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Nanoemulgel Formulation of Marjoram (*Origanum majorana L.*) Essential Oil with Potential as Anti-Inflammatory and Anti-Hyperuricemia

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Abstract
The prevalence of hyperuricemia has increased in several countries, including Indonesia. Uric acid will continuously be deposited in the form of crystals in the joint cavity, which can cause inflammation. One natural ingredient potentially treating this problem is marjoram essential oil (*Origanum majorana* L.). Marjoram essential oil (MEO) contains various terpene compounds that have the potential to reduce pro-inflammatory mediators and uric acid levels. Terpene compounds are known to be able to be delivered through the skin, but their penetration levels are still very limited. The development of pharmaceutical preparations such as nanoemulgel is needed to enhance the penetration of the active components in marjoram essential oil. This research aimed to optimize the components that make up nanoemulgel, namely polysorbate 80 surfactants and PEG 400 co-surfactant, to produce a nanoemulgel preparation of marjoram essential oil with optimum physical characteristics and potential as an anti-inflammatory and anti-hyperuricemia in the rat. The optimum composition of Polysorbate 80 surfactant and PEG 400 co-surfactant is 9.958: 6.042. It resulted in the optimal formula for MEO 1% nanoemulsion, which had an average globule size of 22.94 nm with a polydispersity index of 0.65 and MEO 2% nanoemulsion: 142.4 nm with a polydispersity index of 0.91. MEO nanoemulsion could be dispersed into a gel base and become an MEO nanoemulgel, which has inhibitory power against xanthine oxidase, whereas 2% MEO nanoemulgel has an IC 50 of 25.30±2.57 ppm and has greater anti-inflammatory power than 1% MEO nanoemulgel.

Keywords: Hyperuricemia; Inflammation; Marjoram; Nanoemulgel

1. INTRODUCTION

Hyperuricemia is a condition that increases uric acid levels in the blood. Deposits of monosodium urate crystals in the joint cavity can cause inflammation, known as gout (Manampiring, 2011). The prevalence of hyperuricemia in several countries tends to increase (Kumar et al., 2018; Liu et al., 2015; Roman, 2019). The prevalence of hyperuricemia has shown a notable increase in several countries over recent years. According to Kumar et al. (2018), there is a growing trend in hyperuricemia rates, with an observed increase of approximately 2% per year in specific populations. Liu et al. (2015) report that hyperuricemia prevalence has risen significantly, particularly in Asian countries, where rates have escalated from around 8% to over 20% in specific demographics within a decade.

Additionally, Roman (2019) highlights that some regions, particularly in North America and Europe, have experienced increases in hyperuricemia rates, indicating a worrying trend that correlates with rising obesity rates and changes in dietary habits. Based on the results of a 2015 study also show that Indonesia has the second-highest prevalence of hyperuricemia at 18% in the Southeast Asia Region (Smith, 2015). According to Indonesian basic health research (Riskesdas), in 2018, the prevalence of joint disease due to acute and chronic hyperuricemia reached 19.77% for the age group over 65 years (Balitbangkes, 2018).

Colchicine and other nonsteroidal anti-inflammatory drugs are clinical treatments used for inflammation in gout, while allopurinol and febuxostat are used to treat hyperuricemia. On the other hand, long-term use can cause adverse side effects, such as gastrointestinal disorders, a high risk of liver and kidney damage, and the risk of cardiovascular disease (Ghang et al., 2022; Yokose et al., 2019). Thus, finding new, natural, and safe drugs for anti-hyperuricemia and anti-inflammatory therapy in gout is very important. One example of a natural product that has the potential to overcome this problem is marjoram (*Origanum majorana* L.) essential oil.

