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Physical Quality Test And Antibacterial Activity Of Anti-Acne Cream Preparation Of Neem Leaf Extract (Azadirachta Indica A.Juss) Against Propionibacterium Acnes

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Abstract

Acne is a common skin disease caused by Propionibacterium acnes bacterial infection. Neem leaves (Azadirachta indica A.Juss) are known to contain active compounds such as azadirachtin, nimbin, nimbidin, and quercetin, which have antibacterial properties. This study aims to formulate neem leaf extract into a cream preparation, test its physical quality, and determine its antibacterial activity against Propionibacterium acnes. The research method used is a laboratory experiment. Neem leaf extraction was carried out by maceration method using 96% ethanol solvent. Cream preparations were made in four variations of extract concentration, namely 30%, 35%, 40%, and 45%. Physical quality tests of the preparations included organoleptic tests, homogeneity, pH, viscosity, and spreadability. Antibacterial activity tests were carried out using the diffusion method. The results showed that all cream formulas met the standards for good physical quality preparations. The results of the pH, viscosity, and spreadability tests met the requirements. The antibacterial activity of the cream preparation against Propionibacterium acnes was classified as strong, with an average inhibition zone of 28.94 mm at a 30% concentration, 19.78 mm at a 35% concentration, 33.93 mm at a 40% concentration, and 36.77 mm at a 45% concentration. The conclusion of this study is that neem leaf extract can be formulated into a cream preparation that meets physical quality standards and is effective in inhibiting the growth of Propionibacterium acnes bacteria.

Keywords: ***Antibacterial Activity, Azadirachta Indica, Cream Formulation, Neem Leaf, Propionibacterium Acnes.***

INTRODUCTION

Acne is the most common skin problem in Indonesia, especially among adolescents, with a prevalence of up to 80-90% among those aged 15-18. As stated by Nurhadi Wartiningsih (2021), "One of the most common skin diseases suffered by people is acne." Propionibacterium acnes bacterial infection triggers this condition through the production of lipase, which breaks down triglycerides into free fatty acids, causing inflammation and blackheads.

The skin, as the largest organ, is susceptible to external stimuli, worsening acne due to P. acnes colonization. The prevalence of acne vulgaris rose from 8,563 per 100,000 people in 1990 to 9,790 in 2021. Antika (2020) explains, "Propionibacterium acnes is a normal bacterial flora on human skin that produces lipase that breaks down into triglycerides... causing inflammation and the formation of comedones."

Acne treatment relies on antibiotics such as doxycycline, but prolonged use can lead to resistance. Yu et al. (2024) reported, "53 (56.4%) and 52 (55.3%) isolates were susceptible to erythromycin and clarithromycin, respectively," indicating high resistance to macrolides. Erythromycin and clarithromycin resistance was high, while doxycycline remained effective despite the risk of disrupting the skin microbiota.

Topical anti-inflammatories such as nicotinamide or benzoyl peroxide are used, but natural alternatives with minimal side effects are needed. Kamala Permana (2020) stated, "Continuous use of antibiotics can lead to resistance." The dependence on synthetic antibiotics is pressing for a replacement with effective herbal remedies against P. acnes.

This study aims to formulate an anti-acne cream from neem leaf extract (Azadirachta indica A. Juss.), test its physical quality, and antibacterial activity against P. acnes at a concentration of 30-45%. The urgency of overcoming increasing antibiotic resistance, while its novelty in a stable cream formula with azadirachtin, nimbin, nimbidin, quercetin, and optimal concentration variations has not been widely explored. Andani (2021) found, "neem leaf extract was able to inhibit the growth of Propionibacterium acnes with an inhibition zone diameter of... 30% is 19.6 mm".

RESEARCH METHODS

Types and Methods of Research

This study used a quantitative laboratory experimental study, in which the independent variable, neem leaf extract (Azadirachta indica A. Juss.) concentrations of 30%, 35%, 40%, and 45%, were manipulated to observe its effect on the dependent variables, namely the physical quality of the cream and its antibacterial activity against Propionibacterium acnes. According to Sugiyono (2021), the experimental method involves testing hypotheses through variable control to produce empirical data that can be measured statistically. This approach aligns with Sudaryono (2021) who emphasized quantitative research to test causal relationships through precise measurements such as the diameter of the inhibition zone.

