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Formulation of Nanoemulsion Gel *Pogostemon cablin* Benth. and Anti-acne Activity

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Abstract

Patchouli oil (*Pogostemon cablin* Benth.) contains patchouli alcohol, a compound known for its antibacterial properties, including activity against *Propionibacterium acnes* (*Cutibacterium acnes*), a key bacterium implicated in the pathogenesis of acne. This study aims to evaluate the anti-acne potential of a nanoemulsion gel formulation of patchouli oil using three different gelling agents: HPMC (F1), Na CMC (F2), and Carbopol 940 (F3). Each formulation was assessed for its physicochemical characteristics, including color, odor, texture, pH, adhesion, spreadability, viscosity, particle size, polydispersity index (PDI), and zeta potential. All formulations appeared milky white, retained the characteristic aroma of patchouli oil, and exhibited a smooth gel texture. Among the three, F1 demonstrated the highest viscosity, while F3 exhibited superior adhesion. The average pH value (6.19 ± 0.23) was within the acceptable range for topical application. In vivo evaluations indicated that F3 exhibited the most significant reduction in erythema and edema, with minimal irritation potential. Additionally, F3 received the highest respondent satisfaction rating (77.6%), indicating better acceptance. Based on these findings, Carbopol 940 is identified as the most suitable gelling agent for the development of a patchouli oil nanoemulsion gel with promising anti-acne efficacy and favorable respondent acceptability.

Keywords: acne, essential oil, nanoemulsion, topical.

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Formulasi Gel Nanoemulsi *Pogostemon cablin* Benth. dan Aktivitas Anti Jerawat

Abstrak

Minyak nilam (*Pogostemon cablin* Benth.) mengandung senyawa aktif patchouli alkohol yang dikenal memiliki aktivitas antibakteri, termasuk terhadap *Propionibacterium acnes* atau *Cutibacterium acnes*, penyebab utama jerawat. Penelitian ini bertujuan untuk mengevaluasi efektivitas antijerawat dari sediaan nanoemulsi gel minyak nilam dengan tiga variasi basis gel: HPMC (F1), Na-CMC (F2), dan Carbopol 940 (F3). Ketiga formula diuji berdasarkan karakteristik fisik seperti warna, aroma, tekstur, pH, daya lekat, daya sebar, viskositas, ukuran partikel, indeks polidispersitas, dan zeta potensial. Semua formula berwarna putih susu, beraroma khas minyak nilam, dan memiliki tekstur lembut. F1 memiliki viskositas tertinggi, sedangkan F3 memiliki daya lekat paling besar. Nilai pH rata-rata sebesar 6,19 ± 0,23 yang sesuai dengan pH kulit. Uji aktivitas menunjukkan bahwa F3 paling efektif dalam menurunkan eritema dan edema, serta menunjukkan iritasi yang sangat ringan. Lebih lanjut, F3 mendapatkan tingkat kepuasan responden tertinggi (77,6%). Hasil ini menunjukkan bahwa basis gel Carbopol 940 merupakan pilihan terbaik untuk formulasi nanoemulsi gel minyak nilam sebagai sediaan antijerawat yang efektif dan diterima baik oleh responden.

Kata Kunci: jerawat, minyak esensial, nanoemulsi, topikal

1. Introduction

Adolescents are often impacted by acne vulgaris, a chronic inflammatory condition that affects the pilosebaceous follicles and can influence self-esteem. Acne results from obstructions or inflammation in the sebaceous glands.[1,2] *Propionibacterium acnes* is acknowledged as a key bacterial factor contributing to acne development and is often identified as a principal cause of acne. Topical antibiotics are frequently prescribed for this condition; Long-term use could increase the risk of bacterial resistance and skin barrier irritation. Careful consideration is required when selecting antibiotics for acne treatment. Antibacterial resistance affects the effectiveness of treatment and could be disseminated among bacteria through cross-contamination.[3]

Patchouli oil exhibits antibacterial properties due to its constituents, notably saponins, flavonoids, tannins, glycosides, terpenoids, and steroids.[4] Patchouli oil, comprising 50-60% patchouli alcohol, has demonstrated the potential in inhibiting acne-causing bacteria. Previous studies indicated that patchouli oil exhibits inhibitory activity or a minimum inhibitory concentration (MIC) against bacterial growth, affecting *P. acnes* at a concentration of 10% and S. aureus in gel formulations containing 5-35% patchouli oil, both correlating with its anti-acne properties.[5] Emulgel formulations with 0.2%, 0.3%, and 0.4% patchouli oil demonstrated activity against *P. acnes*, a Gram-positive bacterium.[6]