While the term "natural" does not inherently guarantee safety, marjoram (*Origanum majorana* L.) essential oil may offer a more favorable safety profile than conventional anti-hyperuricemic and anti-inflammatory medications. Historically, marjoram has been used for centuries in traditional medicine, indicating its relative safety when used appropriately (Bouyahya et al., 2021). Unlike many conventional gout medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, which can cause gastrointestinal issues, kidney damage, or elevated blood pressure, marjoram essential oil is associated with fewer adverse effects. The phytochemicals found in marjoram, particularly carvacrol and thymol, possess anti-inflammatory and antioxidant properties without the harmful side effects common to synthetic drugs (Gheitasi et al., 2021).

Marjoram essential oil (MEO) contains terpene compounds, such as sabinene, p-cymene, thymol, and components with a high percentage are carvacrol and terpinene-4-ol (AytaÇ, 2020; Busatta et al., 2017; Raina & Negi, 2012). Research shows that MEO can act as an anti-inflammatory agent by reducing pro-inflammatory agents such as TNF-α, IL-1β, IL-6, and IL-10, which are high in hyperuricemia sufferers (Arranz et al., 2015; Sah et al., 2016; Zha et al., 2022). Another study showed that the administration of carvacrol, one of the largest components of MEO, showed results similar to allopurinol treatment. The study showed that treatment with carvacrol (at doses of 20 and 50 mg/kg) resulted in a significant decrease in serum uric acid and C-reactive protein (CRP) levels, similar to the effects of allopurinol treatment. While both treatments effectively lowered these markers, the higher dose of carvacrol (50 mg/kg) had a slightly more pronounced effect on serum uric acid levels. Additionally, no signs of kidney damage were observed in the carvacrol-treated groups (Riaz et al., 2022).

Some of these studies indicate that MEO has the potential to be developed into pharmaceutical preparations. One of the preparations developed for MEO is nanoemulgel with the consideration that the target delivery of the active substance is to reduce inflammation and

uric acid, which accumulates in joint fluid and bones. One of the formulations developed for MEO is nanoemulgel, which was chosen for its ability to improve the targeted delivery of active hydrophobic compounds. Nanoemulgel combines the benefits of a nanoemulsion, which enhances the solubility and bioavailability of hydrophobic substances, with a gel base that provides a cooling effect to soothe pain. The essential oil in the nanoemulsion also acts as a permeation enhancer, facilitating deeper penetration of the active compounds into the skin, allowing them to reach the joint fluid and bones where uric acid accumulates and inflammation occurs (Bilia et al., 2014).

Terpinene-4-ol and carvacrol have a molecular weight of less than 400 Daltons and a Log P of less than 1-4 (Dionisio et al., 2018). Therefore, they have great potential for drug delivery through the skin. Apart from that, developing nanoemulgel preparations has several advantages, such as reducing the risk of drug interactions, reducing the risk of first-pass metabolism, and applying the preparation directly to the inflammatory area (Priani, 2022).

Surfactants and co-surfactants are two essential components in nanoemulsion preparations. Polysorbate 80 and PEG 400 are known to have been successful as surfactants and co-surfactants in producing nanoemulsion preparations with active ingredients of essential oil. Polysorbate 80 and PEG 400 were chosen as surfactants and co-surfactants based on numerous studies demonstrating their effectiveness in producing stable nanoemulsions with active essential oil ingredients. Polysorbate 80 is crucial for reducing the interfacial tension between oil and water phases, thereby enhancing the stability and solubility of hydrophobic compounds. PEG 400 complements this by acting as a co-surfactant, improving the emulsification process and facilitating better dispersion of the active ingredients. This combination has produced consistent and effective nanoemulsion formulations in various studies, making them essential components for the current nanoemulsion formulation with essential oil as an active pharmaceutical ingredient (Dasawanti et al., 2022; Purwanto, 2021). Solubility tests were conducted in various solvents that can act as surfactants and co-surfactants further to support the selection of these surfactants and co-surfactants. These included Tween 20 as a surfactant and propylene glycol, glycerol, and ethanol as co-surfactants. The results of these solubility tests helped confirm the superior performance of Polysorbate 80 and PEG 400 in enhancing the solubility and stability of MEO.

