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Evaluation Of Papaya Latex Protease Activity (Carica Papaya L.) Against Propionibacterium Acnes And Its Formulation In Peel-Off Gel Mask

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Abstract:
Background:
Papaya sap contains protease enzymes which are quite high compared to other chemical contents. Protease hydrolyzes protein peptide bonds into oligopeptides and amino acids so that it can break down dead skin cells, help clean necrotic tissue on the skin surface, then slowly stimulate the growth of new skin tissue thereby improving skin health and brightness. This makes papaya latex protease potentially applicable in facial masks. This facial mask is formulated with papaya latex protease, besides functioning to brighten the skin, it can also prevent acne caused by bacteria. This is supported by other research which proves that papaya leaf extract can inhibit the growth of acne-causing bacteria, Propionibacterium acne. Among the various types of facial masks, peel-off gel masks are practical in terms of their use, namely when they are finished being applied, they are simply peeled off without needing to be washed. Thus, this research aims to isolate and test the protease activity of papaya sap which is then applied in a peel-off gel mask
Materials and Methods: *This research was carried out in several stages, namely isolating the protease enzyme from papaya sap using ammonium sulfate precipitation and testing its activity using the Kunitz method. Then, the isolated protease is formulated into a peel-off gel mask. Protease activity in inhibiting Propionibacterium acne was tested before and after being prepared as a peel-off gel mask.*
Results: *The results of the protease activity test showed that each fraction resulting from ammonium sulfate precipitation had protease activity. Then, by testing the activity against Propionibacterium acne, it was discovered that the peel-off gel mask formula containing 0-80% ammonium sulfate fraction protease had higher inhibitory activity than the formula containing papaya sap.*
Key Words: *papaya latex, protease, peel-off gel mask, Propionibacterium acne*

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I. Introduction

The papaya plant (*Carica papaya* L.) is widely used as a medicinal ingredient (1), starting from the roots, stems, flower buds, fruit, seeds, skin and sap (2). Papaya sap contains protein levels that are quite high compared to other chemical contents. The protein content is a protease enzyme which has proteolytic activity of up to 2655 units/g at pH 5.5 (3). Proteases are able to break down polypeptide molecules, thus playing an important role in various biological processes in both physiological and pathophysiological conditions (4). The protease enzymes in papaya consist of papain, chymopapain and lysozyme (2). Papain and kimopapain, have been shown to help reduce inflammation and heal burns (5). Protease hydrolyzes protein peptide bonds into oligopeptides and amino acids so that it can break down dead skin cells, help clean necrotic tissue on the outer skin surface (epidermis), then slowly stimulate the growth of new skin tissue thereby improving skin health and brightness (6).

The health and brightness of the skin encourages people to use papaya as a component in facial masks. There are various types of facial masks, namely Sheet masks, Clay masks, Mud masks, Peel off masks, Wash off masks, Exfoliating masks, and Sleeping masks. A peel-off mask or peel-off gel mask is a practical type of facial mask, because after it dries, you just need to peel it off, without needing to wash it. This peel-off gel mask is formulated with the addition of various natural ingredients to improve its function, for example it is formulated with papaya leaf extract, and has been proven to inhibit the growth of acne-causing bacteria, *Propionibacterium acne* (7).

Various previous studies have utilized secondary metabolites of papaya plants to identify their pharmacological functions and effects. Different from previous research, this research will test one of the primary metabolites of the papaya plant, namely protease as an anti-bacterial Propionibacterium acne, which is then applied in a peel-off gel mask.

II. Material And Methods

Material

Materials used in this research include: papaya latex (papaya obtained from Cianjur), Propionibacterium acne (School of Life Sciences and Technology, Bandung Institute of Technology), Ammonium sulfate, pH 7.6 phosphate buffer, 0.1M sodium hydroxide, Mueller Hinton Broth (MHB), Mueller Hintin Agar (MHA), N-dimethyl casein substrate, Clindamycin, trichloroacetate solution (TCA), Polyvinyl Alcohol, Polyvinyl Pyrrolidone, Carbopol 940, Propylene glycol, Ethanol 95%, Tri-ethanolamine, DMDM Hydantoin, Aquades

Methods

Extraction of Papaya Sap

The sap is tapped directly from the papaya tree from the young fruit, approximately 2.5-3 months old. The sap can be taken by scraping a knife against the fruit (8).

