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IN VITRO EVALUATION OF ANTIBACTERIAL PROPERTIES AND FORMULA OPTIMIZATION OF TURMERIC RHIZOME (*CURCUMA DOMESTICA* V.) NANOEMULSION CREAM

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Turmeric rhizome (Curcuma domestica V.) has broad-spectrum antibacterial effects due to its curcumin content. Topical application of turmeric extract has limitations, which is curcumin was hydrophobic, low solubility and bioavailability. A concentration of 5% turmeric extract in nanoemulsion cream was selected. Optimization of the turmeric rhizome extract nanoemulsion cream base was carried out using Factorial Design. The formula was divided into 4, namely F_1, F_A, F_B, and F_{AB} with Tween 80 emulsifier (35 gr - 45 gr) and PEG 400 co-emulsifier (14 gr - 24 gr). A conventional turmeric cream was also made as a comparison. The resulting nanoemulsion had a particle size between 138.20 and 287.37 nm. The evaluation of the nanoemulsion cream showed values in accordance with topical preparation standards with a p-value <0.05 for each evaluation result, where the pH was 4.32-4.87 (4.5-6.5), viscosity values of 3638–8954 cPs (2000–50000 cPs), spreadability values of 4.32–4.87 (5–7 cm), and adhesion values of 31.59–97.00 seconds (>1 second). In the Freeze and Thaw stability test, all four formulas showed good stability. The results of the release test using the Franz Diffusion Cell showed the highest cumulative curcumin content in F_1 (14.2457 µg/cm^2) and the lowest in F_{AB} (2.3799 µg/cm^2). The penetration rate (flux) showed the highest flux value in F_1 (7.1218 µg/cm^2.hour^{-1}) and the lowest flux value in F_{AB} (1.1900 µg/cm^2.hour^{-1}). Compared to conventional turmeric cream, the results show that the formulation with a nanoemulsion system produces a good cream with good stability and more stable and controlled penetration of the curcumin.

Keywords: *antibacterial, cream, Curcuma domestica V., curcumin, nanoemulsion*

INTRODUCTION

The development of antibacterial products from natural ingredients has increased in line with the growing problem of side effects from topical antibacterial drugs. In empirical traditional medicine, turmeric rhizome (*Curcuma domestica* V.) is used to treat skin wounds[1]. Turmeric rhizome (*Curcuma domestica* V.) has broad-spectrum antibacterial effects due to its curcumin content[1]. Curcumin in turmeric rhizome (*Curcuma domestica* V.) has been proven to have significant wound healing properties[2]. Research on turmeric rhizome extract (*Curcuma domestica* V.) has shown effective antibacterial activity. Research by Wu et al. (2024) states that in the concentration range of 0.1-10 mg/mL, turmeric extract has broad-spectrum antibacterial activity[2].

Topical application of turmeric extract in topical wound healing has obstacles, where the physicochemical properties of curcumin are hydrophobic and have very low solubility in aqueous media, making it unsuitable for topical application to wound areas due to its low bioavailability[3]. The production of topical preparations of turmeric extract in the form of nanoemulsions can be a solution to overcome these obstacles by increasing solubility, protecting the curcumin compound from hydrolysis degradation reactions, and increasing the bioavailability of the active substance when applied to wound areas[3].

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The manufacture of nanoparticle-based topical cream preparations has been widely developed in the field of pharmacy because it has advantages such as better skin penetration so that the effects of active substances can be delivered better, has long-lasting efficacy, more stable preparation quality, and good protection against ultraviolet exposure[4].

Curcumin-loaded nanoemulsion from turmeric rhizome has been widely conducted, including a study by Saari, et. al. (2020), which developed nanoemulsions for checmical curcumin delivery through the skin. In their study, nanoemulsion curcumin showed good permeability and stability of curcumin. In this study was focus to developed a nanoemulsion cream formulation using standardized turmeric rhizome extract for antibacterial effect and optimaizing the emulsifier and co-emulsifier for optimal topical application.

This study is an experiment to find the optimal formula for a nanoemulsion cream preparation of turmeric rhizome extract (*Curcuma domestica* V.) as an antibacterial agent. To obtain the optimal nanoemulsion cream formula, *in vitro* antibacterial activity testing was conducted to determine the concentration of extract in the nanoemulsion cream that provided the best antibacterial activity. Then it was followed by optimization of the cream base used, namely Tween 80 as an emulsifier and PEG 400 as a co -emulsifier, with evaluation of the turmeric rhizome extract nanoemulsion cream based on pH, viscosity, spreadability, adhesion, *Freeze and Thaw* stability test, and curcumin release test.

MATERIALS AND METHOD

Materials

Turmeric rhizome powder (*Curcuma domestica* V.) obtained from Bumi Herbal Dago, Bandung, 96% ethanol, VCO (Trivico®), cetyl alcohol, citric acid, liquid paraffin, propyl paraben, PEG 400, glycerine, methyl paraben, Tween 80, distilled water, citric acid, agar broth medium (all chemicals obtained from Nitra Kimia Laboratory), *Staphylococcus aureus* (ATCC6538) and *Escherichia coli* (ATCC25922) bacteria.

Instruments

Water bath (Thermo®), beaker (Pyrex®), vial bottle, Ultrasonicator (Qsonica), 100 g tube bottle, UV-Vis Spectrophotometer (Libra-Biochorm), Particle Size Analyzer (Horiba Scientific), Viscometer (*Brookfield*), dropper pipette, petri dish, pH meter (Lutron®), stirring rod, vial bottles, 100g ointment pots, digital analytical scales (OHAUS®), dark glass bottles, parchment paper, aluminium foil, porcelain dishes (Iwaki®), *Franz* Diffusion Chamber (Alterlab), cellulose acetate membrane paper for analysis (Membrane Solutions).

Sample Preparation

Preparation of Turmeric Rhizome (Curcuma domestica V.) Extract Samples

Turmeric rhizome extract (Curcuma domestica V.) 250 g was prepared using a maceration method with 2.5 L of 96% ethanol (ratio 1:10). Maceration was carried out for 2 days (48 hours) at a temperature of 25ºC, followed by re-maceration for 2 times. The solvent was evaporated using a rotary evaporator at the temperature of 40ºC-50ºC until a thick extract in the form of turmeric rhizome extract was obtained.

Standardization of turmeric rhizome (Curcuma domestica V.) extract

Standardization of turmeric rhizome extract refers to the Indonesian Herbal Pharmacopoeia[5]. Macroscopic testing, moisture content testing using the toluene distillation method, total ash content testing, acid-insoluble ash content testing, and qualitative and quantitative curcumin testing on turmeric rhizome extract were conducted.

Optimization of Nanoemulsion Concentration of Turmeric (Curcuma domestica V.) Extract for Antibacterial Testing

The preparation of turmeric extract nanoemulsion was carried out by mixing Tween 80 and PEG 400 with citric acid. The oil phase was then prepared by mixing turmeric rhizome extract in VCO. The oil phase was then added to the water phase with stirring using a magnetic stirrer at a speed of 1000 rpm for 15 minutes at a temperature of 25ºC. Then, methyl paraben was added to the mixture. The stirring speed was increased to 1200 rpm for 15 minutes. The mixture was then ultrasonicated at 20 kHz for 30 minutes to form a nanoemulsion of turmeric rhizome extract.

Table 1: Composition of Turmeric Extract Nanoemulsion.

Materials	Composition (gr)		
	Turmeric NE 2.5%	**Turmeric NE 5.0%**	**Turmeric NE 7.5%**
Turmeric Extract	2.5	5.0	7.5
VCO	15	15	15
Tween 80	35	35	35
PEG-400	14	14	14
Methyl paraben	0.2	0.2	0.2
Citric acid	0.2	0.2	0.2
Distilled Water	100	100	100

Antibacterial activity testing of turmeric rhizome extract nanoemulsion

Antibacterial activity testing was performed using the agar well diffusion method. Standard suspensions of test bacteria, namely *Staphylococcus aureus* (ATCC6538) and *Escherichia coli* (ATCC25922), were prepared with gentamycin cream (Sagestam®) as a positive control. Incubation was carried out at 37°C for 24 hours. After the incubation period was complete, the average diameter of the inhibition zone was measured.

