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Formulation and Testing of Antioxidant Activity of Papaya Fruit (*Carica papaya* L.) Extract Spray Gel Using the DPPH Method

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Abstract

Methanol extract of papaya fruit possesses natural antioxidant activity that can be utilized to counteract free radicals. Spray gel is a formulation with low viscosity that can remain in contact for an extended period when sprayed on the skin. This study aims to formulate a spray gel made from papaya fruit extract and evaluate its antioxidant activity using the DPPH method. The papaya fruit extract was prepared using the maceration method with methanol as the solvent. Three spray gel formulas with papaya fruit extract concentrations of 1%, 2%, and 3% were tested for their physical properties. The testing revealed that Formula III (with a 3% extract concentration) met the appropriate pH criteria (5.67 ± 0.058), the desired viscosity (41.1 ± 2.84 cps), and the fastest drying time (< 5 minutes). The antioxidant activity test yielded the lowest IC50 value for Formula III at 330.307 ppm, followed by Formula II (IC_{50} = 374.08 ppm) and Formula I (IC_{50} = 455.21 ppm). All three formulas exhibited good sprayability and spreadability, but their antioxidant activity was relatively weak. Formula III with a 3% extract concentration is considered the best option based on physical criteria and antioxidant activity, contributing to developing a product that can protect the skin from UV damage.

Keywords: antioxidant activity; Carica papaya L.; DPPH; papaya, spray gel.

INTRODUCTION

UV rays can cause the formation of free radicals that damage the skin and increase oxidative stress (Sari, 2015). Antioxidants can prevent cellular damage and oxidative stress caused by exposure to ultraviolet light (Ebtavanny et al., 2021). Even at low concentrations, antioxidants can halt oxidation (Andarina & Djauhari, 2017). Antioxidants can stop oxidation reactions by binding to highly reactive molecules and free radicals (Santi et al., 2021). The antioxidant content of papaya *(Carica papaya* L.) includes beta-carotene, phenols, flavonoids, and vitamin C, which are effective against free radicals (Pratiwi & Wahdaningsih, 2018). Methanol extract from papaya (*Carica papaya* L.) shows protective potential against skin damage with a low IC_{50} value. The lower the IC_{50} value, the higher the antioxidant activity. The IC_{50} value indicates the strength of the antioxidant: categorized as very strong if below 50 ppm, moderately strong in the range of 50-100 ppm, moderate at 100-150 ppm, and weak if between 150-200 ppm. An IC_{50} value greater than 200 ppm indicates that antioxidant activity is less active (Molyneux, 2004).

Spray gel is a formulation with low viscosity that provides benefits in dermatology with a combination of gel properties and practical sprayability. This preparation facilitates application and maintains skin moisture while reducing the risk of contamination. This study aims to develop a spray gel derived from methanol extract of papaya (*Carica papaya* L.) and evaluate its antioxidant activity using the DPPH method (Butar, 2018). Testing was conducted with variations in extract concentrations (1%, 2%, and 3%) to evaluate physical characteristics such as pH, viscosity, and antioxidant activity.

Antioxidant compounds capture these free radicals, converting them into less reactive forms and reducing potential toxicity (Molyneux, 2004). When DPPH receives hydrogen radicals or electrons, stable diamagnetic molecules are formed. When antioxidants interact with DPPH, either through transfer or with hydrogen radicals on DPPH, the free radical character of DPPH becomes inactive (Molyneux, 2004). By analyzing various spray gel formulations, this study aims to find the most effective concentration of papaya extract to enhance antioxidant activity and physical properties, thereby contributing to developing skincare products that can protect the skin from UV damage.

