OPTIMIZATION OF SOURSOP (*Annona muricata* L.) LEAF EXTRACT IN NANOEMULGEL AND ANTIACNES ACTIVITY TEST AGAINST *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* BACTERIA

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Abstract

Soursop leaf extract (*Annona muricata* L.) has antibacterial activity with active components of alkaloids, flavonoids, steroids, and saponins. This research aims to optimize the use of VCO, Tween 80, and PEG 400 in the nanoemulsion system, which will be added Carbopol 940 as a gelling agent to form a nanoemulgel and to determine antibacterial activity. By utilizing the simplex lattice design method in Design Expert 13.0, the nanoemulsion formula has been successfully optimized involving a mixture of VCO, Tween 80, and PEG 400. A total of 14 different nanoemulsion formulas were produced through this process. The process of making nanoemulsion is carried out by mixing extracts, oils, surfactants and cosurfactants, homogenized using a vortex, then sonicated. Evaluation of % transmittance, emulsification time, and pH were carried out to determine nanoemulsion physical properties. The nanoemulsion optimal formula was evaluated for particle size, polydispersity index, and zeta potential and made into nanoemulgel with the addition of Carbopol 940. The optimal formula has a composition of 10.86% VCO, 67.33% Tween 80, 21.81% PEG 400 form homogeneous and clear nanoemulsion with % transmittance of 91.97±1.11%, emulsification time of 56.42±0.72 seconds, and pH of 5.67±0.24. Particle size, polydispersity index, and zeta potential of the optimal formula were 229.47±38.79 nm, 0.41±0.10, and -39.13±0.19 mV, respectively. Evaluation of nanoemulgel physical properties showed homogeneous, pH value of 5.83±0.24, spreadability of 5.57±0.25 cm, adhesive force of 3.80±0.25 seconds, viscosity of 11479.33±167.49 cP, antibacterial activity against *P. acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* with inhibition zone diameters of 9.67±0.47 mm, 7.33±0.47 mm, and 5.67±0.47 mm. respectively. Nanoemulgel soursop leaf extract has suitable physical properties, and antibacterial activity is in the medium category.

Key Words: Soursop leaf extract, Nanoemulgel, Antibacterial, Simplex Lattice Design

INTRODUCTION

The skin, as the outermost layer of the body, plays a crucial role as a hydrophobic barrier, housing nerve endings and maintaining body temperature regulation. The skin harbors microorganisms, including *Staphylococcus aureus*, *P. acnes*, and *Staphylococcus epidermidis*, as part of the natural human microbiota. Certain microorganisms are harmful and can cause disorders like acne (Claire and Lake, 2018). Acne, scientifically referred to as acne vulgaris, is a prevalent inflammatory disorder that develops in the pilosebaceous unit, primarily affecting teenagers and young adults. Acne commonly manifests on the arms, back, chest, and (Cong *et al.*, 2019).

Acne can arise from intricate interplays among different variables, including microorganisms like *S. aureus*, *P. acnes*, and *S. epidermidis*. While these bacteria often do not induce skin issues under normal circumstances, they can pose a problem when there is an alteration in the skin's health. The sebaceous glands and sweat glands within the skin generate sebum and various other fluids.

Sebum is a compound composed of salt, urea, amino acids, water, and fatty acids. The bacteria on the skin utilize these compounds as a nutritional source. Increased sebum production or changes in skin state might lead to heightened invasiveness of germs. Bacteria play a crucial role in the development of acne through an inflammatory chemotactic mechanism, where they react to chemical alterations in their immediate surroundings. Furthermore, bacteria can also contribute to the production of lipolytic enzymes, which modify the proportion of sebum that forms a solid mass. Consequently, the obstruction of sebaceous gland ducts can lead to the development of acne (Claire and Lake, 2018).

