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Research Article

Test Of *Anti-Acne* Activity In Gel Peel Off Mask Preparation Of Papaya Leaf (Carica Papaya L.) Extract On Rabbit Backs Induced With *Propionibacterium Acnes* Bacteria

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Abstract

Acne involves inflammation and *Propionibacterium acnes* infection. Papaya (*Carica papaya* L.) leaves, containing antibacterial karpain alkaloids and anti-inflammatory flavonoids, offer potential treatment. This study aimed to formulate and evaluate papaya leaf extract peel-off gel masks for anti-acne efficacy on P. acnes-induced rabbits. Extracts were obtained via maceration using 96% ethanol. Formulations containing 5%, 10%, and 15% extract were compared against clindamycin (positive control) and a negative control. Evaluations included physical quality (organoleptic, pH, viscosity, drying time, spreadability, adhesion), irritation, and antibacterial efficacy tests. Results indicated the 15% formulation was optimal, demonstrating homogeneity, pH 5-6, spreadability of 5 cm, drying time of 17 minutes, viscosity of 3167 cps, and adhesion of 6.22 seconds, without causing irritation. In vivo observations confirmed that the 15% concentration effectively accelerated acne healing and dried pus. Thus, papaya leaf extract peel-off masks exhibit significant anti-acne activity.

Keywords: (*Carica papaya* L.), *anti-acne*, *propionibacterium acnes*

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1 Introduction

Skin characteristics vary among individuals and are classified into four types: dry, normal, oily, and combination skin, based on water and oil content. Facial skin is highly susceptible to external substances, including anti-acne treatments. One of the most common facial health issues in adolescents is acne, which develops when follicular keratinization is disrupted and sebum production increases, causing an obstruction in sebum flow. Acne triggers include genetics, hormonal activity during the menstrual cycle, stress, overactive sebaceous glands, hygiene, diet, and cosmetic usage [1]. Furthermore, acne is caused by the bacterium *Propionibacterium acnes*, which contributes to comedo formation and inflammation stimulated by bacterial metabolic products [2].

Propionibacterium acnes is a normal flora of the skin, particularly on the face, and has a relatively slow growth rate. It plays a role in the pathogenesis of acne, leading to inflammation. Acne treatment can involve chemical antibiotics or traditional medicine utilizing papaya leaves (*Carica papaya* L.).

Papaya is a plant known for its antibacterial properties. Papaya leaf extract contains carpaine alkaloids and flavonoids that can inhibit peptidoglycan production in bacterial cells and lower the target organism's immunity. The chemical family known as carpaine alkaloids includes substances with antibacterial activity, the ability to poison protoplasm, damage and penetrate bacterial cell walls, and precipitate bacterial cell proteins. The mechanism of action for flavonoids functions as an inhibitor, preventing bacterial DNA replication and transcription. Flavonoids have the capability to disrupt bacterial cell walls and bind to extracellular proteins [3].

Aside from conventional acne treatments, chemical antibiotics such as clindamycin can also be utilized. Clindamycin is a lincosamide antibiotic used to treat severe bacterial infections by inhibiting bacterial reproduction. By preventing bacterial ribosomes from synthesizing proteins, clindamycin stops bacteria from building peptide chains. Since *Propionibacterium acnes* is inhibited and killed by clindamycin, it is effective as an acne treatment [4].

Papaya leaf extract has been formulated into a dosage form for ease of use, specifically as a peel-off gel mask. The peel-off mask format was selected because it provides better moisturization compared to other mask types, removes dead skin cells, and nourishes the face. A peel-off gel mask is a practical formulation that can be lifted directly after drying to remove impurities adhering to the skin surface.

2 Method

2.1 Tools (Instruments)

The instruments used in this study included an incubator, autoclave, mortar and pestle, scale, water bath, stirring rod, dropper pipette, spatula, porcelain dish, vortex mixer, centrifuge tube, analytical balance, glassware, viscometer, spreadability tester, and pH strips.

2.2 Materials

The sample material used was papaya leaves (*Carica papaya* L.) collected from Bayur Village, Raja Basa, Bandar Lampung City. The test subjects were white male local rabbits weighing 1.5–3 kg and aged 3–5 months. *Propionibacterium acnes* bacteria and Brain Heart Infusion (BHI) media were also utilized.

2.3 Preparation of Papaya Leaf Extract

The maceration extraction technique was employed using 96% ethanol as the solvent. For maceration, a ratio of 1:10 was used, involving soaking 500 grams of simplicia powder in 5,000 cc of solvent (Putri et al., 2020).