MATERIALS AND METHODS

Materials and Equipment

In this study, the equipment used includes an analytical balance, blender, spatula, stirring rod, scoop, maceration vessel, filter paper, flannel cloth, porcelain dish, water bath, parchment paper, magnetic stirrer, hot plate, glassware, mica plastic, watch glass, digital pH meter, viscometer, UV-Vis spectrophotometer, cuvette, measuring pipettes of various sizes, and dropper pipettes. The materials used in this study include ¾ ripe orange papaya, methanol, Carbopol 940, propylene glycol, DMDM hydantoin, triethanolamine (TEA), distilled water, DPPH, and analytical grade ethanol.

Extract Preparation

Papaya fruit (*Carica papaya* L.) that has ripened to approximately ¾ of its orange color was cleaned with clean water, then peeled and its seeds removed. The papaya fruit was cut into slices about 2-3 mm thick and then dried in an oven at 60°C. After drying, the papaya slices were blended until they became papaya powder (Meutia, 2013). The papaya powder was sieved with mesh no. 40 to obtain a uniform size.

250 mg of dried papaya powder was macerated with 1 liter of methanol solvent in a closed container. The maceration process was stirred thrice daily and lasted 3 x 24 hours (Pratiwi & Wahdaningsih, 2018). The maceration result was then filtered to separate the filtrate from the residue. The filtrate was heated in a water bath at 60°C until a thick extract was formed.

Formulation of Spray Gel

The spray gel formula used refers to the research by Akib et al. (2023), titled “Formulation and Characterization of Spray Gel Containing Ethanol Extract of Bitter Melon Fruit (*Momordica charantia* L.).” The spray gel formula is shown in Table 1.

Table 1. Spray Gel Formula of Papaya Fruit Extract (*Carica papaya* L.).

Ingredients	Concentration (%)		
	Formula I	Formula II	Formula III
Papaya Fruit Extract	1%	2%	3%
Carbopol 940	0.1%	0.1%	0.1%
Propylene Glycol	15%	15%	15%
TEA (Triethanolamine)	0.5%	0.5%	0.5%
DMDM Hydantoin	0.6%	0.6%	0.6%
Distilled water	ad 100 mL	ad 100 mL	ad 100 mL

Dissolve all Carbopol 940 in distilled water on a hotplate at 70°C until a gel mass forms, stirring it with a magnetic stirrer. Then, mix TEA, propylene glycol, and DMDM Hydantoin in a separate container. Add the mixture to the gel mass and stir with the magnetic stirrer until homogeneous. Next, add the papaya fruit extract and stir thoroughly. After mixing, pour the preparation into spray bottles (Marlina et al., 2021).

Organoleptic Test

The organoleptic test was conducted by directly observing the spray gel’s texture, color, and scent (Kamishita et al., 1992).

Homogeneity Test

The gel was sprayed onto a transparent glass to check for unmixed particles. If no coarse granules were found, the spray gel was considered homogeneous.

pH Test

Approximately 0.1 grams of papaya fruit extract spray gel was mixed with 100 mL of distilled water. Then, the pH meter electrode was dipped into the solution. The pH range was expected to be between 4.5 and 6.5 (Departemen Kesehatan Republik Indonesia, 1977).

Viscosity Test

Around 75 mL of the spray gel was placed into a beaker and its viscosity was measured using a rotor viscometer number 3 (Hayati et al., 2019). The spray gel viscosity values should range between 25 and 250 cps (Akib et al., 2023).

Sprayability and Spreadability Test

The spray gel was sprayed from a distance of 5 cm. The evaluation criteria were as follows: If the spray gel did not spray at all, it was rated as "very poor." If the spray came out as droplets or clumps, it was rated as "poor" If it sprayed but produced large particles, it was rated as "fair." If it sprayed evenly with fine particles, it was rated as "good" (Hayati et al., 2019) (Kamishita et al., 1992).

Drying Time Test

The spray gel was sprayed onto paper, and the drying time was observed using a stopwatch. The drying time for the spray gel was expected to be no longer than 5 minutes (Kamishita et al., 1992).