Optimization of Tween 80 and PEG 400 Base in Nanoemulsion Cream of Turmeric Rhizome (Curcuma domestica V.) Extract

Preparation of nanoemulsion cream from Turmeric Rhizome (*Curcuma domestica* V.) Extract with optimization based on Tween 80 and PEG 400. The optimized composition is based on the research by Pratiwi, 2024[6], with modifications to the active ingredients. The preparation of the nanoemulsion is the same as in the previous stage using a magnetic stirrer at a speed of 1000 rpm for 15 minutes and increased to 1200 rpm for 15 minutes and then the mixture ultrasonicated at 20 kHz for 30 minutes. After that, with the addition of 3% xanthan gum and 1% glycerine as thickening agents to make the nanoemulsion cream preparation. Xanthan gum and glycerine are mixed gradually into the nanoemulsion by stirring using a magnetic stirrer at a speed of 1000 rpm for 15 minutes at a temperature of 25ºC.

Table 2: Composition of Nanoemulsion Cream from Turmeric Extract with Optimization of Tween 80 and PEG 400**.**

Composition of Nanoemulsions (gr)				
Materials	**F_1**	**F_A**	**F_B**	**F_{AB}**
Turmeric Extract	7.71	7.71	7.71	7.71
VCO	15	15	15	15
Tween 80	35	45	35	45
PEG-400	14	14	24	24
Methyl paraben	0.20	0.20	0.20	0.20
Citric acid	0.05	0.05	0.05	0.05
Distilled Water	75.9	65.9	65.9	55.9
Composition of Turmeric Extract Nanoemulsion Cream (gr)				
Materials	**F_1**	**F_A**	**F_B**	**F_{AB}**
Xanthan gum 3%	4.62	4.62	4.62	4.62
Glycerine 1%	1.54	1.54	1.54	1.54
Cream (gr)	**154**	**154**	**154**	**154**
Turmeric extract (%)	**5**	**5**	**5**	**5**

Production of Turmeric Rhizome Extract Cream as a Comparison

Turmeric Extract Cream is made with an optimal cream base composition from the research by Abduraffi et al. 2015[7], modified in its active ingredients. The cream is made by heating each oil phase and water phase in a porcelain dish based on the composition of each ingredient in Table 3. The melting temperature is 70ºC above a water bath. Stirring is carried out by adding the oil phase to the water phase while grinding little by little until a cream mass is formed. Next, turmeric rhizome extract is added, then stirred again until the cream is homogeneous.

Table 3: Ingredients of Turmeric Rhizome Extract Cream.

Ingredients	**Ingredients composition (gr)**
Turmeric rhizome extract	5
Stearic acid	12
Cetyl Alcohol	2
TEA	3
Glycerine	8
Methyl paraben	0.2
Distilled water	100

Testing the characteristics of nanoemulsion from turmeric rhizome extract (F_1, F_A, F_B, and F_{AB})

Characterization testing of turmeric extract nanoemulsions was carried out using a particle size analyser to measure particle size, zeta potential, and polydispersity index. The test was conducted by dispersing 1 µL of 4 turmeric extract nanoemulsion formulas into 100 mL of distilled water, then analysing 3 mL of the solution for particle size, particle size distribution/polydispersity index using a Particle Size Analyzer, and zeta potential using a Zeta Zizer.

Testing the Formulation Characteristics of Turmeric Rhizome Extract Nanoemulsion Cream

a) Organoleptic and Homogeneity Tests

Organoleptic testing is conducted by physically examining the cream through visual observation of its texture, colour, and smell. Homogeneity is examined visually by placing the preparation between two pieces of glass and observing for coarse particles or inhomogeneity.

b) Spreadability Test

The test was conducted by pressing two glass plates onto 0.5 g of the preparation and measuring its spread on the glass surface with each addition of weight, namely 50, 100, 150, and 200 g. The spread diameter of the formula was calculated from the average length of the diameter of several edges[19].

c) pH test

The pH test began with calibrating the pH meter using pH 4 and pH 7 buffer solutions. The pH meter was dipped into the cream and the pH value indicated by the pH meter was recorded.

d) Viscosity test

Viscosity testing of samples was measured using a Brookfield Viscometer. 50 g of cream was placed in a container and the height of the container was adjusted so that the motor could move. The measurement results displayed on the device were recorded.

e) Cream Type Test

Testing of the nanoemulsion cream type of turmeric rhizome extract was carried out by dispersing the cream into the water phase and oil phase. In the water phase, if the preparation is soluble, then the preparation is classified as an oil-in-water (O/W) cream type.

f) Stability test (*Freeze and Thaw*)

The stability testing of turmeric rhizome extract nanoemulsion cream was conducted using the freeze and thaw method by storing the sample at 4°C for 24 hours in 3 days and at 40°C for 24 hours in 3 days. The testing was conducted for 3 cycles in 9 days, then the organoleptic properties, pH, spreadability, and viscosity of the preparation were observed.

g) In Vitro Release Test of Curcumin Active Substance from Nanoemulsion Cream of Turmeric Rhizome (Curcuma domestica V.) Extract

The curcumin release test was conducted using the Franz diffusion cell method. A total of 1.5 g of Formula F_1, F_A, F_B, F_{AB}, and Turmeric Cream were tested for release at intervals of 15, 30, 60, and 120 minutes. The release test was conducted using a cellulose

membrane soaked in a phosphate buffer solution with a pH of 5.5 to simulate the pH of the skin. The samples were placed in the Franz diffusion apparatus. Next, stirring was performed using a magnetic stirrer at a speed of 250 rpm. Subsequently, 2 mL samples were taken at each time interval. The samples were then analysed with a UV spectrophotometer for quantitative testing of curcumin in turmeric extract nanoemulsion cream. The curcumin content was measured at λ_{max} of 422 nm, based on the calibration curve obtained from the laboratory measurements.

- The calculation of curcumin concentration in the sample used a linear equation from the curcumin calibration curve[8]

$$Y = aX + b$$

- Calculation of the cumulative amount of curcumin in the penetrated sample per diffusion area (µg/cm^2) [8]

$$Q = \frac{\{Cn\ x\ V + \sum_{i=1}^{n-1} x\ Ci\ x\ S\}}{A}$$

- Calculation of Penetration Speed per unit of time (Flux)[8]

$$J = \frac{Q}{t} \quad \mu g/cm^2.hour^{-1}$$

Data Analysis of Antibacterial Activity Test Results and Characteristic Test Results of Nanoemulsion Cream from Turmeric Rhizome Extract (Curcuma domestica V.)

a) Analysis of Antibacterial Test Results of Nanoemulsion from Turmeric Rhizome (*Curcuma domestica* V.) Extract

One-way ANOVA analysis was used to analyse the differences in the effectiveness of turmeric rhizome extract nanoemulsion formulations with concentration ranges of 0%, 2.5%, 5%, and 7.5% on the antibacterial activity of *Staphylococcus aureus* and *E. coli*. A p-value < 0.05 indicates a significant difference between the samples tested, followed by a Tukey post-hoc test.

b) Data Analysis of Test Results for the Characteristics of Nanoemulsion Cream Preparations from Turmeric Rhizome (*Curcuma domestica* V.) Extract

Formula optimization using factorial design was performed using Design Expert Version 13 (free trial) to obtain the effects and interactions between two factors at two levels on each response. The effect and interaction data from each factor were analysed using two-way ANOVA. A p-value < 0.05 indicates a significant effect on the physical properties and stability of the preparation. The data recorded were spreadability, pH, viscosity, and adhesion of the evaluated nanoemulsion cream formulations of turmeric extract (*Curcuma domestica* V.).