Data Analysis Instruments and Techniques

The research instruments included laboratory equipment such as an incubator, spectrophotometer, viscometer, pH meter, and Petri dishes for agar diffusion tests, as well as SPSS version 26 software for statistical analysis. Data analysis techniques included the Shapiro-Wilk normality test, Levene's homogeneity test, and One-Way ANOVA followed by Tukey HSD to compare antibacterial activity between formulas at a significance level of $\alpha = 0.05$. Emzir (2021) stated that valid instruments must be tested for reliability to ensure measurement accuracy in experimental research, while Creswell and Creswell (2022) recommended descriptive and inferential statistical analysis to interpret quantitative data from experimental designs.

Population and Sample

The study population was a pure culture of Propionibacterium acnes ATCC 11827 bacteria and fresh neem leaf plant material from Surakarta, while samples were taken purposively with four cream formulas (F1-F4) each in triplicate for physical and antibacterial quality tests. Purposive sampling techniques according to characteristics are justified by Sugiyono (2021) for experimental research where samples are selected based on specific criteria such as the purity of bacterial cultures. Sudaryono (2021) added that the triplicate sample size is adequate to reduce bias and increase the validity of hypothesis testing results.

Research Procedures

The procedure begins with plant identification at the Sebelas Maret University Herbarium Laboratory, preparation of simplicia through sorting, washing, chopping, oven drying at 50°C, and grinding, followed by standardization of simplicia (organoleptic, water content <10%, total ash). Maceration extraction with 96% ethanol for 3x24 hours, extract standardization, phytochemical screening, preliminary antibacterial test, O/W cream formulation with stearate base, propylene glycol, triethanolamine, and physical quality testing (organoleptic, homogeneity, pH 4.5-6.5, viscosity 5,000-10,000 cP, spreadability 5-7 cm), as well as agar diffusion antibacterial test. This flow logically follows Creswell and Creswell (2022) who suggest a stepwise procedure from data collection to analysis for quantitative design, and Emzir (2021) who emphasizes a systematic sequence in pharmaceutical experiments for replicability.

RESULTS AND DISCUSSION

Neem Leaf Material Collection

Neem leaf samples (Azadirachta indica A.Juss) were collected in Gunung Pati District, Semarang Regency. Neem leaf sorting was carried out by selecting young, fresh leaves and then separating or removing contaminants in the form of dirt attached to the leaves.

Determination

Plant determination aims to determine the truth and suitability of the identity of the neem leaf samples (Azadirachta indica A.Juss) used in the research, to avoid errors in taking materials and to avoid mixing materials with other plants during collection.material. The determination of the neem

leaf plant (Azadirachta indica A.Juss) was carried out at UPF Yankerstrad dr. Sardjito Tawangmangu, showing that the plant studied was indeed a neem leaf plant (Azadirachta indica A.Juss).

Drying Materials and Making Neem Leaf Powder

The results of drying the material in the simplicia powder are known that neem leaves (Azadirachta indica A.Juss) with a wet weight of 5,000 grams are dried and a dry weight of 2,000 grams is obtained. The percentage of dry weight to wet weight is 40% and it is known that neem leaves (Azadirachta indica A.Juss) with a dry weight of 2,000 grams, are then ground and a fine powder weight of 1,000 grams is obtained. The percentage of dry simplicia weight to fine powder weight is 50%.

Standardization Test of Neem Leaf Simplicia

Determination of Drying Loss of Neem Leaf Powder

The drying shrinkage result of 9.7% meets the requirements of the Indonesian Herbal Pharmacopoeia if a simple powder shows no more than 10%. If it is too high, it can change the chemical composition of the simple so that it reduces the quality of the simple and is easily overgrown by bacteria.

Determination of Water Content of Neem Leaf Powder

Determining the water content of powdered medicinal plants aims to provide a minimum limit for the range of water content within the powdered medicinal plants. Moisture content determination is performed using a moisture balance. The test results yielded a water content of 3.06%.

Making Neem Leaf Extract

Neem leaf extract was made using maceration. A total of 1,000 grams of neem leaf powder was extracted using 10 liters of 96% ethanol solvent with a ratio of 1:10. The maceration was carried out for 3 days with occasional stirring and then filtered. The macerate obtained was evaporated using a rotary evaporator at 50°C to separate the filtrate from the solvent. The evaporation results were then thickened using a water bath at 60°C to obtain a thick extract. The resulting thick extract was 175.40g.

