

Antibacterial Activity of Young Papaya Fruit Extract (*Carica papaya* L.) Against Acne-inducing *Propionibacterium acnes*

Aktivitas Antibakteri Ekstrak Buah Pepaya Muda (*Carica papaya* L.) terhadap Bakteri *Propionibacterium acnes* Penyebab Jerawat

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Abstract. Acne is one of the skin disorders that often attracts the attention of teenagers and young adults. The inappropriate use of antibiotics in the treatment of acne can lead to resistance, thus requiring alternatives from natural ingredients that have the ability as antibacterials. This study was conducted to test the antibacterial properties of young papaya fruit extract against the growth of *P. acnes* and determine the optimal concentration of young papaya fruit extract in stopping the growth of *P. acnes*. Antibacterial testing used the pitting method with a completely randomized design (CRD). Data analysis using Kolmogorov-Smirnov test, One Way Anova test, and Duncan test showed that the treatment of young papaya fruit extract concentration had a positive effect on the growth of *P. acnes* and there were significant differences with other treatments. The results of testing the antibacterial activity of young papaya fruit extract with a concentration of 20%, 40%, and 60% produced an average inhibition zone sequentially of $4,60 \pm 0,54$ mm, $7,60 \pm 0,54$ mm, and $10,80 \pm 0,83$ mm so that the 60% concentration can provide optimal results in stopping the growth of *P. acnes* because it forms the largest inhibition zone. Thus, this study proves that young papaya fruit extract has antibacterial properties so that it can be applied as a natural acne medicine.

Keywords: acne; young papaya fruit; inhibition zone; health policy

Abstrak. Jerawat merupakan salah satu gangguan kulit yang sering menarik perhatian para remaja maupun dewasa muda. Penggunaan antibiotik yang salah dalam pengobatan jerawat dapat mengakibatkan resistensi sehingga membutuhkan alternatif dari bahan alami yang memiliki kemampuan sebagai antibakteri. Penelitian ini dilakukan untuk menguji sifat antibakteri ekstrak buah pepaya muda terhadap pertumbuhan *P. acnes* dan mengetahui konsentrasi ekstrak buah pepaya muda yang optimal dalam menghentikan pertumbuhan *P. acnes*. Pengujian antibakteri menggunakan metode sumuran dengan Rancangan Acak Lengkap (RAL). Analisis data menggunakan Uji Kolmogorov-Smirnov, Uji One Way Anova, dan Uji Duncan menunjukkan bahwa perlakuan dari konsentrasi ekstrak buah pepaya muda berpengaruh positif terhadap pertumbuhan *P. acnes* dan terdapat perbedaan signifikan terhadap perlakuan lainnya. Hasil pengujian aktivitas antibakteri ekstrak buah pepaya muda dengan konsentrasi 20%, 40%, dan 60% menghasilkan rerata zona hambat secara berurutan yaitu $4,60 \pm 0,54$ mm, $7,60 \pm 0,54$ mm, dan $10,80 \pm 0,83$ mm sehingga konsentrasi 60% dapat memberikan hasil yang optimal dalam menghentikan pertumbuhan *P. acnes* karena membentuk zona hambat paling besar. Dengan demikian, penelitian ini membuktikan bahwa ekstrak buah pepaya muda memiliki sifat antibakteri sehingga dapat diaplikasikan sebagai obat jerawat secara alami.

Kata kunci: jerawat; buah pepaya muda; zona hambat; kebijakan kesehatan

INTRODUCTION

Skin is a constituent member of the body at the outermost part that covers the surface of the body (Chandrawati dan Susanti, 2023). Everyone wants to have smooth, healthy, and fresh skin to appear more confident, but the skin is not always clean and will be free from disease or disorders (Mundriyastutik dan Habibah, 2022). According Pinartin *et al.* (2023), a skin disorder that often attracts the attention of teenagers and young adults is acne. One of the factors that cause inflammatory acne is bacterial infection (Mulyani *et al.*, 2017). The bacteria that most often infect the skin and are directly involved in acne inflammation is *Propionibacterium acnes* (Hikma *et al.*, 2023).