2.4 Determination of Moisture Content

The Sterling-Bidwell instrument was assembled. Then, 10 g of weighed papaya leaf powder was added to the distillation flask with xylene solvent until the powder was completely submerged. The

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moisture content percentage was calculated based on the volume indicated on the instrument scale using the formula:

$$\text{Moisture content} = \frac{\text{Volume of water}}{\text{sample of content}} \text{ X } 100\%$$

2.5 Formulation of Peel-Off Gel Mask Preparations

Table 1. Formulation of Papaya Leaf Extract Peel-Off Gel Masks

Material	Requirement (%)	Function	F1 (%)	F2 (%)	F3 (%)	K- (%)	K+ (%)
Papaya leaf extract	-	Active Ingredient	5	10	15	-	-
Clindamycin	-	Comparator	-	-	-	-	0.15
PVA	1 - 10	Gel Base	10	10	10	10	10
HPMC	2 - 4	Gel Base	1	1	1	1	1
Propylene glycol	1 - 15	Humectant	10	10	10	10	10
Methylparaben	0.02 - 0.3	Preservative	0.2	0.2	0.2	0.2	0.2
Ethanol 96%	-	Solvent	15	15	15	15	15
Distilled Water	-	Solvent	Ad 10 mL	Ad 10 mL	Ad 10 mL	Ad 10 mL	Ad 10 mL

- **Note: F1, F2, F3:** Extract Formulations; **K-:** Negative Control (Basis only); **K+:** Positive Control (Clindamycin)

2.6 Preparation of Peel-Off Gel Mask Dosage Form

Materials and equipment were prepared. Each component was weighed. First, PVA was prepared using hot distilled water and stirred in a hot mortar until fully expanded. Separately, HPMC was allowed to expand in a heated mortar with hot water, then added to the PVA mixture and stirred until homogeneous. Methylparaben was added and stirred until completely dissolved, followed by the gradual addition of propylparaben into the PVA and HPMC combination. Finally, glycerin was added and stirred until fully dissolved. Papaya leaf extract was added at concentrations of 5%, 10%, and 15%. The extract was first dissolved with sufficient hot water to form a slurry, then added to the mask base and stirred until homogeneously mixed [3].

2.7 Identification of Papaya Leaf Extract Compounds

2.7.1 Alkaloid Identification

To test the filtrate, one or two drops of Mayer's reagent were added to 0.5 grams of the material, which was dissolved in ethanol, acidified with drops of HCl, and filtered. A positive reaction is indicated by the presence of a white or yellowish precipitate upon addition of Mayer's reagent [4].

2.7.2 Flavonoid Identification

The presence of flavonoids is indicated if an orange, red, or yellow color appears after adding 3 drops of concentrated HCl to 0.5 grams of the sample, following the addition of 2 mg of magnesium powder.

2.7.3 Tannin Identification

1 gram of papaya leaf powder and extract was dissolved in 100 mL of hot water, cooled, and filtered for the tannin test. Tannins are declared positive if the reaction with 1% FeCl3 reagent produces a blackish-blue color in the test tube containing 5 mL of filtrate.

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2.8 Physical Quality Evaluation of Peel-Off Gel Mask Preparations

2.8.1 Organoleptic Test

The color, aroma, and texture of the papaya leaf extract peel-off gel mask were evaluated as part of the organoleptic test [3].

2.8.2 Homogeneity Test

The homogeneity test on the peel-off gel mask formulation was used to determine if the ingredients were evenly mixed. The test was conducted by placing 0.1 grams of the preparation on transparent glass to observe for any unmixed portions. Homogeneity is indicated by the absence of lumps or coarse granules [3].

2.8.3 pH Test

A pH meter was used to measure the pH of the peel-off gel mask. The pH meter electrode was dipped into the preparation for measurement. The monitor displayed the pH findings. The formulation met the skin pH requirements of SNI 16-4399-1996, within the range of 4.5 to 8.0. The skin feels slippery and dry if the preparation is alkaline (above skin pH range), whereas skin irritation may occur if the preparation is acidic (below skin pH range) [3].

2.8.4 Spreadability Test

A 20x20 cm glass plate was carefully covered with 1 gram of gel. After adding a 125-gram load and covering it with another glass plate, the diameter was measured after 1 minute. The requirement for topical medication is a spreadability diameter of 5-7 cm [3].

2.8.5 Drying Time Test

A layer of 0.7 grams with a thickness of 1 mm was applied to a glass slide. It was left to dry until it could be peeled off. The time required was determined, with the requirement that the preparation dries in no more than 30 minutes [3].

