Effect of Phenolic Levels on the Activity of Bacteria Propionibacterium Acnes in Cassava Extract Ointment Preparations Manihot Esculenta

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Cassava peel is an abundant agricultural by-product that contains bioactive secondary metabolites with potential pharmaceutical applications. This study aimed to evaluate the effect of phenolic content on the antibacterial activity of cassava peel extract formulated as a topical ointment against Propionibacterium acnes. Cassava peel was extracted using maceration with 96% ethanol and formulated into ointments at three extract concentrations, namely 2% (FI), 4% (FII), and 6% (FIII). Phenolic content was determined using the Folin–Ciocalteu method with UV–Vis spectrophotometry, while antibacterial activity was assessed using the agar well diffusion method. The extraction process produced a yield of 12.52% and phytochemical screening confirmed the presence of flavonoids, saponins, tannins, and phenolic compounds. Phenolic content increased with extract concentration, with values of 0.74 ± 0.04 mg GAE/g for FI, 1.15 ± 0.14 mg GAE/g for FII, and 1.36 ± 0.08 mg GAE/g for FIII. All ointment formulations met physical quality requirements, including homogeneity, acceptable pH range, spreadability, adhesion, and consistent organoleptic properties. Antibacterial testing showed that all formulations exhibited strong inhibitory activity against Propionibacterium acnes. The largest inhibition zone was observed in FI at 16.33 ± 2.56 mm, followed by FII at 12.00 ± 1.81 mm and FIII at 11.50 ± 2.64 mm. The negative control showed no antibacterial activity, confirming that inhibition was attributed to the cassava peel extract. Statistical analysis indicated a significant effect of phenolic content on antibacterial activity ($p < 0.05$). These findings demonstrate that cassava peel extract ointment has strong antibacterial potential against Propionibacterium acnes, and that antibacterial effectiveness is influenced not only by phenolic content but also by formulation characteristics affecting compound diffusion. The ointment shows promise as a natural topical antibacterial candidate for acne treatment.

Keywords: Antibacterial, Cassava Peel, Ointment, Phenolic, Propionibacterium acnes

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1. Introduction

Indonesia is seen as a country that relies on the agrarian sector, where most of its people depend on plant cultivation activities as a source of livelihood. Agriculture occupies a strategic position as the foundation of the national economy, especially since most of the food needs and a number of industrial commodities are sourced from this sector. One of the commodities that is widely developed is cassava, a plant that is easy to cultivate in various land conditions, has abundant availability, and provides a fairly high economic value for farmers. Cassava, which belongs to the group of taproot bulb plants with developing branches, is capable of producing large quantities of bulbs. This high production volume automatically produces a large amount of cassava peel waste as well. Although it is an easily decomposed organic waste, disposal without treatment can cause environmental problems due to its high moisture content so that the decay process takes place quickly. Behind this potential problem, cassava peel actually has a fairly high content of nutrients and carbohydrates. However, the existence of linamarin that can decompose into cyanide makes this ingredient need to be handled appropriately. In addition, cassava peel also contains a number of bioactive

metabolites such as flavonoids, tannins, and saponins that are valuable in the pharmaceutical field Borges and Bresson, (2004).

Phenolic compounds found in cassava peel and leaves are part of secondary metabolites that are recognized to have strong antioxidant activity. Its chemical structure allows the release of hydrogen atoms that are able to neutralize free radicals, thus preventing oxidative damage to cells. Phenolic compounds also form stable phenoxic radicals, making them natural antioxidant agents that function to protect biological tissues from various forms of oxidative stress. To find out the phenolic content of a material, one of the commonly used analysis methods is UV-Vis spectrophotometry. This method was chosen because it is able to provide accurate analysis results in a relatively short time by utilizing the absorption of ultraviolet and visible light to detect the presence of bioactive compounds. In addition to antioxidant activity, phenolic compounds have antibacterial abilities that can inhibit the growth of microorganisms through enzyme inhibition mechanisms, membrane damage, and disruption of bacterial cell metabolic processes Cushnie and Lamb, (2005).