Propionibacterium acnes are Gram positive facultative anaerobes and part of the normal skin flora, but can become pathogenic when colonized in the pilosebaceous follicles (Thiboutot, 2014;

McLaughlin *et al.*, 2019). These bacteria produce hydrolytic enzymes that can cause pilosebaceous follicle damage, one of which is lipase (Fauziyah *et al.*, 2020). Lipase then breaks down sebum triglycerides and becomes free fatty acids which can be a good growth trigger for *P. acnes*. If the pilosebaceous follicular duct is blocked due to an increase in sebum, it can cause blackheads (Teresa, 2020) and will develop into inflammatory acne (Sifatullah dan Zulkarnain, 2021).

Antibiotics are one of the most effective methods to treat acne (Xu dan Li, 2019). Tetracycline, erythromycin, dan clindamycin are some of the antibiotics commonly used to treat acne and function to reduce the population of *P. acnes* (Hamka dan Hardyanty, 2021). However, incorrect use of antibiotics can cause resistance that makes acne difficult to cure and stops the development of bacteria (Sholih *et al.*, 2015; WHO, 2023). Overuse of antibiotics can cause or the emergence of other resistant pathogenic bacteria that can reduce or even lose the effectiveness of a drug (Dessinioti dan Katsambas, 2022). Therefore, to reduce antibiotic resistance and prevent side effects, there is a need for alternatives with high potential as antibacterials (Anggreni dan Yowani, 2022).

Papaya plants (*Carica papaya* L.) are natural ingredients that can be utilized as antibacterials (Hermyati *et al.*, 2021). One of the uses of papaya plants using papaya fruit pulp is that it can be used as a skin health product in the form of soap which can be used as a natural facial cleansing product (Yuniarti *et al.*, 2021) and liquid soap as a product that helps maintain skin moisture (Amir *et al.*, 2023). Active compounds such as papain enzymes, alkaloids, tannins, flavonoids, saponins, steroids, and phenol compounds cause papaya plants to have antibacterial properties (Mahatrinny *et al.*, 2014; Yuliastuti *et al.*, 2019). Puspita *et al.* (2021) by applying ethanol 70% extract of California young papaya fruit flesh aged 3-4 months as a cream preparation formula with a concentration of 1% and 3%, the results of their research show that flavonoid content is the highest active compound found in young papaya fruit extract.

Based on the background that has been described, some secondary metabolite compounds possessed by papaya plants (*C. papaya* L.) are able to inhibit bacterial growth so that this plant can be recommended as a natural antibacterial alternative. Parts of the papaya plant have also been widely researched, one of which is the flesh of the fruit. Therefore, this study aims to test the antibacterial properties of young papaya fruit extract and determine the optimal concentration in stopping the growth of *P. acnes* so that it can be used as an alternative to natural acne treatment.

MATERIALS AND METHODS

This type of experimental research applies the Completely Randomized Design (CRD) method which was carried out from December 17, 2023 - February 06, 2024 at the Microbiology Laboratory, Biology Study Program, Faculty of Mathematics and Natural Sciences, State University of Surabaya. The research materials used include California young papaya fruit aged 3-4 months (obtained from Papaya Plantation in Clumpit Village, Pagelaran District, Malang City), *P. acnes* strain ATCC 11828 (obtained from the Microbiology Laboratory of Brawijaya University which has been stored in the Microbiology Laboratory, FMIPA, Unesa), Nutrient Agar (NA) media [Merck cat no. 1.05450.0500], ethanol 70%, clindamycin antibiotic 300 mg, distilled water, NaCl 0,9%, and alcohol 70%. Research tools used include petri dish, erlenmeyer, test tube, glass stirring rod, iron spatula, beaker glass, micropipette, microtip (blue tip and yellow tip), digital balance, analytical balance, autoclave (Tomy Kogyo ES-215), incubator (Lab-Line Instruments 302-1), hot plate (Cimarec), blender, rotary evaporator (Buchi R-215), laminar air flow (LAF), vortex, UV-Vis spectrophotometer (Shimadzu UV-1800), cork borer (0,5 cm), ruler, and spirit lamp.