2.8.6 Viscosity Test

Using a Brookfield Viscometer at room temperature, the viscosity of the gel preparation was measured using a volume of 100 mL in a 250 mL beaker. After the spindle was submerged in the gel, the viscosity result of the peel-off mask could be viewed on the viscometer monitor [5].

2.8.7 Adhesion Test

Applying 1 g of the peel-off gel mask to the skin of the arm and waiting for it to dry served as the adhesion test. A stopwatch was used to calculate how long the dried mask took to completely detach. The adhesive strength of a properly prepared mask is greater than 4 seconds [6].

2.8.8 Irritation Test

The irritation test was conducted by applying the preparation for approximately 15 minutes to the inner arm of 10 different respondents, and any irritation reaction was observed [7].

2.9 Preparation of Bacterial Suspension

Using the McFarland standard, a *Propionibacterium acnes* suspension was prepared with concentrations of *Propionibacterium acnes* isolates were cultured on Brain Heart Infusion (BHI) media and incubated in a jar (designed for anaerobic conditions) for 24 hours. The bacteria were then centrifuged for 15 minutes at 5000 rpm using BHI media. The sediment was mixed with 10 mL of 0.9% NaCl, homogenized, and centrifuged again for 15 minutes at 5000 rpm. The sediment was re-dissolved with 10 mL of 0.9% NaCl. This process was repeated up to three times. Furthermore, the bacterial sediment was dispersed in physiological NaCl solution meeting McFarland turbidity standards [8].

2.10 Antibacterial Activity Testing

The time required for the *Propionibacterium acnes* infection on the rabbit's back skin to heal after receiving the peel-off gel mask was used to test the healing rate. The peel-off gel mask was applied twice daily over a period of 14 days.

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2.11 Data Analysis

In this study, the one-way ANOVA test was used for data processing and analysis. One-way ANOVA was employed to compare the antibacterial efficacy of papaya leaf extract against *Propionibacterium acnes* at each dose, utilizing visual criteria of erythema, pus, drying, and healing [9]. Subsequently, a Post Hoc test was performed to determine if there were significant differences between the groups.

3 Result and Discussion

3.1 Results of Papaya Leaf Extraction

Maseration was employed to extract the papaya leaves. To prepare the papaya leaf extract using 96% ethanol solvent, 500 grams of papaya leaf powder were weighed and placed into a maceration bottle with 5,000 mL of 96% ethanol. The mixture was left for 5 days with intermittent stirring. The resulting extract was filtered through a flannel cloth and then concentrated at 50°C using a rotary evaporator until a thick extract was obtained. Table 2 presents the results of the papaya leaf extraction.

Based on the results shown in Table 2, 500 g of papaya leaf powder macerated with 96% ethanol yielded 67 g of thick extract, resulting in a yield of 13.4%. This indicates that a higher yield value corresponds to a greater amount of extract produced.

Table 2. Yield Percentage of Papaya Leaf Extract

Powder Weight (g)	Extract Weight (g)	Yield (% w/w)
Papaya Leaf 500 g	67 g	13.4%

3.2 Results of Moisture Content Determination

To determine the moisture content, 10 grams of papaya leaf powder were placed into a distillation flask and submerged in xylene solvent. The Sterling-Bidwell apparatus was assembled to determine the moisture content based on the volume indicated on the instrument's scale. The results of the moisture content determination are shown in Table 3.

Table 3. Moisture Content Determination Results

Initial Weight (g)	Final Weight (g)	Yield (% w/w)
Papaya Leaf 500 g*	9.7 g	97%

**Note: The text description mentions 10 g, while the table lists 500 g. The calculation follows the text context.*

Based on the results in Table 3, the moisture content of the simplicia was measured to determine the amount of water contained within it. Standard requirements indicate that simplicia must have a moisture content of less than 10%. However, the findings of this study indicate that the moisture content of the papaya leaves was 97%, which falls within the range obtained in this specific observation.

3.3 Results of Phytochemical Identification of Papaya Leaf Extract

Chemical analysis was conducted to determine the chemical composition of the papaya leaf extract. Table 4 presents the findings of the extract identification.

Table 4. Phytochemical Identification Results of Papaya Leaf Extract

Chemical Constituent	Reagent	Result	Conclusion
Alkaloids	Conc. HCl + 2 drops Mayer	White precipitate formed	+
Flavonoids	Mg + 3 drops conc. HCl	Yellow precipitate formed	+
Tannins	Hot water + 3 drops 1% FeCl3	Blackish-blue color formed	+

Note: (+) = contains compound; (-) = does not contain compound.

