extract was not suitable as an active ingredient in spray gel preparations based on Carbopol and HPMC as gelling agents. Organoleptic testing of the melinjo leaf ethanol extract spray gel formulation showed no significant differences in appearance, odor, and color before and after the cycling test. The results also showed no bubbles present in the preparation. The homogeneity test indicated the presence of small particles derived from the melinjo leaf extract, suggesting that the preparation was not homogeneous before and during the cycling test. However, the pH test showed a slight increase in pH compared to the value before the cycling test. These changes may occur due to temperature fluctuations during storage and variations in air humidity.

## CONCLUSION

The conclusions of this research are the antioxidant activity of 70% ethanol extract of melinjo leaves and spray gel preparation with a combination of Carbopol and HPMC is very weak, with IC50 values of 771.80 ppm and 3501.89 ppm respectively, and total phenolic content of 46.237 mgGAE/g and 0.88 mgGAE /g. The combination of Carbopol 940 and HPMC is not suitable as a base for spray gel for 70% ethanol extract of melinjo leaves because it does not meet the parameters required for an effective preparation. This research recommends fractionating the extract to achieve greater antioxidant potential. Furthermore, it is recommended to use another gelling agent when formulating a 70% tenol extract gel spray from melinjo leaves.

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