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Formulation of Nanoemulsion Gel Cayenne Pepper (*Capsicum frutescens*) Extract as an Anti-inflammatory in Gout Arthritis Pain

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ABSTRACT: Cayenne pepper fruit extract has anti-inflammatory effects and could be a natural remedy of arthritis gout pain. The research aims to find the optimal formula for cayenne pepper extract nanoemulsion gel to improve its permeation capability and assess its effect on inflammation in mice induced by 1.5% carrageenan. Extraction using the maceration method followed by standardizing the extract. The optimal nanoemulsion formula is determined by constructing a pseudo-ternary diagram of the nanoemulsion base. The optimal formula obtained was 80% aquadest, 2% VCO, 18% SMIX (surfactant and cosurfactant). The cayenne pepper extract nanoemulsion is integrated with a gel base followed by evaluation. The extract nanoemulsion has been proven to form globules with a size of 54.4 nm, zeta potential of -23.6 mV, and polydispersity index of 0.403. Exudate volume and TNF-α levels were measured using the air pouch method to assess for anti-inflammatory activity. In comparison to other treatment groups, rats treated with chili pepper extract nanoemulsion gel had the lowest average exudate volume, with a statistically significant difference (DNMRT; P <0.05), according to the results of an ANOVA test combined with Duncan's post-hoc test. Meanwhile, the test for TNF-α levels did not show any significant differences.

Keywords: **arthritis gout pain; cayenne pepper; nanoemulsion; anti-inflammatory activity.**

Introduction

Gouty arthritis is a common form of inflammatory arthritis that occurs due to the deposition of monosodium urate (MSU) crystals in the tissues [1]. MSU crystals in the tissue activate NLRP3 to activate IL-1β which will induce pro-inflammatory factors such as TNF-α, IL-6, and IL-8, causing an inflammatory reaction [2]. Symptoms of gouty arthritis are characterized by acute and severe joint pain, redness, and swelling that may occur in both acute and chronic conditions [3]. Gout is the most frequently observed inflammatory arthritis in adults, being three to four times more common than rheumatoid arthritis [4]. The global prevalence of gout has been reported at 1–4%, while its incidence ranges from 0.1% to 0.3% [5].

Symptoms of pain in gout arthritis are usually treated with NSAIDs (Nonsteroidal Anti-Inflammatory Drugs), colchicine, and corticosteroids. The drug is generally available in oral dosage form, but oral drugs have many side effects such as irritating the gastrointestinal system, nausea, and bone thinning [6]. Topical administration of drugs will be effective because it reduces side effects when compared to oral administration [7]. However, topical preparations have challenges in the ability of permeation to reach the target. In addition, existing topical drugs such as diclofenac sodium gel have disadvantages such as causing skin dermatitis, and are not effective for single treatment [8]. Preparations with nanotechnology from natural ingredients can be a solution to these problems.

The transdermal route provides a non-invasive approach for administering drugs into systemic circulation, significantly reducing various constraints associated with oral and intravenous methods. These include quick systemic clearance, hepatic first-pass metabolism, and the pain that comes with invasive treatments such as injections. Furthermore, this method promotes regulated drug release and increases patient compliance. However, the stratum corneum, becomes a barrier that often inhibits the entry of drugs into the body via transdermal drug delivery [9]. A carrier is needed to deliver the drug compound through the skin barrier, one of which is nano-emulsion which allows the drug to penetrate the deepest layers of the skin and target tissues such as synovial joints. The more

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lipophilic nature of nano-emulsion helps to increase skin permeability, resulting in better drug absorption through the skin, avoiding liver metabolism, fewer systemic side effects, and higher patient acceptance [10]. Several studies have found that entrapment of nonsteroidal anti-inflammatory drugs (NSAIDs) such as ketoprofen [11], indomethacin [12], aceclofenac [13], and celecoxib [14] into nanoemulsion-based formulations significantly enhances transdermal penetration. This improvement is mainly due to the nanoscale droplet size that allows drug transport across the stratum corneum phospholipid membrane, resulting in deeper skin penetration and better transdermal therapeutic efficacy.