RESULT AND DISCUSSION

Standardization of Turmeric Rhizome (*Curcuma domestica* V.) Extract

Standardization testing of turmeric rhizome extract was conducted to evaluate the quality of the extract obtained and ensure its compliance with the standards established in the Indonesian Herbal Pharmacopoeia[5].

Table 4 shows that the turmeric rhizome extract contained curcumin. Overall, the results of testing the turmeric rhizome extract showed that the quality of the turmeric rhizome extract was standardized and the results met the standards.

Table 4: Results of turmeric rhizome (*Curcuma domestica* V.) extract standardization.

Turmeric extract parameters	Results (%)	IHP category
Water Content	16.6 ± 0.0234	< 10%
Ash Content	0.2832 ± 0.0022	< 0.4%
Acid Insoluble Ash Content	0.0944 ± 0.0007	< 0.1%

Testing the Antibacterial Activity of Nanoemulsion from Turmeric Rhizome (Curcuma domestica V.) Extract

The results of antibacterial activity testing are shown in **Table 5**. Statistical analysis using ANOVA, followed by Tukey's post hoc test, showed a significance value of p-value < 0.05, where the nanoemulsion system with each concentration produced different inhibition zone values. Curcumin in turmeric extract has antibacterial effects due to its ability to denature bacterial cell proteins[9]. Based on the test results, it can be concluded that the higher the concentration of turmeric rhizome extract in the nanoemulsion system, the greater the bacterial inhibition zone formed[10]. These results are in line with research by Magani, et. al. 2020, where testing of chitosan nanoparticles on S. aureus and E. coli bacteria showed that the higher the concentration of the active substance, the greater the antibacterial activity as seen from the inhibition zone produced[11].

a) Results of Base Optimization of Tween 80 and PEG 400 in Nanoemulsion of Turmeric Rhizome Extract

Base optimization in turmeric rhizome extract nanoemulsions was carried out by creating four formulas: F1, FA, FB, and FAB. Variations in Tween 80 concentration ranged from a low concentration of 35% to a high concentration of 45%. PEG 400 was used at a low concentration of 14% and a high concentration of 24%. The purpose of optimizing the emulsifier and co-emulsifier was to determine the effect of different amounts of base added to the nanoemulsion system on the characteristics of turmeric rhizome nanoemulsion and the characteristics of turmeric rhizome nanoemulsion cream[7]. In the formulation, there was addition of citric acid at low concentrations to maintain pH stability and as an antioxidant agent to prevent the instability of turmeric rhizome extract in the nanoemulsion cream.

The four formulas are visually yellowish to reddish yellow in colour, bright and liquid. The pH of the preparation must be within the pH range of the skin[12]. A pH level that is too high or too low can cause skin problems such as irritation, dryness, or other skin problems[13].

b) Results of Particle, Polydispersity Index (PDI) and Zeta Potential Measurements

The results of testing the average particle size of four nanoemulsion formulas extracted from turmeric rhizomes showed test values that met the standards. Based on **Table 6**, all four formulas had particle sizes within the nanoparticle size range, with value ranging from 138.20 nm to 287.37 nm, which are consistent with the nanoparticle size between range of 20-500 nm[14]. Small particle sizes can improve the penetration of active substances into the skin[15]. This study developed a formulation for antibateria topical so the particle sizes ranging from 20-500 nm were a crucial role to effectively penetrate the stratum corneum and reach the epidermis and dermis where wound healing processes occur. Nano-sized particle facilitated the rapid transport of curcumin for active antibacterial agents to the infected area. Thus, the turmeric rhizome extract nanoemulsion cream not only maximized the penetration of curcumin but also accelerated the wound healing process by enhanced the stability and bioavailability of curcumin on the skin.

The average Polydispersity Index (PDI) of the four nanoemulsion formulas of turmeric rhizome extract had a PDI values within acceptable range between the standard PDI range of 0.01-0.7[16]. An appropriate PDI indicates that the particle distribution is fairly narrow and monodisperse[17]. According to the standard guidelines, a PDI values typically signifies a well-distributed, homogeneous and good uniformity in the nanoemulsion system, to ensuring that the nanoemulsions can deliver the curcumin effectively.

Table 5: Inhibition Zone of Turmeric Rhizome Extract Nanoemulsion.

No.	Bacteria	Inhibition Zone (mm)				
		Positive Control	**Turmeric NE 2,5%**	**Turmeric NE 5%**	**Turmeric NE 7,5%**	**Remarks**
1	*Staphylococcus aureus*	28,93 ± 0,49	9,22 ± 1,46^{c}	15,71 ± 0,75^{b}	24,42 ± 1,43^{a}	*p-value < 0,05*
2	*Escherichia coli*	37,21 ± 0,79	20,31± 1,14 ^{c}	25,49 ± 2,32 ^{b}	29,99 ± 0,88 ^{a}	*p-value < 0,05*

Note: The inhibition zone values are marked with letters (a, b, c), where values with the same letter are not statistically different, and those with different letters are significantly different according to the Tukey's test.

Table 6: Results of Particle, Polydispersity Index (PDI) and Zeta Potential Measurements.

Formula	Particle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
F_1	229.73 ± 1.12	0.038 ± 0.02	- 47.30 ± 0.35
F_A	242.00 ± 2.91	0.248 ± 0.18	- 33.40 ± 0.61
F_B	287.37 ± 10.27	0.220 ± 0.05	- 60.33 ± 2.36
F_{AB}	138.20 ± 1.81	0.329 ± 0.15	- 46.37 ± 3.15
Note: (n=3, the recurrence value is shown by ± SD).			

Based on zeta potential measurements, the four nanoemulsion formulas of turmeric rhizome extract produced ranged zeta potential values between -33 and -60. Zeta potential measurements aim to determine the stability of nanoparticles, with zeta potential values smaller than -30 mV and greater than +30 mV having good electrostatic stability to prevent the aggregation or coalescence in nanoemulsion system[18]. The negative zeta potential value observed in this study indicated that the nanoemulsion of turmeric extract had a good electrostatically stable. This strong repulsion between particle ensured that the emulsions did not easily aggregate each other which was ctirical for maintaining the physical stability of the formulation over time.

The optimizing Tween 80 and PEG 400 also influence to the differences particle size produced by each formula. In the formulation of turmeric rhizome extract nanoemulsion, particle size is important parameter to determine the efficiency of curcumin penetration into the skin. When compared Formula F1 and FAB it can be observed that both formula produced smaller particle size than FA and FB. These result showed that increasing the concentration of Tween 80 and PEG 400 reduced particle size. This result consistent with the studied by Saari, et. al. (2020), which formulated a nanoemulsion using synthetic curcumin, where the increasing ratio Tween 80 and PEG 400 significantly affected the particle size .

c)Test Results of the Characteristics of Nanoemulsion Cream from Turmeric Rhizome Extract

The results of testing the characteristics of nanoemulsion cream from turmeric rhizome (*Curcuma domestica* V.) extract are shown in **Table 7**. The testing was conducted on four nanoemulsion cream formulas from turmeric rhizome (*Curcuma domestica* V.) extract (F_1, F_A, F_B, F_{AB}) and turmeric extract cream. The evaluation of the creams aimed to assess the quality of each formulation and ensure that the formulas met the standards for topical preparations[19].

Of the four nanoemulsion cream formulas containing turmeric extract, formula F1 produced the highest pH value (5.70± 0.01). All four formulas and turmeric creams were within the standard pH range for topical preparations, which is between 4.5 and 6.5[20]. Inconsistent pH in topical preparations can irritate the skin or cause other skin problems, so the final pH of the cream must be suitable for the pH of the skin[20].