The preparation of young papaya fruit extract begins with the way young papaya fruit is washed thoroughly using running water and drained. Next, the papaya fruit flesh is grated and then dried for 3 days (Puspita *et al.*, 2021). The dried samples were then blended and filtered to obtain 40 g of simplisia powder. Simplisia was macerated for 2 x 24 hours in a ratio of 1: 4 (40 g of young papaya fruit powder and 160 mL of ethanol 70%). Each maceration was stirred in the first 2 hours for 10 minutes (Yuliastuti *et al.*, 2019). The filtrate results from the first and second maceration were then mixed and put into a rotary evaporator to separate the solvent from the extract until a thick extract of 12 g of young papaya fruit flesh was obtained.

The concentrations of young papaya fruit extract tested were 20%, 40%, and 60%. The 20% concentration required 2 g of extract, the 40% concentration required 4 g of extract, and the 60% concentration required 6 g of extract. Each concentration of extract was dissolved using sterile distilled water to a volume of 10 mL.

Making bacterial growth media, namely NA media powder as much as 10 g is put into 500 mL of distilled water (20 g / 1000 mL). NA media was cooked on a hot plate until evenly dissolved. Next, NA media was sterilized using an autoclave at 121° C for 15 minutes. After that, the NA media was allowed to stand at room temperature and then put into the refrigerator ($\pm 4^{\circ}$ C).

Preparation of bacterial culture (rejuvenation) was carried out by inoculating one ose of *P. acnes* then streaked into a test tube containing NA slant. The rejuvenated bacteria were then incubated in an incubator at 37° C for 24 hours. Calculation of bacteria (suspension) was made from the results of rejuvenation of *P. acnes* taken as much as one ose and put into 10 mL of NaCl 0,9% solution in a test tube. Bacterial turbidity was calculated using a Uv-Vis spectrophotometer at a wavelength of 625 nm (Sukandar *et al.*, 2014) to produce an absorbance value of 0.08-0.13 A or equal to 1.5×10^8 bacterial cells/mL. (Gunarti *et al.*, 2021).

Antibacterial testing was carried out using the pitting method. A bacterial suspension of 1 mL was put into a petri dish followed by pouring 15 mL of liquid NA media into the petri dish. Petri dish is placed and moved slowly by rotating so that the bacterial suspension with the media can be homogeneous then the media is allowed to stand until solid. The media that has been solid then in each petri dish three wells are made using a cork borer. Each well contains 30 μ L with clindamycin (positive control), sterile distilled water (negative control), and young papaya fruit extract using a micropipette. Next, the media was incubated in an incubator at 37° C for 14-15 hours. Tests were carried out as many as 5 repetitions at each concentration.

Observation of antibacterial activity is known from the appearance of an inhibition zone around the pits. Measurement of inhibition zone diameter using a ruler with centimeter unit. The results of measuring the diameter of the inhibition zone were calculated using the following formula (Harti, 2015).

$$\frac{(Dv-Ds)+(Dh-Ds)}{2} \quad (1)$$

Description:

Dv: Vertical Diameter (mm)

Dh: Horizontal Diameter (mm)

Ds: Well Diameter (mm)

The data obtained were then analyzed using the SPSS (Statistical Product and Service Solutions) statistical program version 23.0 for windows. The data was tested using the Kolmogorov-Smirnov test to determine the normality of the data, then analyzed using the One Way Anova test to determine the effect of each treatment, and continued with the Duncan test to compare the results obtained from each treatment.

RESULTS

The results of testing the antibacterial activity of young papaya fruit extract (*C. papaya* L.) using concentrations of 20%, 40%, and 60% by the pitting method showed the presence of an inhibition zone around the pitting hole, indicating that young papaya fruit extract has an effect in stopping the growth of *P. acnes*. The formation of the zone of inhibition is shown in Figure 1.

