

Solvent Types of Pandan Leaves (*Pandanus amaryllifolius* Roxb.) Extraction Effect on the *Cutibacterium acne* Activity and Development of its Spot Cream

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Abstract: Acne vulgaris is a skin disease that is caused by *Cutibacterium acnes*. *Pandanus amaryllifolius* Roxb. contains flavonoids that have antibacterial activity. The aims of this study are to determine the effect of the ethanol concentration on the pandanus leaf extraction in terms of total flavonoid content produced and antibacterial activity against *C. acnes*, and to evaluate the spot cream made from pandanus leaf extract. The total flavonoid content of the extract was measured by spectrophotometry, and its antibacterial activity was tested using the microdilution method. The extract with 96% ethanol solvent has a total flavonoid content of 385.758 ± 7.910 mgRE/g extract and the smallest MIC₅₀ value of 3.565 mg/mL. The MIC₅₀ value was used as a reference for the cream dose in the acne patch cream formulation. The spot cream obtained was tested for accelerated stability for 4 weeks. The cream obtained has a soft texture, a pale yellow color, and a distinctive pandanus odor. The spot cream is homogeneous and has a pH of 5. The viscosity of the cream decreased from 9896.75 ± 116.42 cPoise in week 0 to 8660.25 ± 170.51 cPoise in week 4. The spot cream has good spreadability and stickiness

Keywords: acne spot cream; *Cutibacterium acnes*; flavonoids; *Pandanus amaryllifolius* Roxb.; solvent extraction.

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1. Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous tissue caused by *Cutibacterium acnes* [1]. Acne is the 8th most common disease worldwide, with a prevalence of 9.38% according the Global Burden of Disease Study in 2010 [2]. Acne can affect sufferers both socially and physically. The impact of acne on quality of life includes stress, depression, and psychosocial disorders [3].

Topical treatments for acne, such as benzoyl peroxide, topical antibiotics, topical retinoids, azelaic acid, and dapson, are first-line treatments for mild to moderate acne[4]. Benzoyl peroxide is a bactericidal and oxidizing agent. However, benzoyl peroxide can cause skin irritation, peeling, itching, and redness. Topical retinoids can cause skin irritation [5].

Therefore, alternative therapies are needed to treat acne that can minimize the occurrence of side effects.

As anti-acne compounds, plants can act through several mechanisms of action, namely antibacterial, anti-inflammatory, antioxidant, and antiandrogenic[6]. *Pandanus amaryllifolius* is an aromatic plant in the Pandanaceae family, commonly found in tropical and subtropical regions. Pandan leaves are used as a treatment for skin diseases in South Asia[7]. Research from Purwantiningsih *et al.* shows that phenolic compounds and flavonoids can function as antibacterials [8]. Quercetin, catechin, apigenin, luteolin, kaempferol, genistein, fisetin, rutin, naringin, and mirisetin are some of the flavonoids found in pandan leaves [9]. Research by Subrata and Lawrence shows that flavonoids from *the Pandanus amaryllifolius leaf extract exhibit antibacterial activity against Enterococcus faecalis by inhibiting nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism* [10]. To optimize therapeutic efficacy, the active compounds in pandanus leaves need to be extracted first to facilitate dosage and drug administration.

Extraction is the process of attracting soluble chemical compounds to separate them from insoluble materials using liquid solvents. Maceration is a simple extraction method that is carried out by immersing dry extract powder in a liquid extractor [11]. In the extraction process, the type of solvent is the main parameter that affects efficiency, as it determines two important factors: the solubility of target compounds and penetration into the matrix [12].

Some studies show that variations in the liquid concentration during the extraction process affect the total flavonoid content obtained. In this study, pandanus leaves will be extracted using the maceration method with different concentrations of ethanol solvents (10%, 25%, 50%, 70%, and 96%). The total flavonoid content of the leaves will be measured. The extracts were also analyzed qualitatively by thin-layer chromatography. This qualitative TLC analysis was carried out to confirm the flavonoid content of the pandan leaf extract obtained. The extract with the best antibacterial activity was selected and used as the active ingredient of the spot cream. The smallest MIC₅₀ value of the microdilution results determined the selection of extracts with the best antibacterial activity.

2. Materials and Methods

2.1. Materials.

The materials used in this study are 1 kg of dry *Pandanus amaryllifolius* Roxb. leaves, 96% ethanol (CV. Progo Mulyo, Indonesia), filter paper, rutin standard (Aldrich®), silica plate (Merck TLC Silica gel 60 F254), ethanol analytical grade (Smart-Lab®), ethyl acetate analytical grade (Emsure®), formic acid analytical grade, sodium acetate, AlCl₃, agar, Brain Heart Infusion media (Merck®), *Cutibacterium acnes*, disposable petri dish (Labware® Charuzu), multi-well microplate 96 wells (Iwaki®), white tip, yellow tip, blue tip (Biologix®), 5 mL pipette tips (Gilson®), plastic wrap, aluminum foil, distilled water, stearic acid, cetyl alcohol, liquid paraffin, glycerin, triethanolamine, methylparaben, and propylparaben (PT. Brataco Chemika, Indonesia).

2.2. Determination of dried extract.

The determination of dried extract was carried out at the Department of Pharmaceutical Biology, Gadjah Mada University. The determination of dried extract aims to confirm that the extract used in this study is *Pandanus amaryllifolius* Roxb. leaves.