The administration of oil-based components in topical preparations may result in irritation.[7] The addition of surfactants, cosurfactants, and gelling agents is necessary to improve the acceptability of nanoemulsion administration.[8] Due to the high volatility of patchouli oil, appropriate delivery methods are required to prevent evaporation upon exposure to light and air. Consequently, stable microemulsions characterised by low viscosity that enhance skin contact represent an effective strategy for improving occlusion and extending contact duration with the skin. The improvement of occlusion and the duration of contact with the skin can be achieved. 6,8–10 Gelling agents, including HPMC, Na CMC, and Carbopol 940, are utilized primarily to reinforce the gel structure and improve viscosity. The disadvantages involve substantial quantities, resulting in challenges during the gel application. Additionally, gels containing microemulsions are irritating when applied continually, causing the skin to become red (erythema) or swollen (edema).[11] A previous study has been conducted on the formulation of spray gel using patchouli oil with a Carbopol 940 base. This study continues the previous research by modifying the base variation and evaluating the effectiveness of the formulation as an anti-acne agent.[12] Similarly, the use of patchouli oil as a wound healing agent for cuts in rabbits has been previously studied, utilizing the antibacterial properties of patchouli oil. [13] The current study was developed to formulate Patchouli oil in a microemulsion gel and to evaluate its anti-acne efficacy and irritation potential utilizing rabbit models. Research on patchouli oil continues to be developed, particularly through formulation strategies to improve penetration. The choice of nanoemulsion gel formulation is based on its role as an anti-acne treatment. Nanoemulsion gels are widely used in transdermal drug delivery systems to enhance drug absorption through the skin. The enhancement of bioavailability and drug absorption, as well as the rapid penetration and solubilization of hydrophobic active compounds, are advantages of nanoparticles. Based on previous research findings, this study aims to evaluate the anti-acne potential of a nanoemulsion gel formulation of patchouli oil using three different gelling agents: hydroxypropyl methylcellulose (HPMC, F1), sodium carboxymethyl cellulose (Na CMC, F2), and Carbopol 940 (F3).

2. Method

2.1. Research Tools

The equipment used in this study included a set of glassware (Iwaki Pyrex), a Brookfield RV viscometer (D220), a magnetic stirrer (Scilogex MS-H280 Pro), a particle size analyzer (SZ100), a centrifuge (Gemmy), an analytical balance (Ohaus), a pH meter (SI Analytics Handy Lab 100), a Malvern Zeta sizer Nano ZS, a set of spreadability testing equipment, a set of adhesion testing equipment, a set of homogeneity testing equipment, a laminar air flow (Suzhou Antai Air Tech Co., LTD, type HF 100), a 100–1000 µL micropipette, an incubator (Binder), Petri dishes, a Bunsen burner, a rounded loop, calipers, syringes (5 mL and 1 mL), alcohol swabs (One Med), scissors (Joyko), a ruler (Butterfly), razor blades (Gillette Goal), and markers (Snowman).

2.2. Materials

The materials used in this study included technical-grade patchouli oil (PT. Happy Green, Jakarta). The additives, all of technical grade, are HPMC, Na CMC, Carbopol 940, isopropyl myristate, PEG 400, Tween 80, Nipagin, Nipasol, Butylated Hydroxy Toluene (BHT), Triethanolamine (TEA), Aquadest (PT. Indrasari, Semarang), alcohol pad, *Propionibacterium acnes* bacterial suspension (Faculty of Medicine, Microbiology, Diponegoro University, Semarang), and Medi-Clin gel (PT. Surya Dermato Medica Laboratories).

2.3. Procedure

2.3.1. The formulation of nanoemulsion gel

The Nanoemulsion gel was prepared by adding the aqueous phase (PEG 400) to the oil phase (patchouli oil, isopropyl myristate, and Tween 80) and heating it to 70°C. The mixture was stirred magnetically at 550 rpm for 30 minutes or until a clear yellow nanoemulsion was formed. Patchouli oil nanoemulsion gel was prepared by integrating gelling agents, HPMC, Na CMC, and Carbopol at a concentration of 3%. The composition of Carbopol 940 was neutralized by adding TEA to achieve the desired pH, making it suitable for skin application. The nanoemulsion was manually incorporated.[14]

2.3.2. An anti-acne test

An Anti-acne test was performed on five rabbits that were intradermally induced with a 0.2 mL suspension of *P. acnes* at a concentration of $3x10^9$ CFU/mL. The testing procedure has obtained ethical clearance from the Faculty of Health, UDINUS, with approval number 634/EA/KEPK-Fkes-UDINUS/III/2024. Observation continued until acne occurred on the rabbit's skin, accompanied by redness and edema. The erythema diameter of the induced area was measured, and the animals wer patchouli oil nanoemulsion gel (NG), comprising: (a) NG with HPMC base; (b) NG with Na CMC base; (c) NG with Carbopol 940 base; (d) Medi-Klin gel (positive control-clindamycin gel); and nanoemulsion gel without active substances for 15 consecutive days, administered twice daily (the morning and evening).[15] The measurement of the reduction in erythema diameter on the back acne of the rabbit was conducted.[16]