Based on the findings in Table 4, the study identified the chemical composition of the papaya leaf extract. First, the extract tested positive for alkaloids, indicated by the formation of a white precipitate

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using concentrated HCl and Mayer's reagent. Second, it tested positive for flavonoids, indicated by a yellow precipitate using Mg and concentrated HCl. Third, it tested positive for tannins.

3.4 Results of Papaya Leaf Extract (*Carica papaya* L.) Peel-Off Gel Mask Preparation

3.4.1 Organoleptic Test of Peel-Off Mask

The smell, color, and form of the papaya leaf extract peel-off mask were observed. Table 5 presents the findings for organoleptic observation.

(Note: Table 5 data was not fully provided in the source text, but the description follows).

Peel-off gel mask preparations were produced based on the findings in Table 6 (referenced in text), derived from Formula I (positive control/clindamycin), Formula II (negative control), Formula III (5% concentration), Formula IV (10% concentration), and Formula V (15% concentration). Formula I and Formula II had different colors and smells; specifically, Formula I smelled like soap, whereas Formula II was odorless. Formula III (5%), Formula IV (10%), and Formula V (15%) had different color intensities due to the extract concentration but shared the same aroma and form. The variation between the five formulas was caused by the concentration of papaya leaf extract.

3.4.2 Homogeneity Test of Peel-Off Mask

This test aimed to determine the homogeneity of the peel-off mask. The results of the homogeneity test for the five formulas indicated that no coarse particles were present in the gel preparations. The test results showed that every peel-off mask formula applied to a glass plate demonstrated homogeneous results across Replications 1 to 3.

3.4.3 pH Test of Peel-Off Mask

The pH test was conducted by dipping a pH stick into the papaya leaf extract peel-off mask preparation; the color change on the stick indicated the pH value. Table 7 displays the pH test results for the five formulas.

Table 7. pH Test Results of Papaya Leaf Extract Peel-Off Mask

Formula	pH Test Replication 1	pH Test Replication 2	pH Test Replication 3
Formula I	6	6	6
Formula II	5	5	5
Formula III	5	5	5
Formula IV	5	5	5
Formula V	6	6	6

Based on Table 7, the pH values of the five formulas ranged from 5 to 6. This falls within the topical preparation pH range suitable for skin (4.5 to 6.5). Preparations that are too alkaline can cause dry skin, while preparations that are too acidic may cause skin irritation.

3.4.4 Spreadability Test

A round glass with a diameter of 15 cm was used for the spreadability test. Another glass was placed on top of the mask and left for 1 minute. After measuring the spread diameter, a 100-gram load was added and left for one minute before measuring again. Table 8 displays the spreadability test observations.

Table 8. Spreadability Test Results

Formula	Rep 1 (cm)	Rep 2 (cm)	Rep 3 (cm)	Average ± SD
Formula I	5.0	5.0	5.1	5.003 ± 0.0557
Formula II	5.6	5.7	5.8	5.7 ± 0.1
Formula III	5.5	5.7	5.8	5.667 ± 0.1528
Formula IV	5.6	5.5	5.4	5.5 ± 0.1
Formula V	5.4	5.5	5.7	5.533 ± 0.1528

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Based on Table 8, the spreadability results were: Formula I (5.0 cm), Formula III (5.6 cm), Formula IV (5.5 cm), and Formula V (5.5 cm). It was determined that spreadability can be influenced by different extract concentrations. However, in this study, the average spreadability was approximately 5 cm. A decrease in spreadability is caused by an increase in molecular unit size due to solvent absorption, which retains liquid and increases flow resistance. All spreadability results fell within the 5–7 cm range required for topical treatments, ensuring ease of application.

3.4.5 Drying Time Test

The drying time test aimed to determine how long the preparation takes to dry on the skin surface. Table 9 displays the results.

Table 9. Drying Time Test Results

Formula	Rep 1 (min)	Rep 2 (min)	Rep 3 (min)	Average ± SD
Formula I	18	18	19	18.33 ± 0.577
Formula II	17	18	17	17.33 ± 0.577
Formula III	18	17	17	17.33 ± 0.577
Formula IV	16	16	17	16.33 ± 0.577
Formula V	15	15	16	15.33 ± 0.577

Based on Table 9, Formula V dried faster compared to Formula I, II, III, and IV. Formula V (15% concentration) had the fastest drying time (15.33 min). The results indicate that the peel-off masks met the criteria of drying in no more than 30 minutes.

3.4.6 Viscosity Test Results

Testing was performed using a viscometer with spindle 64 at 12 RPM. Table 10 shows the viscosity results.