Nanotechnology-based transdermal preparations can increase the ability of active ingredients to permeate through skin tissue because the size of the active substance globule is smaller than the skin pores [9]. In addition, the nanoemulsion gel dosage form has been shown to allow drugs to be applied directly to swollen areas and then penetrate the skin layer to reach the target so that the drug works faster and is easier to use [10].

Natural medicines have been shown to have fewer side effects than synthetic drugs [11]. Natural ingredients that have the potential to be anti-inflammatory in gouty arthritis are cayenne pepper plants. Cayenne pepper (*Capsicum frutescens*) has been shown to have anti-inflammatory effects produced by capsaicin compounds [12]. Capsaicin in the extract can reduce the secretion of pro-inflammatory cytokines. In addition, capsaicin shows effective inhibition (95%) of prostaglandin formation [15]. Moreover, based on clinical observations, *Capsicum frutescens* oleoresin has been shown to relieve pain and enhance physical hand function, with good tolerance and few adverse effects. These results suggest its potential as a low-cost, natural medicinal agent, making it a good option for formulation into various topical dosage forms [16].

Previous study has shown that the anti-inflammatory effect of capsaicin is comparable to diclofenac tested on mice, but when cayenne pepper extract is made into a gel dosage form, its inflammatory inhibition ability is weak when compared to Na diclofenac gel [7]. Despite the low anti-inflammatory effectiveness of *C. frutescens* extract in conventional gel formulations, its potential in a nanoemulsion-based formulation is yet unknown. For this extract, the lack of nanoemulsion-based methods constitutes a major research gap. Given the proven benefits of nanoemulsion systems in enhancing drug transport across the skin barrier, the development of a nanoemulsion formulation of *C. frutescens* extract and testing the anti-inflammatory activity of the nanoemulsion transdermally is a logical and necessary next step.

With the potential for drug development, researchers are interested in formulating cayenne pepper extract nanoemulsion gels and testing the effectiveness of the anti-inflammatory activity of cayenne pepper extract nanoemulsion gels in vivo on experimental animals by measuring the levels of the inflammatory biomarker TNF-α which is commonly found in gouty arthritis pain inflammation.

Methods

Materials

Cayenne pepper (*Capsicum frutescens*) was obtained from Padang, West Sumatra, Indonesia. Technical n-hexane (Bratachem, Indonesia), ethyl acetate pro analysis (Merck, Germany), toluene pro analysis (Merck, Germany), capsaicin standard (MarkHerb, Indonesia), TLC plate (Merck, Germany), Virgin Coconut Oil (VCO) (CV Siti Nurbaya, Indonesia), Tween 80 (Bratachem, Indonesia), PEG 400 (Bratachem, Indonesia), carbopol 940 (Bratachem, Indonesia), distilled water (Bratachem, Indonesia), triethanolamine (Bratachem, Indonesia), DDY strain mice, carrageenan, TNF-α ELISA kit (Bioassay Technology Laboratory, China), sesame oleum, 1% diclofenac sodium gel (Haleon, Indonesia), mouse feed (RatBio, Indonesia).

Extraction and Standardization

Cayenne pepper fruits (5 kg) were collected and subsequently washed, dried, sorted, blended, and sieved to obtain fine particles. Weighed 644.12 grams of chili pepper simplicia powder, then macerated using 5000 ml of n-hexane solvent for 3 days then remacerated 2 times and stirred periodically. The soaking results were filtered and the filtrate was evaporated using a rotary evaporator (BUCHI Rotavapor R-210, Switzerland) until a thick extract was obtained. The extract was standardized by conducting thin layer chromatography (TLC) testing, measuring drying loss, water content, and total ash content.

Nanoemulsion Base Optimization

The preparation of the nanoemulsion base begins with the selection of oil, surfactants, and cosurfactants that make up the nanoemulsion. The selection is carried out by testing the miscibility of the extract in each component to be used, namely VCO, Tween 80, and PEG 400. The nanoemulsion is made by the titration method based on the pseudo-pic-phase diagram of the mixture of oil, water, and surfactant: cosurfactant (S:KS) that has been selected.