2.3. Extraction.

Extraction optimization of *Pandanus amaryllifolius* Roxb leaf extract was carried out with various ethanol concentrations (10%, 25%, 50%, 70%, and 96%). The weight of the dry extract of pandan leaves for one maceration is 200 grams. Dilution of 96% ethanol with distilled water to the required ethanol concentration is used as the solvent of extraction. The ratio of the dry extract's weight to the solvent's volume is 1:10 w/v. Maceration was carried out for 3 x 24 hours, with the first 6 hours stirred. Remaceration is done once by filtering the macerate, then using the same ethanol concentration as the solvent, with the solvent volume half that of the first extraction. Remaceration was carried out using a 1:5 w/v dry extract-to-solvent ratio for 1 day. All macerates were collected and then evaporated with a water bath until a thick liquid extract was obtained. The extract obtained was then freeze-dried to remove solvents and obtain the extract powder.

2.4. Qualitative test by thin-layer chromatography.

Thin-layer chromatography analysis was performed using the macerate obtained from the sample extraction. The chromatography vessel to be used was first saturated with the mobile phase. Samples were spotted with 5 μ L, with 0.1% rutin standard at 2 μ L on a silica gel 60 F₂₅₄ plate. The plate was eluted with a mobile phase of ethyl acetate:formic acid:water (100:13.5:10). After elution, the plate was observed under visible light and UV366. Then, the plate was sprayed with the AlCl₃ reagent and heated in the oven for \pm 15 minutes to detect the presence of flavonoids in the extract.

2.5. Total flavonoid content test.

Freeze-dried extracts were determined for total flavonoid content based on the colorimetry method.

2.5.1. Determination of the wavelength of maximum absorbance of rutin.

Two mL of the 1000 ppm rutin stock solution was pipetted and put into a 5 mL volumetric flask. Ethanol of analytical grade was added to obtain rutin with a concentration of 400 ppm. Rutin at a concentration of 400 ppm was pipetted into a test tube to a volume of 0.5 mL, then 1.5 mL ethanol, 0.1 mL AlCl₃ 10%, 0.1 mL sodium acetate 1 M, and 2.8 mL distilled water were added. The test solution was homogenized by vortexing and incubated for 30 minutes at room temperature. The absorbance of the test solution was measured at a wavelength of 400–800 nm using a UV-Vis spectrophotometer.

2.5.2. Preparation of rutin standard curve.

The stock solution of rutin was taken with a micropipette as much as 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 mL and then put into a 5 mL volumetric flask and added ethanol analytical grade up to the mark so as to obtain rutin levels of 100, 150, 200, 250, 300, 350, and 400 μ g/mL, respectively. Each solution was taken up in 0.5 mL with a micropipette and placed into a test tube. Each was added 1.5 mL ethanol, 0.1 mL AlCl₃ 10%, 0.1 mL sodium acetate 1 M, and 2.8 mL distilled water. The standard solution was homogenized by vortexing and allowed to stand for 30 minutes at room temperature. The absorbance of the standard solution was measured at the wavelength of maximum absorbance using a UV-Vis spectrophotometer.

The blank solution was prepared without the addition of AlCl_3 . After obtaining the absorbance, a calibration curve was made, and the test solution levels were calculated. The graph is made with the X-axis representing the concentration of rutin in ppm and the Y-axis representing the difference in absorbance between the test solution and a blank solution.

2.5.3. Preparation of sample solution.

The test solution was prepared for the extract by carefully weighing approximately 100 mg of freeze-dried extract, placing it in a conical tube, and adding 5 mL of ethanol at the same concentration as during maceration. The sample was vortexed and ultrasonicated for 10 min until completely dissolved. The sample was centrifuged at 8000 rpm for 5 minutes, and the supernatant was taken. Samples of pandan leaf extract were diluted by taking 1 mL of the sample and placing it in a 5 mL volumetric flask, then adding 96% ethanol. Pandan leaf extract samples with 10%, 25%, 50%, and 70% ethanol were not diluted because the data obtained were already interpolated.

2.5.4. Determination of total flavonoid content in the sample solution.

The test procedure for total flavonoid content was carried out by separately pipetting 0.5 mL of the sample test solution and adding 1.5 mL of ethanol, 0.1 mL of AlCl_3 10%, 0.1 mL of sodium acetate 1 M, and 2.8 mL of distilled water. The sample test solution was homogenized by vortexing and allowed to stand at room temperature for 30 minutes. The sample test solution was measured for absorbance at the wavelength of maximum absorbance using a UV-Vis spectrophotometer. The blank solution was prepared the same way without adding AlCl_3 . The total flavonoid content in the samples was determined using the calibration curve equation.

2.6. Antibacterial activity test with microdilution method.

The antibacterial activity test of *Pandanus amaryllifolius* Roxb. leaf extract with variations in the distillation liquid was carried out using the microdilution method with BHI broth. The Petri dishes were incubated for 24 hours under anaerobic conditions. Aseptically, one inoculation needle of *C. acnes* culture was inoculated into 5 mL of BHI broth media in a 15 mL test tube. The test tube was incubated for 1-2 hours at 37°C until the OD_{600} of *C. acnes* reached 0.08-0.1, with a bacterial count of 1×10^8 CFU/mL [13]. Preparation of bacterial test solution by taking 1 mL of bacteria with an OD_{600} of 0.08-0.1, then diluting to 5 mL using BHI broth media. According to the Clinical Microbiology Procedures Handbook, the final concentration of test bacteria in each well is 3×10^5 to 5×10^5 CFU/mL [14]. The diluted suspension was added up to 40 μL per well, corresponding to a final test bacteria concentration of 4×10^5 CFU/mL in each well.