Antioxidant Activity Test Using the DPPH Method

- Preparation of DPPH Stock Solution
  Weigh 5 mg of DPPH powder and dissolve it in ethanol p.a. using a 50 mL volumetric flask covered with aluminum foil to achieve a DPPH stock solution concentration of 100 ppm (Angelia et al., 2022).
- Determination of Maximum Wavelength and DPPH Absorbance (Blank)
  Add 1 mL of 100 ppm DPPH stock solution to ethanol until it reaches the mark in a 5 mL volumetric flask and mix until homogeneous. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength range of 400-600 nm (Patria & Soegihardjo, 2013).

- Preparation of Papaya Fruit Extract Spray Gel Stock Solution
  Weigh 5 mg of the spray gel extract and mix it with ethanol p.a. in a 10 mL volumetric flask to achieve a stock solution concentration of 500 ppm.
- Determination of Operating Time (OT)
  Place 50 µL of the spray gel stock solution with a concentration of 500 ppm into a 5 mL volumetric flask. Then, add 1 mL of 100 ppm DPPH stock solution and gradually add ethanol until it reaches the mark in the flask. Measure the absorbance every minute for 30 minutes. Use the stable absorbance value as the operating time.
- Absorbance Measurement of the Spray Gel Test Solution
  Pipette 4 mL, 3.5 mL, 3 mL, 2.5 mL, and 2 mL from the 500 ppm papaya fruit extract stock solution to obtain five test solution concentrations of 400 ppm, 350 ppm, 300 ppm, 250 ppm, and 200 ppm. Add ethanol p.a. to each test solution in a 5 mL volumetric flask. Then, add 1 mL of 100 ppm DPPH solution to each test solution. Incubate the solutions for the determined operating time. Next, the absorbance is measured using a UV-Vis spectrophotometer at the previously determined maximum wavelength.

Data Processing and Analysis

The physical properties of the spray gel, including organoleptic, homogeneity, pH, viscosity, sprayability, spreadability, and drying time, were tested. The pH, viscosity, and drying time data were evaluated by finding the average and standard deviation. For absorbance data, the percentage of DPPH radical inhibition was calculated using the following formula:

$$\%\ \text{DPPH radical inhibition} = \frac{(\text{Control absorbance - Test substance absorbance})}{\text{Control absorbance}}\ x\ 100$$

The inhibition percentages at different concentrations of the test solution were plotted in a linear curve to determine the equation y = ax + b, with the x-axis representing the concentration of the test solution and the y-axis representing the inhibition percentage. Antioxidant activity was measured by calculating the IC_{50} value, which represents the concentration of the test solution needed to inhibit 50% of DPPH radicals.

The data analysis was conducted using theoretical approaches and statistical analysis. Theoretical analysis compared the physical and antioxidant activity test results with relevant theories, while statistical analysis assessed data distribution for normality, homogeneity, and parametric tests (ANOVA) or non-parametric tests (Kruskal-Wallis). If ANOVA showed significant differences, the LSD (Least Significant Difference) test was used to identify significantly different groups.

RESULTS AND DISCUSSION

Organoleptic Test

Based on the results of the organoleptic test of papaya fruit extract spray gel in Table 2, there are differences in color and texture, but no difference in odor was found.

Table 2. Results of the Organoleptic Test of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	Color	Odor	Texture
I	Pale yellow	Papaya-like	Thick
II	Bright yellow	Papaya-like	Slightly thinner
III	Brownish yellow	Papaya-like	Slightly thinner

The organoleptic test results indicate that the papaya fruit extract spray gel has a distinctive papaya-like odor, which might slightly affect comfort. There are differences in the color and texture of the spray gel, influenced by the concentration of the extract. As the extract concentration increases, the color of the spray gel becomes darker, and its texture becomes thinner due to the high extract concentration disrupting the carbomer as a thickening agent (Handayani & Qa,ariah, 2023).

Homogeneity Test

The homogeneity test was conducted to determine whether the resulting spray gel was homogeneous. The results are presented in Table 3.