Soursop leaves (*Annona muricata* L.) possess inherent antibacterial properties. The presence of bioactive compounds in it, such as glycosides, steroids, alkaloids, terpenoids, tannins, flavonoids, and eugenol, contribute to its antibacterial, anti-inflammatory, and antioxidant properties (Vijayameena *et al.*, 2013). The methanol extract derived from soursop leaves exhibited notable antibacterial efficacy at a dosage of 150 mg/mL, effectively inhibiting the development of *S. aureus* germs. The efficiency of the extract in inhibiting the growth of these bacteria is demonstrated by the presence of an inhibitory zone with a diameter of 14.1 mm(Haro *et al.*, 2014). The inhibitory zone refers to the region surrounding the site of extract application where the growth of bacteria is visibly suppressed or halted. The results indicate that soursop leaf extract possesses antibacterial activity against *S. aureus* at specific doses.

Enhancing the efficacy and user-friendliness of semi-solid formulations can be achieved by integrating nanoemulsions with gel matrices. This study tries to tackle many challenges that may occur when applying nanoemulsions topically, including issues like poor viscosity and dispersion that can hinder the efficient transport of medications into the skin(Elmarzugi *et al.*, 2015). The dispersion of the nanoemulsion into a gel foundation is anticipated to enhance the formulation of the topical medication. Nanoemulsions offer several benefits due to their enhanced stability and superior ability to act as carriers, particularly for hydrophobic medicines(Kute and Saudagar, 2013). The gel basis offers enhanced viscosity, hence facilitating more efficient application to the skin.

This study involved examining the optimization of nanoemulsion components, specifically VCO, tween 80, and PEG 400, and evaluated the response variables of % transmittance, emulsification duration, and pH using the simplex lattice design approach.

RESEARCH METHODOLOGY

Material

Soursop leaves (*Annona muricata* L.) were collected from Karanganom district, Klaten (Indonesia) and the species was determined and assured at the Faculty of Pharmacy, Gadjah Mada University. The following chemicals were purchased from Sigma: ethanol, VCO, tween 80, PEG 400, distilled water, Carbopol-940, nipagin, nutrient agar (NA), blood agar, NaCl, DMSO. The bacteria *S. aureus*, *P. acnes*, and *S. epidermidis* were collection of Laboratorium of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta.

Extraction of Soursop leaf

The production of soursop leaf extract involves the maceration technique, where 300 grams of dry powder is combined with 3000 mL of 70% ethanol in a 1:10 ratio. The mixture is submerged in an opaque container for 2 days, with intermittent agitation every 12 hours. The liquid extract was further concentrated using a rotary evaporator at a controlled temperature of 40 °C and then further condensed in a water bath at temperatures below 65 °C. The procedure yields a concentrated ethanol extract from soursop leaves (Vijayameena *et al.*, 2013).

Ternary Diagramming

The oils, surfactants, and co-surfactants that have been chosen based on the solubility test are graphed on a ternary phase diagram. VCO (VCO), Tween 80, PEG 400 were combined in different ratios (1:9; 2:8; 3:7; and 4:6) to create a total of 16 ternary phase compositions (**Table 1**). A blend of VCO, tween 80, and PEG 400 is then left undisturbed for 24 hours to facilitate the process of separation. The prepared substance is subsequently diluted in 25 mL of distilled water to assess its clarity using a UV-Vis spectrophotometer. The nanoemulsion region is depicted by generating a ternary phase diagram utilizing the Prosim software (Mardiyanto *et al.*, 2018).

Table 1. Ternal Phase Comparison *Virgin coconut oil*, Tween 80, and PEG-400

Oil : Smix	Oil (%)	Surfactant (%)	Cosurfactant (%)
1:9	10	80	10
	10	70	20
	10	60	30
	10	50	40
2:8	20	70	10
	20	60	20
	20	50	30
	20	40	40
3:7	30	60	10
	30	50	20
	30	40	30
	30	30	40
4:6	40	50	10
	40	40	20
	40	30	30
	40	20	40

Optimization and Formulation of Nanoemulsions with Simplex Lattice Design

The upper and lower threshold values of oil, surfactant, and co-surfactant are derived from the ternary diagram in the preceding phase. The dependent variables (response) of transmittance, emulsification time, and pH were evaluated using Design Expert 13 software with the Simplex Lattice Design approach. This analysis resulted in obtaining 14 designs of nanoemulsion formulations. The % transmittance response, emulsification time, and pH were reevaluated to determine the optimal formulation for the nanoemulsion. The optimal formula was assessed by examining key attributes such as % transmittance, emulsification time, and pH, which were then compared to the projected values using Simplex Lattice Design (Mardiyanto *et al.*, 2018).

