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IN VITRO ANTIBACTERIAL ACTIVITY OF CARICA PAPAYA LEAF EXTRACT-LOADED ACNE PATCHES AGAINST *Propionibacterium acnes*

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ABSTRACT

Acne vulgaris is a prevalent skin condition that is characterized by inflammatory lesions. Propionibacterium acnes is a key bacterium implicated in its pathogenesis. This study aimed to investigate the antibacterial potential of *Carica papaya* leaf extract against *P. acnes* and its efficacy when formulated into acne patches. Ethanolic extracts of *C. papaya* leaves were prepared at concentrations of 20%, 25%, and 30%. These extracts were incorporated into acne patch formulations and evaluated for antibacterial activity using the paper disc diffusion method. Clindamycin 1% was used as a positive control and 70% ethanol was used as a negative control. The results demonstrated that all concentrations of the *C. papaya* leaf extract exhibited significant antibacterial activity against *P. acnes*. The 30% concentration demonstrated the largest zone of inhibition, which was comparable to that of the positive control. These findings suggest that *C. papaya* leaf extract-based acne patches are promising natural alternatives for the treatment of acne vulgaris.

Keywords: Papaya leaves (*Carica papaya* L), Propionibacterium acne, Acne patch, Inhibitory power

INTRODUCTION

Studies in Southeast Asia show that the prevalence of acne is 40-80%, with the 2017 PERDOSKI data mentioning acne as the third most common skin disease in Indonesian clinics and hospitals. At the age of 14-17 years, the prevalence is very high, at 83-85% in women and 95-100% in men (Yusuf *et al.* 2020). Acne vulgaris damages the self-confidence of every individual, especially adolescents. This is very disruptive to one's appearance. Acne vulgaris, characterized by follicular hyperkeratinization, increased sebum production, and colonization by *Propionibacterium acnes*, is a prevalent dermatological condition. Reducing the population of *P. acnes* or its metabolic by products is a common therapeutic strategy for acne treatment (Sifatullah *et al*., 2021).

The increasing prevalence of antibiotic resistance has prompted the search for alternative therapies including natural products with antibacterial properties. Numerous studies have demonstrated the presence of flavonoids, tannins, saponins, and alkaloids in the ethanolic extracts of papaya leaves. These phytoconstituents have been shown to inhibit the growth of *Propionibacterium acnes*, suggesting their potential as natural remedies for acne. (Nor, 2018).

Papaya leaf extracts contain antimicrobial components, including flavonoids, alkaloids, and tannins. The membrane is damaged by lipophilic flavonoids, which increase permeability and interfere with bacterial metabolism (Baskaran *et al.*, 2012; Prasetya *et al.*, 2018). Papaya leaf extract (*Carica papaya* L.) has been shown to inhibit the growth of

Propionibacterium acnes colonies, as evidenced by an increased zone of inhibition. A study by Veronica *et al*. (2023) reported zones of inhibition diameters of 13.2 mm, 21.6 mm, and 25 mm at concentrations of 30%, 40%, and 50%, respectively.

Topical preparations are the most attractive choice for dermatological therapy because of their local application, which allows for direct penetration and absorption into the skin. Among the topical formulations, patches represent an innovative approach that enhances safety, compliance, and patient comfort. Additionally, patches offer the advantages of occluding acne lesions, preventing bacterial spread, and minimizing contamination (Yulianti *et al*., 2021).

RESEARCH METHODS

Equipment and Materials

Oven (Memmert), micropipette, Petri dishes (Pyrex), autoclave (Tommy), Erlenmeyer flask, incubator (Memmert), caliper (Krisbow), pH meter (Mettler Toledo-F20), thermometer, electronic balance (Acis AD-300i), and a percolator. The materials used were papaya leaves and 70% v/v analytical grade ethanol (CV. Mustika Lab), *Propionibacterium acnes* bacteria, 150 mg clindamycin capsules (Proteknis), nutrient agar media (Oxoid), acetic acid, sulfuric acid, potassium dichromate, hydrochloric acid, zinc powder (Zn), magnesium powder, concentrated hydrochloric acid (CV. Mustika Lab), ferric chloride ($FeCl_3$) (CV. Mustika Lab), distilled water (CV. Brataco Indonesia), hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), propylene glycol (CV. Mustika Lab), and PEG 400.