The spreadability of the four formulas was quite good but did not meet the standard spreadability of cream, which is 5-7 cm[21]. The FAB formula produced the highest spreadability value and was close to the standard spreadability value (4.87 ± 0.11). The overall viscosity met the standard for good viscosity (2000-50000 cPs)[21]. The adhesion produced in the four nanoemulsion cream formulas showed good adhesion (>1 second). Testing of good cream adhesion shows that the longer the adhesion to the skin, the greater the active substance will be absorbed into the skin[8]. Overall, when compared to conventional turmeric cream, nanoemulsion cream produces better cream characteristics.

d)The Effect of Tween 80 and PEG 400 Base Optimization on the Characteristics of Turmeric Rhizome Extract Nanoemulsion Cream

Formula optimization aims to optimize the composition of turmeric extract nanoemulsion

cream formulations based on the testing results of the characteristics of turmeric rhizome extract nanoemulsion creams[6]. The result of effect Tween 80 and PEG400 base optimization are shown in **Table 8**.

Using statistical analysis with two-way ANOVA, the base variation in the nanoemulsion formula of turmeric rhizome extract had a significant effect on the characteristics of the resulting cream. A p-value < 0.05 indicates a significant effect on the physical properties of pH, spreadability, viscosity, and adhesion of each formula[6]. Optimization of the base in Tween 80 and PEG 400 and their combination produced a p-value < 0.05, where Tween 80 and PEG 400 had an effect on the evaluation results of the cream characteristics in each formula of the turmeric rhizome extract nanoemulsion cream.

Based on the results of the Two-Way ANOVA analysis in **Table 8**, the Tween 80 (A) factor had a significant effect on two responses, namely viscosity and adhesion. The PEG 400 (B) factor had a significant effect on pH, spreadability, viscosity, and adhesion. The interaction between the two factors, Tween 80 and PEG 400 (AB), showed a significant effect on pH, spreadability, viscosity, and adhesion.

Formula optimization on Tween 80 and PEG 400 bases in terms of pH response, viscosity, adhesion, and spreadability is illustrated in **Fig**. 1. To obtain the optimal composition of each nanoemulsion cream formula from turmeric rhizome extract, a contour plot was created and the area that met the standard physical characteristics of the desired cream was selected[22].

The contour plot for each response shows the effect of the response, with blue indicating the lowest value and red indicating the highest value in the test results.

The contour plot area obtained was then combined in an overlay plot in **Fig. 2.** The yellow area in the plot is the area with the optimum composition modelled based on the response data for pH, spreadability, viscosity, and desired adhesion[23].

The predicted composition of Tween 80 and PEG 400 in turmeric rhizome extract nanoemulsion cream that meets the cream characteristic test requirements is 40 g of Tween 80 and 19 g of PEG 400. In the formulation of nanoemulsion cream from turmeric rhizome extract with base optimization in Tween 80 and PEG 400, the formulation results closest to the yellow area (optimal area) were obtained for formulas F_1 and F_{AB} with compositions of 35 and 45 grams of Tween 80 and 14 and 24 grams of PEG 400, respectively.

Table 7: Results of the Evaluation of Nanoemulsion Cream from Turmeric Rhizome Extract.

Formula	Organoleptic	Homogeneity	Cream type	pH	Spreadability (cm)	Viscosity (cPs)	Adhesion (second)
F_1	Semi-solid, bright yellow colour, turmeric aroma	Homogeneous	O/W	5.70 ± 0.01	4.46 ± 0.06	3638 ± 55.8	31.59 ± 0.92
F_A	Semi-solid, bright yellow colour, turmeric aroma	Homogeneous	O/W	5.53 ± 0.02	4.32 ± 0.03	5766 ± 72.6	96.67 ± 1.53
F_B	Semi-solid, bright yellow colour, turmeric aroma	Homogeneous	O/W	5.34 ± 0.03	4.69 ± 0.07	8954 ± 32.7	75.67 ± 2.08
F_{AB}	Semi-solid, bright yellow colour, turmeric aroma	Homogeneous	O/W	5.45 ± 0.10	4.87 ± 0.11	7506 ± 87.9	97.00 ± 2.00
Turmeric Cream 5%	Semi-solid, bright yellow colour, turmeric aroma	Homogeneous	O/W	5.35 ± 0.07	3.88 ± 0.06	9565 ± 5.30	175.33 ± 2.31

Note: O/W (Oil-in-Water).

Table 8: The Results of Statistical Analysis of Effect Base Optimization

Respon	pH		Spreadability (cm)		Viscosity (cPs)		Adhesion (second)	
	F-Value	***P-Value***	***F-Value***	***P-Value***	***F-Value***	***P-Value***	***F-Value***	***P-Value***
Model	26.35	0.0002	31.77	< 0.0001	3668.6	< 0.0001	986.85	< 0.0001
Tween 80 (A)	0.7075	0.4247	0.2185	0.6526	80.91	< 0.0001	1947.15	< 0.0001
PEG400 (B)	55.63	< 0.0001	81.68	< 0.0001	8692.61	< 0.0001	514.36	< 0.0001
AB	22.72	0.0014	13.41	0.0064	2232.28	< 0.0001	499.03	< 0.0001

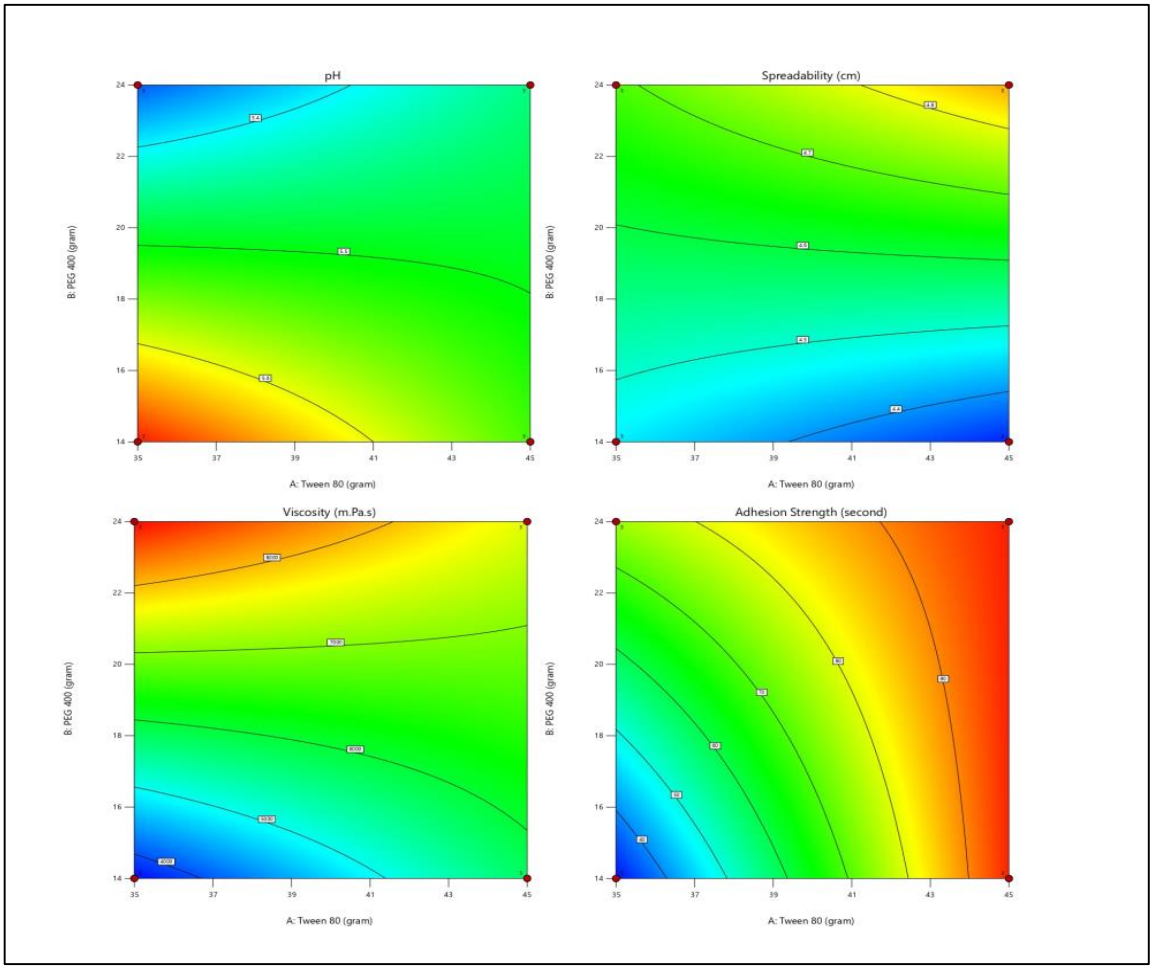


Fig. 1: Contour Plot of pH, Viscosity, Adhesion, and Spreadability Responses.