Preparation of Soursop Leaf Extract Nanoemulsion

The nanoemulsion was prepared using a mixture VCO, tween 80, and PEG 400A solution containing 50 mg of soursop leaf extract was prepared by dissolving it in VCO (**Table 2**.). The mixture was then vortexed for 5 minutes and sonicated for 10 minutes until it became homogeneous. Tween 80 was added to the mixture, which was then mixed using vortex for 5 minutes and sonicated for 10 minutes. The last component added was PEG 400, followed mixed using vortex for 5 minutes and sonication for 10 minutes (Fithri *et al.*, 2017).

Table 2. SLD experimental design of soursop leaf extract nanoemulsion formula

Formula	Extract (mg)	VCO (%)	Tween 80 (%)	PEG 400 (%)
Run 1	50	20	70	10
Run 2	50	13.33	63.33	23.33
Run 3	50	10	60	30
Run 4	50	30	60	10
Run 5	50	13.33	73.33	13.33
Run 6	50	10	80	10
Run 7	50	17	66.67	16.67
Run 8	50	30	60	10
Run 9	50	23.33	63.33	13.33
Run 10	50	10	80	10
Run 11	50	10	70	20
Run 12	50	10	60	30
Run 13	50	20	70	10
Run 14	50	20	70	10

Evaluation of Nanoemulsions

Transmittance Percent

10 µl of Smix, a combination of extracts, oils, surfactants, and cosurfactants, was mixed with 100 mL of distilled water at a ratio of

1:10000. The % transmittance at a wavelength of 650 nm was measured using a UV-Vis Spectrophotometer (Pratiwi, 2021).

Emulsification time of Nanoemulsions

A 20 µL of nanoemulsion was disseminated into 12.5 mL of distilled water using a magnetic stirrer set at a speed of 150 rpm. The minimum quantity necessary to achieve a homogeneous mixture or milk without the presence of oil clumps is documented (Indrati *et al.*, 2020).

Table 3. Formulation of nanoemulgel soursop leaf extract

Material	Composition (% w/w)
Nano emulsions	20
Carbopol-940	2
Triethanolamine	1
Nipagin	0.2
Distilled water	Ad 100

pH of Nanoemulsions

The pH of the nanoemulsion is determined by immersing the pH meter electrode into the nanoemulsion using a pH meter. Exactly 100 µl of Smix is combined with 5 ml of distilled water. The mixture is homogenized by flipping it for a duration of 1 minute. The pH meter readings are taken after a 5-minute interval to ensure that the values have reached a stable state and are not fluctuating further (Pratiwi, 2021).

Particle Size, Zeta Potential, and Polydispersity Index (PDI)

The nanoemulsion was diluted with distilled water at a ratio of 1:100 and analyzed using a Particle Size Analyzer (PSA). Triangular measurements were conducted on three separate occasions (Mardiyanto *et al.*, 2018).

Preparation of Nanoemulgel Containing Soursop Leaf Extract

The nanoemulgel is prepared according to **Table 3**, by dispersing 2.5 grams of Carbopol 940 in distilled water as a gelling agent, and then adding nanoemulsions while maintaining continuous stirring. Triethanolamine and nipagin are introduced and agitated until a uniform mixture is achieved (Nikam et al., 2018).

Nanoemulgel Evaluation

Homogeneity of Nanoemulgel

0.25 grams of nanoemulgel was evenly distributed at the top, center, and bottom of the glass item. Next, a tactile examination is conducted to assess the drawbacks of the preparation (Elmarzugi *et al.*, 2015).

pH Analysis of Nanoemulgel

For this experiment, 0.5 grams of the substance is mixed with 5 ml of distilled water, and then the pH of the probe is immersed for a duration of 1 minute. The pH of the nanoemulgel is ascertained by observing the color alteration in the pH stick(Ting *et al.*, 2020).

Test for Viscosity of Nanoemulgel

The process involves measuring 100 mL of nanoemulgel preparation using a viscometer. The numerical measurement outcome will be displayed on the screen once the viscosimeter scale has reached a stable state and is read. The experiment was replicated thrice (Mulia *et al.*, 2018).