In this research, optimizing surfactant and co-surfactant concentrations was important to obtain a stable nanoemulsion system. Dispersing the nanoemulsion system into the gelling agent is then carried out to obtain a higher viscosity so that the preparation can be in contact with the skin for longer.

This study aims to evaluate the anti-hyperuricemia and anti-inflammatory efficacy of the optimum nanoemulgel formulation containing marjoram essential oil (MEO) in reducing inflammation. The effectiveness of MEO nanoemulgel preparations as anti-hyperuricemia can then be seen from the concentration of 50% inhibition of xanthine oxidase (IC50) enzyme activity by MEO nanoemulgel preparations in vitro. Furthermore, this will be assessed by

measuring the decrease in inflammation volume in carrageenan-induced rats and comparing it to standard treatments.

2. MATERIAL AND METHODS

2.1. Materials

Materials used in this study were marjoram essential oil (Darjeeling, Marjoram Oil Egypt® (PSD02485), polysorbate (polysorbate) 80 (Petronas), polyethylene-glycol (Mandakhalikar et al.) 400 (Petronas), aquadest (Multi Kimia Raya Nusantara), DMDM-Hidantoin (Clariant), Triethanolamine (Multi Kimia Raya Nusantara), Carbophol 940 (Dunia Kimia Jaya), Xanthine (Merck), Karagenan (Sigma), Voltaren® emulgel, Pirofel® gel, Sumifun® Arthritis Herbal Gel.

Tools used in this study were gas chromatography-mass spectrometry (GC-MS) Shimadzu GCMS-2010 Plus, spectrophotometer Uv-vis Shimadzu 1280, particle size analyzer (Malvern MAL1275495), viscosimeter Brookfield, pH meter Trans instrument HP 9000, plethysmometer.

2.2. Methods

2.2.1 Solubility test of MEO in pre-emulsion system

The solubility examination commenced by blending 1 mL of MEO with 1 mL of the carrier substance using a vortex mixer. The carriers under consideration include constituent surfactants and cosurfactants, which exhibit potential for use in nanoemulsions. These include polysorbate 80, polysorbate 20, polyethylene-glycol 400, ethanol, propylene-glycol, and glycerin.

In this solubility (or miscibility) test, the ability of marjoram essential oil (MEO) to mix with other components in the pre-emulsion system was assessed visually. After mixing, the absence of phase separation indicates that the MEO is miscible with the other components. While a simple visual observation was used in this case, future studies could incorporate more quantitative methods, such as turbidity measurements or centrifugation, to provide more detailed data on the miscibility of the oil within the system.

2.2.2 Preparation of MOE nanoemulsion

Three primary components are needed to create an emulsion: the oil phase, the aqueous phase, and the surfactant. This study determined surfactants and cosurfactants based on the solubility test results. Polysorbate 80 was chosen as the surfactant, and Polyethylene Glycol 400 as the cosurfactant.

The process involved combining 1% (0.2 mL) of MOE in a 20 mL glass vial, followed by a 5-minute mixing. Subsequently, 16% (3.2 mL) of Smix (Polysorbate 80 and PEG 400) were combined with the oil phase MOE for 5-minute mixing, sonicated for 10 minutes with Branson 2800-MH ultrasonicator (sonication power was 100, frequency was 40 kHz, and amplitude was 42 μm), and then incubated for 5 minutes in a water bath at 40°C. This procedure was repeated in three cycles. Distilled water was used as the aqueous phase ad 100% (20.0 mL).

The Smix ratio (surfactant: co-surfactant) was optimized within the 1-15% range using Design Expert 10 software.

2.2.3. Evaluation of MOE oil nanoemulsion

2.2.3.1. Measurement of the transmittance value

The transmittance from nanoemulsions was measured at a wavelength of 650 nm using spectrophotometry to assess the clarity of nanoemulsions, with distilled water as the reference (Purwanto, 2021).

