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Effectiviness of *Persea americana Mill* Fruit Facial Skin Mask and Rice Flour in Inhibiting *Propionibacterium acnes*

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Abstract
Skin diseases were caused by microorganisms such as *Propionibacterium acnes* bacteria, which triggered acne vulgaris or acne. Treating acne using antibiotics at inappropriate doses could cause resistance, so alternative natural treatments were needed. This research aimed to test the effect of a combination of avocado skin and rice flour on the growth of *P. acnes*, formulated as a powder mask. This experimental research used the Completely Randomized Design (CRD) method with treatment concentrations of 10%, 15%, 20%, positive control, and negative control. This research included five repetitions. Data analysis used the Kolmogorov-Smirnov Test, One Way ANOVA Test, and Duncan Test, which showed that the data were normally distributed, all combination treatments of avocado peel powder and rice flour affected inhibiting *P. acnes* bacteria, and each treatment was significantly different. A 10% concentration produced an average clear zone of 6.35 ± 0.48 mm, 15% produced 8.00 ± 0.68 mm, and the most optimal concentration, a 20% concentration, produced 11.05 ± 0.37 mm. Thus, it was proven that combining avocado skin powder and rice flour inhibited *P. acnes* bacteria and could be used as an alternative natural acne treatment.

Keywords: Bacteria; clear zone; combination.

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INTRODUCTION

The outermost layer covering the human body was called the skin. The skin's structure was complex and varied depending on climate, age, gender, race, and location of the skin on the body. The skin comprised the epidermis, dermis, and subcutis (Pramadiyani *et al*., 2023). The specific function of the skin was to wrap the entire body's surface (Adhisa & Megasari, 2020). In addition, the surface of human skin contained many nutrients necessary for organism growth, such as fat, nitrogen-containing materials, and minerals. As a result, infections frequently occurred on human skin due to bacteria, fungi, and viruses (Rahayu, 2016). This caused skin diseases such as acne vulgaris or acne (Sifatullah & Zulkarnain, 2021).

Acne was a skin condition caused by a buildup of oil on the skin, which blocked facial pores, allowed bacteria to multiply, and caused inflammation (Efrata *et al*., 2018). According to Habibie and Aldo (2019), acne was a pile of oil glands on dirty skin. When this dirt mixed with dust, sweat, and other impurities, the bacteria inside the blackheads created a pile of fat and black spots, commonly called blackheads, which caused inflammation, usually resulting in pain.

Most acne vulgaris was caused by the bacteria *Propionibacterium acnes*. *P. acnes* was a Gram-positive bacterium that belonged to the *Corynebacteria* group in terms of morphology and organization. These bacteria were part of the normal skin flora but were not toxigenic (Zahrah *et al*., 2019). These bacteria, which were normal flora found on human skin, caused the formation of lipase, which broke down triglycerides—one of its parts—sebum, which was broken down into free fatty acids. *P. acnes* bacteria used these free fatty acids to multiply, causing inflammation and the formation of blackheads, which were factors that caused acne vulgaris (Marliana *et al*., 2018).

Antibiotics were often employed in the treatment of acne, with commonly used antibiotics including clindamycin, erythromycin, doxycycline, minocycline, benzoyl peroxide, and retinoids

(Adyani, 2019). However, the use of these drugs had an impact, such as irritation, and prolonged or long-term use could cause the emergence of antibiotic resistance (Dreno *et al*., 2018) Resistant bacteria were more challenging to overcome, and they also required high doses, which led to harmful effects and high costs (Garcia *et al*., 2018). Therefore, other alternatives were needed to treat acne, such as using plants that grew in the surrounding environment.