Bacteria are microorganisms that are very abundant in the environment and have a diversity of structures and functions. Based on the composition of their cell walls, bacteria are differentiated into gram-positive and gram-negative. Gram-positive bacteria have a relatively simpler cell wall structure and tend to be more susceptible to various antibacterial agents. One of the gram-positive bacteria that plays an important role in skin health problems is Propionibacterium acnes. These anaerobic bacteria contribute to the inflammatory process of acne, especially when excess oil production, clogged pores, and the presence of dirt lead to the development of blackheads. Propionibacterium acnes produces a variety of chemical compounds and enzymes that damage the skin layer, causing irritation and inflammation, as well as worsening acne conditions Davis and Stout, (1971).

As an opportunistic pathogen, Propionibacterium acnes has the ability to produce lipases that can break down lipids on the skin's surface into free fatty acids, thus creating conditions that favor the development of inflammation. Concerns arise when bacteria become increasingly resistant to synthetic antibacterial drugs commonly used in acne therapy. This condition encourages the need for natural active ingredient alternatives that are safe, effective, and have minimal side effects. Cassava peel extract is considered to have the potential as an antibacterial agent due to its secondary metabolite content. Its use in the form of an ointment is considered appropriate because this semi-solid preparation is easy to apply, allows the active ingredients to be evenly distributed, and provides sufficient adhesion to the skin without interfering with normal physiological processes. The homogeneous basic structure of the ointment also helps to maximize the contact between the active ingredient and the surface of the skin Reo, Berhimpon and Montolalu, (2017); Putri, Hardiansyah and Supriyanta, (2020).

Based on the chemical potential of cassava peel and the increasing need for safer natural antibacterial ingredients, extraction using the maceration method with ethanol is planned as an initial stage. The extracts obtained were then formulated into ointment preparations and tested for antibacterial activity against Propionibacterium acnes. This effort is expected to produce an alternative acne treatment based on natural ingredients that is not only effective but also more environmentally friendly and safer than synthetic materials.

This research is important because until now the use of cassava peel waste is still limited and is more directed to animal feed or disposed of without adequate processing, so it has the potential to cause environmental problems. In fact, based on chemical studies, cassava peel contains phenolic compounds and other secondary metabolites that have the potential to be developed as pharmaceutical active ingredients. On the other hand, the increasing cases of acne and the emergence of bacterial resistance to synthetic

antibacterials demand the development of safer and more sustainable alternatives to natural material-based therapies.

Previous studies have generally only focused on testing the antibacterial activity of cassava peel extract in vitro or on the identification of secondary metabolite content only. Previous studies have not integrated phenolic content analysis with antibacterial effectiveness in the form of applicable pharmaceutical preparations. In addition, the relationship between variation in extract concentration, phenolic levels in preparations, and antibacterial response to Propionibacterium acnes has rarely been studied quantitatively and comprehensively.

Research gaps are also seen in the limitations of studies that tested cassava peel extract in the form of ointment preparations with a complete evaluation of physical qualities, such as homogeneity, pH, spreadability, and adhesion, as a prerequisite for safety and convenience of topical use. Some previous studies have not linked the physical quality parameters of preparations to their biological effectiveness, so the practical implications as an antiacne product are still not strong.

Therefore, this study was conducted to fill the gap by studying in an integrated manner the effect of phenolic levels on the antibacterial activity of Propionibacterium acnes in cassava peel extract ointment preparations. This approach is expected to strengthen the scientific basis for the use of cassava peel waste as a safe, effective, and value-added topical antibacterial candidate, while contributing to the development of sustainability-oriented natural ingredient-based pharmaceutical preparations.

2. Methods

The research activities were carried out at the Botanical Laboratory of the Department of Biology, Faculty of Mathematics and Sciences, University of Lampung and the Integrated Laboratory of Malahayati University. The entire series of experiments was carried out in the period from May to June 2025. The equipment used consists of a rotary evaporator, mortar, oven, incubator, pH meter, waterbath, petri dish, test tube, erlenmeyer, autoclave, UV-Vis spectrophotometer, as well as an ointment physical test device.
The ingredients used include cassava peel (Manihot esculenta), 96 percent ethanol solvent, PEG 400, PEG 4000, propylene glycol, nipagin, aquaade, NA media, Folin-Ciocalteu reagent, Na2CO3, and Propionibacterium acnes bacteria.