Table 1. Surfactant: cosurfactant composition.

Oil +(S+CS) (%)	20	20	20
(S:CS) Ratio	1:1	2:1	3:1
Oil : (S+KS) Ratio	1:9	1:9	1:9
Aquadest (%)	80	80	80
Total	100	100	100

The preparation of the three-phase diagram begins by determining the ratio of surfactants and cosurfactants first according to Table 1. Then continued by determining the ratio of each material according to Table 2 [17].

Characterization of Nanoemulsion

Characterization of the nanoemulsion base and nanoemulsion extract is carried out by measuring the size of globules, polydispersity index, and zeta potential of the nanoemulsion using a Particle Size Analyzer (PSA) (Horiba scientific SZ-100, Japan) with the dynamic light scattering method. The principle of the tool is measuring particles that experience Brownian motion [18].

Nanoemulsion Morphology Test

The nanoemulsion was dripped onto a Cu grid (formvar/Carbon 400 mesh support film) and left for 1 minute, then the excess nanoemulsion was absorbed and left to dry, then observed using a Transmission Electron Microscope (TEM) (Jeol JEM-1400, Japan) [19].

Making of Nanoemulsion Gel from Cayenne Pepper Extract

The preparation of the cayenne pepper extract nanoemulsion gel formula was carried out using the nanoemulsion and gel base incorporation method. Cayenne pepper extract (concentration in nanoemulsion 0.5%) was mixed with virgin coconut oil, and then a mixture of Tween 80 and PEG 400 was added according to the optimal A1 base formula. The mixture was homogenized using a magnetic stirrer (IKA C-MAG HS 7, China) for 5 minutes and then titrated with distilled water for 10 minutes at a temperature of 50°C and a stirring speed of 1000 rpm. Then the sonication process was carried out (Elmasonic S 40 H, Germany), at 40°C, for 30 minutes. Carbopol 940 is mixed into hot water at 80-100°C and then left for 24 hours to expand. Triethanolamine is added and then homogenized until a clear gel base is formed. The nanoemulsion that has been made is inserted into the gel base while being homogenized with a magnetic stirrer. The ratio between nanoemulsion and gel base used refers to the literature is 3:1.

Organoleptic Test

The organoleptic evaluation of the gel includes assessment of its shape, color, and odor through sensory observation [20].

pH Test

The pH measurement was carried out using a pH meter (Mettler Toledo SevenCompact S210, Indonesia). The cathode part of the pH meter was dipped into the nanoemulsion gel and then the measured pH value was viewed on the screen until a stable value was obtained [20].

Table 2. Base composition.

Formula	A	B	C	D	E	F
Oil + (S+KS) (%)	20	30	40	50	60	70
	1:9	1:9	1:9	1:9	1:9	1:9
	3:7	3:7	3:7	3:7	3:7	3:7
Oil : (S+KS) Ratio	5:5	5:5	5:5	5:5	5:5	5:5
	7:3	7:3	7:3	7:3	7:3	7:3
	9:1	9:1	9:1	9:1	9:1	9:1
Aquadest (%)	80	70	60	50	40	30
Total	100	100	100	100	100	100

The preparation must be tolerable by the skin pH, which is 4.5-6.5 [21].

Viscosity Measurement

The tool used in this test is a Brookfield viscometer (DV2T, USA), as much as 50 mL of the nanoemulsion gel preparation was put into a tube-shaped container and then the appropriate spindle was installed [22].

Centrifugation Test

The nanoemulsion gel was centrifuged at 5000 rpm for 30 minutes. Stability measurement can be seen from the absence of separation, sedimentation, creaming, and breaking experienced by the sample after centrifugation (Thermo Scientific MicroCL 21R, Germany) [23].

Freeze and Thaw

Test Observations were made by storing the preparation in 3 cycles, namely one cycle consisting of 24 hours at 4°C followed by 24 hours at 40°C [24].