Plants that could be used to treat acne vulgaris included avocado peel (*Persea americana* Mill) and rice flour (*Oryza sativa* L). Avocado fruit peel (*P. americana* Mill) was used because it contained one of the phenol group compounds, namely flavonoids, which functioned as antibacterials (Sarmila *et al*., 2021). As an antimicrobial compound, the mechanism of action of flavonoid compounds included inhibiting nucleic acid synthesis, cell membrane function, and energy metabolism (Fidyandini & Silviana, 2021). Meanwhile, rice flour was also used in the beauty field as a base for masks because it contained amylose, amylopectin, dextrin, γ-oryzanol, and kojic acid, which could brighten skin color (Sinurat *et al*., 2022). Kojic acid could also be an antibacterial, anti-inflammatory, and antifungal agent (Zilles *et al.*, 2022). Combining plants that contained different active substances was hoped to produce a mutually supportive effect to increase their effectiveness (Rifda & Lisdiana, 2022).

Based on this description, it was necessary to develop new research by combining avocado peel and rice flour in the form of a powder mask to examine and analyze its effectiveness as an antibacterial agent and determine the optimal concentration so that it could inhibit the growth of *P. acnes*, which caused acne. The powder mask became an antibacterial testing formula to facilitate application (Sinurat *et al*., 2022). Powder preparations were chosen because they were resistant to bacterial growth and did not require preservatives due to their low water content (Hendrawati *et al*., 2021).

MATERIALS AND METHODS

This research was classified as experimental research using a completely randomized design (CRD). The study was conducted from April to May 2024 at the IDB Building, Biology Laboratory, Faculty of Mathematics and Natural Sciences, Surabaya State University. The materials used in this study included avocado fruit peels, organic rice, *Propionibacterium acnes* bacteria, commercial powder masks, sterile distilled water, Nutrient Agar (NA) media, 70% alcohol, methylated spirits, and a 0.9% NaCl solution. The tools used in this study included racks and test tubes, ose needles, 10-100 µL micropipettes, microtips (blue tip and yellow tip), Erlenmeyer flasks, syringes, cotton swabs, beaker glasses, glass stirring rods, spatulas, spirit lamps and lighters, Petri dishes, stirrers, an autoclave (Tomy Kogyo ES-215), an incubator (Lab-Line Instruments 301-1), a vortex mixer (VWR 12620-854), a UV-Vis spectrophotometer (Shimadzu, 1900), a ruler, a sieve, a hot plate, rubber bands, a cork borer (6 mm), aluminum foil, a Laminar Air Flow (LAF) cabinet, plastic wrapping, and a chopper/blender.

The first stage of making avocado peel powder was wet sorting, which separated avocado peel powder from impurities or other foreign materials. The second process was washing with running water to remove dirt. The third process involved cutting the sample into 3 to 5 mm pieces. This was done to reduce the sample size for easier drying and to prevent fungal growth. Afterward, the samples were dried for four days in indirect sunlight. Once the samples were dry, dry sorting was performed to separate the *simplisia* from any remaining impurities. Then, the samples were pulverized and sieved to obtain a fine powder.

The process of making rice flour began with washing the rice under running water and then draining it on a dry tray covered with paper. The rice was then air-dried until it became scorched. Afterward, the rice was blended and sifted until it reached a smooth consistency.

The NA media was prepared by weighing 20 g of NA powder and dissolving it in 1 L of distilled water. The solution was then transferred into an Erlenmeyer flask and heated on a hot plate until fully dissolved. The flask was covered with cotton and aluminum foil before being sterilized using an autoclave at 121°C for 15 minutes (Rifda & Lisdiana, 2022).

Bacterial rejuvenation was performed using the streak method, where one ose needle was used to scrape bacteria from a pure culture onto slanted NA media. The media was then incubated for 24 hours at 37°C. The test bacterial suspension was prepared by transferring 1-2 ose needles from the 24-hour bacterial culture into a reconstitution tube containing 10 mL of 0.9% NaCl solution. The turbidity level was then compared to a 0.5 McFarland standard solution (equivalent to 1.5×10^8 CFU/mL). The absorbance of the bacterial suspension was analyzed using a UV-Vis spectrophotometer at a wavelength of 625 nm until an absorbance range of 0.08 to 0.11 was achieved, corresponding to the standard turbidity level (Sukandar *et al*., 2014).