2.2.3.2. pH

The pH of nanoemulsion formulations was determined using a pH meter. After pH 4 and pH 7 buffer solutions were calibrated, the electrode was immersed in the preparation, and the recorded pH value was noted (Talegaonkar & Alabood, 2011).

2.2.3.3. Measurement of globule size distribution

The size of nanoemulsion globules was analyzed using a particle size analyzer. A 1-gram sample of nanoemulsion was dissolved in 100 grams of ultrapure water, and 10 mL of the solution was placed in a cuvette for measurement. The size of nanoemulsion globules was analyzed using a particle size analyzer (Malvern MAL1275495) based on the principle of Dynamic Light Scattering (DLS). A 1 mL sample of nanoemulsion was diluted in 100 mL of ultrapure water to prevent multiple scattering effects. Then, 10 mL of the diluted solution was placed in a cuvette for measurement, where the scattered light from the nanoemulsion droplets was analyzed to determine the globule size distribution (Zhao et al., 2021).

2.2.3.4 Zeta potential

Zeta potential measurements were conducted using a zeta sizer. Zeta potential measurements were performed using a Zetasizer Nano ZS (Malvern MAL1275495) based on electrophoretic light scattering (ELS). A 1 mL sample of the nanoemulsion was diluted with ultrapure water to achieve optimal conductivity and prevent particle-particle interactions. The diluted sample was loaded into folded capillary cells (DTS1070) and placed in the instrument. Measurements were conducted at a controlled temperature of 25°C, and each sample was analyzed in triplicate to ensure reproducibility. The zeta potential values, indicating the stability of the nanoemulsion, were recorded and averaged (Zhao et al., 2021).

2.2.4 Fabrication of MOE nanoemulgel

The MEO nanoemulsion was then dispersed into a gel base with the composition carbophol 940 1%, triethanolamine 0.1%, DMDM-Hydantoin 0.03%. Nanoemulgel preparations with drug loading of 1% MEO and 2% MEO were each made five times in replication. Carbopol 940® was milled with triethanolamine until the gel mass was formed, and then DMDM Hydantoine was added. 50 mL prepared nanoemulsion was introduced into the 100 grams gel base and stirred gently on low speed until homogeneous.

2.2.5. Physical characterization of MOE nanoemulgel

2.2.5.1. Organoleptic test

Organoleptic testing involves a qualitative assessment of the gel preparation, focusing on observable changes in consistency, color, odor, and uniformity. This assessment was conducted through direct observation, allowing for the evaluation of the physical and sensory characteristics of the gel.

2.2.5.2. pH test

The pH of the gel was measured using a pH meter, with the value recorded after calibration (Talegaonkar & Alabood, 2011).

2.2.5.3. Adhesion test

For the adhesion test, 500 mg of the preparation was placed in two bepolysorbate slides and subjected to a 1.0 kg load for 5 minutes. The time it took for the slides to detach was recorded. This method is considered reliable for evaluating the adhesive properties of gel formulations, as it provides a quantitative measure of adhesion under controlled conditions (Purwanto et al., 2023).

2.2.5.4. Spreadability test

Spreadability was assessed by placing 0.5 grams of the gel on a glass cylinder for 1 minute to allow for a consistent application period and minimize variability in the results. The gel spread was measured on all four sides using a calibrated ruler, although a more precise instrument could be used in future studies to enhance accuracy. A 200-gram weight was added to the load until a constant weight was achieved. While we initially measured the spread linearly, presenting the results as area increments per time per force could provide a more comprehensive understanding of spreadability. This approach may be considered in subsequent evaluations to offer a more quantitative assessment of the formulation's performance (Bakhrushina, 2022).

2.2.5.5. Viscosity test

Viscosity was determined by placing the sample in a Brookfield viscometer with an immersed spindle set to a speed of 50 rpm.