DMSO 1% was used to dissolve the extract, based on preliminary tests to determine concentration. The sample was vortexed and ultrasonicated until it was completely dissolved. The sample was centrifuged at 10000 rpm for 3 minutes, and the supernatant was used as the test sample. The extract was prepared in a series of levels so as to cover the MIC_{50} value.

The test sample consisted of 40 μL of test bacteria, 50 μL of test sample, and 110 μL of BHI broth media. The sample control consisted of 50 μL of the test sample and 150 μL of BHI broth. Bacterial growth control, as a negative control, consists of 40 μL of test bacteria and 160 μL of BHI broth. The negative control consists of 40 μL of test bacteria, 50 μL of 1%

DMSO, and 110 µL of BHI broth. Microplates were incubated at 37°C for 16-18 hours under anaerobic conditions in a jar incubator. The microplate was read at OD₆₀₀ using a microplate reader. Antibacterial activity was determined by the percentage inhibition of the extract against *C. acnes* using the following formula.

$$\% \text{ inhibition} = \frac{(\text{absorbance of negative control} - (\text{absorbance of sample test} - \text{absorbance of sample control}))}{\text{the absorbance of negative control}} \times 100 \quad (1)$$

The data obtained were analyzed by probit regression to obtain the MIC₅₀ value with the SPSS application.

2.7. Formulation of topical cream.

Pandan leaf extract, which has the highest antibacterial activity and the lowest MIC₅₀ value, will be formulated into an acne patch cream. This cream formulation refers to the optimal formulation reported by Puspitasari *et al.* with modifications [15]. The formulations of the cream base and the pandan leaf extract cream are shown in Table 1.

Table 1. Formulation of pandan leaf extract topical cream.

Excipients	Composition (g/100 g)	Function
Pandan leaf extract	0.3565	Active ingredient
Stearic acid	10	Oil base
Cetyl alcohol	3	Oil base
Liquid paraffin	2	Emollient
Glycerin	10	Humectant
Triethanolamine	0.5	Emulgator
Methylparaben	0.2	Preservative
Propylparaben	0.05	Preservative
Aquadest	Ad 100	Solvent

2.8. Evaluation of the physical stability of the topical cream.

The stability test was conducted using the accelerated stability method based on ICH Q1A (R2). This test is conducted to measure the quality of the cream produced if the cream is in certain environmental conditions. The test was conducted by placing the cream in a climatic chamber at 40°C and 75% RH for 4 weeks. Evaluation of the cream's physical properties was conducted weekly from week 0 to week 4. Evaluation of the cream's physical properties included organoleptic tests, homogeneity, viscosity, pH, spreadability, and stickiness.

2.8.1. Organoleptic test.

The organoleptic test is based on observations of the physical appearance of the spot cream [16].

2.8.2. Homogeneity test.

The homogeneity test is performed by applying a specified amount of cream preparation to an object glass. The preparation must exhibit a homogeneous composition and no noisible coarse grains [17].

2.8.3. pH measurement.

The pH measurement was carried out using Supelco® pH indicator strips. The pH tolerance range of the cream ranges from 4.5-6.5 [18,19].

2.8.4. Viscosity test.

The viscosity test was carried out to determine the level of viscosity of the cream preparation using a Brookfield viscometer. Viscosity measurement is carried out by installing the RV-7 spindle. The spindle is immersed to a certain extent during preparation, and the speed is set to 00 rpm for 15 seconds at 25°C [17].

2.8.5. Spreadability test.

The spreadability test was carried out by weighing 0.5 grams of the cream preparation and placing it in the middle of a round glass placed on a millimeter block of paper. Next, the other glass was placed on top of the cream and left for 1 minute to measure the spread's diameter. The diameter of the spreading cream was measured. Next, an additional weight of 50 g was added to the glass, which was allowed to stand for 1 minute, and the diameter of the spread formed was recorded. The process continued by adding 50 g loads each time until the total load reached 250 g, and the diameter of the spreading cream was recorded after 1 minute. Good spreadability is 5-7 cm [16,19].

2.8.6. Adhesion test.

A total of 250 mg of cream preparation was placed on an object glass that had been determined. Another object glass was placed on top of the preparation and given a load of 1 kg for 5 minutes. The test glass was placed on the test device and given a load of 80 grams. The attached load was dropped, and the time until both glasses were released after the load was dropped was recorded. Good adhesion is more than 1 second [16,19].

3. Results and Discussion

3.1. Determination of dry extract.

The pandan leaves (*Pandanus amaryllifolius* Roxb.) were determined at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University. The results of the determination of simplisia based on Certificate Number 51.9.1/UN1/FFA.2/S1/PT/2023 show that the plant used is *Pandanus amaryllifolius* Roxb., belonging to the tribe Pandanaceae.

3.2. Extraction.

In the extraction process, the type of liquid is the main parameter that affects its efficiency, as it can determine two important factors: the solubility of the target compound and its penetration into the matrix (Rostagno and Prado [12]). The ethanol distillation solution is prepared by diluting 96% ethanol with distilled water to the required ethanol concentration. The results of ethanol dilution are measured using an alcoholmeter to ensure the ethanol concentration.

This study used a variant of ethanol as the extraction solvent. The ratio of the simplest's weight to the liquid's volume was 1:10 b/v. Maceration was carried out for 3 x 24 hours with occasional stirring for the first 6 hours, then allowed to stand for the next 18 hours. Furthermore, maceration was carried out for 1 day with a solvent volume of 1 liter. After the

extract is freeze-dried, the characteristics of the extract, as well as the weight and yield of the extracted fragrant pandanus leaves at varying ethanol concentrations, are shown in Table 2.

Table 2. Extraction yield.