2.3.3. Irritation test

The irritation tests were conducted on 3 New Zealand rabbits and were approved by the Health Research Ethics Committee of Dian Nuswantoro University, Semarang, with the reference number 637/EA/KEPK-Fkes UDINUS/VII/2024. This test was divided into three groups based on the formulations: 1 (HPMC), 2 (Na CMC), and 3 (Carbopol 940). The dorsal area of the rabbits was shaved and divided into five sections, with 0.5 g of the formulation applied to each section. The erythema and edema were evaluated at 1, 24, 48, and 72 hours. The selection of test animals conformed to defined inclusion criteria, encompassing a body weight range of 1.6–2 kg and the presence of healthy skin, marked by the lack of lumps or erythema. The exclusion criteria included rabbits in poor health, those with wounds on their dorsal region, or rabbits suffering from illnesses. The requirements comply with the Indonesian Food and Drug Authority (2014)[11] standards. The study commenced with an acclimatization phase for the rabbits, ensuring they remained devoid of stress. Observations were conducted over a two-day interval, following which the dorsal skin of the rabbits was shaved. After shaving treatment, the rabbits were permitted a one-day acclimatization period to ascertain that no irritation ensued from the shaving technique. An aqueous preparation was applied as the control without treatment in order to compare the skins of rabbits that received therapy and those that did not.

2.3.4. The hedonic test

The Hedonic test involved the observation of color, aroma, shape, texture, and perception, and was applied to the hands of 30 participants. The test was conducted with informed consent from the participants. The criteria for respondents included individuals aged 18 to 35 years, regardless of gender, who were in good physical and mental health, not experiencing skin allergies or sensitivities, and were willing to participate.[17] Respondents with sensitive skin were excluded from participation. The participants were instructed to complete a questionnaire developed by the researchers to collect their insights on the patchouli oil nanoemulsion gel formulation. The questionnaire assessed three key attributes: aroma, texture, and color, using a scoring system. Panelists provided feedback on their preference for the patchouli oil nanoemulgel formulations with different gel bases, considering factors such as packaging, odor, appearance, and texture. [18] The scoring system ranged from 1 to 6, with one representing "strongly dislike," 2 e," 3 as "dislike," 4 as "somewhat like," 5 as "like," and 6 as "strongly like." The final scores were calculated as an average percentage.[19]

3. Result

3.1. Formulation of nanoemulsion gel

Patchouli oil was incorporated into a nanoemulsion gel using three different bases: HPMC (Formula 1), Na CMC (Formula 2), and Carbopol 940 (Formula 3). Organoleptic observations were conducted to assess the physical characteristics of the formulation using sensory evaluation. This included examining color, shape, and odor to determine whether any changes occurred after the formulation process. Organoleptic and homogeneity assessments indicated that all three formulations, 1, 2, and 3, had the characteristic scent of patchouli oil, milky white hue, and a semisolid consistency, and all three developed formulations showed the absence of coarse particles and were verified to be homogenous. Viscosity with F1 demonstrating higher than F3 and F2. The pH test is

performed to assess whether the pH is suitable for skin application. The mean pH of the three formulations was 6.19 ± 0.23. Topical formulations should have a pH range of 4.5 to 7, similar to that of the skin. An excessively acidic or alkaline pH may disrupt the skin barrier, leading to irritation. The spreadability, adhesiveness and viscosity tests were related to absorption and contact time with the skin. The test outcomes were displayed in Table 1.

The spreadability test was conducted to evaluate the ability of the semisolid formulation to spread easily without significant pressure, ensuring smooth application without causing discomfort. An optimal spreadability range for gel formulations is between 5-7 cm. A higher spreadability value enhances the distribution of the active ingredient on the skin. All three formulations exhibit adhesion and spreadability within the specified range. Meanwhile, the adhesion test of the gel aims to evaluate the interaction between the gel and the skin. A longer adhesion time indicates a stronger bond between the gel and the skin, allowing for higher drug absorption. An optimal gel adhesion time is more than 4 seconds. The viscosity test is essential to determine the thickness of a gel formulation. The viscosity value reflects the resistance of a liquid to flow. The highest viscosity is observed in formulations with gel bases in the following order: HPMC, Carbopol 940, and Na CMC. Viscosity is related to the thickness of the nanoemulsion formulation. A thicker formulation remains on the skin for a longer duration. However, viscosity can also affect the comfort of application.

The next test performed was particle size analysis, which evaluates particle size, molecular mass distribution, and electrostatic stability of a particle dispersion in the sample using a Particle Size Analyzer (PSA), along with the polydispersity index and zeta potential (Table 2). Particle analysis aims to determine the particle size distribution in the sample. Measurements are performed using a particle size analyzer (PSA). An optimal nanoemulgel particle size is <200 nm. 20 Meanwhile, according to previous research, the ideal droplet size for patchouli oil nanoemulsion formulations ranges between 100-200 nm.[21] The results show that all three formulations fall within the specified range. Polydispersity Index (PDI) is used to assess the particle size distribution in a formulation. A lower PDI value (<0.7) indicates a more uniform particle size distribution, whereas a higher PDI value suggests a broader particle size distribution.[22] In this study, formulation F1 (HPMC) had the lowest PDI value (0.18 ± 0.02), indicating a more uniform particle size distribution than other formulations. Zeta potential is used to evaluate the electrostatic stability of the formulation. A zeta potential value greater than ±30 mV indicates good stability, as the repulsive forces between particles prevent aggregation. The results of this study showed that all three formulations had zeta potential values lower than -30 mV, indicating that the particles in the formulation exhibit good colloidal stability and are less likely to aggregate. Based on the obtained zeta potential values, the nanoemulgel formulation of patchouli oil in all formulas has the potential to prevent particle aggregation, thereby contributing to better formulation stability.