Isolation of Protease Enzymes from young papaya sap

The papaya sap resulting from tapping is then precipitated gradually with ammonium sulfate saturation of 0-20%, 20-40%, 40-60%, 60-80%. The amount of ammonium sulfate weighed is based on the ammonium sulfate saturation table (9). Starting from 20% saturation, the tapping sap is added with a certain amount of ammonium sulfate (adjusting the desired final volume). The addition of ammonium sulfate was done in small increments while stirring gently using a magnetic stirrer for 30 minutes. Then centrifugation was carried out at a speed of 3000 rpm for 30 minutes. The resulting precipitate is stored. Meanwhile, to the supernatant, ammonium sulfate was added with a saturation of 20-40%. Next, for saturation of 20-40% to 60-80%, do the same as for saturation of 0-20% (10). The precipitate from each stage was dissolved in phosphate buffer pH 7.6 and then dialyzed in the same buffer. This sample is hereinafter referred to as the fractionation result.

Protease activity test using the Kunitz method

A total of 100 µL of sample (fractionation result) was added with 0.5 mL of N,N-dimethyl casein substrate (20 mg/mL in pH 7.6 0.1 M phosphate buffer) and 0.9 mL of pH 7.6 phosphate buffer; 0.1 M. The mixture was incubated at 37ºC for 30 minutes. Incubation was stopped by adding 0.5 mL of 8% trichloroacetate (TCA) solution, then centrifuged at 12,000 rpm for 30 minutes. The absorbance of the supernatant was measured at a wavelength of 280 nm (11). As a blank, the enzyme sample is replaced with water, and treated like a sample. Test the antibacterial activity, Propionibacterium acne, using the disc diffusion method

A total of 5 µl of Propionibacterium acnes bacterial suspension was put into a petri dish, then sterile MHA was added. Then homogenize and leave to solidify. After that, three paper discs were placed with the help of tweezers. The paper discs were each dripped with phosphate buffer solution (as a negative control), clindamycin (as a positive control), and samples resulting from 0-80% ammonium sulfate fractionation. Next, it was incubated at 37°C for 18 – 24 hours and the bacterial growth was observed. If an inhibition zone is formed; The inhibition zone was measured with a caliper (12–14).

Peel-off Gel Mask Formulation

Peel-off gel masks are formulated by mixing several ingredients gradually. The first stage, Polyvinyl Alcohol (PVA) and Polyvinyl pyrrolidone (PVP) are developed in distilled water at a temperature of 80ºC in a separate container, stir until homogeneous, mix the PVP into the PVA container, then stir with constant stirring until homogeneous (mass 1). In the second stage, Carbopol was dissolved in distilled water and DMDM Hyndantoin was dissolved in propylene glycol, then added to carbopol and stirred until homogeneous (mass 2). The third stage, mass 2 is added to mass 1, stirred until homogeneous (mass 3). Then, the sample is added to mass 3, stirred until homogeneous (7.15–20). There are two formulations, where formula 1 contains papaya sap, and formula 2 contains the results of 0-80% ammonium sulfate fractionation. The peel-off gel maker formula is shown in Table 1.

Table 1. Formulation Gel *Peel-off* Mask

Ingredients	Concentration (%)
Sample	10
Polivinil Alkohol	12
Polivinil Pirolidon	1
Carbopol 940	0,5
Propilenglikol	2
Etanol 95%	17 ml
Tri-etanolamin	7 tetes
DMDM Hydantoin	0,6
Akuades	Up to 100 gram

Peel-off gel mask evaluation (7.15–20)

Evaluation of peel-off gel mask preparations includes testing pH, viscosity, spreadability and drying time.

The pH of the mask preparation is measured using a pH meter, where the pH must match the skin's pH, namely 4.5-6.5.