Research Procedure

1. **Plant Determination**

   The determination and identification of papaya leaves (*Carica papaya* L.) was conducted at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung.

2. **Preparation of Percolation Method Extract**

   The study utilized papaya leaves (*Carica papaya* L.) were used as the plant material. Extraction was performed using the percolation method with 70% ethanol as the solvent.

3. **Phytochemical screening**

   a. Flavonoid

      0.3 grams of the extract was added to 20 ml of methanol-water (18:2) and heated to boiling. The solution was filtered under hot conditions and concentrated until the volume reached one-third, after which a small amount of magnesium metal and 1–2 drops of concentrated hydrochloric acid were added if an orange color was formed. It contains flavonoids (Indriaty *et al*., 2023).

   b. Alkaloid

      0.5 grams of the extract was placed in a test tube and 2 mL of chloroform and 2 mL of ammonia were added. The mixture was shaken and 2N HCl was added. After filtration, the clear portion was divided into three parts and placed in separate test tubes. Dragendorff's reagent, Mayer's reagent, and Wagner's reagent were added to each tube, respectively. The formation of a red or orange precipitate with Dragendorff's reagent, a white precipitate with Mayer's reagent, and a brown precipitate with Wagner's reagent indicate a positive test for alkaloids (Kursia *et al*., 2016).

   c. Tanin

      The extract (0.5 g) was added to 10 mL distilled water. The filtrate was then filtered and transferred to a test tube. Three drops of $FeCl_3$ were added. A dark green or bluish-black color indicates a positive result (Nor, 2018).

d. Saponin

The extract (0.5 g) was mixed with 10 mL warm or hot water. The mixture was shaken vigorously and observed for foam stability for 5–10 minutes (Kursia *et al.*, 2016).

4. Acne Patch Formulation

The formulation used in this study is presented in **Table I**.

Table I. Acne Patch Formula of Papaya Leaf Ethanol Extract (*Carica papaya* L)

	Function	Formula			
		F0	F1	F2	F3
Papaya leaf ethanol extract	Active ingredient	-	20%	25%	30%
HPMC	Polymer	1,2 g	1,2 g	1,2 g	1,2 g
PVP	Polymer	0,4 g	0,4 g	0, 4 g	0,4 g
Propylene Glycol	Enhancer	1 mL	1 mL	1 mL	1 mL
PEG 400	Plasticizer	2 mL	2 mL	2mL	2 mL
70% Ethanol	Solvent	Ad 20 mL	Ad 20 mL	Ad 20 mL	Ad 20 mL
Papaya leaf ethanol extract	Active ingredient	-	20%	25%	30%

Description
F0: Control formulation (without extract)
F1: Formulation containing 20% extract (w/v)
F2: Formulation containing 25% extract (w/v)
F3: Formulation containing 30% extract (w/v)

5. Acne Patch Preparation

HPMC was dissolved in 4 mL of distilled water and PVP was dissolved in 2 mL of distilled water. The two dissolved solutions were then combined. The papaya leaf extract, previously dispersed in 70% ethanol, was added to the polymer mixture. The mixture was stirred until homogeneous, followed by addition of propylene glycol and PEG 400. Ethanol 70% was added to bring the total volume to 20 mL, and the mixture was stirred until it was homogeneous. The mixture was then transferred to a 5 cm diameter Petri dish and placed in an oven at 40°C until dry. Once dry, the patch was removed from the Petri dish.

6. Evaluation of Acne Patch Preparation

a. Organoleptic Test

The organoleptic evaluation consisted of visual assessment of the odor, color, and surface characteristics of the patch.

b. pH Test

The pH was measured using a digital pH meter. Gel (1 g) was dissolved in distilled water to a final volume of 10 mL. The electrode on the pH meter was first washed with distilled water, then calibrated on a standard solution of pH 4 and pH 7, and measurements were taken. pH should be by the pH of the skin, which is 4.5-6.5 (Yulita *et al*., 2019).

c. Weight Uniformity Test

A random sample of three patches from each formulation was weighed. The mean weight of patches in each group was determined (Nitiariksa & Iskandar, 2021).

d. Patch Thickness Test

Calipers were used to measure the thickness of the fabricated patches. The results indicated that the thickness of all patches was below the specified 1 mm limit, satisfying the predefined thickness criterion (Arifin & Ibrahim, 2018).