Solvent	Characteristics	Weight (g)	Yield (%w/w)
Ethanol 10%	Color: Brown Odor: Typical pandan Taste: Tart	23.785	11.89
Ethanol 25%	Color: Brown Odor: Typical pandan Taste: Tart	20.122	10.06
Ethanol 50%	Color: Brown Odor: Typical pandan Taste: Tart	22.528	11.26
Ethanol 70%	Color: Brown Odor: Typical pandan Taste: Tart	24.282	12.14
Ethanol 96%	Color: Greenish brown Odor: Typical pandan Taste: Tart	19.440	9.72

3.3. Thin-layer chromatography.

Thin-layer chromatography analysis is performed using the macerate from the sample extraction. The chromatography vessel to be used is first saturated with the mobile phase. Saturation of the vessel aims to optimize the mobile-phase development process, equalize the vapor pressure of the mobile phase, and produce good compound separation spots [20]. Samples were bottled at 5 μ L each side by side with 0.1% rutin standard at 2 μ L on silica gel 60 F254. The rutin standard was chosen because the target compound is a flavonoid. The plate was eluted with the mobile phase ethyl acetate:formic acid: water (100:13.5:10). The mobile phase was chosen based on the polarity of the target compound. After elution, the plate was observed under visible light and UV366. Then, the plate was sprayed with an $AlCl_3$ reagent and heated in the oven for \pm 15 minutes to detect the presence of flavonoids in the extract. Thin-layer chromatography results with rutin standards before and after spraying with $AlCl_3$, visible under visible light and UV at 366 nm, are shown in Figures 1 and 2.

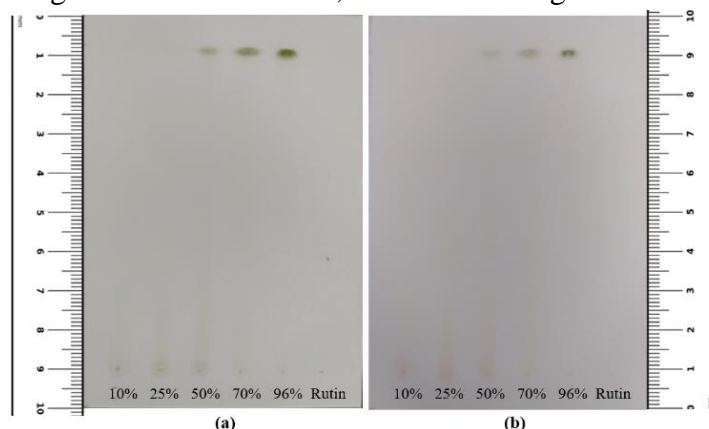


Figure 1. Results in visible light (a) before spray; (b) after spray $AlCl_3$.

Figure 1 shows the TLC profile in visible light, while Figure 2 shows the flavonoids shown in UV light 366 nm. Thin layer chromatography profiles show that pandan leaf samples with 10%, 25%, 50%, 70%, and 96% ethanol distillation liquid have flavonoids at R_f of 0.19 and 0.63, which are marked by fluorescence after being sprayed with $AlCl_3$ reagent. The rutin standard has an R_f of 0.54. The flavonoid compound at point (1) in pandan leaves in the TLC results is thought to be a flavonoid glycoside, which has a less polar nature than rutin.

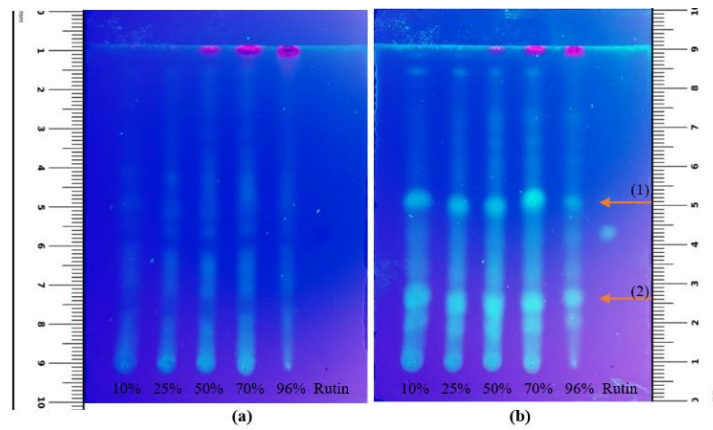


Figure 2. Results in UV light 366 nm (a) before spray; (b) after spray AlCl_3 .

Rutin and flavonoid content in pandan extracts are seen in a thin yellow color and fluorescence at 366 nm after being sprayed with AlCl_3 because they form a stable acidic chelate complex between AlCl_3 and the keto group at C4 and with the hydroxyl group at C5 and at C3' and C4' on rutin. Flavonoids generally react with AlCl_3 in the keto group at C4 and with the hydroxyl group at C5 [21]. Figure 3 shows the reaction mechanism of flavonoids in general with AlCl_3 .

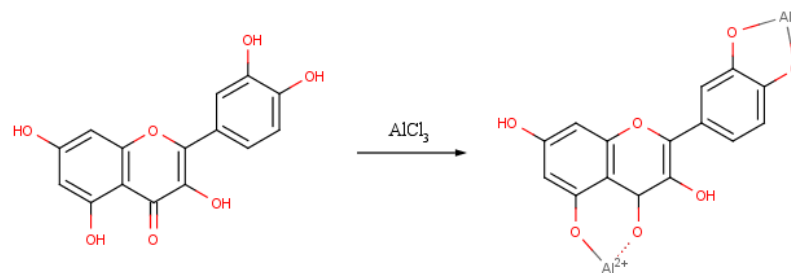


Figure 3. Mechanism of the reaction of flavonoid with AlCl_3 .