3.2. Anti-acne effectiveness test

An antibacterial activity test was performed by applying a *P. acnes* suspension to the dorsal area of rabbits to induce acne. Acclimatization was three days prior to allow for a reduction in stress and to facilitate the animals' adaptation to the new cage.[23] Initial administration occurred within 30 minutes, followed by applications at various time intervals for day 15, which were conducted on the same rabbits assessed on day 1. Diameter of erythema was subsequently measured at one, two, and six hours post-application. The rabbits' extensive back surface area and skin pigmentation were the primary factors for their selection as experimental subjects for

Table 1 Spreadability, Adhesiveness, and Viscosity of Patchouli Oil Nanoemulsion Gel

Replication	Spreadability (cm)			Adhesiveness (seconds)			Viscosity (dPas)		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
1	6.20	4.10	3.65	3.83	11.86	16.88	410	150	320
2	6.30	4.05	3.50	3.92	11.55	14.26	400	140	310
3	6.30	4.05	3.50	3,9	11.03	15.19	390	130	300
Average ± SD	6.2 ± 0.06	4.1 ± 0.02	3.5 ± 0.09	3.9 ± 0.04	11.5 ± 0.40	15.4 ± 1.30	400 ± 0.10	140 ± 0.10	310 ± 0.10

Description:
F1: Patchouli oil nanoemulsion gel formulation with HPMC base
F2: Patchouli oil nanoemulsion gel formulation with Na CMC base
F3: Patchouli oil nanoemulsion gel formulation with Carbopol 940 base

Table 2 Particle Size Analyzer, Polydispersity Index, and Zeta Potential Results

Description	Average ± SD		
	F1	F2	F3
Particle Size Analyzer	101.90 ± 0.49	129.90 ± 0.80	151.00 ± 0.90
Polydispersity Index	0.18± 0.02	0.39 ±0.07	0.32±0.03
Zeta Potential	-35.35	-42.14	-44.85

Description:
F1: Patchouli oil nanoemulsion gel formulation with HPMC base
F2: Patchouli oil nanoemulsion gel formulation with Na CMC base
F3: Patchouli oil nanoemulsion gel formulation with Carbopol 940 base

observing acne. The rabbits' dorsal area was shaved and subsequently disinfected in seven designated areas prior to receiving intradermal injections of 0.2 mL at each site, utilizing a suspension of P. A fresh standard bacterial suspension for induction was prepared.[15,24] The effectiveness of the antibacterial agent was evaluated by administering a *P. acnes* suspension to the dorsal region of rabbits to induce acne. Nanoemulsion gel formulation of patchouli oil using HPMC, Na CMC, and Carbopol 940 as bases is considered to have anti-acne activity if it reduces acne, as indicated by a decrease in erythema/redness on the rabbit's back. The five treatments were compared to evaluate differences in erythema reduction and analyzed using a one-way ANOVA test, followed by the Post Hoc Tukey test (Figure 1).

The study used a positive control (Medi-Klin gel), a nanoemulgel without an active ingredient, and nanoemulgel formulations with different gel bases, including HPMC, Na CMC, and Carbopol 940. The positive control, Medi-Klin gel, is a topical formulation containing 1% clindamycin, an antibiotic with both bacteriostatic and bactericidal properties that targets acne-causing bacteria by inhibiting protein synthesis.[25] The positive control was used to evaluate whether the patchouli oil nanoemulgel formulations with different gel bases demonstrated comparable or superior efficacy in reducing erythema and edema, which are clinical acne indicators. The findings indicated a significant difference between the patchouli oil nanoemulgel and the significantly negative control group (nanoemulgel without an active ingredient), as evidenced by a p-value <0.05. This result suggests that the patchouli oil component in the nanoemulgel exhibits anti-acne activity. Statistical analysis further revealed that the nanoemulgel formulation with Carbopol 940 as a gel base demonstrated the highest efficacy in reducing erythema, as indicated by a significant p-value ($p<0.05$), followed by formulations with HPMC and Na CMC as bases. Overall, the study compared nanoemulsion formulations containing patchouli oil with those without active ingredients to determine whether the nanoemulsion contributed to erythema reduction. The results demonstrated that the nanoemulgel formulation without an active ingredient did not significantly affect acne treatment. Thus, it can be concluded that patchouli oil possesses antibacterial activity, which is further enhanced when formulated as a nanoemulsion gel.