The study population is cassava husks from Pekon Mataram, Gading Rejo District, Pringsewu Regency. Samples were taken using purposive sampling techniques with the criteria of fresh and undamaged cassava peels. The independent variable is the variation in the concentration of cassava peel extract in the ointment preparation. The bound variables were phenolic levels and antibacterial activity against Propionibacterium acnes. The concentration of cassava peel extract is expressed in percent according to the formulation of the ointment. Phenolic levels were measured using a UV-Vis spectrophotometer and expressed as mg GAE/g. Antibacterial activity was assessed based on the inhibition zone around the wells in the NA media.

The fresh cassava peel is cleaned, dried, and ground into simplicia powder. The extraction process is carried out through maceration using 96 percent ethanol for two times 24 hours, then evaporated until a concentrated extract is obtained. The extracts are tested for phytochemical screening for the presence of phenolics, flavonoids, tannins, and saponins. Determination of phenolic levels is carried out through the Folin-Ciocalteu reaction and absorbance readings at maximum wavelengths.

The ointment preparations are made on the basis of PEG 4000 and PEG 400 using three concentrations of extracts, then organoleptic, homogeneity, pH, dispersibility, and adhesion are tested. Antibacterial testing was carried out by the sewage diffusion method on a medium that has been inoculated with

Propionibacterium acnes. The inhibition zone was measured after 24-hour incubation. The data was tested for normality using Shapiro-Wilk. If the data is normally distributed and homogeneous, the analysis is followed by a one-way ANOVA to see the differences between the formulas, then a follow-up LSD test if there are significant differences.

3. Results and Discussion

Results

Total Phenolic Levels of Cassava Peel Extract Ointment Preparation

The determination of the total phenolic level was carried out using the Folin–Ciocalteu method with a UV-Vis spectrophotometer at a maximum wavelength of 753 nm. Testing was carried out on three variations of cassava peel extract ointment concentrations, namely 2%, 4%, and 6%, with each treatment repeated three times. The results of the measurement of total phenolic levels in cassava peel extract ointment preparations are presented in Table 1.

Table 1. Total Phenolic Levels of Cassava Peel Extract Ointment Preparation

Formula	Extract Concentration (%)	Phenolic levels (mg GAE/g) ± SD
FI	2%	0.74 ± 0.04
FII	4%	1.15 ± 0.14
FIII	6%	1.36 ± 0.08

Based on Table 1, it can be seen that the total phenolic levels increase along with the increase in the concentration of cassava peel extract in the ointment preparation. The FIII formula (6%) has the highest phenolic content, while the FI formula (2%) has the lowest phenolic content. (Rudiyat, Yulianti and Indra, 2020; Rohimah and Tuti, 2021; Rustanto, Kusumaningrum and Rasyid, 2022).

Antibacterial activity against Propionibacterium acnes

The antibacterial activity test was carried out using the well diffusion method by measuring the diameter of the inhibition zone formed around the well after incubation for 24 hours at a temperature of 37°C. Cassava peel extract ointment was tested against Propionibacterium acnes bacteria and compared with positive controls (mupirocin ointment) as well as negative controls (ointment base without extract).
The results of the antibacterial activity test can be seen in Table 2.

Table 2. Antibacterial Activity of Cassava Peel Extract Ointment Preparations against Propionibacterium acnes

Sample	Buffer Zone (mm) ± SD	Category Hambat
FI (2%)	16.33 ± 2.56	Strong
FII (4%)	12.00 ± 1.81	Strong
FIII (6%)	11.50 ± 2.64	Strong
Positive Control	15.50 ± 3.27	Strong
Negative Control	–	None

Based on Table 2, all cassava peel extract ointment formulas show antibacterial activity with strong inhibition category against Propionibacterium acnes. Negative control showed no inhibition zone, which indicates that the antibacterial activity comes from the cassava peel extract content(Sangi *et al.*, 2008; Siregar, 2009; Susanti, Primadiamanti and Ulfa, 2022).