The antibacterial activity test was carried out using the healthy diffusion method. One mL of bacterial suspension was transferred into a Petri dish. Then, 15 mL of molten NA media (cooled to approximately 40-42°C) was added. The mixture was gently swirled in a figure-eight motion to ensure even distribution (Sinurat *et al*., 2022). Once the media solidified, three wells were created in each Petri dish using a cork borer with a diameter of 6 mm. These wells were then filled with a negative control solution (sterile distilled water), a positive control solution (commercial mask), and a mask solution with a predetermined concentration. A micropipette was used to add 30 µL of each solution to the wells. The plates were then incubated for 24 hours at 37°C (Adha & Ibrahim, 2021). The test was performed five times.

Antibacterial activity was determined by measuring the clear zone formation around the wells, which indicated bacterial growth inhibition. The inhibition zone was measured using a ruler in millimeters, and the clear zone diameter was calculated using the appropriate formula (Harti, 2015).

$$D = \frac{(Dv-Ds) + (Dh-Ds)}{2}$$

where: D: average diameter of clear zone (mm); Dh: horizontal diameter (mm); Dv: vertical diameter (mm); Ds: diameter of the well (mm)

The data obtained consisted of the average apparent zone diameter. Its normality was tested using the Kolmogorov-Smirnov Normality Test, and subsequently, a One-Way Analysis of Variance (ANOVA) was conducted to determine the effect of the treatments used. The ANOVA results showed significant findings. Furthermore, Duncan's test was used to compare the results of each treatment. Statistical tests on the data obtained were performed using the SPSS for Windows version 25.0 program.

RESULTS

The results of the antibacterial activity testing showed that concentrations of 10%, 15%, and 20% in the combination of avocado fruit peel powder masks and rice flour inhibited *P. acnes* bacteria in Nutrient Agar (NA) media. This was indicated by the clear zone formed around the wells (Figure 1).

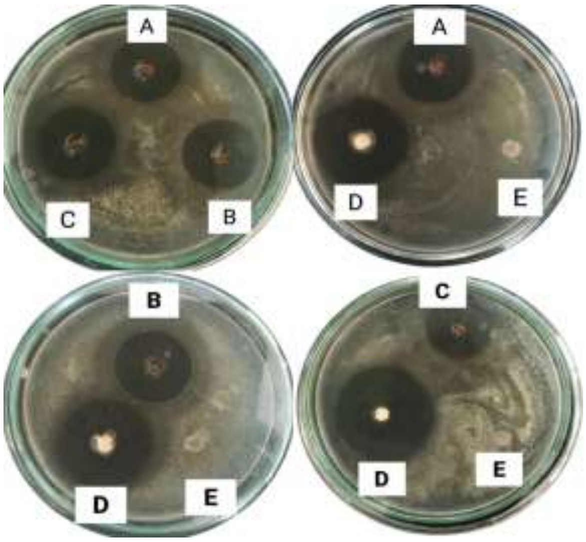

Figure 1. Antibacterial activity test result of combination of avocado peel and rice flour against *Propionibacterium acnes*; (A) concentrate 10% powder mask with a combination of avocado skin and rice flour; (B) concentrate 15% powder mask with a combination of avocado skin and rice flour; (C) concentrate 20% powder mask with a combination of avocado skin and rice flour; (D) positive control (commercial powder mask); (E) negative control (sterile distilled water).

The diameter of the inhibition zone was compared with the antibacterial inhibition category. The analysis was performed using SPSS (Statistical Product and Service Solutions) version 25.0 for Windows. The data were tested using the Kolmogorov-Smirnov Test, and the significance value of 0.126 was greater than the α value (0.05), indicating that the data were normally distributed.

Furthermore, a one-way ANOVA test was conducted to assess the effect of antibacterial treatment activity. The ANOVA test results showed that the p-value was less than α, with a p-value (significance) of 0.000 < α (0.05). Thus, the combined powder mask of avocado peel and rice flour at various concentrations significantly affected the growth of *P. acnes* bacteria.

Duncan's test was then used to analyse the data to determine the optimal concentration of the avocado peel and rice flour combination powder mask in inhibiting the growth of *P. acnes* (Table 1).