Extract Standardization

Determination of water content of extract

Determination of the water content of neem leaf extract using a moisture balance. The results showed that the water content of the extract was 6.26%.

Determination of ash content of extract

Ash content was determined by weighing 2 g of the extract and placing it in a previously tared crucible. It was then heated in a furnace at a temperature gradually increased to 600°C for 3 hours, then cooled in a desiccator, resulting in a result of 4.22%.

Ethanol-free testing

1 gram of neem leaf ethanol extract was put into a test tube, 2 mL of concentrated sulfuric acid (H_2SO_4) and 2 drops of acetic acid (CH_3COOH) were added, then heated and the ethanol-free result was obtained.

Phytochemical Screening Test of Neem Leaf Extract

Screening is a preliminary stage in phytochemical research that aims to provide an overview of the compound groups contained in the plant being studied. The phytochemical screening method used is the tube test method, which involves observing the color reaction using a color reagent.

Table 1. Phytochemical Screening Test Results

Compound Test	Reagent	Results Based on Literature	Results	Information
Alkaloid	*Mayer*	There is a yellowish white precipitate.	There is a yellow sediment.	+
	Wagner	There is orange sediment	There is orange sediment	+
	Dragendorff	There is orange sediment	There is orange sediment	+
Flavonoid	Mg metal + concentrated HCL	A blackish red color appears	A blackish red color appears	+
Saponin	Aquadest	Forms a stable foam	A stable foam is formed.	+
Tannin	FeCl3 1%	There is a greenish brown color.	Forms a greenish brown color	+
Steroid	concentrated H2SO4	A green or blackish blue color is formed	A blackish green color is formed	+

Preliminary Test Results of Neem Leaf Extract

Preliminary tests were conducted using various extract concentrations: DMSO (negative control), 30%, 35%, 40%, and 45%, and clindamycin (positive control). These concentration variations aimed to determine the effect of each concentration of the neem leaf extract anti-acne cream formula on the test bacteria.

Table 2. Preliminary Results of Neem Leaf Extract

Table of Average Inhibition Zone of Neem Leaf Extract

Replication	Inhibition Zone Diameter (mm)					
	30%	35%	40%	45%	Control +	Control -
1	08.59	15.1	20.01	24.19	38.08	0
2	08.24	12.64	19.78	22.96	37.98	0
3	05.79	13.1	19.76	26.28	34.41	0
Average	07.54	13.61	19.85	24.48	36.82	0
Elementary School	2,647	5,450	3,211	3,495	4,481	0

Antibacterial Data Analysis of Neem Leaf Extract

The data obtained were then subjected to data analysis. Data analysis was performed using SPSS 23, using the One-Way ANOVA (Analysis of Variance) test and followed by a Post Hoc Test with the Tukey method. The test aimed to determine whether there were significant differences between neem leaf extract concentrations of 30%, 35%, 40%, and 45% with the positive control, kindamycin. The purpose of the normality test was to determine whether the data were normally distributed.

Table 3.Data Normality Test Results

Tests of Normality

	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistics	df	Sig.	Statistics	df	Sig.
extract_result	.296	13	.003	.838	13	.020

The One Sample Shapiro-Wilk test results obtained significance for all samples exceeding Sig>0.05. Negative controls were not included in this data processing because the results were static, namely 0, so they were automatically removed by the system. All treatment groups were said to have normal data because their significance value was p>0.05.

Preparation of Anti-Acne Cream Preparation from Neem Leaf Extract

After weighing, the ingredients in the formula are separated into two groups, namely the oil phase (liquid paraffin, adeps lanae, stearic acid) and the water phase (triethanolamine, DMDM hydantoin). Each phase is heated at a temperature of 60-70° C in a water bath. The oil phase is separated into a hot mortar and added to the water phase then stirred until cooled to form a creamy mass, between mixing the oil and water phases add extracts with different variations in each formula.