3.4. Total flavonoid test.

3.4.1. Determination of maximum wavelength (λ_{max}).

The maximum wavelength was determined using a UV-Vis spectrophotometer over the 200-700 nm range. Based on the study of Pękal & Pyrzyńska, the calculation of flavonoid glycoside content that reacts with AlCl_3 uses a wavelength of 410-430 nm [22]. The maximum wavelength obtained in this study was 416.0 nm, as shown in Figure 4.

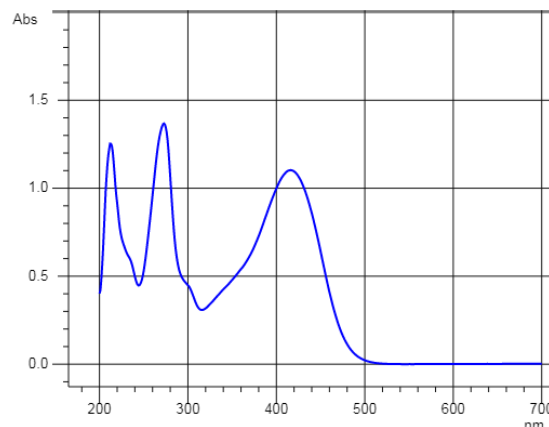


Figure 4. Rutin maximum wavelength.

3.4.2. Determination of rutin standard curve.

Flavonoids react with $AlCl_3$ to form a stable acidic chelate complex between $AlCl_3$ and the keto group at C4 and with the hydroxyl group at C5 as well as at C3' and C4' [21]. The addition of sodium acetate in testing total flavonoid content is a sliding reagent that maintains the wavelength in the visible light region [23]. The standard curve for the rutin assay is shown in Figure 5. Determination of the flavonoid standard curve (rutin) obtained the equation $y = 0.0031x - 0.1808$.

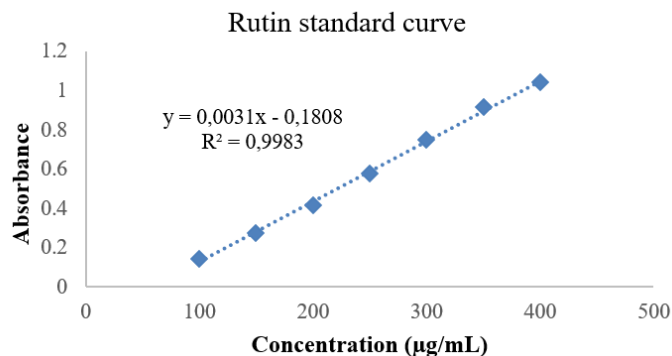


Figure 5. Flavonoid standard curve (Rutin).

3.4.3. Determination of total flavonoid content of *Pandanus amaryllifolius* roxb leaf extract.

The determination of total flavonoid content was calculated using the linear regression line equation $y = 0.0031x - 0.1808$ obtained from the rutin calibration curve, so that the total flavonoid content in the sample can be known. The y value was substituted with the absorbance obtained from the formula for calculating total flavonoid content. The total flavonoid content was determined in triplicate, and the average was calculated. Data from the determination of total flavonoid content can be seen in Figure 6. Total flavonoid levels in pandan leaf extracts are expressed in units of mgRE/g, meaning that 1 mg of rutin is equivalent to 1 g of extract. The highest total flavonoid levels in pandan leaf extracts, with variations in distillation liquid, were observed with 96% ethanol solvent, with a mean \pm SD of 385.758 ± 7.910 mgRE/g.

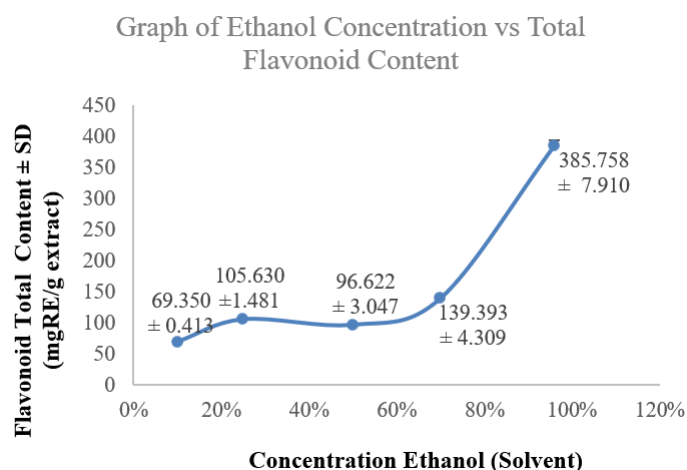


Figure 6. Graph of ethanol concentration against total flavonoid content.

Data analysis of the results of determining the total flavonoid content of *Pandanus amaryllifolius* Roxb. leaf extract was tested for normality using the Shapiro-Wilk at the 95% confidence level. The results of ANOVA showed significant differences in total flavonoid levels across distillation liquids. This is in accordance with the solvent, which is the main

parameter affecting the process efficiency, namely the solubility of the target compounds and their penetration into the Pandanus leaf extract matrix. A study by Sari *et al.* states that the higher the concentration of ethanol used, the higher the content of secondary metabolites produced because ethanol is an organic solvent with a high portion of hydroxyl groups, which will more easily penetrate the thick layer of plant cell boundaries to extract secondary metabolites in these cells [24]. Ethanol has a lower surface pressure than water, so it can penetrate the plant cell wall more easily. Ethanol can break hydrogen bonds that play an important role in maintaining the structure of plant cell walls [25]. This statement emphasizes that ethanol concentration during distillation is one factor that influences the effectiveness of pandan leaf extraction, as measured by TLC and total flavonoid content.