2.2.6. Anti-hyperuricemia in vitro testing

Isolation of xanthine oxidase enzyme from 250 mL of fresh cow's milk. 250 mL of fresh cow's milk is heated to 30ºC. 81.68 g NaCl was added and centrifuged at 4000 rpm for 30 minutes. The supernatant obtained was fractionated using 40% ammonium sulfate and stored at 40C. Centrifuged at 4000 rpm for 75 minutes using a centrifuge. The supernatant and residue obtained were used as a sample of the xanthine oxidase enzyme. Dissolve the residual fraction using 0.05 M phosphate buffer pH 7.5 to 250 mL.

All samples produced were measured for their inhibitory activity against xanthine oxidase. Tests were carried out using a spectrophotometer under aerobic conditions. 1 mL of

the test solution with a concentration of (20-50 ppm) was added with 2.9 mL of 0.05 M phosphate buffer, pH 7.5, and 0.1 mL of xanthine oxidase enzyme solution in phosphate buffer, pH 7.5. After pre-incubation for 15 minutes, the reaction was started by adding 2 mL of 12 ppm xanthine substrate solution. The mixed solution was then incubated for 30 minutes. The reaction was stopped by adding 1 mL of 1 N HCl and then measuring the absorption at the maximum wavelength determined using a spectrophotometer. Testing has done five replications. One unit of xanthine oxidase is the enzyme required to produce 1 mM uric acid per minute at the optimum temperature. The formula can calculate the inhibitory activity of xanthine oxidase:

$$\text{Percentage of Inhibition (\%)} = \frac{(A-B)-(C-D)}{(A-B)} \text{ x } 100\% \quad \text{.....(1)}$$

Equation 1. Calculation of the inhibitory activity of xanthine oxidase. Description: Enzyme activity without sample (A), control for A, without sample and enzymes (B), sample activity (C), and activity of the sample without the enzyme (D) (Wahyuni, 2016).

2.2.7. Anti-inflammation in vivo testing

The study was conducted with ethical clearance (EC No. 533/YP-NA/KEPK/STIFAR/EC/ VIII/2023) obtained before the experiments. A total of 25 male white mice were randomly selected and divided into 4 groups, each consisting of five of 3 month old male white mice with a body weight of 200-300 grams. Before being tested, rats were acclimatized for 7 days and fasted for 18 hours while still being given drinking water. On the day of the test, the right rear leg of the rat was smeared according to the following treatment groups:

Negative control group : 200 mg nanoemulgel base

Positive control group : 200 mg Voltaren® Emulgel

Normal control group : Nothing was given

Test group 1 : 200 mg MOE 1% nanoemulgel

Test group 2 : 200 mg MOE 2% nanoemulgel

After treatment, 0.1 ml of carrageenan solution was injected into the right hind leg of all groups except the normal control group. The volume of the right hind leg of mice was measured at 0, 30, 60, and 90 minutes after carrageenan injection (Vt) (Mansouri et al., 2015).

3. RESULTS AND DISCUSSION

3.1. Identification of marjoram essential oil (MEO) content by GC-MS

In the initial research stage, the researcher selected raw materials for marjoram (*Origanum marjorana* L.) essential oil. The percentage of each compound in the marjoram essential oil (MEO) was determined by analyzing the GC-MS chromatogram (Figure 1).

The figure presents the GC-MS spectra of Marjoram Essential Oil (MEO), where each peak corresponds to a different volatile compound present in the oil. The x-axis represents the retention time (in minutes), which indicates the time it takes for each compound to pass through the GC column. In contrast, the y-axis represents the compounds' intensity (or abundance),

showing their relative concentration based on the area under each peak. Each peak in the chromatogram represents a specific compound, which was identified by comparing the mass spectra of the detected compounds with a reference library. By comparing these peaks to known spectra in the database, we confidently identified the compounds in the MEO.