3.3. Irritation Test

An irritation assessment was conducted on the dorsal skin using the patch test methodology and indicated by erythema and edema. Erythema (redness) occurs due to blood vessel swelling in the irritated area, while edema (swelling) results from the expansion

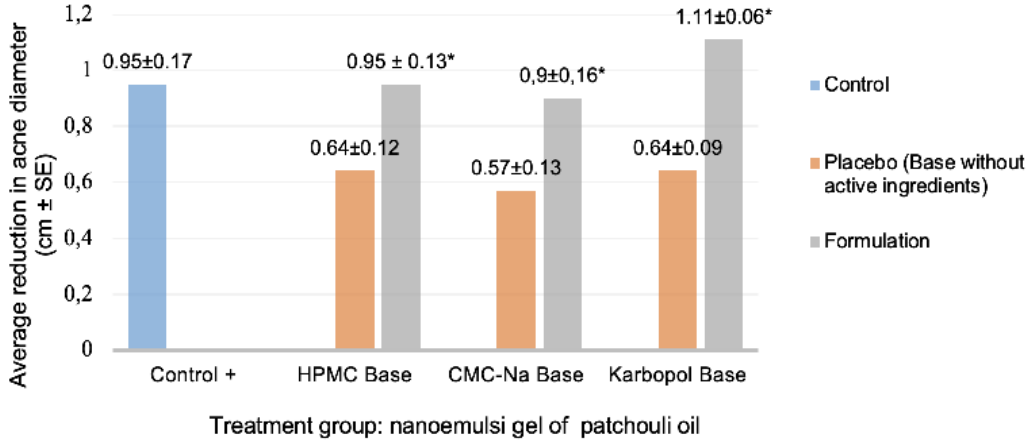

Figure 1. Acne diameter mean reduction (cm ±SE)

of coagulated plasma in the injured area. This test assessed irritation on the dorsal skin of rabbits. The process entailed dividing the dorsal portion of each rabbit into five segments, each measuring 2x3 cm, with a 1 cm gap between the treatment zones, as illustrated in Figure 2.

The results of the irritation test for patchouli oil gel nanoemulsion preparations indicated no signs of irritation, such as erythema and edema, at the first hour. Therefore, observation was extended to the 24, 48, and 72 hours. The first hour was calculated four hours after the preparation was applied. Additionally, the calculation is performed utilizing the primary irritation index formula. The primary irritation index scores results are presented in Table 3.

3.4. Hedonic Test

The hedonic test was conducted by involving panelists to evaluate the patchouli oil nanoemulsion based on fragrance, texture, color, stickiness, and oily feel of each composition when applied to the panelists' palms. The study solicited the panellists to provide their evaluations of their preference levels for the patchouli oil nanoemulgel formulations with varying bases. The results of the hedonic test are presented as percentages in Table 4. Subsequently, the percentage was calculated and statistically analyzed using the general linear model univariate. The patchouli oil microemulgel formulation was found to be the most preferred by panelists based on statistical analysis, with the highest percentage of preference. The panelists' assessment of stickiness and oily sensation was analyzed descriptively. The hedonic test was conducted with 30 panelists who completed a questionnaire evaluating the formulations. The formulation color received the highest preference score across all samples (>80%), indicating that the product's appearance was well accepted by the panelists. There was no significant difference ($p>0.05$) among the three formulations; however, F3 remained the most preferred overall. The results indicated that the nanoemulsion gel formulation with a Carbopol 940 base received the highest preference, with a favorability score of 77.6%, outperforming the other two formulations.

4. Discussion

Patchouli oil was formulated into a nanoemulsion gel using three distinct bases: HPMC (Formula 1), Na CMC (Formula 2), and Carbopol 940 (Formula 3). HPMC was selected as a base due to its characteristics, including its function as a viscosity-enhancing agent, stability within a skin pH, high spreadability, and ability to form a turbid gel while being nonirritant. Additionally, Na CMC was chosen as a gelling agent due to its low spreadability, moderate viscosity, and ability to form a clear gel.26,27 Another research demonstrated that topical preparations, specifically spray gels using HPMC and Carbopol 940 as bases, exhibited favorable physical characteristics.28 Carbopol 940, as a gel base, enhances the viscosity and stability of the formulation.9 The gel base functions as an agent that improves the structural network of the gel by elevating viscosity. This improvement allows for the effective absorption of active ingredients into the skin while maintaining both thermodynamic and kinetic stability. The three bases were evaluated to determine the optimal base selection and their effectiveness as anti-acne agents in nanoemulsion gel formulations containing patchouli oil.