Preparation of Test Animals

The Test animals were male mice of the DDY strain (weight 20-25 grams, age 2.5-3 months) obtained from Animal House, Padang, West Sumatra. Before being used for testing, the animals were acclimatized for 7 days in the animal maintenance room. Mice were fed Rat-Bio and drank tap water ad libitum. The mouse treatment procedure has received ethical approval from the Ethics Commission of the Faculty of Pharmacy, Andalas University with number 52/UN16.10.D.KEPK-FF/2024.

The treatment group of mice was divided into 4 groups consisting of 3 replications each group. The first group was the group that was not given the preparation, the second group was the group that was given Na diclofenac gel, the third group was the group that was given cayenne pepper extract gel preparation, and the fourth group was the group that was given cayenne pepper extract nanoemulsion gel preparation.

Inflammatory Conditioning in Mice

The dorsal hair (back) of the mice were shaved. Air sacs were made by placing the mice in a chamber and anesthetized. After being unconscious, the mice were removed from the chamber, the dorsal/back area was shaved, and the entire area was wiped with 70% ethanol. Room air was taken as much as 5-6 ml with a syringe and injected subcutaneously into the back of the mice, then 1.5% carrageenan was injected. After 24 hours, the mice were reanesthetized and 1.5% carrageenan was injected again as much as 1 ml [25].

Gel Preparation Administration in Treatment Groups

Diclofenac Na gel, cayenne pepper extract gel, and nanoemulsion gel were applied evenly to each group after inflammatory conditioning on the back once a day for 4 days. The air sacs were cut sagittally about 2 cm using scissors and the skin was held with tweezers. Exudate was taken using a syringe and the volume of exudate was recorded.

TNF-α ELISA

Test Mice blood samples were taken through the jugular vein which was then prepared as a serum for measuring TNF-α concentration using the Sandwich ELISA method with an ELISA reader (Bio-Rad, USA).

Data Analysis

Exudate volume and TNF-α concentration data were analyzed statistically by one-way analysis of variance (ANOVA) with a 95% confidence level. The results of the analysis that had significant differences were continued with Duncan's Multiple Range Test (DNMRT; $P < 0.05$).

Table 3. Standardization results of cayenne pepper extract.

Quality	Extract Obtained	Extract Quality Standards
Organoleptic	Thick extract, reddish brown color, distinctive odor, spicy taste	Thick extract, reddish brown color, distinctive odor, spicy taste [26]
TLC	Rf : 0.45	Rf : 0.51 [26]
Yield	11.058%	Not less than 10.8% [26]
Water Content	1.098% ± 0.016	No more than 12% [26]
Total Ash Content	5.384% ± 0. 007	No more than 6.6% [26]

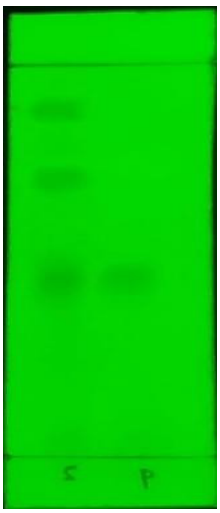

Figure 1. TLC plate under 254 nm UV light.

Result and Discussion

The identification results at the Andalas Herbarium (ANDA) of Andalas University stated that the sample was *Capsicum frutescens*. The extract standardization carried out included organoleptic, TLC test, yield, water content, and total ash content. The results of the extract standardization are listed in Table 3.

Organoleptically, a thick reddish-brown extract was obtained, with a distinctive odor, and a spicy taste. The thick extract was obtained as much as 71.227 grams from a total of 644.12 grams of cayenne pepper powder with a yield of 11.058%. Determination of the water content of the extract gravimetrically showed a result of 1.098% ± 0.016. The results of determining the total ash content were 5.384% ± 0.007. Thin layer chromatography results show spots that are parallel to the capsaicin reference standard (Figure 1). All standardization results have met the requirements Suplemen I Farmakope Herbal Indonesia [26].