Table 1. Clear Zone Diameter Results of Antibakterial Activity Test.

Treatment	*Clear Zone* Diameter (mm)
A (10% powder mask combination)	6.35 ± 0.48^{b}
B (15% powder mask combination)	8.00 ± 0.68^{c}
C (20% powder mask combination)	11.05 ± 0.37^{d}
D (Positive control)	12.35 ± 0.65^{e}
E (Negative control)	00.0 ± 0.00^{a}

Notes: The results of the letter notation a, b, c, d, and e indicated significant differences in each treatment from the Duncan test with a significance level (α) of 0.05.

Duncan's test showed that all treatments were significantly different, as evidenced by the acquisition of different notations in each treatment. This indicates that each concentration of the combined powder mask had a distinct effect on inhibiting *P. acnes* growth. Therefore, it can be concluded that the combination of avocado fruit peel (*Persea americana* Mill) and rice flour in a powder mask formulation effectively inhibits the growth of *P. acnes*.

DISCUSSION

Based on the study's results, the 20% concentration treatment with treatment code C produced the largest apparent zone diameter (11.05 ± 0.37 mm) compared to other concentrations of avocado fruit peel and rice flour combination powder masks. At a concentration of 15%, with treatment code B, the average clear zone was (8.00 ± 0.68 mm). The lowest average value of the clear zone in the concentration treatment was at 10% concentration with treatment code A, which was (6.35 ± 0.48 mm). The control treatment that produced the most significant average value was the positive control at (12.35 ± 0.65) with treatment code D. In the adverse control treatment, namely with treatment code E, there was no inhibitory activity against the growth of P. *acnes bacteria*, so it did not have an average value or value (00.0 ± 0.00).

Antibacterial activity testing aimed to determine whether a solution contained substances with potential antibacterial properties. Based on the study's results, it was observed that the combination of avocado and rice peel plant samples contained antibacterial substances, as evidenced by the appearance of a clear zone around the well. The formation of a clear zone indicated the presence of antibacterial compounds in a solution with various mechanisms of action that affected the damage or death of bacterial cells. The average diameter of the clear zone generated from treatment A at 10% concentration was 6.35 mm, treatment B at 15% concentration was 8.00 mm, and treatment C at 20% concentration was 11.05 mm. According to Davis and Stout (1971), the inhibition zone diameter category of 11.05 mm at a concentration of 20% fell into the strong inhibition zone diameter category. The significant inhibition at 20% concentration was due to the higher concentration of the solution, resulting in a more significant number of active compounds that worked optimally to inhibit the growth of P. *acnes bacteria*. Thus, the powder mask made from the combination of avocado fruit peel and rice flour inhibited the growth of acne-causing bacteria, namely P. *acnes*.

The suitable diffusion method was used in this study because it had several advantages, such as measuring the clear zone area more effectively. This was because bacteria moved not only on the surface of Nutrient Agar (NA) media but also at the bottom of the media (Retnaningsih *et al*., 2019). A clear zone was formed when a solution with a concentrated concentration in the wells diffused into the NA with a lower concentration. This phenomenon was called diffusion. Additionally, its implementation was relatively easy and practical (Nadi *et al*., 2020). However, there were also disadvantages, such as difficulty in making wells, which could cause the Nutrient Agar (NA) media to crack or break around the wells, potentially interfering with the research process and affecting the formation of clear zones (Nurhayati *et al*., 2020).

The positive control used in this study was a commercial powder mask. The commercial powder mask exhibited the most significant inhibitory activity. This was because the positive control contained lemon, which has antibacterial compounds such as flavonoids, tannins, saponins, terpenoids, citric acid, essential oils, and limonoids (Batubara & Lindawati, 2019). These active compounds were similar to those found in the avocado peel and rice flour combination powder mask, namely flavonoids, saponins, and tannins. Flavonoids in lemons acted as antimicrobial compounds by altering the physical and chemical properties of the cytoplasm and denaturing the cell wall through hydrogen bonds (Dewi *et al*., 2020). Tannin compounds in lemons inhibited reverse transcriptase and DNA topoisomerase enzymes, which were responsible for transcription and replication in bacteria, thereby preventing bacterial cell formation (Ngajow *et al*., 2013).