Table 4.Neem Leaf Extract Cream Formulation

Material	Cream Formula (%)				
	F 0	FI	F II	F III	F IV
Neem leaf extract	-	30%	35%	40%	45%
Stearic Acid	13	13	13	13	13
TEA	1.5	1.5	1.5	1.5	1.5
Adeps Lanae	3	3	3	3	3
Liquid paraffin	25	25	25	25	25
DMDM hydantoin	0.5	0.5	0.5	0.5	0.5
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

The cream formulation in the table above was made with 4 different concentrations for further organoleptic testing, homogeneity testing, pH testing, viscosity testing, and spreadability testing.

Physical Quality Test of Neem Leaf Extract Anti-Acne Cream Preparation

Organoleptic Test

The purpose of organoleptic testing is to examine the physical appearance of a preparation. Organoleptic testing of this gel preparation includes shape, color, and odor.

Table 5.Organoleptic Test Results

Formula	Replication	Color	Form	Smell
Base	**1**	**White**	**Semi-solid**	**Neutral (scentless)**
FI	1	Green	Semi-solid	Typical neem leaves
	2	Green	Semi-solid	Typical neem leaves
	3	Green	Semi-solid	Typical neem leaves
F II	1	Green	Semi-solid	Typical neem leaves
	2	Green	Semi-solid	Typical neem leaves
	3	Green	Semi-solid	Typical neem leaves
F III	1	Green	Semi-solid	Typical neem leaves
	2	Green	Semi-solid	Typical neem leaves
	3	Green	Semi-solid	Typical neem leaves
F IV	1	Green	Semi-solid	Typical neem leaves
	2	Green	Semi-solid	Typical neem leaves
	3	Green	Semi-solid	Typical neem leaves

Homogeneity Test

The purpose of the homogeneity test is to determine whether the resulting mixture is truly mixed with all of its constituent ingredients. This test is performed by applying the cream preparation to a watch glass, then covering it with another glass object and observing for the presence of coarse particles. Based on the results of the homogeneity test, all formulas exhibited good homogeneity.

Table 6. Homogeneity Test Results

Formula	Replication	Homogeneity
Base	**1**	**Homogeneous**
FI	1	Homogeneous
	2	Homogeneous
	3	Homogeneous
F II	1	Homogeneous
	2	Homogeneous
	3	Homogeneous
F III	1	Homogeneous
	2	Homogeneous
	3	Homogeneous
F IV	1	Homogeneous
	2	Homogeneous
	3	Homogeneous
FV	1	Homogeneous
	2	Homogeneous
	3	Homogeneous

pH test

The pH test aims to determine whether a cream preparation is acidic, basic, or neutral. The pH test is also performed to prevent skin irritation when applied to the skin.

Table 7. pH Test Results

Formula	pH Test Results			Average	Elementary School
	1	2	3		
F0	6.34	6.34	6.34		
F1	5.06	5.06	5.07		
F2	5.55	5.60	5.50	5.56	0.4
F3	5.45	5.46	5.47		
F4	5.41	5.42	5.37		

Viscosity Test

Viscosity testing is performed using a Brookfield viscometer. The purpose of this test is to determine the thickness of the cream preparation. A cream that is too thin will result in a short adhesion time, resulting in poor active ingredient delivery. A cream that is too thick can cause discomfort during application.

Table 8.Viscosity Test Results

Formula	Replication	Viscosity (cp)	Average	Elementary School
F0	1	11890		
	2	11890	11,890	2,647
	3	11890		
F1	1	11820		
	2	11210	11,500	5,450
	3	11470		
F2	1	11820		
	2	11820	11,746	3,211
	3	11600		
F3	1	11880		
	2	11880	11880	3,495
	3	11880		
	1	10490		
F4	2	10520	10,510	4,481
	3	10520		

Based on the results of the viscosity test table, it shows that each formula has a different viscosity. F0 has an average viscosity of 11,890 cp. F1 has an average viscosity of 11,500 cp. F2 has an average viscosity of 11,746 cp.,F3 has an average viscosity of 11,880 cp and F4 has an average viscosity of 10,510 cp.

Spread Power Test

Spreadability testing is conducted to determine the cream's ability when applied to the skin and to observe the spread of the cream preparation. The wider the spreadability of the preparation, the more the active ingredient can diffuse into the skin. The spreadability test requires a 0.5 gram cream sample, placed on a 15 cm diameter round glass and a weight placed on it, wait for 1 minute, then measure. The measurement begins without a load then adds a load weighing 50 grams to 250 grams. The requirement for the spreadability test is 5-7 cm (Rahmatullah et al., 2020).