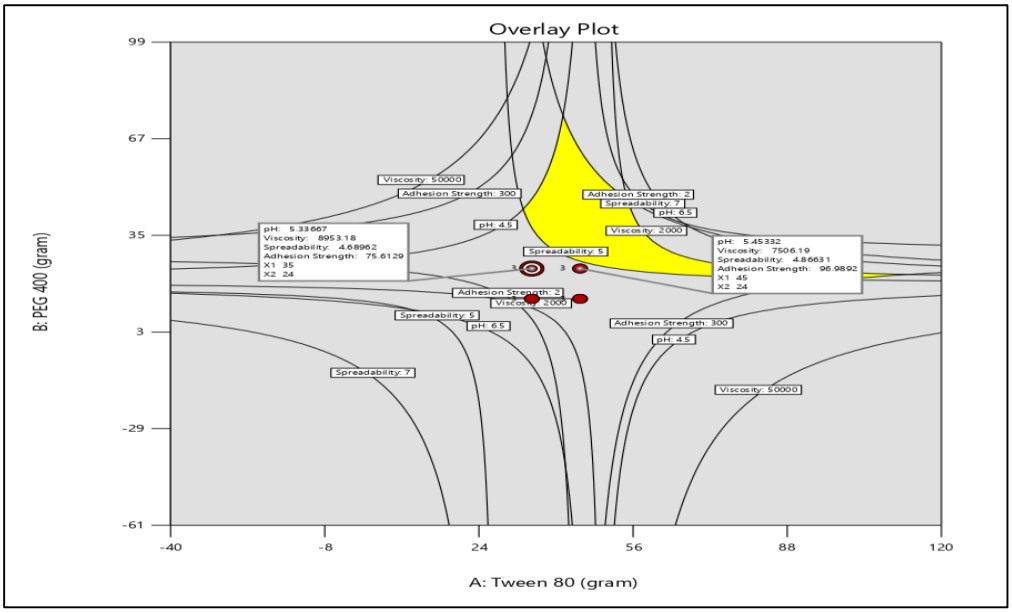


Fig 2: Overlay Plot on pH Response, Viscosity, Spreadability, and Adhesion.

Stability Testing of Nanoemulsion Cream from Turmeric Rhizome Extract and Turmeric Extract Cream

The stability tests for the nanoemulsion cream formula from turmeric rhizome extract and turmeric extract cream are shown in **Fig. 3**. The stability tests were conducted using the freeze and thaw method for 3 cycles by storing each cream preparation at 4°C and 40°C for 3 days. This stability test aims to detect physical instability in the cream as seen from changes in pH, viscosity, and spreadability while being stored[8].

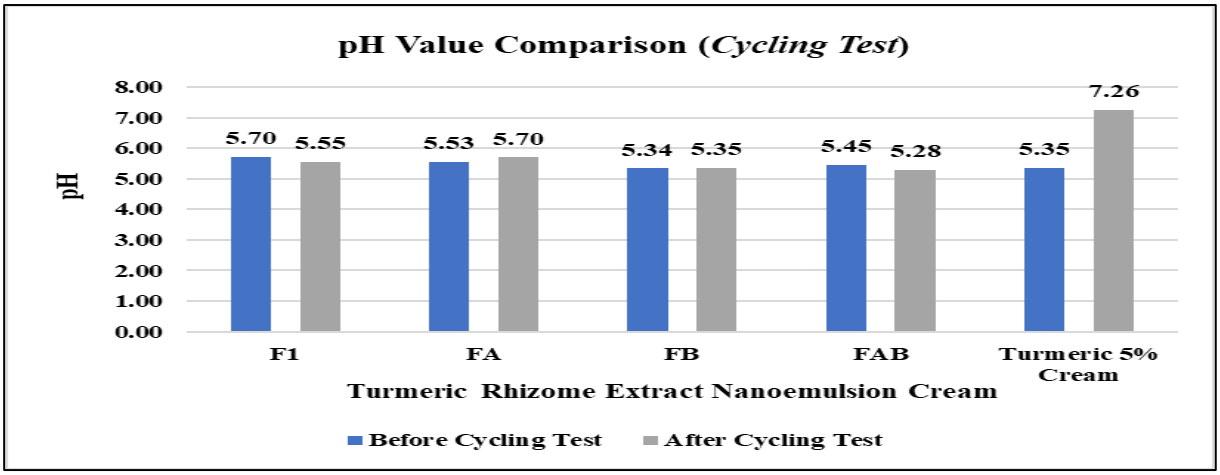
In the stability test with pH parameters, the nanoemulsion cream formulas of turmeric rhizome extract (F_1, F_A, F_B, F_{AB}) did not experience extreme pH changes. In comparison, the turmeric extract cream experienced instability with a significant increase in pH. This difference occurred because the hydrophilic groups in the nanoemulsion cream system remained stable. At low temperatures (4^{o}C), the hydrophilic groups freeze, and at high temperatures (40^{o}C), the hydrophilic groups return to their original state, surrounding the droplets in the preparation and maintaining a high steric barrier between droplets, thereby preventing changes in the pH of the preparation[24]. Conversely, in turmeric extract creams, the possibility of curcumin degradation due to heating may change the pH of the preparation.

In the viscosity stability test, shown in **Fig. 3**, the nanoemulsion cream formulas (F_1, F_A, F_B, F_{AB}) did not experience significant changes in viscosity. Changes in viscosity during the cycling test (freeze and thaw) may occur because when tested at 4°C, the hydrophilic phase will freeze and the particles will tend to shrink, causing the lipophilic phase globules to come together, resulting in an increase in viscosity. When tested at 40°C, the hydrophilic phase will melt again and spread throughout the system. To maintain the stability of the emulsion system, the contribution of surfactants and co-surfactants is crucial in determining the final viscosity of the preparation[6].

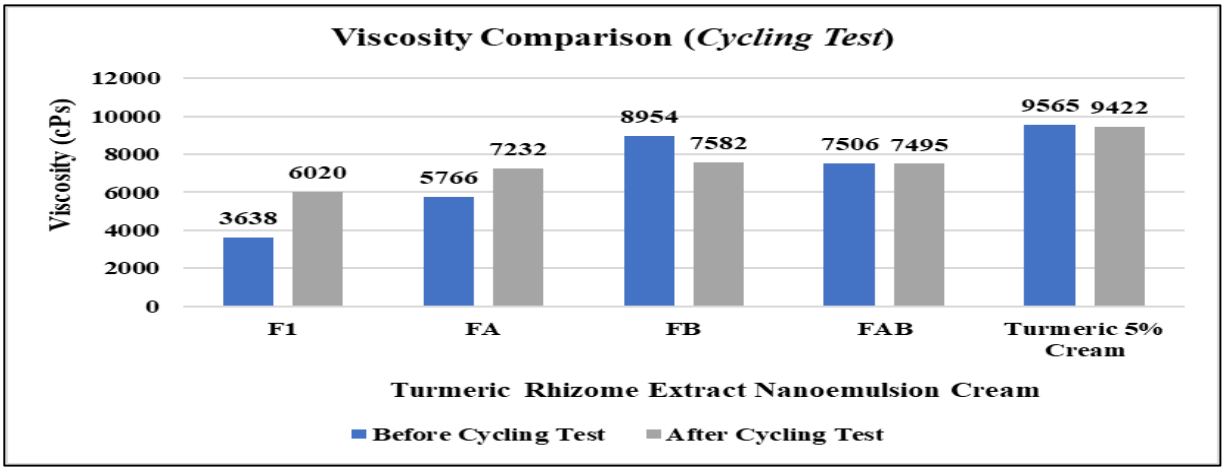
In the spreadability stability test, the spreadability value of all formulations did not reach 5-7 cm. However, the nanoemulsion cream formulations from turmeric rhizome extract (F_1, F_A, F_B, F_{AB}) produced relatively stable test results because the nanoemulsion structure was still able to maintain an even distribution of droplets.