Test for dispersion of Nanoemulgel

The test protocol is applying 0.5 grams of nanoemulgel onto a circular glass with a diameter of 15 cm. An additional glass is placed on top and allowed to sit for a duration of 1 minute. The current measurement involves determining the diameter of the dispersion of the preparation. Subsequently, an extra burden of 100 grams is introduced, and the specimen is allowed to remain undisturbed for an additional 1 minute prior to measuring the diameter that has achieved stability (Mulia *et al.*, 2018).

Adhesion Test of Nanoemulgel

The test technique commences by applying 0.5 grams of nanoemulgel onto a circular glass with a diameter of 15 cm. An extra layer of glass is placed on the top and allowed to remain for a duration of 1 minute.

The current measurement involves determining the diameter of the preparation's spread. Subsequently, an extra burden of 100 grams is applied, and the specimen is allowed to remain undisturbed for 1 minute prior to measuring the diameter that has achieved stability(Mulia *et al.*, 2018).

Antibacterial Activity Test

Quantitative antibacterial activity studies are conducted using the sumuran diffusion method. Each test bacteria suspension, consisting of 200 µL of *P. acnes* on blood agar media and 200 µL of *S. aureus* and *S. epidermidis* on nutrient agar media (NA), was inoculated. The substrate is punctured with a pasteur pipette of 6 mm in diameter. Exactly 20 µL of extract solution, 20 µL of soursop leaf extract nanoemulgel solution, 20 µL of 1% mycin gel solution as a positive control, and 20 µL of nanoemulgel solution without extract as a negative control were aseptically placed into the designated wells using a laminar air flow (LAF) system. The incubation process for *P. acnes* bacteria was conducted under anaerobic conditions at a temperature of 37°C for a duration of 48 hours. On the other hand, *S. aureus* and *S. epidermidis* bacteria were incubated at the same temperature for a period of 24 hours. The evaluation is conducted through the observation of inhibitory zones that are generated, which are then interpreted by identifying clear areas that indicate the absence of bacterial growth. The experiment was conducted on three separate occasions (Wijayanti *et al.*, 2021).

Optimum Formula Approach

The recorded data involved the test results of % transmittance, emulsification time, and pH on nanoemulsions containing soursop leaf extract. In the data analysis stage, the Design Expert application is used by applying the Simplex Lattice Design method. The purpose of this step is to establish the optimum formula based on the results of the analysis that has been done. This process allows the discovery of formulas that provide optimal results based on pre-tested variable settings.

Inhibition Zone Approach

The antibacterial activity of the inhibitory zone was measured using a caliper three times at different positions, and the measurement results were averaged. In this way, a more accurate and representative value of the inhibitory zone size that reflects the antibacterial activity of the sample can be obtained.

Statistical Approach

The data of nanoemulsion characteristics were analyzed statistically using Design Expert software with the Simplex Lattice Design method. The prediction results obtained were verified and analyzed using one sample t-test with the help of SPSS software, with a confidence level of 95%. This step allows further evaluation of the accuracy of the predictions generated by the statistical model used.

RESULT AND DISCUSSION

Soursop Leaf Extraction

In the extraction process, 400 grams of simplisia powder were extracted using maceration method, resulting in a dry extract weighing 33.34 grams. The extraction yield is calculated as 8.34%, calculated from the weight of the extract obtained against the weight of the simplisia used. The selection of maceration method was chosen because of its simple uniqueness and ease of implementation. 70% ethanol was chosen as a universal solvent because of its ability to extract polar and nonpolar compounds with optimal activity (Tambun *et al.*, 2021).

Solubility of Extracts

Prior to formulation, dry extracts undergo solubility testing, as solubility is essential in identifying the appropriate carrier. High solubility enhances the usability of extracts for therapeutic reasons and aids in the creation of ideal nanoemulsions. Mardiyanto *et al* (2018) found that soursop leaf extract

demonstrated the greatest solubility in VCO (6 mg/mL), Tween 80 (18 mg/mL), and PEG 400 (9 mg/mL) based on test findings. Thus, the three constituents chosen were oil, surfactant, and cosurfactant phases. Generally, the lower the amount of solvent used to dissolve the extract, the higher the solubility of the extract in that solvent.