Table 9. Spread Power Test Results

Formula	Replication	Spread power results (cm)
Base	1	5.5
FI	1	5.2
	2	5.5
	3	6.1
F II	1	5.0
	2	5.6
	3	6.1
F III	1	6.0
	2	6.1
	3	6.0
F IV	1	5.5
	2	5
	3	5.8

Antibacterial Activity Test of Neem Leaf Extract Anti-Acne Cream Preparation

The antibacterial activity of the neem leaf extract anti-acne cream was tested using a disc diffusion method. The principle of the disc diffusion method is to impregnate the test substance onto a paper disc, which is then placed on a medium homogenized with bacteria until an inhibition zone is obtained around the disc.

Antibacterial testing of the neem leaf extract anti-acne cream preparation was carried out with various concentration variations, namely 0% (negative control), 30%, 35%, 40%, 45% and mediclin (positive control). This concentration variation aims to see the effect of each concentration of the neem leaf extract anti-acne cream formula on the test bacteria. The media used was Nutrient Agar (NA), NA media was chosen because it has good nutritional content for the culture of most bacteria, especially Propionibacterium acnes, in addition NA is also neutral so it does not cause any effect on the antibacterial test.

Table 10. Antibacterial Activity Test Results of Anti-Acne Cream Preparations

Table of Average Zone of Inhibition of Neem Leaf Cream Preparations

Replication	Inhibition Zone Diameter (mm)					
	30%	35%	40%	45%	Control +	Control -
1	26.8	17.5	30.4	32.9	51.5	0
2	28.12	15.84	34.7	39.7	43.68	0
3	31.9	26.0	36.68	37.7	43.8	0
Average	28.94	19.78	33.93	36.77	46.33	0
Elementary School	2,647	5,450	3,211	3,495	4,481	

Antibacterial Activity Data Analysis of Neem Leaf Extract Anti-Acne Cream Preparation

The data obtained were then analyzed using a One-Way ANOVA (Analysis of Variance) test followed by a Post Hoc Test with the Tukey method. The test aimed to determine whether there were significant differences between the anti-acne cream concentrations of 30%, 35%, 40%, and 45% and the positive control, Medi-Klin anti-acne medication. The purpose of the normality test was to determine whether the data were normally distributed.

Table 11. Data Normality Test Results

Tests of Normality

		Kolmogorov-Smirnova			Shapiro-Wilk		
	treatment	Statistics	df	Sig.	Statistics	df	Sig.
formula_result	f1	.345	3	.	.840	3	.213
	f2	.319	3	.	.884	3	.338
	f3	.303	3	.	.909	3	.415
	f4	.307	3	.	.904	3	.397

The One Sample Shapiro-Wilk test results obtained significance for all samples exceeding Sig>0.05. Negative controls were not included in this data processing because the results were static, namely 0, so they were automatically removed by the system. All treatment groups were said to have normal data because their significance value was p>0.05.

The next stage is to carry out a Test of Homogeneity of Variance to test whether the samples taken have the same variance.

Table 12.Results of the Homogeneity of Variance Test

Test of Homogeneity of Variances			
formula_result			
Levene Statistics	df1	df2	Sig.
7,253	3	8	.011

The results of the Homogeneity of Variances test were 0.011>0.05, thus concluding that the data came from populations with the same variance or were homogeneous. Furthermore, all data were analyzed using a one-way ANOVA.

Table 13. One Way Test (ANOVA) Results

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,397	3	.799	13,252	.002
Within Groups	.482	8	.060		
Total	2,879	11			

Analysis using One Way ANOVA shows a significance result of 0.000<0.05, so H0 is accepted, so it can be concluded that the hypothesis is proven true that there is a difference in the effectiveness of antibacterial power in the neem leaf extract anti-acne cream preparation. The function of the One Way ANOVA test is to differentiate the average between groups from an experiment that has samples of more than 2 groups.