The freeze-thaw test was highly relevant in assessing the resistance of the nanoemulsion cream during temperature fluctuations that frequently occur during the storage period. Based on this freeze-thaw test, all formulations turmeric rhizome extract nanoemulsion cream ((F_1, F_A, F_B, F_{AB}) demonstrated stable results even temperature fluctuations. The addition of appropriate emulsifier and co-emulsifiers helped maintain the integrity of the cream. When compared to the 5% conventional turmeric cream, the conventional cream result more significant physical changes were

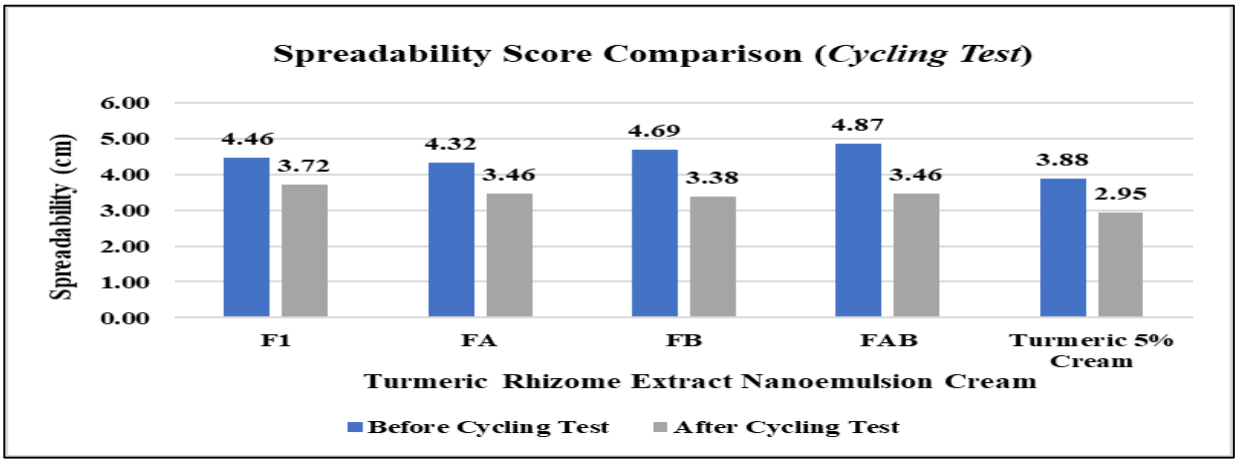
observed during the test, as indicated by the increased viscosity, reduced spreadability, and significant changed in pH. These result showed that conventional cream less stable under temperature fluctuations during storage.



(a)



(b)



(c)

Fig. 3: Stability Test Results (Freeze and Thaw) of Turmeric Rhizome Extract Nanoemulsion Cream, where each sample was stored at 4°C for 24 hours to 40°C for 24 hours. Stability Test involves compared the average values of pH, viscosity, and spreadability at the start and end of the freeze-thaw cycles.

Results of Curcumin Release Testing in Nanoemulsion Creams from Turmeric Rhizome Extracts and Turmeric Extract Creams

Testing of curcumin release in turmeric extract nanoemulsion cream was conducted using a Franz diffusion cell. The testing method referred to the research by Dewi et al. (2020)[20]. The test was conducted by placing the nanoemulsion cream formulation on a membrane that resembled skin conditions between the donor and receptor compartments. The receptor medium used for testing was a pH 5.5 phosphate buffer because this solution could describe the physiological conditions of the skin. The curcumin concentration in the formula F_1, F_A, F_B and F_{AB} was determined using the calibration curve equation, as shown in **Fig. 4.**

Based on **Fig. 5(a)**, the cumulative amount of curcumin released per diffusion area shows that the amount of curcumin released in each formula differs. In general, the amount of curcumin released increases with diffusion time. F_1 (14.2457 ± 0.153 µg/cm²) has a higher cumulative amount of curcumin than the F_A (3.4528 ± 0.016 µg/cm²), F_B (6.5879 ± 0.150 µg/cm^2) and F_{AB} (2.3799 ± 0.123 µg/cm^2) formulations. These results indicate that curcumin release in the F_1 formulation is higher per unit of time.

F_1 with low viscosity causes the highest release of curcumin due to low adhesion, so that curcumin is released more quickly from the cream base matrix. Meanwhile, F_A, F_B, and F_{AB} creams have high viscosity, resulting in high adhesion in the cream base matrix, which causes the curcumin in the cream to be retained longer, so that the curcumin is released gradually.

Compared to 5% turmeric cream (1.9854 ± 0.062 µg/cm^2), the curcumin release rate in the initial stage was very high (burst release), but in the final stage, the curcumin level did not accumulate significantly. The cream in the form of a nanoemulsion had a cumulative level that was initially released slowly, but in the final stage, the accumulated curcumin was quite high.

Based on **Fig. 5(b),** the curcumin flux graph indicates the speed of movement of dissolved active substances through the membrane (8). F_1 formula (7.1218 ± 0.081 µg/ cm^2.hour^{-1}) had the highest flux value with controlled curcumin release over time. Meanwhile, the F_{AB} formula (1.1900 ± 0.061 µg/cm^2.hour^{-1}) has the lowest flux value, where it is possible that more curcumin is bound to the cream base matrix.

Compared to 5% turmeric cream (0.9927 ± 0.031 µg/cm^2.hour^{-1}), 5% turmeric cream produced a very high flux in the initial stage, but in the final stage, the flux value decreased. It indicates that curcumin was released very quickly at the beginning but was unable to maintain the curcumin released with long-term penetration. Therefore, the form of nanoemulsion cream, it is expected to penetrate on the skin with slowly and controlled and provide more optimal effect compared to 5% turmeric cream.

Based on the results of curcumin penetration flux per unit time in the turmeric extract nanoemulsion cream formula (F_1, F_A, F_B, F_{AB}), it can be seen that the nanoemulsion cream form produces a more stable curcumin penetration rate and controlled release compared to conventional turmeric cream.

The results of the calculation of the cumulative curcumin release rate and the curcumin flux value per unit of time from the turmeric extract nanoemulsion cream show results that are in line with previous research by Saari, et. al. 2020[25], where the results of the topical delivery profile of turmeric nanoemulsion can improve the stability of the nanoemulsion and the bioavailability of curcumin in the skin with controlled and stable cumulative release.

The results of the release studies between nanoemulsion cream and conventional cream, reveal a significant difference in the release profiles. The nanoemulsion cream exhibited higher cumulative release of curcumin compared to the conventional 5% turmeric cream. This difference is crucial as it suggest that the nanoemulsion cream system offer more controlled and sustained release of curcumin over time.

This findings, impact the clinical efficacy of the antibacterial cream because more stable and controlled release of curcumin allows for prolonged antibacterial effects. The higher concentration of curcumin could be available to provide a longer-lasting antibacterial effect on the skin. Compared to the conventional trumeric cream, with its rapid release and burst effect in the first release, may result the shorter

duration of curcumin action and potentially limiting its curcumin efficacy for long term therapeutic and might only maintain antibacterial activity for shorter period and not sustain a sufficient concentration of the curcumin to reduce the bacteria over the extended time.