3.5. Antibacterial test.

3.5.1. Preparation of *Cutibacterium acnes* bacteria suspension.

A bacterial growth medium is needed to maintain the nutrition and survival of bacteria. Brain Heart Infusion (BHI) agar medium was used as a culture medium for testing bacteria. Bacteria *Cutibacterium acnes* from the glycerol stock were streaked on Petri dishes containing sterile BHI agar media. The petri dish was incubated at 37°C for 24 hours under anaerobic conditions in an incubator jar. Aseptically, one inoculum needle of the *C. acnes* culture was inoculated in 5 mL of BHI broth media in a 15 mL test tube. The test tube was incubated for 1-2 hours at 37°C until the OD₆₀₀ of *Cutibacterium acnes* reached 0.08-0.1, with a bacterial count of 1×10^8 CFU/mL [13]. Preparation of bacterial test solution by taking 1 mL of bacteria with an OD₆₀₀ 0.08-0.1, then diluting to 5 mL using BHI broth media. The use of bacteria in the exponential phase in antimicrobial activity tests is important because cell division occurs regularly at a constant rate, and growth proceeds in a balanced, rapid manner [26].

3.5.2. Sample preparation of *Pandanus amaryllifolius* roxb. leaf extract.

The concentration of DMSO used to dissolve the extract is based on preliminary tests in the form of concentration determination. The DMSO concentration used was 1%, as it dissolves the extract and lacks antibacterial activity against *C. acnes*. The test sample was prepared by dissolving the sample in DMSO and diluting it with sterile distilled water to a final DMSO concentration of 1%. The extract was prepared in a series of levels so as to include the MIC₅₀ value.

MIC₅₀ was chosen as the minimum level that inhibits the growth of test bacteria by 50%. Studies by Levison & Levison found that long-term exposure to antibiotics can increase the development of antibiotic resistance, leading bacteria to evade their effects and rendering antibiotics ineffective against them. Mechanisms of bacterial antibiotic resistance include enzymatic degradation of antibiotics, alteration of antibiotic-targeted bacterial proteins, changes in bacterial membrane permeability, reduced antibiotic penetration, efflux pumps, biofilm formation, and horizontal gene transfer. Biofilm formation is influenced by the microbes' ability to communicate, known as quorum sensing. In their communication, microbes use chemical signals called autoinducers to detect population size, thereby coordinating their behavior and regulating expression in response to changes in population density [28]. In addition to biofilms, pathogenic traits affected by quorum sensing include the ability of motile microbes to invade other cells, toxin production, antibiotic resistance, and pigment production. Microbes that form biofilms have very complex structures that can protect cells from

unfavorable environmental conditions for growth, such as antibiotics, drought, and other factors, making them more resistant. Currently, the approach to overcoming infection by pathogenic microbes is to disrupt microbial communication (quorum sensing) so that the pathogenic properties of microbes can be eliminated, while maintaining microbial growth to avoid antibiotic resistance[29,30].

3.5.3. Antibacterial test with microdilution method.

The data were analyzed using probit regression in SPSS to estimate the MIC₅₀ value, and the results are shown in Table 3. The highest antibacterial potential is exhibited by fragrant pandanus leaf extract, obtained via 96% ethanol distillation, with an MIC₅₀ of 3.565 mg/mL.

Table 3. MIC₅₀ Value of *Pandanus amaryllifolius* roxb. leaves extract.

Solvent	MIC ₅₀ value (mg/ml)
Ethanol 10%	30.281
Ethanol 25%	15.078
Ethanol 50%	16.795
Ethanol 70%	15.538
Ethanol 96%	3.565

The results of the Welch test and Brown-Forsythe test resulted in a Sig. 0.000, which indicates that there is a significant difference in the MIC₅₀ value in the variation of solvent. An extract is categorized as an antibacterial substance into 3 groups: strong (MIC < 100 µg/mL), moderate (100 < MIC < 625 µg/mL), and weak (MIC > 625 µg/mL)[31]. Pandan leaf extract is categorized as a weak antibacterial agent. This can be caused by the extraction process that is not followed by fractionation, so there are still many impurities in the extract. The highest total flavonoid content and the lowest MIC₅₀ value are owned by pandanus leaf extract with 96% ethanol distillation liquid. The extract was chosen as the active substance in the cream preparation.

3.6. Formulation of anti-acne topical cream.

Pandan leaf extract with 96% ethanol solvent was formulated into an acne spot cream. The formulation of this cream preparation is based on the optimal formulation results reported by Puspitasari *et al.* with modifications [15]. The base formulation is not added with active substances; the sample formulation is added with active substances dissolved in distilled water according to the formula. The dose of pandanus leaf extract cream used is in accordance with the MIC₅₀ value of 3.565 mg/mL obtained from the microdilution antibacterial test.

3.7. Evaluation of the physical stability of the topical cream.

Shelf time is one of the requirements that must be met in pharmaceutical products. The shelf life of drugs is strictly regulated, so the stability test stage is a critical stage. The shelf life displayed on the pharmaceutical product label indicates that the product's integrity, quality, and potency are guaranteed for use within that time period [32]. The stability test was conducted using the accelerated stability method based on the ICH Q1A(R2) guidelines [33]. This test is conducted to measure the quality of the cream produced under specific environmental conditions. The test was conducted by placing the cream in a climatic chamber at 40°C and 75% RH for 4 weeks. Evaluation of the cream's physical properties was conducted weekly from week 0 to week 4. In this study, the stability test was conducted by evaluating the cream's

physical properties, including organoleptic tests, homogeneity, viscosity, pH, spreadability, and stickiness.