Discussion

This thesis research aims to identify the antibacterial activity of neem leaf extract (Azadirachta indica A.Juss) in a cream preparation. This research stage begins with plant determination to ensure the correct identity of the material to be used. The results of the neem plant determination indicate that the sample used is (Azadirachta indica A.Juss). This is very important to ensure the presence of ingredients in the neem plant that have potential bioactivity including antioxidant properties, antibacterial, and activity that fights diabetes.(Hanafi et al., 2020).

After the determination was made, young neem leaves were collected because young neem has a higher concentration of beneficial compounds, such as azadirachtin (a natural anti-insecticide and anti-inflammatory agent), flavonoids, and saponins. These compounds play an important role in the cream's properties, for example, to relieve skin inflammation, fight bacteria, and heal minor wounds. As the leaves age, the concentration of these compounds tends to decrease (Handoyo & Pranoto, 2020). The neem leaves obtained were 5000 g and then dried, resulting in 2000 g of dried leaves and 1000 g of fine powder. As shown in Table 4.1, it shows that the loss of water content in the leaves. The drying shrinkage test for the simplex obtained in Table 4.2 obtained a result of 9.7% tested using a heated crucible and then dried and the results were observed, according toNur Fadhila et al., (2022)The drying shrinkage value is not more than 10%. For the water content test itself, the result was 3.06%, which according toNur Fadhila et al., (2022)A good water content value is 10%. To determine the ash content obtained, it was 1.43%. This test was carried out to provide an overview of the total amount of ash obtained and the material remaining after high-temperature annealing.

After standardization, extraction was carried out using 96% ethanol, the powder used in maceration was 1000 grams and dissolved in 1 L of ethanol. Maceration was carried out for 3 days while stirring occasionally and after that filtering was carried out for the next evaporation process using a rotary evaporator and water bath to thicken the extract. The extract obtained obtained an extract of175.40 gr with a yield of 11.69%.

Based on the results in table 4.9, it can be seen that the phytochemical test on neem leaf extract shows that the neem leaf extract (Azadirachta indica A.Juss) contains active compounds of dragendorff, wagner, and mayer alkaloids. Flavonoids, tannins, steroids, and saponins are positive. In the alkaloid test, a number of extracts are put into a test tube and dripped with 2N HCl, aiming to

extract alkaloids from the simplex. Alkaloids are basic so that with the addition of HCl, salt will form, then heated to break the bonds between alkaloids that are not in the form of salts, then cooled. The filtrate is divided into 3 parts and added to each reagent of mayer, wagner, and dragendorff (La et al., 2020). From the three test tubes, it was shown that the neem leaf extract in the Mayer reagent showed a positive result indicated by the formation of a yellow color, the Wagner reagent showed a positive result indicated by the presence of an orange precipitate, while the Dragendorff reagent showed a positive result indicated by the presence of an orange precipitate (Ferdinan et al., 2021). In the flavonoid test, a number of extracts were put into a test tube, dissolved in hot methanol, then added with magnesium powder, then added concentrated HCl. The purpose of adding magnesium powder and concentrated HCl was to reduce the glycosidic bond with flavonoids. In order for flavonoids to be identified, the glycosidic bond with flavonoids in plants must be broken by reducing the bond, which results in the neem leaf extract containing flavonoids positive because it formed a blackish red color (Muthmainah, 2017). Saponin is a surface active compound that is easily detected by its ability to form foam. This foam indicates the presence of glycosides that have the ability to form foam in water that is hydrolyzed into glucose and other compounds. The glycosidic bond components contained in saponins cause these compounds to tend to be polar. In the saponin test, a number of extracts were placed in a test tube, added with hot water, then cooled and shaken for 30 seconds, and the tube was left standing for 30 minutes. The presence of saponins was positive because the tested sample formed foam (Wahidah et al., 2021). Based on the test results, it showed that the neem leaf extract contained saponins positively, because there was foam. In the tannin test, a number of extracts were placed in a test tube and added with FeCl3. The addition of FeCl3 aims to determine the presence of phenol groups, the presence of phenol groups is indicated by a greenish-brown color. The test showed that the neem leaf extract contained tannins positively, indicated by a greenish-brown color (Khafid et al., 2023). In the steroid test, a number of extracts were placed in a test tube, dissolved in chloroform, added acetic anhydride and concentrated H2SO4.The addition of acetic anhydride aims to form acetyl derivatives, while the addition of concentrated H2SO4 (concentrated sulfuric acid) aims to hydrolyze water, which reacts with the acetyl derivatives to form purplish-red or blackish-green rings. A positive test for steroids is indicated by a color change to green or blackish-blue. The test showed a blackish-green color, thus confirming the presence of steroids in the neem leaf bark extract (Khafid et al., 2023).