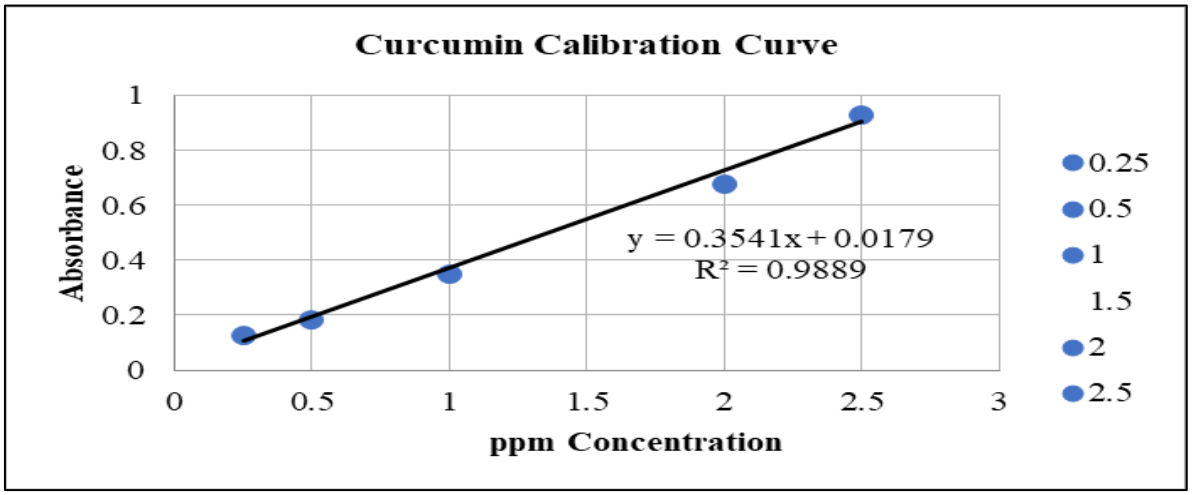
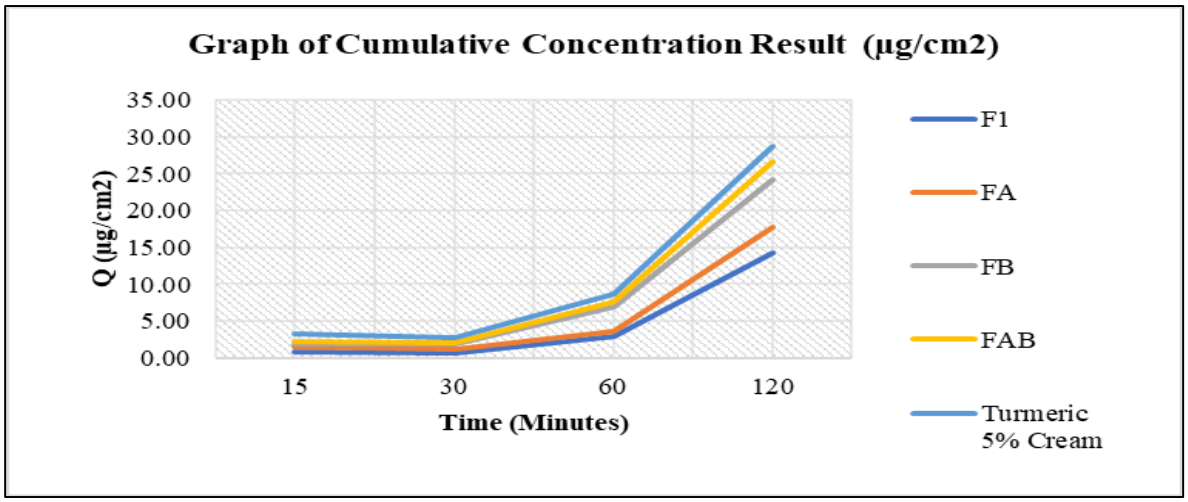
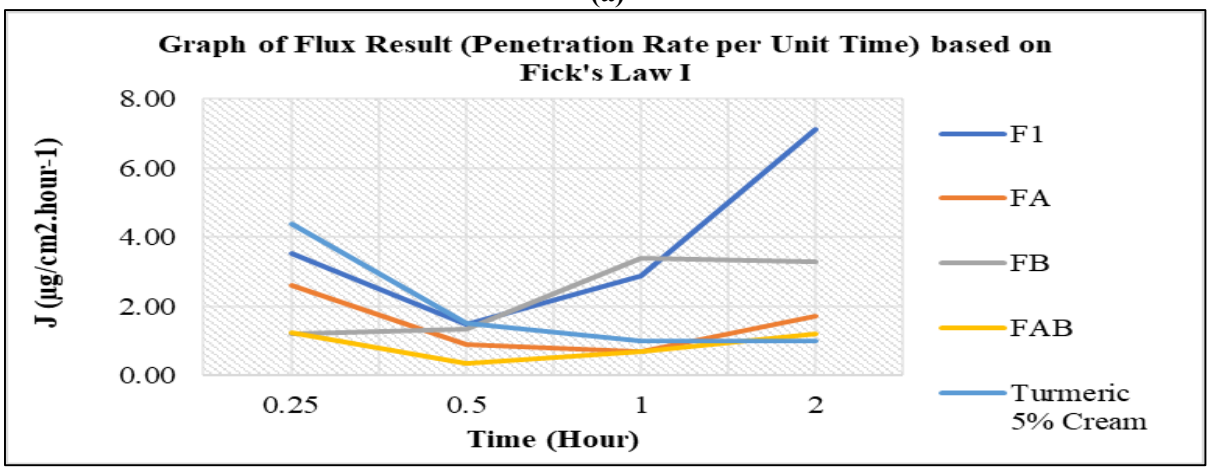


Fig. 4: Curcumin Calibration Curve.



(a)



(b)

Fig. 5: Curcumin Release Results Graph: (a) Curcumin Cumulative Graph; (b) Curcumin Flux Graph.

Conclusion

The 5% turmeric rhizome extract nanoemulsion cream formula provides optimal antibacterial activity with high effectiveness and good nanoemulsion stability. Optimized Formula F1 showed favorable characteristics as an antibacterial topical cream, supported by evaluations of cream properties, stability and curcumin release. Compared to the conventional 5% turmeric cream, the nanoemulsion cream offers enhanced performance.

This study opens new possibilities for further development of more sustainable and effective natural compound. Future clinical trials are recommended to assess the long term safety and effectiveness of thus nanoemulsion cream.