Surfactants and cosurfactants were selected based on the active substance (cayenne pepper extract) miscibility test because surfactants and cosurfactants play an important role in reducing surface tension in the system. The surfactants and cosurfactants selected were tween 80 and PEG 400. Tween 80 was selected as a surfactant due to its nonionic nature, stability against pH and ionic strength variations, recognized safety and biocompatibility, and its ability to produce smaller emulsion droplets compared to other nonionic surfactants [27]. PEG 400 was selected because it is a stable polymer that is widely used in formulations and has non-toxic and non-irritating properties. In addition, PEG 400 has a fairly wide nanoemulsion formation area when combined with tween 80. The results of the miscibility test showed that all selected materials met the requirements as seen in Figure 2. The homogeneity of the mixture characterizes the mixing of surfactants and cosurfactants with the active substance. The selected oil, surfactant, and cosurfactant phases showed homogeneity when mixed so that they were able to form clear and stable nanoemulsions [28].

The test results of 3 surfactant and cosurfactant ratios

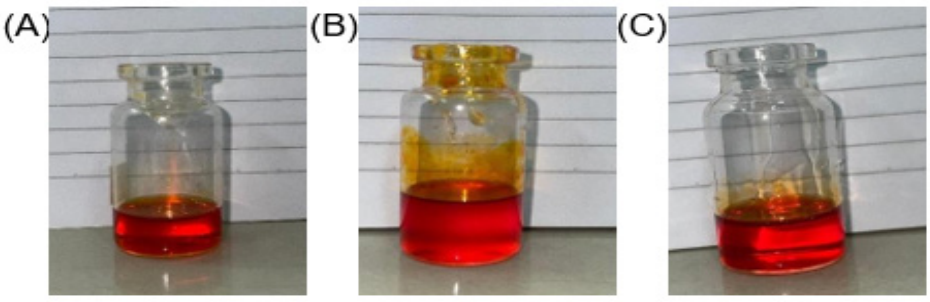

Figure 2. Mixing test of extract with VCO (A); Extract with PEG 400 (B); Extract with Tween 80 (C).

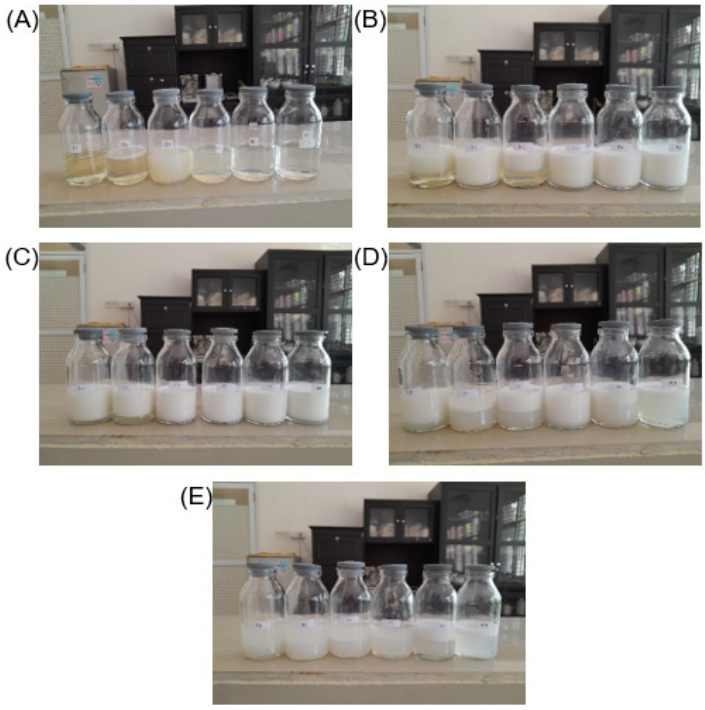

Figure 3. Optimization of nanoemulsion base formula oil:SMIX ratio 1:9 (A); oil:SMIX ratio 3:7 (B); oil:SMIX ratio 5:5 (C); oil:SMIX ratio 7:3 (D); oil:SMIX ratio 9:1 (E).