3.7.1. Organoleptic test.

The results of organoleptic observations of the cream base and pandan cream are listed in Tables 4 and 5, respectively. The results of weekly organoleptic observations did not show significant differences in the cream preparations. The cream base and pandan cream preparations are stable in organoleptic observations.

Table 4. Organoleptic testing results of the topical cream base.

Time	Texture	Color	Odor
Week 0	Soft	White	Odorless
Week 1	Soft	White	Odorless
Week 2	Soft	White	Odorless
Week 3	Soft	White	Odorless
Week 4	Soft	White	Odorless

Table 5. Organoleptic testing results of pandan topical cream.

Time	Texture	Color	Odor
Week 0	Soft	Pale yellow	Typical pandan, sweet aroma
Week 1	Soft	Pale yellow	Typical pandan, sweet aroma
Week 2	Soft	Pale yellow	Typical pandan, sweet aroma
Week 3	Soft	Pale yellow	Typical pandan, sweet aroma
Week 4	Soft	Pale yellow	Typical pandan, sweet aroma

3.7.2. Homogeneity test.

The homogeneity test is performed to determine whether all cream components are well mixed. The preparation must exhibit a homogeneous composition and no noisible coarse grains [17]. The homogeneity test results showed that the cream base and pandan cream formulas did not show any coarse grains on the transparent glass object. The cream base and pandan cream look homogeneous before and after accelerated stability testing.

3.7.3. pH measurement.

The pH tolerance range of the cream ranges from 4.5 to 6.5. If the cream's pH is below 4.5, it is acidic and can irritate the skin. If the pH of the cream is above 6.5, the cream is alkaline and can cause dry and flaky skin [18,19]. The pH measurements of the cream base and the pandan leaf total cream preparation are shown in Figure 7. The pH measurement results show that the cream base and pandan cream formulas fall within the cream pH range of 4.5-6.5. The pH of the cream base and pandan cream remained unchanged before and after storage under accelerated stability test conditions.

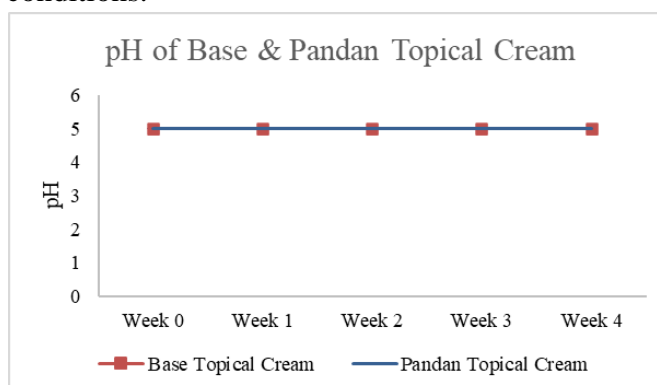


Figure 7. Graph of pH measurement results of cream base and pandan topical cream.

3.7.4. Viscosity test.

The viscosity test was conducted to determine the viscosity level of the cream preparation using a Brookfield viscometer. The selection of the spindle number. 7 was based on the torque value obtained from the experiment between 20% and 80%. A good cream viscosity range is between 2000 and 50000 Cps [34]. The viscosity test results obtained can be seen in Figure 8.

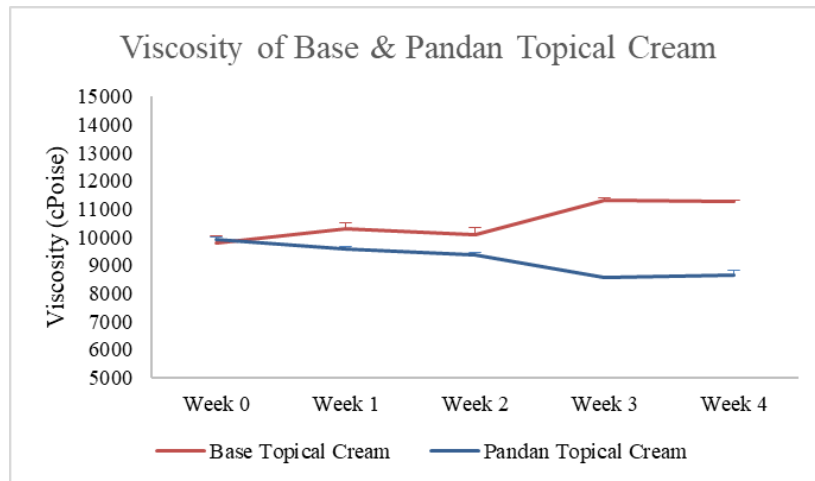


Figure 8. Graph of viscosity test results of cream base and pandan topical cream.

The viscosity of the cream base increases every week under accelerated stability conditions due to accelerated coalescence accompanied by evaporation of water from the cream during storage in the climatic chamber [35]. The decreased viscosity of pandan cream can be attributed to increased molecular motion as the storage temperature increases, reducing the contact time between molecules and loosening their bonds [36]. Based on Labconco® guidelines, freeze-dried extract powder is hygroscopic, so exposure to moisture during storage can cause it to absorb more water and become less stable. In addition, the pandan leaf extract used contains flavonoid glycosides, which are more hydrophilic than flavonoid glycosides [38]. This is influenced by flavonoid glycosylation, which increases hydrophilicity and decreases the log P value [39]. The extract's hydrophilic nature can attract water from the environment, reducing the viscosity of the pandan cream over time. Although viscosity changes, the viscosity of the cream base and pandan cream remains within the good cream viscosity range, namely 2000-50000 cP [34].