REFERENCES

1. W. Apriliantisyah, I. Haidir, Y. Sodiqah and M. F. M. Said, "The inhibitory activity of turmeric extract (*Curcuma domestica* Val.) against *Staphylococcus aureus* and *Escherichia coli*", ***Fakumi Med J***, 2(10), 694–703 (2022).
2. H. Wu, Z. Liu, Y. Zhang, B. Gao, Y. Li, X. He, J. Sun, U. Choe, P. Chen and R. A. Blaustein, L. Yu, "Extract and its antimicrobial activities and free radical scavenging capacities", ***Foods***, 13(10), 1–13 (2024).
3. A. E. Krausz, B. L. Adler, V. Cabral, M. Navati, J. Doerner, R. Charafeddine, D. Chandra, ... and A. J. Friedman, "Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent", ***Nanomedicine: Nanotechnology, Biol Med,*** 11(1), 195–206 (2015).
4. R. Ahdyani, S. Rahayu, I. Zamzani andika, "Review: Development of nanoparticel-based delivery system in herbal cosmesetics", ***J Curr Pharm Sci***, 4(1), 2598–2095 (2020).
5. Depkes, "Indonesian Herbal Pharmacopoeia", 2 (2017).
6. D. M. N. Pratiwi, "The effect of nanoemulgel formulation of purified extract of binahong leaves (*Anredera Cordifolia* (Ten) Steenis) on anti-inflammatory activity", ***Masters thesis, Sanata Dharma University*** (2024).
7. A. M. Dermawan, L. P. I. Kusharyanti, "Anti acne cream effectivity of methanol extract of *Impatiens balsamina* L. Leaves", ***Tradit Med J***, 20(3), 127–33 (2015).
8. D. I. Putri, Formulation of angkak extract and its penetration, stability and saftey tests", ***Masters Thesis, Setia Budi University,*** (2018).
9. C. Dai, J. Lin, H. Li, Z. Shen, Y. Wang and J. Shen, "Review: The natural product curcumin as an antibacterial agent: Current achievements and problems", ***Antioxidants,*** 11(3), 459 (2022).
10. M. Górski, J. Niedźwiadek, A. Magryś, "Antibacterial activity of curcumin: A natural phenylpropanoid dimer from the rhizomes of *Curcuma longa* L. and its synergy with antibiotics", ***Ann Agric Environ Med***, 29(3), 394-400 (2022).
11. A. K. Magani, T. E. Tallei and B. J. Kolondam, "Antibacterial test of chitosan nanoparticles against *Staphylococcus aureus* and *Escherichia coli*", ***J Bios Logos***, 10(1), 7–13 (2020).
12. N. Salsabila, S. Indratmoko, A. Tenri, "Development of hand and body lotion with chitosan nanoparticles and *Spirulina Sp*. as antioxidants", ***J Pharm UMUS***, 2(01), 11–20 (2021).
13. S. Dianmurdedi, "Formulation of *Gamma oryzanol* nanocream using high-energy emulsification method with variations in stirring speed", ***Masters Thesis, Syarif Hidayatullah University,*** (2018).
14. T. Sunarni, I. Kuncahyo, R. Herowati, "Optimization of nanoparticles ionic gelation method with crosslink agent on fraction of *Stelechocarpus burahol* leaves", ***J Adv Pharm Technol Res***, 15(4), 270–5 (2024).
15. M. Y. Koroleva, E. V. Yurtov, "Nanoemulsions: The properties, methods of preparation and promising applications", ***Russ Chem Rev***, 81(1), 21–43 (2012).
16. N. Ningsih, S. Yasni, S. Yuliani, "Nanoparticle of red mangosteen peel extract synthesis and the functional characteristics of its encapsulated product", ***J Tecnol and Food***, 28(1), 27–35 (2017).
17. X. Li, X. Ye, J. Qi, R. Fan and G. Guo, "EGF and curcumin co-encapsulated nanoparticle/hydrogel system as potent skin regeneration agent", ***Int J***

Nanomedicine, 11(3), 993–4009 (2016).
18. M. Abdassah, "Nanoparticle with ionic gelation", ***J Farmaka***, 15(1), 45–52 (2017).
19. B. Pudyastuti, M. Marchaban, R. Kuswahyuning, "The effect of xanthan gum concentration on the physical stability of VCO", ***J Pharm Sci Community***, 12(1), 6–14 (2015).
20. N. P. Y. A. Dewi, N. L. G. W. Pebriani, P. A. Duarsa, P. C. I. Warnaya, I. Candraningrat, C. I. S. Arisanti, "Formulation and release testing of ethanol extract of guava leaf cream with acne prevention potential", ***J Chem***, 14(2), 119-125 (2020).
21. W. A. Ningrum, W. Wirasti, Y. W. Permadi, F. F. Himmah, F. Ulfa, "Formulation test of dutch eggplant extract nanoparticle as antioxidants", ***J Ilm Kesehat***, 14(1), 99-104 (2021).
22. S. Sisna, S. H. Yuliani, "Optimization of tween 80 and cetyl alkohol in hand sanitizer cream formulation with *Centella asiatica* L. extract", ***J Pharmascience***, 10(2), 273-287 (2023).
23. I. G. M. Suradnyana, I. K. Wirata and N. M. Suena, "Optimization of gelling agent and humectant handsanitizer gel of lime leaf essential oil (*Citrus Amblycarpa* (Hassk.) Ochse.)", ***J Medicam***, 6(1), 16–22 (2020).
24. M. A. Artigas, Y. L. Pérez and O. M. Belloso, "Curcumin-loaded nanoemulsions stability as affected by the nature and concentration of surfactant", ***Food Chem***, 266, 466–474 (2018).
25. N. H. M. Saari, L. S. Chua, R. Hasham and L. Yuliati, "Curcumin-loaded nanoemulsion for better cellular permeation", ***Sci Pharm***, 88(44), 1–12 (2020).

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نشرة العلوم الصيدلية
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التقييم المختبري لخصائص مضادّة البكتيريا وتحسين صيغة كريم النانو-مستحلب لريزومات الكركم (Curcuma domestica V.)

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يمتلك ريزوم الكركم (Curcuma domestica V.) تأثيرات مضادّة للبكتيريا واسعة الطيف بفضل احتوائه على مادة الكركمين. إلا أن التطبيق الموضعي لمستخلص الكركم يواجه بعض المحددات، إذ إن الكركمين مادة كارهة للماء ذات ذوبانية وتوافر حيوي منخفضين. وقد تم اختيار تركيز ٥% من مستخلص الكركم ضمن كريم نانو-مستحلب. أُجريت عملية تحسين قاعدة كريم النانو-مستحلب لمستخلص ريزوم الكركم باستخدام التصميم العاملي .(Factorial Design) وقُسّمت الصيغ إلى أربع صيغ هي F1 : و FAو FBوFAB، باستخدام المستحلب Tween 80 بتركيز (٣٥–٤٥ غرامًا) والمساعد المستحلب PEG 400بتركيز (١٤–٢٤ غرامًا). كما تم تحضير كريم كركم تقليدي للمقارنة.

أظهرت المستحلبات النانوية الناتجة أحجام جسيمات تراوحت بين ١٣٨,٢٠ و٢٨٧,٣٧ نانومتر. وأظهرت نتائج تقييم كريم النانو-مستحلب قيماً متوافقة مع معايير المستحضرات الموضعية، مع قيمة p أقل من ٠,٠٥ لكل نتائج التقييم، حيث تراوح الأسّ الهيدروجيني (pH) بين ٤,٣٢ و٤,٨٧ (المعيار: ٤,٥–٦,٥)، وتراوحت قيم اللزوجة بين ٣٦٣٨ و٨٩٥٤ سنتيبواز (المعيار: ٢٠٠٠–٥٠٠٠٠ سنتيبواز)، وقيم قابلية الانتشار بين ٤,٣٢ و٤,٨٧ سم (المعيار: ٥–٧ سم)، وقيم الالتصاق بين ٣١,٥٩ و٩٧,٠٠ ثانية (المعيار: أكثر من ثانية واحدة).

وفي اختبار الثبات بالتجميد والذوبان(Freeze and Thaw) ، أظهرت جميع الصيغ الأربع ثباتًا جيدًا. كما أظهرت نتائج اختبار التحرر باستخدام خلية انتشار فرانز (Franz Diffusion Cell) أن أعلى محتوى تراكمي للكركمين كان في الصيغة F1 بمقدار ١٤,٢٤٥٧ ميكروغرام/سم²، وأدناه في الصيغة FABبمقدار ٢,٣٧٩٩ ميكروغرام/سم². وأظهرت معدلات النفاذية (Flux) أعلى قيمة تدفق في الصيغة F1بمقدار ٧,١٢١٨ ميكروغرام/سم²·ساعة⁻¹، وأدنى قيمة في الصيغة FAB بمقدار ١,١٩٠٠ ميكروغرام/سم²·ساعة⁻¹.

وبالمقارنة مع كريم الكركم التقليدي، أظهرت النتائج أن الصيغة المعتمدة على نظام النانو-مستحلب تُنتج كريمًا ذا جودة جيدة وثبات مرتفع، بالإضافة إلى تحقيق نفاذية أكثر استقرارًا وتحكمًا لمادة الكركمين.