The organoleptic and homogeneity tests indicated that all three formulations exhibited the distinctive

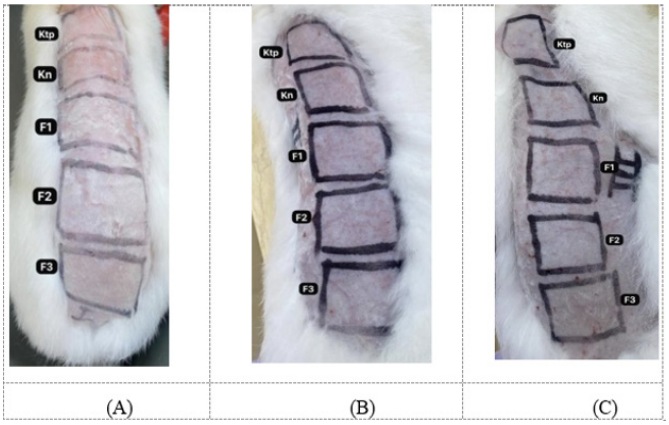

Figure 2. Dorsal area of the rabbit prior to treatment

Description:
Ktp: Control without treatment
Kn: Negative control (patchouli oil nanoemulsion)
F1: Patchouli oil nanoemulsion gel with HPMC base
F2: Patchouli oil nanoemulsion gel with Na CMC base

Table 3. Primary irritation index score of patchouli oil nanoemulsion gel

Formulation	Index Irritation	Irritation Response Category
Negative control	0.1	slight
Formulation 1(HPMC base)	0.3	negligible
Formulation 2 (Na CMC base)	0.1	negligible
Formulation 3 (Carbopol base)	0.7	negligible

aroma of patchouli oil. All three exhibited a milky white hue and possessed a semisolid texture, with Formula 3 demonstrating a lower viscosity in comparison to formulas 2 and 1. The three formulations exhibited no coarse particles and demonstrated homogeneity. These results are consistent with previous research, which indicates that, organoleptically, patchouli oil has a characteristic odor, a dark color, and becomes turbid when combined with other ingredients. [29]

The pH measurements were conducted to guarantee user comfort while preventing any irritation. The mean pH of the three formulations was 6.19 ± 0.23. The pH range indicated 4.5-7.0 is well-tolerated by the skin. These results indicate that the pH of the patchouli oil nanoemulsion gel with the varied bases falls within the acceptable range. The pH of the skin must be adjusted to avoid irritation caused by an extremely acidic pH, while an excessively alkaline pH for topical application might occur in dry skin. If the preparation has an excessively low pH, it may lead to skin irritation, whereas a high pH can result in dry and scaly skin.[17]

The spreadability and adhesiveness tests were related to absorption time and contact time with the skin. The test results were presented in Table 1. Based on the observation, the formulation with an HPMC base demonstrated a wider spreading area than those with a Na CMC and Carbopol 940 base. This is attributed to the thinner texture of HPMC, which is more fluid, whereas Na CMC and Carbopol 940 are thicker. Formulations with a thin texture exhibit reduced adhesion, shortening the contact time between the active substance and the application site.[30] Spreadability refers to a product's capacity to be applied smoothly and evenly on the intended surface. Difficulties in their formulation may impact impacted efficthe high fluid formulations. Furthermore, a fluid formulation might use an adhesive at the application site, which could shorten the time that the active ingredient and the site are in touch.[31] Similarly, regarding adhesiveness, the HPMC-based gel showed lower values than the other two gel bases. The subsequent factors will impact the absorption of the active ingredient. Optimal gel adhesiveness should exhibit elevated values, as effective adhesiveness on the skin signifies a strong bond between the skin and the gel, thereby enhancing drug absorption.[32] In summary, the absorption of the active ingredient is most effectively achieved in formulations utilizing Na CMC and Carbopol 940 as their bases.

The viscosity test measures the degree of consistency or thickness of a formulation carried out with a rheometer and presented in Table 1. Skin moisturizer viscosity values range from 200 to 500 dPas, in accordance with the Indonesian National Standard standards. A high viscosity value enhances stability by preventing aggregation and coalescence.33 Higher viscosity value means a thicker formulation. The use of high-viscosity formulations affects the application on the skin. The viscosity test results of the three formulations using HPMC, Carbopol 940, and Na CMC bases showed varying viscosities, with HPMC having the highest viscosity, followed by Carbopol 940 and Na CMC. Optimum viscosity refers to a formulation condition that is neither too thick nor too fluid. According to the research results, Carbopol 940 offers an optimal viscosity for the patchouli oil nanoemulgel formulation, maintaining a balanced consistency that is neither excessively thick nor too fluid. Viscosity plays a crucial role in determining spreadability and enhancing application properties. This study is consistent with previous research, indicating that Carbopol 940 is an effective gelling agent for nanoemulgel formulations. It produces gels with an appropriate viscosity for transdermal delivery, further confirming its effectiveness as a gelling agent.[34]

Table 4. Hedonic Test

Parameters (n=30)	Percentage of panelists' preference based on the gel base		
	F1 (HPMC base)	F2 (Na CMC base)	F3 (Carbopol 940 base)
Aroma	68.3	70.0	69.4
Textur	74.4	76.1	78.3
Color	83.3	85.6	85.0
Average Percentage (%)	75.5	77.2	77.6