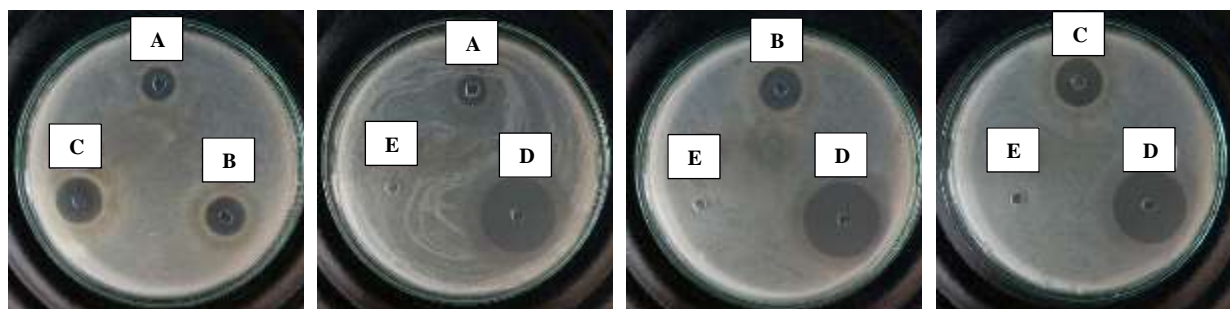


Figure 1. Antibacterial activity test results of young papaya fruit extract (*Carica papaya* L.) against *Propionibacterium acnes*; (a) extract concentrations 20%; (b) extract concentrations 40%; (c) extract concentrations 60%; (d) positive control (clindamycin antibiotic); (e) negative control (sterile distilled water)

The results of the analysis of the average inhibition zone quantitatively using the Kolmogorov-Smirnov test with a significance value of $0.20 > \alpha$ value (0.05) indicates that the data is normally distributed. Furthermore, the data were analyzed using the One Way Anova test with a significance value of $0.00 < \alpha$ value (0.05) indicating the effect of each treatment in inhibiting *P. acnes*. After that, continued using the Duncan test to compare the results obtained in each treatment and obtained the analysis results in Table 1.

Table 1. Antibacterial activity test results of young papaya fruit extract (*Carica papaya* L.) against *Propionibacterium acnes*

Treatment	Mean of Inhibition Zone Diameter (mm) \pm SD*	Percentage of Inhibition Zone Diameter (%)
Negative control (sterile distilled water)	0.00 \pm 0.00 ^a	0
Extract concentrations 20%	4.60 \pm 0.54 ^b	23.96
Extract concentrations 40%	7.60 \pm 0.54 ^c	39.58
Extract concentrations 60%	10.80 \pm 0.83 ^d	56.25
Positive control (clindamycin antibiotic)	19.20 \pm 0.57 ^e	100

Note: *)Different letter notations indicate that the results obtained are significantly different based on Duncan test ($\alpha=0.05$).

The results of Duncan test prove that the treatment between positive control and negative control has a significant difference with the treatment of each concentration of young papaya fruit extract. This can be seen from the different letter notations in each treatment so that the overall comparison made has a significant difference.

DISCUSSION

The results of testing the antibacterial activity of young papaya fruit extract (*C. papaya* L.) are known to have antibacterial properties because it is able to produce an inhibition zone around the well. The average at 20% concentration is $4,60 \pm 0,54$ mm with letter notation (b), 40% concentration is $7,60 \pm 0,54$ mm with letter notation (c), and 60% concentration is $10,80 \pm 0,83$ mm with letter notation (d). The negative control treatment did not form an inhibition zone ($0,00 \pm 0,00$) with letter notation (a) and the average of the positive control was $19,20 \pm 0,57$ mm with letter notation (e). The difference in the notation results between treatments, both the treatment of the extract concentration and the treatment of the control followed by different letters indicates that there is a significantly different effect. Thus, the different letter notations mean that there are real or significant differences in each treatment.