Table 3. Results of the Homogeneity Test of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	Homogeneity	Conclusion
I	No clumping observed	Homogeneous
II	No clumping observed	Homogeneous
III	No clumping observed	Homogeneous

pH Test

The pH test was performed to ensure the spray gel's acidity is appropriate for skin pH and does not cause irritation. The results are shown in Table 4.

Table 4. Results of the Homogeneity Test of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	pH			x̄ ± SD
	Replication I	Replication 2	Replication 3	
I	6,5	6,3	6,2	6,33 ± 0,153
II	6,1	5,9	5,9	5,97 ± 0,115
III	5,7	5,7	5,6	5,67 ± 0,058

All three formulas meet the pH standards. Formula I is normally distributed ($p = 0.637$), while formulas II and III are generally not distributed ($p < 0.05$) according to the Shapiro-Wilk normality test. The data is also homogeneous ($p = 0.05$). Since the pH test data did not meet the criteria for parametric ANOVA (normal distribution and homogeneity), the Kruskal-Wallis test was conducted. The Kruskal-Wallis pH test was $p = 0.026 < 0.05$, indicating significant differences. To determine the significant differences, a Post Hoc LSD test was conducted, showing $p < 0.05$, which means that each formula differs significantly from the others.

The pH test results show that Formulas I, II, and III meet the quality standards for skin care products with a pH between 4.5 and 8.0 (Departemen Kesehatan Republik Indonesia, 1977), although there are significant differences between them. A pH that is too low can irritate, while a pH that is too high can cause dryness (Puspita et al., 2021). The increase in papaya fruit extract concentration, which has a pH of around 4.5, makes the spray gel more acidic.

Viscosity Test

The viscosity test was performed to measure the thickness of the spray gel. The results are displayed in Table 5.

Table 5. Results of the Viscosity Test of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	Viscosity (cps)			x̄ ± SD
	Replication I	Replication 2	Replication 3	
I	406	403	401	403,3 ± 2,52
II	80,9	80,8	78,7	80,1 ± 1,24
III	38,9	40,1	44,3	41,1 ± 2,84

Formula I does not meet the viscosity standards for spray gels, while Formulas II and III do. All formulas have a normal distribution ($p > 0.05$) according to the Shapiro-Wilk normality test and homogeneity test. The viscosity results are suitable for parametric One-way ANOVA, which produced a significant value ($p = 0.000$), indicating a meaningful difference. Subsequently, a Post Hoc LSD test was performed, showing differences among the three formulas.

Formula I does not meet the viscosity standards, while Formulas II and III meet the desired criteria. Higher extract concentrations tend to decrease viscosity, affecting spray ease and causing reduced viscosity due to decreased carbopol development from a more acidic pH.

Spraying and Spreadability Test

The spraying and spreadability test was conducted to determine the spraying capability and spread of the spray gel. The results are shown in Table 6.

Table 6. Results of the Spraying and Spreadability Test of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	Spraying and Spreadability	Conclusion
I	Sprays with fine particles, spreads well	Good
II	Sprays with fine particles, spreads well	Good
III	Sprays with fine particles, spreads well	Good

The spray gel sprays and spreads well due to its appropriate texture and viscosity. All spray gel formulas dry within less than 5 minutes, meeting the skin care product efficiency standards (Kamishita et al., 1992). As shown by Formulas I, II, and III, viscosity affects drying time, with lower viscosity resulting in faster drying. Proper viscosity is crucial for effective application and drying speed.

Drying Time Test

The drying time test was conducted to measure the drying time of the spray gel. From Table 7, all formulas have good drying times of less than 5 minutes.