The selection of gelling agents in topical nanoemulsion formulations must consider critical factors such as viscosity, spreadability, adhesion, homogeneity, and the release profile of the active substance. Adjustments to the gelling agent composition are essential to optimize the formulation, ensuring it meets therapeutic quality requirements while maintaining user convenience.[35] There are no significant differences; however, their selection should be based on the formulation's characteristics and intended function. [26,36] HPMC exhibited superior spreadability and viscosity among the three bases evaluated, followed by Carbopol 940 and Na CMC. However, HPMC demonstrated the lowest adhesion. Meanwhile, Na CMC showed the lowest viscosity, which may influence the formulation's application duration and result in a thinner consistency. Carbopol 940 exhibits a viscosity level between that of HPMC and Na CMC, with characteristics indicating high adhesion, which influences its application on the skin. Given that the nanoemulsion gel formulation is intended for anti-acne efficacy testing, the selection criteria prioritize a formulation with moderate viscosity, controlled spreadability, and high adhesion. Excessively low viscosity may compromise the formulation's effectiveness, while overly wide spreadability could lead to uneven application, particularly on localized acne lesions, potentially reducing the absorption efficiency of the active substance. An optimal formulation should facilitate smooth application on the skin, ensuring ease of use and enhanced user comfort.[37] However, effectiveness testing must be conducted on all three formulations to ensure anti-acne efficacy.

The next test performed was particle size analysis, which examines particle size, molecular mass distribution, and electrostatic stability in a dispersed sample using the Particle Size Analyzer (PSA) method. This analysis also includes the pH, which is the pH, the pI, and the zeta potential. The results obtained from this method confirm that the sample is well-dispersed in the solvent, causing the particles to move randomly along with the solvent molecules, in accordance with Brownian motion. The average particle size of the patchouli oil nanoemulsion gel nanoparticles is shown in Table 2. The particle size analysis confirmed that all three formulations fell within the specified range. The dimensions of the globule are a crucial determinant of the formulation's effectiveness, since smaller dispersed phases facilitate enhanced contact with the skin, promoting deeper absorption of active substances.[38] This provides insight into the level of uniformity in particle sizes within the nanoemulsion system. The conclusions of all the nanoemulsion gel formulations of patchouli oil exhibited a polydispersity index value of <0.7, signifying that the preparation demonstrates homogeneity in small globule size and homogenous dispersion results. This allows for a limited range of particle sizes, crucial in preventing Ostwald ripening, a process where smaller particles diffuse and merge into larger ones, ultimately leading to coalescence.[23] The stability of nanoemulsion is significantly affected by zeta potential. Particle aggregation was not permitted for zeta potential values exceeding +30 mV or falling below -30 mV. The low values obtained for the zeta potential resulted in aggregates with diminished physical stability.[26] A high zeta potential indicates that repulsive forces prevail among the particles, whereas a low zeta potential value suggests that attractive troops take over, leading to the merging of globules and instability.[39] The findings from the zeta potential measurements indicate that all formulations of patchouli oil nanoemulsion gels exhibit a low tendency for particle aggregation, thereby enhancing stability.

Anti-acne testing using *Propionibacterium acnes* (P. acnes), classified as a natural flora organism present in sebaceous follicles, plays a role in the etiology of acne through its inflammatory activities. Bacterial hydrolysis occurs via the actions of lipase, protease, neuraminidase, and hyaluronidase, all contributing to inflammation.[40,41] *P. acnes* hydrolyzes triglycerides to release free fatty acids that can induce acne through the induction of bacterial suspension. Free fatty acids serve as a nutritional source for P. acnes. The greater the available amounts, the more they enhance the multiplication process, leading to elevated levels of reactive oxygen species.[15] The following illustrates the cross-section of the rabbit's dorsal skin prior to and following induction. The observed redness and swelling in the cross-section below suggest the presence of inflammation. The positive control utilized in this study was Medi-Klin gel, which includes 1% clindamycin. Clindamycin demonstrates both bacteriostatic and bactericidal effects on acne-causing organisms by inhibiting protein synthesis.[25] This suggests that if the test group fails to exhibit antibacterial activity against P. acnes, it will not enhance any antibacterial response, consequently impeding acne clearance. The negative control refers to a formulation of emulsion microgel that lacks active principles. The patchouli oil nanoemulsion gel was administered topically to the test animals twice daily over a period of 15 consecutive days. The observations on days 1 and 15 were documented in accordance with the evaluation of the decrease in erythema diameter. Based on anatomical and physiological factors, normal skin undergoes renewal every 14–21 days; consequently, ex vivo anti-acne testing was typically conducted over a period of 15 days to achieve optimal results.16 The healing process of skin affected by acne was demonstrated through specific acne lesions on rabbits' skin that exhibited no signs of redness, swelling, or pus discharge.[16,42] The study results indicated that