The preparation of the anti-acne cream preparation with neem leaf extract is carried out through structured stages. First, all ingredients in the formula are weighed first, then separated into two main phases, namely the oil phase and the water phase. The oil phase consists of liquid paraffin, adeps lanae, and stearic acid, while the water phase consists of triethanolamine (TEA) and DMDM hydantoin. Second, each phase is heated at a temperature of 60-70°C while stirring until evenly distributed; the oil phase is then added to a hot mortar, after which the heated water phase is also added and stirred continuously until the mixture cools and a homogeneous cream mass is formed. Next, neem leaf extract as an active ingredient is added at the stage of mixing the oil and water phases, with different concentration variations in each formula (i.e. 30%, 35%, 40%, 45% in formulas F2-F5, while F0 is used as a control without extract).

After that, a physical quality test was conducted on the cream preparation. Based on the results of organoleptic test observations based on the shape of the positive control, formulas F0, F1, F2, F3, and F4 were semi-solid creams. According to color, formula F0 was white, formulas F1, F2, and F3 were green due to the addition of neem leaf extract, and F4 was dark green due to the addition of the largest amount of extract. Based on odor, formula FO had a distinctive cream base odor, formulas F1, F2, F3, and F4 had a distinctive neem leaf extract odor. Homogeneity testing is very important because the method of application of the cream will be applied to the surface of the skin, so that the active ingredients of the cream can provide a therapeutic effect on every part of the skin that is smeared with the cream. Based on the results of the homogeneity test table, it shows that all formulas did not contain coarse particles in the preparation, so it can be said that this cream preparation formula is homogeneous and meets the requirements. A good cream shows homogeneous particles (Adnan et al., 2021). Next, a pH test was conducted to determine whether the resulting cream is acidic or basic, seen

from the pH value obtained. In topical preparations, pH is related to the feel when applied. A pH that is too acidic can irritate the skin, and if it is too alkaline, it can cause scaly skin (Ghina et al., 2022). Based on pH test results, each formula has a different pH value. F0 has a pH value of 6.34..Formula Fl with 30% extract concentration has an average pH value of 5.06, formulation F2 with 35% extract concentration has an average pH value of 5.55, formulation F3 with 40% concentration has an average pH value of 5.46 and formulation F4 with 45% concentration has an average pH of 5.40. According to Irmanesia, increasing the concentration of active ingredients will lower the pH value in the preparation. This can be seen in F2, F3, and F4 whose pH values are smaller than F1. The high pH value in the base can be caused by the high concentration of triethanolamine, but after adding extracts with different concentrations the pH value decreases. Changes in the pH of the cream at different concentrations are caused by chemical interactions between its constituent components, especially active ingredients, preservatives, emulsifiers, and pH regulators (buffers), where changes in the amount or concentration of each ingredient can shift the balance of hydrogen ions (H^+) and hydroxide (OH^-) in the system. If the concentration of acidic ingredients (such as salicylic acid) is increased, there will be an increase in the number of H^+ ions which causes the pH to decrease (more acidic), conversely, increasing the concentration of basic ingredients (such as triethanolamine) will increase OH^- ions so that the pH increases (more alkaline). In addition, emulsifiers and surfactants at certain concentrations can affect the degree of dissociation of molecules in the water and oil phases of the cream, while the existing buffer system may have a limited buffer capacity so that it is unable to maintain a constant pH when the concentration of other components changes significantly, which ultimately causes fluctuations in the overall pH value of the cream formulation (Tungadi & Pakaya, 2023). However, all formulas meet the skin pH requirements, which are in the range of 4.5-8 (SNI). Based on the results of the pH test, it shows that each formula has a different pH value. F0 has a pH value of 6.34.Formula Fl with 30% extract concentration has an average pH value of 5.06, formulation F2 with 35% extract concentration has an average pH value of 5.55, formulation F3 with 40% concentration has an average pH value of 5.46 and formulation F4 with 45% concentration has an average pH of 5.40. According to Irmanesia, increasing the concentration of active ingredients will lower the pH value of the preparation. This can be seen in F2, F3, and F4 whose pH values are smaller than F1. The high pH value in the base can be caused by the high concentration of triethanolamine, but after adding extracts with different concentrations the pH value decreases. However, all formulas meet the skin pH requirements which are in the range of 4.5-8 (SNI). Based on the results in the viscosity test table, it shows that each formula has a different viscosity. F0 has an average viscosity of 11,890 cp. F1 has an average viscosity of 11,500 cp. F2 has an average viscosity of 11,746 cp.,F3 has an average viscosity of 11,880 cps and F4 has an average viscosity of 10,510 cps. This occurs because the lower the concentration of the active ingredient used, the higher the viscosity value, so the preparation will be more stable because particle movement tends to be difficult with a thicker preparation. Conversely, the higher the concentration of the active ingredient used in the preparation, the lower the viscosity will be (Tungadi et al., 2023). This is seen in F2, F3, and F4 whose viscosity values are smaller than F1. However, all three cream preparation formulas still have viscosity values that meet SNI standards, namely in the range of 2000-50,000 cps. The results of the study showed that each formula has a different spreadability value, this is due to different viscosity values. F3 has the largest spreadability diameter. According to research by Sugiharto and Safitri, (2020) the increasing concentration of active substances increases the spreadability of the preparation, this is because viscosity is inversely proportional to the spreadability test, where the wider the spreadability produced, the smaller the viscosity value produced. In the cream preparation made, the spreadability still meets SNI requirements with a spreadability in the range of 5 - 7 cm. The results of the cream spreadability test show that the higher the concentration of the addition of neem leaf extract, the cream spreadability increases, this is because the addition of neem leaf extract affects the viscosity of the cream preparation.