The viscosity of the peel-off gel mask preparation was measured using a viscometer. A total of 100 ml of the preparation is placed in the stormer viscometer, then set the spindle and desired speed and run, wait a few moments until the viscosity value is read.

The spreadability of the mask preparation was tested by placing 1 gram of the peel-off gel mask preparation on glass A measuring 20x20 cm. Then cover glass A with another glass with code B and place a weight weighing 100 grams on top.

To test the drying time, the peel-off gel mask preparation was applied to the back of the hand and then carefully observed the time it took for the preparation to dry, including when it was applied to form a dry layer.

Test the activity of the peel-off gel mask against Propionibacterium acne using the agar diffusion method

A total of 5 µl of the *Propionibacterium acnes* bacterial suspension was put into a petri dish, then MHA medium was added and homogenized and allowed to solidify. Next, several holes are made in the solidified media using a perforator. In each well, 40 µl of mask preparation, distilled water, mask base and clindamycin were added. Next, incubation was carried out for 24 hours at 37ºC. If a clear zone forms, the clear zone is measured using a caliper.

III. Results And Discussion

One papaya can be tapped several times. In this research, it was found that each tapping produced approximately 10 ml of sap.

Protease in tapped papaya sap was isolated by gradual precipitation using ammonium sulfate. Protease activity in the precipitated fractions that had been dissolved in phosphate buffer pH 7.6 was tested using the Kunitz method. Then it is calculated using equation 1.

The activity unit is the amount of enzyme that causes an increase of 0.001 absorbance units/minute above the blank.

Enzyme activity (µmoles/min/µg)

$$\frac{V\ (\mu L) \times A_{405}\ (cm^{-1})}{\varepsilon \times \text{incubation time (min)} \times \text{enzyme } (\mu g)}$$

Based on the literature, papaya contains various types of proteases (3,4). This is in accordance with the results of this research. Through the Kunitz test, it was found that each fraction had protease activity (Table 2). Thus, to obtain protease from papaya, precipitation can be carried out using 0-80% ammonium sulfate directly.

As confirmation of antibacterial activity, samples resulting from fractionation of 0-80% ammonium sulfate, which had gone through the dialysis process, were tested for their inhibitory power against *Propionibacterium acnes* bacteria. The result was that the sample could inhibit the growth of *Propionibacterium acnes* with an inhibition zone of 9 mm. In this case the sample falls into the medium category, which falls into the inhibition zone range of 6-10 mm.

Papaya sap samples and 80% ammonium sulfate fractionation results were respectively formulated into a peel-off gel mask, called formula 1 and formula 2. Based on the evaluation results, the average results of three repeated measurements, obtained formula 1 (Figure 1), has a pH of 4.9; spreadability 5.4 cm; drying time 20 minutes; and viscosity 2546.7 cPs. Meanwhile, formula 2 (Figure 2) has a pH of 4.7; spreadability 5.5 cm; drying time 15 minutes; and viscosity 2593.5 cPs. From these results, both formula 1 and 2 mask preparations met the requirements, namely being in the skin pH range, namely 4.6-6.5; spreadability is in the range of 5-6 cm; drying time is in the range of 15-30 minutes and viscosity is in the range of 2000-4000 cPs.

Table 2. Protease Activity Result of Ammonium Sulfate Fractionation

Amonium Sulfat saturation (%)	Protease (U/ml) activity
0-20	186,3
20-40	112,3
40-60	86
60-80	154

Papaya and the 0-80% fractionation results in the mask formula still have activity, so the mask preparation was tested against the propionibacterium acnes bacteria. Based on the test results, it is known that the enzyme is still active. Proteases that have been isolated through precipitation of 0-80% ammonium sulfate have higher activity than those still in papaya sap. This is shown by the inhibition zone of mask preparations containing 0-80% fractionation results having an inhibition zone of 25.3 mm (formula 2, Figure 3.C); while the mask preparation containing papaya latex has an inhibition zone of 15.1 mm (formula 1, Figure 3.D). As a comparison for the negative control, 2 comparators were used, namely mask base (F) and distilled water (A).