(1:1, 2:1, 3:1) showed that all surfactant and cosurfactant compositions could mix well to produce a clear and transparent base. However, the ratio that showed the best organoleptic form (visual and homogeneity) was the 1:1 ratio, so that ratio was chosen.

From a total of 30, the clearest and most homogeneous bases that met the organoleptic requirements were formulas A1, B1, C1, and F1 (Figure 3). To select the optimal formula, characterization was carried out. The results of determining the base were visualized in a pseudo-ternary phase diagram using Chemix School software. The area with a red dot on the pseudo-ternary phase diagram in Figure 4 indicates the area that forms a clear and transparent nanoemulsion base. The black dot area indicates the area that experiences phase separation, turbidity, and the formation of macroemulsions.

The stability of the physical form of each formula was observed during storage. From the bases A1, B1, C1,

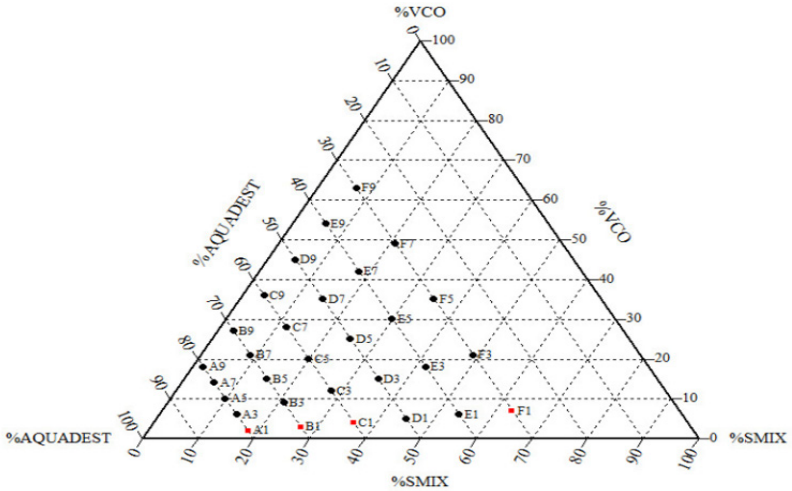

Figure 4. Pseudo-ternary phase diagram on nanoemulsion basis optimization with red dots representing clear areas and black dots representing turbidity or phase separation.

Table 4. Results of base measurement with *Particle Size Analyzer* (PSA).

Sample	Z-Average	Zeta Potential	Polydispersity Index
A1	29.3 nm	-12 mV	0.38
B1	70.7 nm	-6.1 mV	0.467
F1	354.1 nm	-0.1 mV	0.482

Table 5. Measurement of nanoemulsion extract with *Particle Size Analyzer* (PSA).

Z-Average	Zeta Potential	Polydispersity Index
54.4 nm	-23.6 mV	0.403

and F1, only three nanoemulsion bases were selected to be characterized by measuring globule size (Z-average), zeta potential, and polydispersity index. The measurement results can be seen in Table 4.

Formulas A1 and B1 meet the requirements for nanoemulsion particle sizes of 20–200 nm, which can quickly penetrate the skin and deliver active substances deeper and faster [23]. Formula F1 does not meet the particle size requirements.

The ideal nanoemulsion zeta potential value is greater than +25 mV or less than -25 mV. If the zeta potential value is low or close to its flocculation point, it can cause phase separation. The zeta potential of the three bases has not met the requirements for a stable nanoemulsion, but the closest to the ideal value is formula A1 [29]. The PDI of each formulation was less than 0.5, which is within the acceptable range, where values closer to 0 indicate a more uniform particle size distribution and monodisperse system [23]. Of the 3 parameters, formula A1 was chosen with a composition of 2% VCO, 18% SMIX (surfactant and cosurfactant), and 80% distilled water as the optimal formula..

Characterization of cayenne pepper extract nanoemulsion in A1 base was carried out using Particle Size Analyzer (PSA) (Table 5) and Transmission Electron Microscopy (TEM).