3.7.5. Spreadability test.

The spreadability test was conducted to determine the cream's ease of application and use. Cream preparations are expected to spread easily and evenly. Good spreadability is 5-7 cm [16,19]. The results of the spreadability test can be seen in Figure 9.

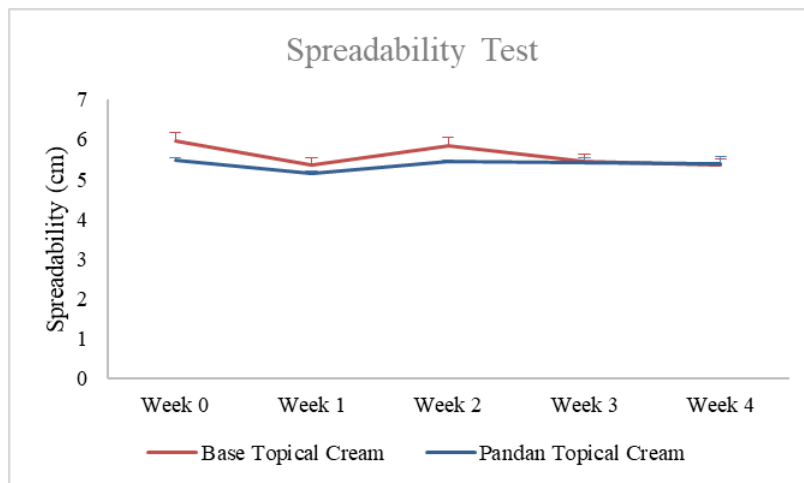


Figure 9. Graph of spreadability test results of cream base and pandan topical cream.

The results of the spreadability test on the cream base and pandan cream showed changes. Although there is a change in spreadability, the cream base and pandan cream remain within the good cream spreadability range 5-7 cm [16, 19].

3.7.6. Adhesion test.

The adhesion test was conducted to determine the cream's adhesion time. The higher the adhesion value, the longer it takes to prepare to adhere to the skin. A good topical preparation can adhere to the skin for sufficient contact time to penetrate and release active substances. Good adhesion is more than 1 second [16, 19]. The adhesion test results obtained can be seen in Figure 10. The adhesion test results on the cream base and pandan cream show changes. Although adhesion changes, the cream base and pandan cream still exhibit good cream adhesion of more than 1 second [16, 19].

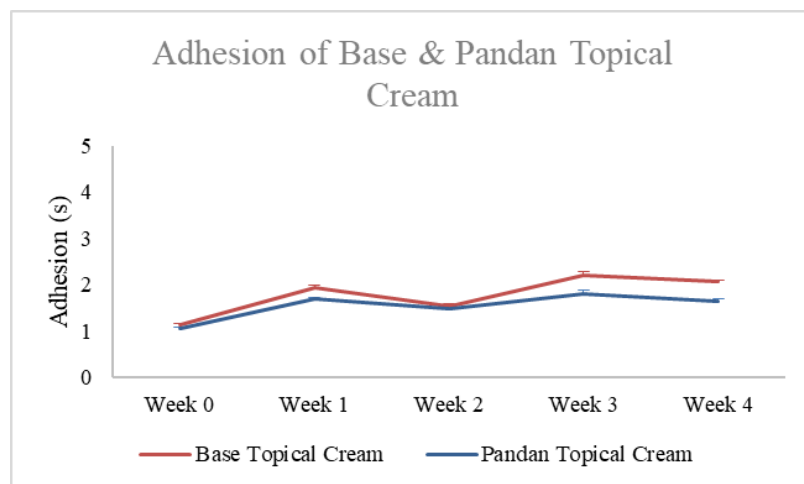


Figure 10. Graph of adhesion test results of cream base and pandan topical cream.

Based on the stability test, the physical test results of the cream base and pandan cream preparations are stable when viewed in several aspects, namely organoleptic, homogeneity, and pH. Although there are significant differences in viscosity, spreadability, and adhesion, these three aspects still meet the standards of a good cream: viscosity of 2000-50000 cP, spreadability of 5-7 cm, and adhesion of more than 1 second. Therefore, the cream base and pandan cream have good stability properties. The stability of the cream preparation can be further improved by optimizing the cream-making method and choosing a more suitable base and emulgator.

4. Conclusions

Research on the effect of the solvent type of pandanus leaf extraction (*Pandanus amaryllifolius* Roxb.) on the antibacterial activity of *Cutibacterium acnes* and its application in a topical cream preparation can be concluded:

The concentration of ethanol as a solvent significantly affects the effectiveness of *Pandanus amaryllifolius* leaf extraction in terms of the TLC profile and total flavonoid content. Ethanol of 96% is the most effective solvent for extracting pandanus leaves related to the highest total flavonoid content (385.758 mgRE/g extract) and antibacterial activity against *Cutibacterium acnes* (MIC₅₀ value of 3.565 mg/mL).

Based on the stability test, the physical test results of the cream base and pandan cream preparations are stable across several parameters, including organoleptic properties, homogeneity, and pH. These three aspects still meet the standards of a good cream.

Author Contributions

Conceptualization, F.N.A. and P.; methodology, C.L., S.U.T.P., and P.; formal analysis, C.L., M.O.V.A., and S.U.T.P.; investigation, C.L., F.N.A., and P.; resources, P.; data curation, C.L.; writing—original draft preparation, C.L. and M.O.V.A.; writing—review and editing, C.L., M.O.V.A., F.N.A., S.U.T.P., and P.; visualization, C.L.; supervision, F.N.A. and P.; funding acquisition, P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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