The results of the negative control test using sterile distilled water showed that no inhibition zone was formed around the well. This is because sterile distilled water does not have antibacterial compounds so it does not have antibacterial ability (Rahayu *et al.*, 2021) and the compounds contained are neutral so they do not affect bacterial growth (Sukadiasa *et al.*, 2023). The results of the positive control test using clindamycin 0,3% antibiotic showed an inhibition zone. According to Kurniawan *et al.* (2018) that clindamycin antibiotics are topical antibiotics commonly used as acne treatment because they are bacteriostatic and even bactericidal depending on the concentration of the drug used. The mechanism of clindamycin antibiotics in stopping bacterial growth is by blocking the synthesis of bacterial proteins that bind directly to the 23S ribosomal RNA (rRNA) subunit of the 50S ribosome of bacteria (Salmi dan Swandi, 2022). The binding causes peptide bonds between amino acids cannot be formed so that bacteria fail to produce the proteins needed and bacterial proliferation will stop (Dallo *et al.*, 2023).

The antibacterial test used is the pitting method because it is more effective to use (Emelda *et al.*, 2021). In addition, the inhibition zone that appears is easier to calculate because the bacterial isolate will move to the bottom of the agar media and not move on the top surface of the agar media so that the resulting inhibition zone will also be wider (Rahman *et al.*, 2022). The difference in the inhibition zone area at several extract concentrations is due to differences in the number of antibacterial compounds contained in the extract (Yusriyani *et al.*, 2023). Nurhamidin *et al.* (2021) also stated that the number of antibacterial compounds contained in the extract affects the inhibition zone area, which results in the ability of antibacterial compounds to control bacteria is also influenced by the large number of compounds contained in the extract. Young papaya fruit is proven to contain alkaloids, tannins, flavonoids, saponins, steroids, and papain enzymes (Paramitha *et al.*, 2021; Hidayat *et al.*, 2023).

The mechanism of action of each antibacterial compound in inhibiting bacterial growth occurs through several stages (Sari dan Asri, 2022). Alkaloid compounds interfere with the constituent parts that form the peptidoglycan layer of bacterial cells resulting in the formation of incomplete bacterial cell walls (Pisacha *et al.*, 2023). Tannin compounds block cell wall permeability by wrinkling the bacterial cell wall, thus stopping the cell from carrying out its life activities and inhibiting its development (Nurhajanah *et al.*, 2020). Flavonoid compounds act through hydrogen bonds to form complex compounds with bacterial extracellular proteins that cause the shape of the cell wall and cytoplasmic membrane of bacteria to become incompatible, thereby inhibiting their biological activities and disrupting the integrity of the cell wall and cytoplasmic membrane, as a result, bacterial cells are lysed and die (Pramiastuti *et al.*, 2020). Saponin compounds play a role in reducing the surface tension of bacterial cell walls which results in increased cell membrane permeability so that the stability of the cell membrane is disrupted and causes the cell cytoplasm to leak and bacterial cells to lysis (Putri *et al.*, 2023). Steroid compounds interact with phospholipid membranes that can be penetrated by lipophilic compounds. This interaction causes cell membrane integrity to decrease and morphological changes occur (Silviana dan Asri, 2022). Finally, the presence of papain enzyme is able to cause bacterial lysis by entering the cell nucleus and breaking down proteins in bacteria so that bacteria cannot carry out their biological activities and cause death (Nugraha *et al.*, 2022).

CONCLUSION

Young papaya (*C. papaya* L.) fruit extract has antibacterial properties so that it has a positive effect on the growth of *P. acnes* because it is able to produce an inhibition zone around the wells. The optimal concentration of young papaya fruit extract to affect the growth of *P. acnes* is 60% concentration with an average inhibition zone of $10,80 \pm 0,83$ mm. The implication of using young papaya fruit extract is that it can be utilized as a natural ingredient for acne treatment.

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