Discussion

The cassava peel (Manihot esculenta) used in this study has gone through a determination process and is declared correct as the plant in question, so that the validity of the test material can be accounted for. The extraction process is carried out by the maceration method using a 96% ethanol solvent because ethanol is universal and is able to attract secondary metabolite compounds both polar and semi-polar, including phenolic compounds, flavonoids, tannins, and saponins.

The results of phytochemical screening showed that cassava peel extract contains flavonoids, saponins, tannins, and phenolics. The presence of phenolic compounds plays a major role in antibacterial activity because these compounds are able to damage the structure of bacterial cell walls through the mechanism of protein denaturation and impaired cell membrane permeability. In addition, phenolic compounds can also act as antioxidants that support the inflammatory healing process in acne.

The results of determining the total phenolic level showed an increase in phenolic levels along with the increase in the concentration of extracts in the ointment preparation. The FIII formula (6%) has the highest phenolic content compared to the FI and FII formulas. This indicates that the higher the concentration of extracts used, the greater the amount of phenolic compounds contained in the ointment preparation. The results of the ANOVA test on phenolic content data showed a p value of < 0.05, which indicates a significant difference in phenolic content between formulas (Victor, 1980; Wahyuni, Afthoni and Rollando, 2022).

In testing the antibacterial activity, the entire cassava peel extract ointment formula was able to inhibit the growth of Propionibacterium acnes with a strong inhibitory category. This antibacterial activity is thought to come from the working combination of phenolic compounds, flavonoids, tannins, and saponins that work synergistically Flavonoids can damage bacterial cell membranes, saponins increase membrane permeability, while tannins play a role in precipitating bacterial cell proteins thereby inhibiting the growth of microorganisms (Syamsuni, 2007; Usma, Anam and Utami, 2023).

Interestingly, although the FIII formula has the highest phenolic levels, the largest inhibition zone is actually seen in the FI formula. This shows that antibacterial activity is not only influenced by phenolic content alone, but also by other factors such as the diffusibility of the ointment in agar media, the viscosity of the preparation, and the ability of the active compound to diffuse optimally. Too high an extract concentration can increase the viscosity of the ointment thereby inhibiting the diffusion of the active compound into the medium Wiritania *et al.*, (2024). Overall, the results of this study show that cassava peel extract ointment preparations have the potential to be used as a natural antibacterial alternative to Propionibacterium acnes, with effectiveness being influenced by the concentration of the extract and the physical characteristics of the preparation.

4. Conclusion

Research shows that cassava peels extracted using the maceration method with 96 percent ethanol produce a yield of 12.52 percent and contain flavonoid compounds, saponins, tannins, and phenolics. These compounds are identified as secondary metabolites that have antibacterial activity. The extract is formulated in the form of an ointment preparation with three variations of concentration, namely FI 2 percent, FII 4 percent, and FIII 6 percent. The entire formula has met the physical quality requirements, demonstrated by the homogeneity of the preparation, pH corresponding to the physiological range of the skin, stable dispersibility and adhesion, and consistent organoleptic characteristics.

The phenolic levels contained in the preparation show the difference between the formulas. The FIII formula has the highest phenolic levels, followed by FII and FI. The results of the antibacterial activity test against

Propionibacterium acnes show that the entire formula is in the strong category. The largest inhibition zone was actually generated by the FI formula of 16.33 mm, higher than FII, FIII, and positive controls. The negative control showed no antibacterial activity, so the inhibitory effect could be attributed to the presence of cassava peel extract. Statistical analysis confirmed significant differences between treatment groups, suggesting that variations in extract concentrations had an effect on antibacterial responses. These findings support the potential of cassava peel extract ointment as a topical antibacterial candidate against Propionibacterium acnes.

As a recommendation, further research needs to be conducted to evaluate the stability of preparations over longer storage periods as well as test the safety of use through skin irritation tests. In addition, testing of antibacterial activity against other clinically relevant microorganisms needs to be developed to expand the potential application of the preparation. Further research is also recommended to explore the optimization of extract concentration and formulation methods to obtain more optimal antibacterial effectiveness.

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