Table 10. Viscosity Test Results

Formula	Rep 1 (cps)	Rep 2 (cps)	Rep 3 (cps)	Average ± SD
Formula I	5,050	5,100	5,150	5,100 ± 5
Formula II	3,060	3,200	3,240	3,167 ± 94.52
Formula III	2,570	2,590	2,620	2,593 ± 25.17
Formula IV	2,680	2,700	2,730	2,703 ± 25.17
Formula V	2,750	2,800	2,820	2,790 ± 35.16

Based on Table 10, viscosity values fluctuated with concentration. Formula I (5,100 cps), Formula II (3,167 cps), Formula III (2,593 cps), Formula IV (2,703 cps), and Formula V (2,790 cps). The masks were categorized as stable and met the requirements of SNI 16-4399-1996, which specifies a range of 2,000 to 50,000 cps.

3.4.7 Adhesion Test

The adhesion test aimed to determine how long the peel-off gel mask adheres to the skin.

Table 11. Adhesion Test Results

Formula	Rep 1 (sec)	Rep 2 (sec)	Rep 3 (sec)	Average ± SD
Formula I	6.18	6.24	6.30	6.22 ± 0.034
Formula II	4.65	4.71	4.77	4.71 ± 0.06
Formula III	4.58	4.62	4.66	4.62 ± 0.04
Formula IV	7.18	7.22	7.26	7.22 ± 0.04
Formula V	4.60	4.64	4.69	4.64 ± 0.045

Table 11 shows that Formula IV (10%) had the longest adhesion time (7.22 seconds), while Formula I was 6.22 seconds. Formula V (15%) had an adhesion time of 4.64 seconds. Variations in adhesion were influenced by the different concentrations used.

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3.4.8 Irritation Test

The irritation test aimed to observe skin reactions (redness and itching) on 10 volunteers after application. Table 12 shows the results.

Table 12. Irritation Test Results

Formula	Vol 1	Vol 2	Vol 3	Vol 4	Vol 5	Vol 6	Vol 7	Vol 8	Vol 9	Vol 10
F1 to F5	-	-	-	-	-	-	-	-	-	-

(Note: Table condensed for clarity; all results were Negative).

Legend: (+) Irritation occurred; (-) No irritation.

Based on Table 12, all five peel-off gel mask formulas showed no signs of irritation. They are considered safe for use, as the pH falls within the skin's pH range of 4.5–8.

3.4.9 Antibacterial Activity Test Results

Healing time observation was conducted by applying the mask twice daily (morning and afternoon) for 14 days on rabbit back skin. The results are shown in Table 13 [1].

Table 13. Antibacterial Activity Observation Results (Infection Observation)

Formula	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
F I	+	n	n	n	n	k	k	s	s	k	k	s	s
F II	+	n	n	n	n	n	n	n	n	n	k	k	s
F III	+	n	n	n	n	n	n	n	n	k	k	s	s
F IV	+	n	n	n	n	n	k	k	s	s	k	s	s
F V	+	n	n	k	k	s	s	s	s	s	s	s	s

Legend: (+): Erythema; (n): Pus; (k): Dry; (s): Healed.

Based on Table 13, the antibacterial activity test showed that the peel-off mask preparations were able to prevent the growth of the test bacteria, marked by the healing of the infection from suppuration to drying of the wound on the rabbit's back. Comparison of effective antibacterial activity among concentrations of 5%, 10%, and 15% showed that the 15% papaya leaf extract (Formula V) was the most effective, demonstrating a faster healing time. The higher the flavonoid content in the peel-off gel mask, the higher the antibacterial effect. Concentrations of 5-10% were less effective, likely because the active ingredient concentration was too low to release sufficient active compounds from the peel-off base.

The peel-off mask applied to the rabbit's back eliminated acne, evidenced by the disappearance of pus formation. Statistical analysis of antibacterial activity showed homogeneity with a significance value of 0.001 (>0.05). One-way ANOVA results indicated significant differences between treatment groups at a significance level of 0.00 (<0.05), followed by the LSD (Least Significant Difference) test. The probability value for the 15% concentration (Formula V) and positive control (Formula I) from day 2 to day 14 had a significance value of <0.05, indicating a significant difference in improvement.

4 Conclusion

Based on the study conducted on papaya leaves (*Carica papaya* L.), the following conclusions can be drawn:

1) The peel-off gel mask containing papaya leaf extract (*Carica papaya* L.) at a concentration of 15% is effective as an anti-acne treatment.

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2) Papaya leaf extract (*Carica papaya* L.) exhibits anti-acne properties when applied to rabbits with acne induced by *Propionibacterium acnes* on the dorsal area.

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