Measurement results showed that the nanoemulsion extract made had met the requirements for particle size and polydispersity index with values of 54.4 nm and 0.403. The drug will be absorbed more easily if the particle size is smaller because it has a larger surface area, thereby accelerating the onset of therapeutic action [30]. Globules formed with a size of less than 200 nm indicate that this formulation can increase the onset of therapeutic action of *C. frutescens* extract. The nanoemulsion of *C. frutescens* extract has a polydispersity index that is close to 0, which is the required value [23]. The low polydispersity index value indicates that the designed dispersion system is more stable over time [31]. The lower the polydispersity index, the more homogeneous the droplet size [32].

The zeta potential value of -23.6 mV was closer to the ideal value when compared to the nanoemulsion base. This is because sonication was not carried out in the manufacture of the nanoemulsion base, while sonication was carried out for 30 minutes in the extract nanoemulsion. The zeta potential value determines whether or not flocculation can occur. It predicts the preparation's storage stability. A high zeta potential promotes stability and

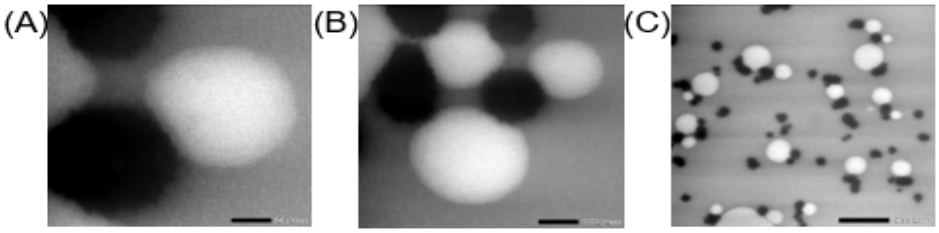

Figure 5. Results of morphological tests using transmission electron microscopy (TEM) of 50 nm (A); 100 nm (B); 500 nm (C).

Table 6. Evaluation of nanoemulsion gel.

Evaluation	Results	Condition
Organoleptic	Thick gel, orange, homogeneous, characteristic odor	-
pH	5.433 ± 0.030	(4.5-6.5) [21]
Viscosity	23,617 ± 0.160 cPs	3000-50.000 cps [35]
Centrifugation	No physical changes occur	No change occurred [19]
Freeze and Thaw	No physical changes occur	No change occurred [19]

prevents flocculation, while a low zeta potential can cause thickening and instability. Stable preparations have zeta potential values ranging from -25 mV to +25 mV. The zeta potential value is -23.6 mV, which is more than -30 mV, suggesting that the preparation may have a repulsive force insufficient to establish colloid physical stability, as stated. If the zeta potential value falls outside of the necessary range, the preparation is likely to produce large particle aggregation and flocculation due to van der Waals forces that cause physical instability [33].

The results of the TEM morphology test in Figure 5 shows that the globules formed are spherical. There are two types of globules, due to the different chemical compositions of each droplet. Dark globules are globules with higher electron density, while globules with lower electron density are lighter in color [34]. prove the formation of nano-sized globules consisting of two forms, namely black and white globules. The black globules are thought to be globules that trap the active ingredients of cayenne pepper extract, while the white globules are globules that do not trap the active ingredients.

Evaluation of the nanoemulsion gel was carried out after the nanoemulsion extract was mixed and homogenized with its gel base. The evaluation results are listed in Table 6. All evaluations have met the requirements according to the literature. Organoleptically, a thick, orange, homogeneous gel with a distinctive odor was obtained. The pH measurement result was 5.433 ± 0,030, which met the requirements for a topical preparation that is safe for the skin (4.5-6.5) [21]. The viscosity measurement result was 23.617 ± 0.160 cPs, which met the requirements for a gel preparation of 3,000-50,000 cPs [35]. The freeze and thaw stability test was carried out for 3 cycles, and the physical changes were observed. The absence of changes in the preparation indicated the stability of the preparation. The centrifugation test on the nanoemulsion gel also showed no physical changes in the nanoemulsion gel.