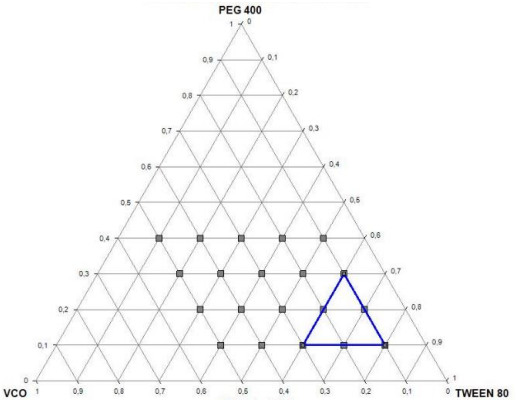

Figure 1. Ternary diagram of *virgin coconut oil*, Tween 80, PEG 400. The area in the blue line indicated a clear solution when the mixture was diluted in distilled water

Ternary phase diagram

Phase diagrams, specifically pseudo ternary diagrams, are employed for the purpose of ascertaining the concentration ranges of several constituents, including VCO (oil), Tween 80 (surfactant), and PEG 400 (cosurfactant). The objective is to detect areas where nanoemulsion is formed and determine the maximum and minimum thresholds for the oil, surfactant, and cosurfactant phases. This procedure is conducted without the inclusion of soursop leaf extract (Elmarzugi *et al.*, 2015).

The nanoemulsion inside the region bounded by the blue line in **Figure 1** is generated spontaneously on the ternary phase diagram. The higher concentration of Tween 80 and PEG 400 can result in the formation of clear or transparent nanoemulsions due to the ability of surfactants and cosurfactants to reduce the interfacial tension of the oil surface, hence enhancing the stability of the nanoemulsions (Mardiyanto *et al.*, 2018).

Optimization of Nanoemulsion Formula with Simplex Lattice Design

The findings of the evaluation for % transmittance response, emulsification time, and pH may be found in **Table 4** and **Figure 2**. The percentage of light transmission ranged from 83.2% to 96.4%. Percent transmittance can be utilized to estimate the size of droplets in nanoemulsions. If nanoemulsions have high transmittance (low absorbance) and appear

Table 4. Experimental Formula Response Data Using *Simplex Lattice Design*

Run	Factor			Response		
	VCO (%)	Tween 80 (%)	PEG 400 (%)	% Transmittant	Emulsification Time	pH
1	20	70	10	85.7	62	5.7
2	13.33	63.33	23.33	94.5	53	5.2
3	10	60	30	86.3	61	5.5
4	30	60	10	82.6	67	6
5	13.33	73.33	13.33	93.4	53	5.2
6	10	80	10	96.4	50	5
7	17	66.67	16.67	92.7	55	5.3
8	30	60	10	80.6	69	6
9	23.33	63.33	13.33	85.1	64	5.7
10	10	80	10	95.2	51	5.1
11	10	70	20	90.8	57	5.4
12	10	60	30	88.6	59	5.5
13	20	70	10	83.2	66	5.8
14	20	70	10	85.7	62	5.7

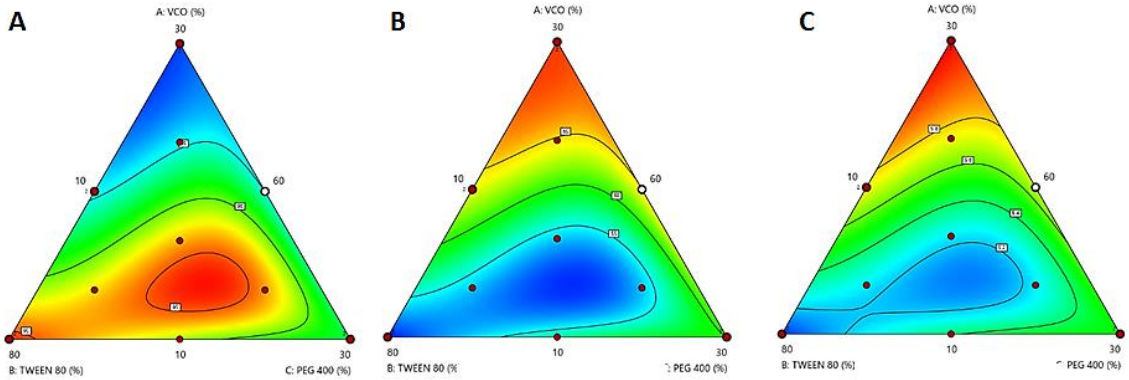

Figure 2. Contour plot responses of transmittance (A), emulsification time (B), and pH (C)