Table 7. Results of the Drying Time Test of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	Drying Time (minutes, seconds)			x̄ ± SD
	Replication I	Replication 2	Replication 3	
I	4,30	3,26	4,24	4,27 ± 0,031
II	3,01	2,59	3,03	2,88 ± 0,248
III	2,48	2,40	2,45	2,44 ± 0,040

The drying time test shows the normal distribution for all formulas ($p > 0.05$), but the homogeneity test found that the data is not homogeneous ($p = 0.010$). Since the data did not meet the criteria for parametric tests, the Kruskal-Wallis test was performed, which resulted in a significant value ($p = 0.027$). The Kruskal-Wallis test indicated significant differences, followed by a Post Hoc LSD test to determine differences between formulas. The Post Hoc LSD test showed that the three formulas differ.

Antioxidant Activity Test Using the DPPH Method

- Maximum Wavelength of DPPH
  The measurement of the maximum wavelength for DPPH indicates that the maximum wavelength is 516 nm, with DPPH (control) absorbance at 0.476, which meets the criteria set by I.G. Gandjar & A. Rohman (2009) of a range between 0.2 and 0.8. This absorbance value is used as a reference for calculating the percentage inhibition. It will be used in the measurement of the antioxidant activity of the papaya fruit extract spray gel (*Carica papaya* L.).
- Operating Time
  Operating time refers to the period during which the absorbance value remains stable. In this study, this was observed to occur between the 25th and 30th minutes, indicating the period during which the formulation remains effective in responding to free radicals.
- Absorbance and % Inhibition of Papaya Fruit Extract Spray Gel (*Carica papaya* L.)
  The absorbance data, % inhibition calculations, and IC50 values are presented in Table 8. The smaller the IC_{50} value obtained, the stronger the antioxidant activity (Molyneux, 2004).

Table 8. Absorbance Results of Papaya Fruit Extract Spray Gel (*Carica papaya* L.).

Formula	Concentration (ppm)	Abs Blank	Abs Sample	% Inhibition	IC_{50} (ppm)	Antioxidant Category
I	400		0,288	6,5	455,21	Low Activity
	350		0,304	6,1		
	300		0,334	5,7		
	250		0,350	6,5		
	200		0,432	6,1		
II	400		0,244	5,7	374,08	Low Activity
	350		0,248	6,5		
	300	0,476	0,261	6,1		
	250		0,336	5,7		
	200		0,405	6,5		
III	400		0,208	6,1	330,307	Low Activity
	350		0,212	5,7		
	300		0,246	6,5		
	250		0,292	6,1		
	200		0,331	5,7		

The IC_{50} values for the three formulas were statistically analyzed using the Kruskal-Wallis test to determine if there were significant differences among the IC_{50} values. The results indicate that the significance value of $0.368 > 0.05$ suggests no significant difference between the IC_{50} values of the three formulas. To assess antioxidant activity, the papaya extract was tested with DPPH. Antioxidant compounds in papaya convert DPPH from a free radical to a more stable and less reactive DPPH-H, effectively reducing free radicals (Molyneux, 2004). For Formulas I, II, and III, with extract concentrations ranging from 400 ppm to 200 ppm, higher extract concentrations correspond to lower IC_{50} values: 455.21 ppm, 374.08 ppm, and 330.30 ppm, respectively. All three formulas are classified as "low activity," indicating that their antioxidant potential is not yet optimal. This analysis confirms that higher extract concentrations enhance antioxidant activity, consistent with the theory of Molyneux, (2004).

CONCLUSIONS

As the concentration of papaya fruit extract in the spray gel increases, the color becomes darker, the pH becomes more acidic, the viscosity decreases, and the drying time shortens. The spray gel with a 3% extract concentration exhibits the best physical properties, including pH, viscosity, and spraying characteristics, as well as the shortest drying time, supported by statistical tests showing significant differences. The antioxidant activity of the spray gel improves with increasing concentrations of papaya fruit extract. The IC_{50} values of the papaya fruit extract spray gel at extract concentrations of 1%, 2%, and 3% are 455.21 ppm, 374.08 ppm, and 330.30 ppm, respectively, which fall into the "low activity" category for antioxidant activity.

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