7. Antibacterial activity test

a. Preliminary preparations

1) Equipment sterilization

The instruments to be sterilized were plugged with cotton wool, wrapped in parchment paper, and tied with a cotton thread. The beakers were simply covered with parchment paper and tied with a cotton thread. All items were then placed in an autoclave to allow for the free circulation of steam. Sterilization was performed at 121°C for 15 minutes to ensure a constant temperature throughout the process.

2) Preparation of positive and negative control solutions

In this study, 70% ethanol was used as a negative control. The use of 70% ethanol as a negative control was aimed at determining whether the solvent itself did not possess antibacterial activity against the test solution used.

3) Preparation of nutrient agar media

Nutrient agar (1.8 g) was weighed. 1.6 g was used for three Petri dishes and 0.2 g for slant agar (for bacterial rejuvenation). The agar was dissolved in 90 mL of distilled water (10 mL for slant agar and 80 mL for 3 Petri dishes) in an Erlenmeyer flask. The media was then sterilized in an autoclave at 121°C for 15 minutes (Nor, 2018).

4) Bacteril replating

10 mL of sterilized agar medium was transferred to a test tube. The tube was then incubated at room temperature (30–45°C) at a 30° angle to solidify. Once solid, a loopful of Propionibacterium acnes was aseptically transferred to the slant agar using a sterile inoculating loop and streaked in a zigzag pattern

5) Preparation of bacterial suspensions

Using a sterile loop, a sample of the test bacteria was aseptically transferred to a tube containing 10 mL of 0.9% NaCl solution. The suspension was mixed until the turbidity matched the McFarland standard (Rizki *et al*., 2021).

b. Antibacterial activity testing

A 1 mL suspension of Propionibacterium acnes was added to 80 mL of sterile nutrient agar. This mixture was then poured into Petri dishes in 20 mL aliquots and allowed to solidify. Three acne patches with concentrations of 20%, 25%, and 30%, along with a positive control of 0.01% clindamycin using pre-printed paper disks, and a negative control of 70% ethanol using paper disks, were placed on Petri dishes using sterile forceps. The procedure was conducted aseptically and the media were incubated at 37°C for 18–24 hours (Nor, 2018).

c. Measurement of inhibition zone

The zone of inhibition was measured using calipers. If a clear zone appeared around the paper disk or acne patch, measurements were taken in the vertical, horizontal, and diagonal directions and the average was calculated. The results are expressed as the diameter (mm).

Data analysis

The results obtained in this study were both descriptive and quantitative. Descriptive data were derived from the organoleptic evaluation tests of the prepared acne patch formulations. Quantitative data from the antibacterial activity test were analyzed using SPSS version 23, which included normality (Shapiro-Wilk or Kolmogorov-Smirnov) and homogeneity tests (Levene's test). If the data were normally distributed and homogeneous, one-way analysis of variance (ANOVA) was performed. If the data were not normally distributed or homogeneous, the Kruskal-Wallis test was performed, followed by the Mann-Whitney U test.

RESULTS AND DISCUSSION

1. Determination

Based on the determination results, No. 56/HB/12/2023, it was confirmed that the plant used was indeed *Carica papaya* L. A total of 500 g of dried papaya leaf powder (*Carica papaya* L) was extracted using the percolation method with 70% ethanol as the solvent. with a yield of 13.53%. The percolation method was chosen because it does not involve heating, thus preventing the degradation of the active compounds and facilitating their identification.

2. Phytochemical Screening

The obtained papaya leaf extract was subsequently subjected to phytochemical screening to identify its constituent compounds through colorimetric reactions. The positive results of the secondary metabolite tests of the papaya leaf extract (*Carica papaya* L) are presented in **Table II**.