Meanwhile, clindamycin (B and F) was used as a positive control (Figure 3). Based on this research, it can be concluded that the protease contained in papaya sap has inhibitory activity against *Propionibacterium acne* bacteria both before and after being formulated with a peel-off gel mask. Formula 2 which contains 0-80% ammonium sulfate fractionation results has higher inhibitory activity compared to formula 1 which contains papaya latex. Then, based on the evaluation of the mask preparation, the addition of papaya latex or its fractionation results, in amounts according to this research, does not interfere with the characteristics of the peel-off gel mask where the pH, spreadability, drying time and viscosity are still within the required range.

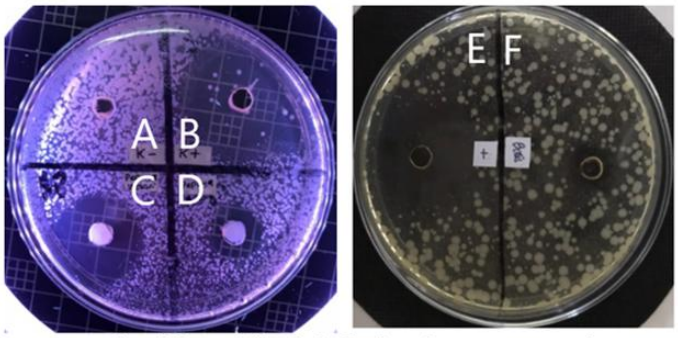

Figure 3. Test Results Inhibition of the feel-off gel mask against Propionibacterium acne; Formula 1 (D), formula 2 (C). positive control, clindamycin (B and E), negative control, mask base (F), negative control, distilled water (A)

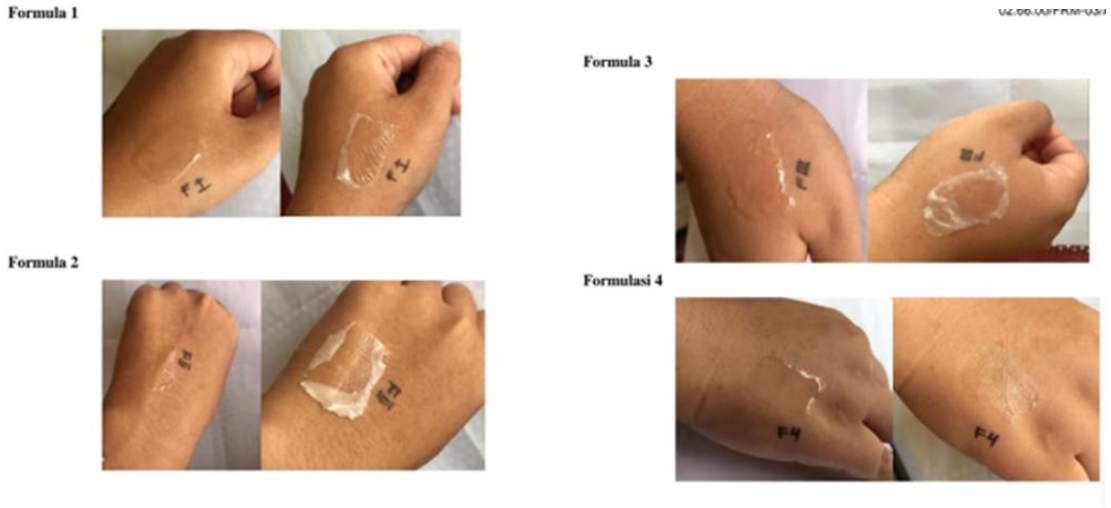

Figure 4. Mask irritation test on skin

IV. Conclusion

Based on this research, it can be concluded that the protease contained in papaya sap has inhibitory activity against Propionibacterium acne bacteria both before and after being formulated with a peel-off gel mask. Then, based on the evaluation of mask preparations, the addition of papaya sap or its fractionation results, in amounts according to this research, meets the requirements for mask preparations.

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