The antibacterial activity of the cream preparation was then tested using the dilution method. Three petri dishes were prepared, and 30 ml of NA media, which had been melted at 45°C, was poured

into each petri dish and allowed to solidify. Insert a sterile cotton swab into the Propionibacterium acnes bacterial suspension medium and then scratch it on the test medium, repeating the scratching process three times. Let the suspension stand for 5 minutes so that the bacterial suspension can penetrate the agar medium. Then, using sterile tweezers, take a paper disc that has been previously soaked in the neem leaf extract anti-acne cream preparation with concentrations of 0%, 30%, 35%, 40%, and 45%, and a positive control using clindamycin. Place it on the surface of the medium at each concentration, repeating three times in each petri dish. Based on the negative control test, the 0% concentration anti-acne cream formula did not have an inhibition zone. The negative control showed a significant difference with various extract concentrations because it showed no inhibition zone. This indicates that the negative control used had no effect on the antibacterial test. The positive control test aims to compare the diameter of the inhibition zone formed by various concentrations of anti-acne creams with clindamycin as a positive control. The antibacterial ability to inhibit microorganisms depends on the concentration and type of antibacterial. The higher the concentration of an antibacterial, the greater the active ingredient content, thus increasing its ability to inhibit bacteria and forming a wider clear zone.

CONCLUSION

This study successfully demonstrated that neem leaf extract (Azadirachta indica A. Juss.) can be formulated into an anti-acne cream preparation with physical qualities that meet standards, including organoleptic (semi-solid, green, distinctive aroma), good homogeneity, pH 5.06-6.34 (safe skin range), viscosity 10,510-11,890 cP, and spreadability 5-6.1 cm. Antibacterial activity against Propionibacterium acnes is categorized as strong, with an average inhibition zone diameter of F1 (30%) 28.94 mm, F2 (35%) 19.78 mm, F3 (40%) 33.93 mm, and F4 (45%) 36.77 mm, significant compared to the control (ANOVA $p < 0.05$). Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and steroids as the main contributors.

However, limitations include the use of a single ATCC culture without resistant clinical isolates, limited long-term stability testing, and the lack of in vivo evaluation on human skin to confirm irritation and clinical efficacy. Suggestions for further research include accelerated stability testing, comparison with clinical isolates, and phase I clinical trials. Practically, the optimized F4 formula has the potential to be developed as an affordable topical herbal alternative for mild-moderate acne therapy, supporting sustainable treatment amidst antibiotic resistance.

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