the nanoemulsion gel of patchouli oil, formulated with a Carbopol 940 base, exhibited the lowest mean percentage of erythema diameter inhibition. The reduction in erythema diameter observed in the patchouli oil gel nanoemulsion demonstrated a statistically significant difference compared to the negative control group (nanoemulsion gel without active substance), with a p-value of less than 0.05. This indicates that patchouli oil gel nanoemulsion (*Pogostemon cablin* Benth.) exhibits anti-acne activity. The positive control medi-klin gel demonstrated a statistically significant difference when compared to the negative group (nanoemulsion gel without active substance), with a value of ($p < 0.05$). Medi-klin serves as a positive control, demonstrating anti-acne activity. The outcomes of the statistical test analysis are detailed with data regarding the percentage of acne diameter reduction (cm ± SE) illustrated in Figure 1. This is consistent with previous studies, which have shown that the components of patchouli oil can be used as an antibacterial agent, including against acne-causing bacteria. [43]

The subsequent irritation test aimed to evaluate the potential occurrence of irritation on the rabbit's skin in comparison to the treatment group. Irritation is an inflammatory phenomenon that occurs on the skin due to exposure to foreign compounds. Symptoms may include heat and edema caused by the dilation of blood vessels in the affected area. Irritation during the application of anti-acne formulations on facial skin is characterized by symptoms such as dryness, pain, cracking, and bleeding. In this study, the negative control used was a patchouli oil nanoemulsion without the addition of a gelling agent. This negative control was intended to determine whether nanoemulsion without gelling agents could induce irritation. During the test, the formulation was applied to the dorsal skin of the rabbits. Each formulation was weighed at 0.5 grams before application, and the application site was covered with sterile gauze and secured with Ultrahold plaster. According to the Indonesian Food and Drug Authority (2014), the use of non-irritant plasters serves to prevent the evaporation of volatile substances. The stability of volatile essential oil components in the patchouli oil nanoemulsion gel is a crucial factor in this formulation. Throughout the test, irritation signs were observed and recorded as a primary irritation index score, which was calculated based on the primary irritation index formula. The test results indicated that no signs of irritation, such as erythema and edema, were observed in the first hour after application. Consequently, observations were extended to the 24th, 48th, and 72nd hours. The first-hour calculation was performed four hours after application, with irritation scores determined using the primary irritation index method (Table 3). During the first-hour observation, no erythema or edema was detected on the rabbits' skin across all replications. However, at the 24-hour observation, mild irritation was observed in the first replication of the negative control group. This was suspected to be due to the dryness of the rabbit's skin during the observation period. Meanwhile, the second and third replications showed no visible signs of erythema or edema. During the 48-hour observation, erythema was distinctly noted in replication 1 within the negative control section. Discussions with the veterinarian suggest that the irritation may have resulted from the rabbit's skin being less healthy prior to treatment, as evidenced by signs of sensitivity and dryness. In replications 2 and 3, the erythema observed was nearly imperceptible. During the 72-hour observation period, slight oedema was noted in replication 3 on section F1. Three patchouli oil nanoemulsion compositions were subjected to a hedonic test, which involved evaluating each one's scent, texture, color, stickiness, and oily feel when applied to the panellists' palms. The hedonic test results regarding the aroma parameter of the patchouli oil nanoemusions formulations indicated that F3, utilising a Carbopol 940 base, was the formulation most favored by participants. The evaluations conducted by 30 panellists yielded average percentage scores of F1 (HPMC) score of 75.5%, F2 (Na-CMC) achieved scores of 77.2% and F3 (Carbopol 940) 77.6%. The univariate test results indicated a significance value ($P>0.05$), suggesting no significant differences among the formulations, with F3 exhibiting the highest preference percentage.

5. Conclusion

The research results indicated a significant difference among three gel base types—HPMC (F1), Na-CMC (F2), and Carbopol 940 (F3)— compared to the positive control. Among these formulations, the most optimal results were obtained from the third formulation, which was a nanoemulsion with a Carbopol 940 gel base. This formulation was characterized by a milky white color, the distinctive aroma of patchouli oil, good homogeneity, a soft texture, and thick consistency. Carbopol 940 demonstrated a viscosity between that of HPMC and Na-CMC, but with higher adhesiveness, offering an advantage in terms of formulation retention on the skin surface. Based on the polydispersity index and zeta potential values, the nanoemulsion with a Carbopol 940 base exhibited uniform particle size distribution and excellent colloidal stability. Regarding biological activity, the patchouli oil nanoemulsion with a Carbopol 940 base showed the lowest percentage of erythema diameter reduction, indicating better anti-inflammatory activity. Irritation testing showed that all formulations fell under the category of very mild irritation, with the Carbopol 940-based formulation

having a primary irritation index value of 0.1 ± 0.5. In preference testing, this third formulation also received the highest acceptance rate, supporting its potential use as a topical preparation for acne treatment.

Conflict of Interest

The authors declare no conflicts of interest.

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