visually clear, it can be inferred that the oil droplets carrying the active substance are in a non-dispersed state within the nanoemulsion formula. The optimum formula is determined using simplex lattice design analysis. The model equation was presented in **Table 5**, represents Y as the response, A as VCO, B as Tween 80, and C as PEG 400.

The link between the percent transmittance reaction to VCO, Tween 80, PEG 400, and the interaction between VCO and PEG 400 exhibits a favorable influence, resulting in an improved transmission percentage. Unlike the interaction observed between VCO and Tween 80, which exhibits a negative response, indicating a drop in the transmittance value at the given percentage of transmittance. The interaction between Tween 80 and PEG 400 resulted in a decrease in % transmittance.

The emulsification time was determined from 14 experiments, with a range of 50-67 seconds. This experiment aimed to determine the duration needed for a uniform mixture to form during the process of dissolving in a medium. This was achieved by visually monitoring the disappearance of nanoemulsion droplets. The simplex lattice design analysis yields a plot of the percent transmittant response, as depicted in Figure 2. The relationship between the response and the variables can be described by a linear model, represented by Equation 2. The equation represents the emulsification time response, denoted as Y, in terms of the variables A (VCO), B (Tween 80), and C (PEG 400). The emulsification time response to VCO, Tween 80, and PEG 400 is influenced by the interaction between each component, resulting in a positive coefficient. This means that a shorter emulsification time is needed.

The pH analysis of nanoemulsions derived from 14 experiments, ranging from 5 to 6, demonstrated that they fell within the desired pH range of 4.5 to 6.5 for skin application. This test is conducted to ensure the safety of nanoemulsion formulations when applied topically. According to the research conducted by Wijayanti *et al* (2021) using simplex lattice design, the best formula may be determined. The correlation between the pH reaction to VCO, Tween 80, and PEG 400, as well as the interaction between Tween 80 and PEG 400, has a beneficial impact on the resultant pH. The interaction between VCO

Table 5. The equation of the responses suggested by the software

Response	Equation
Transmittance	Y = 81.65A + 95.85B + 87.50C + 0.88A – 16.81AB + 1.66AC – 2.74BC
Emulsification time	Y = 67.94A + 50.44B + 59.94C + 18.75AB + 3.24AC + 6.24BC
pH	Y = 6.00A + 5.05B + 5.50C + 0.88A – 0.24AC + 0.46BC

Table 6. Verification of optimization results using one sample t-test

Parameters	Predictions	Verification	Significance	Information
% Transmittance	92.73	91.97 ± 1.16	0.401	Not significantly different
Emulsification Time	54.71	52.76 ± 1.18	0.105	Not significantly different
pH	5.3	5.67 ± 0.24	0.163	Not significantly different

and PEG 400 has a negative response, resulting in a reduction in pH value. The acquired response values are subsequently examined to identify the best formula. The optimal formula for the nanoemulsion was predicted to contain 10.86% VCO, 67.33% Tween 80, and 21.81% PEG 400. The predicted response values were 96.40% transmittance, 54.71 seconds emulsification time, and a pH of 5.3. The desirability value of 0.703, which is close to 1, indicates that this formula is the best solution.

Optimum Formula Verification

This optimum formula is then validated, and the test results are analyzed using a single sample T-test and compared to the predicted value. The P-value was calculated based on the analysis results. There was no substantial difference between the verification results and the software suggestions results ($p > 0.05$) (**Table 6)**. A polydispersity value of 0.5 shows that the particle size distribution is homogeneous. With this value close to zero, the particle size distribution becomes more uniform and can accurately characterize the nanoemulsion formula (Danaei *et al.*, 2018). Potential zeta values greater than +30 mV or less than -30 mV will prevent particle aggregates that can lead to coalescence.