Table II. Phytochemical Screening Test Results

Test	Reagents	literature	Results	Description
Flavonoid	Mg^{+} Concentrated HCl	Orange	Orange	Positive
Alkaloid	a. Mayer b. Dragendorf	a. white precipitate b. Orange to red brown precipitate	a. brown b. Red-brown precipitate	a. negative b. positive
Tanin	$FeCl_3$	blackish-brown	blackish-brown	Positive
Saponin	Warm distilled water	Foam stable for less than 10 minutes	Foam stable for less than 10 minutes	Positive

On alkaloid testing using Mayer's reagent, the results are not in accordance with the literature. Although the Dragendorff test can indicate the presence of alkaloids, negative results in other screening tests do not necessarily mean that the sample is completely negative for alkaloids. Factors such as low levels of alkaloids, different types of alkaloids, sample matrix interference, and procedural errors may affect the test results.

3. Evaluation of Acne Patch Preparations

In this study, organoleptic evaluation utilized human senses as the primary parameter to observe the form, color, and odor of acne patch preparations. The organoleptic results obtained from these preparations showed differences in color and form between the base and the three variations. These differences were caused by the addition of the extract and the varying concentrations of the dark green-black ethanolic papaya leaf extract, which was mixed with other additives, compared to the white base without papaya leaf extract. The base appeared white, formula I had a brownish-green color, formula II had a dark green color, while formula III had a very dark greenish color in **Figure 1**. These color changes were due to the different concentrations of extract added to each formula. It can be concluded that the higher the extract concentration, the darker is the color.

Table III. Acne Patch Evaluation Results

Organoleptic Testing	Base	Formula I	Formula II	Formula III
Form	Patch	Patch	Patch	Patch
Color	white	brownish-green	dark green	blackish green
Odor	distinctive smell	distinctive smell	distinctive smell	distinctive smell
pH	6,04±0,15	5,75±0,01	5,76±0,02	5,76±0,02
Patch thickness	1±0 mm	1±0 mm	1±0 mm	1±0 mm
Weight Uniformity	22,7±0,2 mg	24,6±0,1mg	24,5±0,1mg	24,6±0,1mg

Figure 1. Acne patch papaya leaf extract

A pH meter was used to determine whether the prepared acne patch met the standard pH range of 4.5-6.5, which is compatible with the human skin pH. Differences in pH values among the formulations could be attributed to the addition of the active compound, ethanolic papaya leaf extract. If the pH is too acidic, it can irritate the skin, while an excessively alkaline pH can cause skin scaling. The results were obtained by weighing three patches of each formula, including the base, using an analytical balance. The weight uniformity of the formulas was higher than that of the base, as shown in

Table III. *This difference was due to the addition of the active ingredients.*

4. Antibacterial activity test

The antibacterial activity test results of the ethanolic papaya leaf extract showed moderate to very strong antibacterial activity. The concentration of the extract influenced the size of the inhibition zone formed; the higher the concentration, the higher the content of the active compounds in **Table IV**. The effectiveness of an antibacterial agent in inhibiting bacterial growth depends on the properties of the test bacteria, such as concentration and contact time. An inhibition zone diameter <5 mm indicated weak inhibition, 5-10 mm indicates moderate inhibition, 11-20 mm indicates strong inhibition, and > 20 mm indicated very strong inhibition (Kumowal *et al.*, 2019).

Table IV. Antibacterial Activity Test Results of Acne Patch

Groups	Inhibition zone (mm)
	P. acnes
Formula I	10.83 ± 0.47*#
Formula II	11.40 ± 0.52*#
Formula III	11.83 ± 0.40*#
Positive control	16.60 ± 0.36*
Negative control	0.00 ± 0.00

*p<0,05 against the negative control;
#p>0.05 group of formula II & III againts formula I

The positive control uses clindamycin 0.01% and shows an inhibition zone with an average of 16.6 mm, which indicates that the value is included in the strong category, so that the positive control has antibacterial activity against *Propionibacterium acne* bacteria. The acne patch concentration of 20% was included in the medium category because the average diameter was 10.83 mm, which means that the acne patch with this concentration has antibacterial activity against *Propionibacterium acne*. The 25% acne patch concentration was included in the strong group because the average diameter was 11.4 mm, which means that the acne patch with this concentration has antibacterial activity against *Propionibacterium acne* bacteria. The 30% acne patch concentration was included in the strong group because the average diameter was 11.83 mm, which means that the acne patch with this concentration has antibacterial activity against *Propionibacterium acne* bacteria. The inhibition zone results for all negative controls showed an average inhibition zone of 0 mm. This indicates that the antibacterial activity produced was due to the activity of the papaya leaf extract.