The components in lemons that differed from the powder mask combination of avocado peel and rice flour included citric acid, limonoids, terpenoids, and essential oils. Citric acid in lemons caused acidity and reduced the pH of *P. acnes bacterial* cells, thereby inhibiting bacterial growth. Furthermore, limonoid compounds in lemons altered cell membrane permeability and removed ions from bacterial cells (Dewi, 2015). The terpenoid content in lemons damaged the bacterial cell membrane and reduced cell wall permeability (Nomer *et al.,* 2019). The essential oil content in lemons was lipophilic, allowing it to pass through the bacterial cell wall, causing cell wall damage and, ultimately, bacterial cell death Cahyani *et al.,* 2020).

The antibacterial compounds in the combination of avocado fruit peel and rice flour powder masks absent in lemons included steroid compounds. Steroids in the combination of avocado fruit peel and rice flour powder masks worked by damaging the plasma membrane of bacterial cells, causing cytoplasmic leakage and ultimately leading to the death of *P. acnes* bacterial cells (Rohyati *et al.,* 2015). This explained why the positive control results were more significant than the combined powder mask of avocado peel and rice flour treatment results. Furthermore, more antibacterial compounds in lemons contributed to its superior bacterial inhibition mechanism.

This study used sterile distilled water as a negative control. Based on the test results, sterile distilled water did not show a clear zone, indicating that it did not exhibit antibacterial activity. Using sterile distilled water as a negative control was justified as it contained neutral compounds that did not affect bacterial growth (Gerung *et al*., 2021). Additionally, sterile distilled water did not elicit an inhibitory response against bacterial growth and was safe to use (Henaulu and Kaihena, 2020).

The compounds identified in this study that inhibited *P. acnes* bacteria included flavonoids, alkaloids, tannins, saponins, phenols, polyphenols, steroids, and kojic acid compounds. These compounds exhibited antibacterial activity through various mechanisms, either by inhibiting bacterial growth (bacteriostatic) or by killing bacteria (bactericidal) through interactions with bacterial cell walls, bacterial nucleoids, or cytoplasmic membranes (Rollando, 2019).

Various active secondary metabolite compounds from plant combinations, such as flavonoids, alkaloids, saponins, phenols, tannins, steroids, and kojic acid, exhibited antibacterial activity through their respective mechanisms, often working collaboratively (Rijayanti *et al,* 2014). Among these plants, avocado fruit peel was particularly effective in inhibiting bacteria due to its higher antibacterial content than organic rice. According to Sulistyorini *et al*. (2011), antimicrobials in white rice were not very potent, as bacterial inhibition in white rice was only approximately ±2.24 mm against Pseudomonas sp. bacteria. Davis & Stout (1971) categorized inhibition zones below 5 mm as weak inhibition. In this study, rice flour served primarily as a base material for powder masks. While it contained kojic acid, which had antibacterial properties, its antibacterial potential in isolation was weak. Therefore, potent antibacterial compounds, such as those found in avocado fruit skin, were needed. The selection of plant combinations in antibacterial formulations considered economic feasibility, practicality, taste, and availability of raw materials (Rijal & Asri, 2024).

This study presented an innovative alternative for beauty products derived from natural ingredients to treat acne caused by P. *acnes* bacteria. The results could serve as a reference for further research, such as investigating raw materials for acne medication production. Future studies should focus on purifying the compounds in avocado peel and rice flour, conducting chemical screening tests to determine their potential in industrial-scale anti-acne product manufacturing, and performing durability and shelf-life testing.

CONCLUSION

The combination of avocado peel powder and rice flour inhibited *P. acnes* bacteria by producing a clear zone around the well. The most optimal mask combination of avocado peel powder and rice flour in inhibiting the bacteria was at a 20% concentration, with a clear zone of 11.05 ± 0.37 mm.

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CONFLICT OF INTEREST

There is no conflict of interest.

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