Nanoemulgel Evaluation

An assessment was conducted on nanoemulsion preparations, encompassing visual uniformity, dispersion, adherence, viscosity, and pH. The homogeneity test guarantees the thorough blending of ingredients in the formulation, while the nanoemulgel texture flatness test verifies the consistency of application on the skin. The homogeneity results indicate that no particles or phases segregate when the preparation is applied to the glass object. The pH testing of the Nanoemulgel (pH 5.83) demonstrates that it falls within the acceptable range of normal skin pH (4.5-6.5), hence validating the product's safety for usage (Wijayanti *et al.*, 2021).

Adhesion testing is crucial as it directly impacts the absorption of drugs. Obat can be absorbed by dermal touch. The nanoemulgel formulations containing soursop leaf extract exhibit satisfactory adhesion as they possess a sticking time exceeding 1 second (Sultan *et al.*, 2022). The dispersion test evaluates the extent to which the gel spreads on the skin, which is crucial for determining the rate at which the drug is released and its effectiveness. The test findings indicated a spreading diameter of 5.57 cm, which falls within the specified range of 3-7 cm as stated in the literature. This confirms that the preparation formula achieved a satisfactory dispersion (Okpalaku *et al.*, 2023).

The purpose of the viscosity test is to assess the ease of removal of nanoemulgel preparations from their packaging, as well as to gauge their convenience. The viscosity measurement of the nanoemulgel prepared from soursop leaf extract was found to be 11479.33 centipoise (cP). According to Sultan

Table 7. Antibacterial Test Results of Soursop Leaf Extract dan Nanoemulgel

Bacteria	Inhibition zone (mm)			Inhibition zone (mm)		
	Extract	Control (+)	Control (-)	Nanoemulgel	Control (+)	Control (-)
P. acnes	13	15	0	9.67 ± 0.47	16.33 ± 0.94	0
S. aureus	11	17	0	7.33 ± 0.47	12.33 ± 0.94	0
S. epidermidis	10	17	0	5.67 ± 0.82	10.33 ± 0.47	0

et al (2022), gel preparations are reported to exhibit a favorable viscosity within the range of 900-14000 cP. Conducting pH testing on nanoemulgel preparations is crucial to verify that the gel's pH falls within the typical pH range of the skin, which is around 4.5-6.5 (Lukić *et al.*, 2021).This guarantees the secure application of the gel on the skin without inducing any irritation or discomfort. The maintenance of skin integrity and health is facilitated by maintaining a pH level that is in harmony with the skin condition while utilizing these nanoemulgel formulations.

Antibacterial Activity

The soursop leaf extract exhibited antibacterial activity against *P. acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* bacteria, resulting in inhibitory zones measuring 13 mm, 11 mm, and 10 mm respectively (**Table 7**). The soursop leaf extract nanoemulgel exhibited inhibitory zones measuring 9.67 mm, 7.33 mm, and 5.67 mm on average (**Table 7**). Based on the diameter category, the nanoemulgel formula of soursop leaf extract demonstrated its effectiveness against *P. acnes*, *S. aureus*, and *S. epidermidis* bacteria, including the medium category, as shown by the Davis and Stout inhibitory zones (Sari *et al.*, 2018). This may be attributed to the low concentration of soursop leaf extract present in the nanoemulgel formulation.

CONCLUSIONS

The optimum composition of the nanoemulsion, determined using the simplex lattice design approach, consists of 10.86% VCO, 67.33% Tween 80, and 21.81% PEG 400. The nanoemulsion examination yielded the following results: a transmittance value of 91.97%, an emulsification duration of 52.76 seconds, a pH of 5.67, a zeta potential of -39.13 mV, a particle size of 229.47nm, and a PDI of 0.42. The evaluation of the physical properties of the nanoemulgel resulted in a homogeneous nanoemulgel with a pH value of 5.83±0.24, dispersion power of 5.57±0.25 cm, adhesion of 3.80±0.25 seconds, viscosity of 11479.33±167.49 cP, and medium category antibacterial activity against *P. acnes*, *S. aureus*, and *S. epidermidis*.

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