The parameters of the anti-inflammatory activity test results in this study were exudate volume (in vivo) and TNF-α levels (in vitro). The lower the exudate volume and TNF-α levels, the lower the inflammation produced. Conversely, the higher the volume of exudate and the level of TNF, the higher the resulting inflammation [22,36].

Based on the results of one-way ANOVA statistical analysis on exudate volume data, it is known that there is an effect of the dosage form on the exudate volume that is significantly different ($p<0.05$). Duncan's further test was carried out to see the differences between the four treatments as listed in Table 7. From the results of the Duncan test, the treatment of mice given cayenne

Table 7. Exudate volume measurement results.

Treatment	Exudate Volume (mL)			Average (mL)
	1	2	3	
Carrageenan Injection	1.635	1.710	2.000	1.782 ± 0.193[a]
Carrageenan Injection + Diclofenac Na Gel	1.195	1.138	1.109	1.147 ± 0.043[b]
Carrageenan Injection + Cayenne Pepper Extract Gel	1.265	1.330	1.540	1.378 ± 0.144[c]
Carrageenan Injection + Cayenne Pepper Extract Nanoemulsion Gel	0.910	0.910	0.926	0.915 ± 0.009[d]

Note : Different letters indicate significant subset differences ($p>0.05$) based on Duncan's test.

Table 8. TNF-α Measurement Results.

Treatment	Concentration TNF-α (ng/L)			Average (ng/L)
	1	2	3	
Carrageenan Injection	240.348	160.795	147.964	183.035 ± 50.047[a]
Carrageenan Injection + Diclofenac Na Gel	155.662	141.548	127.434	141.548 ± 14.114[ab]
Carrageenan Injection + Cayenne Pepper Extract Gel	171.060	163.361	145.397	159.939 ± 13.169[ab]
Carrageenan Injection + Cayenne Pepper Extract Nanoemulsion Gel	135.132	127.434	112.036	124.867 ± 11.760[c]

Note : Different letters indicate significant subset differences (p>0.05) based on Duncan's test.

pepper extract nanoemulsion gel showed the smallest average exudate volume and was significantly different from other treatments. The results of the exudate volume measurement showed the effect of cayenne pepper extract nanoemulsion gel in suppressing inflammation in gouty arthritis pain. Previous studies have shown that within 2 hours after administration, diclofenac emulgel exhibited a higher percentage of edema inhibition compared to capsaicin emulgel [37]. When compared to these findings, it was demonstrated that formulations with nanoemulsions can enhance anti-inflammatory effects.

Based on the results of one-way ANOVA statistical analysis on TNF-α levels, it is known that there is no effect of the dosage form on the TNF-α levels that is significantly different (p>0.05). However, Duncan's further test was carried out to see the differences between the four treatments as listed in Table 8. The TNF-α levels data in the cayenne pepper extract gel group and the Na diclofenac gel group were not significantly different. While this suggests a promising therapeutic potential, the lack of significant difference may be influenced by biological variability or the relatively short treatment duration. Future studies are recommended with longer treatment duration and larger sample sizes to confirm the therapeutic efficacy observed. Despite this, this nanoemulsion gel is still a viable choice for topical treatment of gouty arthritis pain in the future.

Conclusion

The optimal formula for making cayenne pepper extract nanoemulsion was obtained at a surfactant and cosurfactant composition ratio of 1:1, which produced a clear and transparent base. The optimal base chosen was formula A1 with 80% aquadest, 2% VCO, and 18% SMIX (surfactant and cosurfactant). From the A1 formula base, a nanoemulsion extract with a size of 54.4 nm, a zeta potential of -23.6 mV, and a polydispersity index of 0.403 was produced. The nanoemulsion gel demonstrated anti-inflammatory activity, as evidenced by a significant reduction in exudate volume in mice induced with 1.5% carrageenan (0.915 ± 0.009 mL, $p < 0.05$), as analyzed using ANOVA followed by Duncan's post hoc test. However, the reduction in TNF-α concentration (124.867 ± 11.760 ng/L) was not statistically significant ($p > 0.05$) when analyzed using the same statistical method.

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