The results of the OneWay ANOVA statistical test in **Table IV** followed by the LSD Post Hoc test for all formulas resulted in significant differences in value, meaning that there were differences in the diameter of the inhibition zone between Formulas I, II,III, and 0.01% clindamycin because the significance value was <0.05. A one-way ANOVA statistical test showed a significant difference between all formulas and the positive control clindamycin 0.01%, with a significance value of 0.00 (p<0.05). However, formula I did not show any significant difference from formulas II and III. This means that the different concentrations of each formula provide significant similarities to the inhibition of *Propionibacterium acne* bacteria. Inhibition was characterized by the presence of a clear zone around the patch, as shown in **Figure 2**.

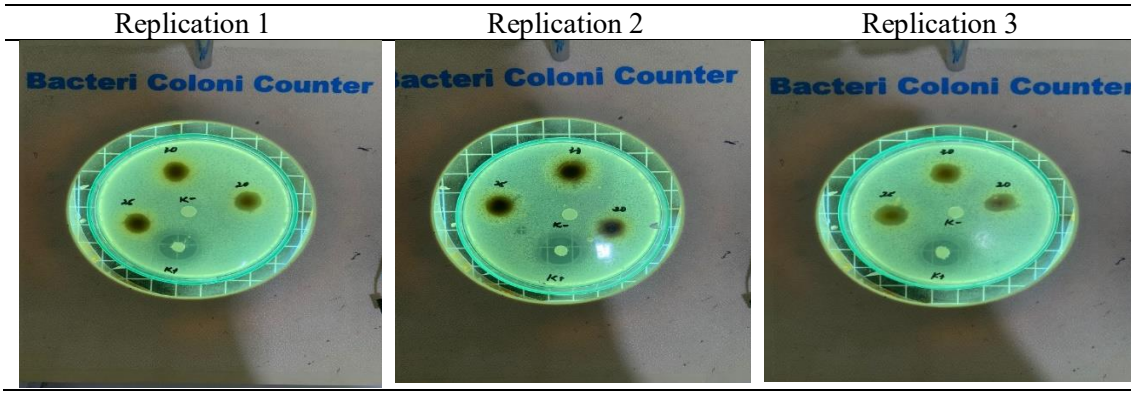

Figure 2. Results of acne patch inhibition test of papaya leaf extract

The inhibitory activity of the ethanolic papaya leaf extract against the growth of Propionibacterium acnes was due to the presence of phytochemically active compounds. The active compounds include flavonoids, tannins, alkaloids, and saponins. Flavonoids

can interact with bacterial DNA. Differences in the polarity of DNA, lipids, and alcohol groups in flavonoids can cause reactions that damage the DNA, leading to bacterial cell rupture (Arifin & Ibrahim, 2018). Alkaloid compounds is (bactericides), which act as antibacterial agents by killing cells. Alkaloids can disrupt the peptidoglycan arrangement in bacterial cells, such that the cell wall is not perfectly formed. Tannin compounds have antibacterial functions that prevent bacterial cells from inhibiting the synthesis of topoisomerase DNA by reverse enzymes. DNA is the target of anticancer and antibacterial drugs, and one of the important factors in the mechanism of cutting DNA strands by topoisomerase I is the volume of the central cavity that can make DNA double strands. Saponin compounds have a role as antibacterials that can increase the permeability of the bacterial membrane, and then die due to the rupture of the bacterial cell wall, and the cell will release components (proteins, nucleic acids, nucleotides, etc.)

CONCLUSION

The ethanol extract of papaya leaves (*Carica papaya* L) was formulated into acne patch preparations at concentrations of 20%, 25%, and 30%. Furthermore, the results of the preparation evaluation, which included organoleptic, pH, patch thickness, and weight uniformity tests, showed that the acne patch preparation from ethanol extract of papaya (*Carica papaya* L) leaves at the three concentrations met the specified evaluation requirements. Furthermore, acne patch preparations also showed antibacterial activity against Propionibacterium acnes, with moderate to strong activity categories.

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