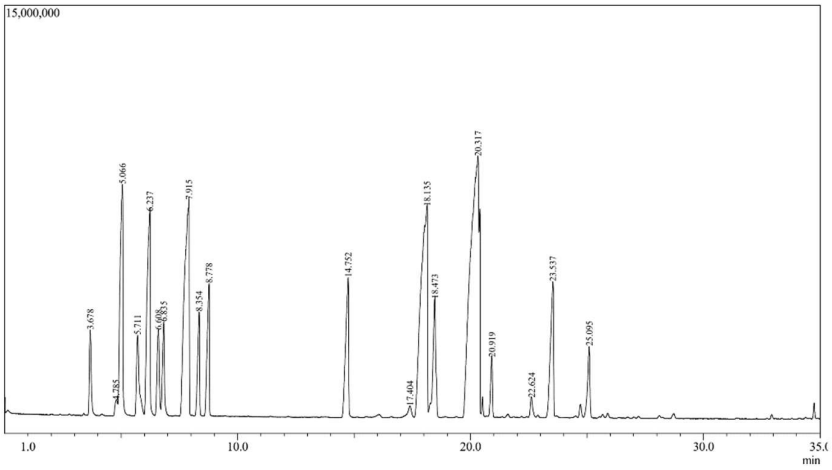

Figure 1. GC-MS spectra of Marjoram Essential Oil (MEO).

Furthermore, the total ion chromatogram (Neoptolemos et al., 2017) reflects the overall composition of the essential oil, confirming that the GC-MS analysis was conducted on MEO. This ensures that the reader can understand that the peaks represent the specific components of MEO, as identified by the retention time and the mass spectra comparison.

The area under each peak (peak area) in the chromatogram corresponds to the relative abundance of the compound, as identified by its retention time and mass spectrum. The ratio of the peak area of each compound to the total peak area of all identified compounds in the sample was calculated to obtain the exact percentage. This method is commonly used to quantify the relative concentration of volatile compounds in essential oils.

MEO essential oil contains 19 components (Table 1), with the 5 largest components being terpinene-4-ol (11.57%), sabinene (10.54%), gamma terpinene (9.60%), trans-sabinene hydrate (9.38%), cis -sabinene hydrate (6.08%).

Previous research shows that marjoram (*Origanum marjorana* L.) essential oil contains some of the largest terpene components such as terpinene-4-ol, sabinene, cis-sabinene trihydrate, gamma-terpinene (Arranz et al., 2015; Busatta et al., 2017). This indicates that the purchased MEO closely matches the characteristics of marjoram (*Origanum majorana* L.) essential oil, strengthening our confidence in using it for further formulation and testing.

3.2 Optimization Smix (Polysorbate 80 and PEG 400) in nanoemulsion containing MEO

Solubility tests demonstrated that when marjoram oil extract (MOE) was mixed in a 1:1 ratio with various solvents, the results were as follows: the sample was soluble, however turbid

in ethanol; phase separation occurred with glycerol; it was also soluble however turbid in Tween 20; soluble and clear in PEG 400; soluble and clear in Tween 80; and it was not soluble with propylene glycol, resulting in phase separation. Based on these results and previous studies that highlighted the efficacy of polysorbate 80 and PEG 400 as surfactants and co-surfactants in nanoemulsion preparations (Dasawanti et al., 2022; Purwanto et al., 2021), the researchers selected these components for their ability to provide enhanced solubilizing capacity and clarity in the formulation.

Table 1. GC-MS analysis results: detected compounds of meo and their corresponding areas.

Peak	*R.Time*	*Area%*	*Name of Compound*
1	3,678	3,76	.alpha.-pinene, (-)-
2	4,785	0,66	l-.beta.-pinene
3	5,066	10,24	sabinene
4	5,711	3,58	.beta.-myrcene
5	6,237	9,08	(+)-2-carene
6	6,608	3,78	l-limonene
7	6,835	4,14	.beta.-phellandrene
8	7,915	9,60	.gamma.-terpinene
9	8,354	4,54	benzene, 1-methyl-4-(1-methylethyl)
10	8,778	5,77	.alpha.-terpinolene
11	14,752	6,08	cis-sabinenehydrate
12	17,404	0,46	4-terpinenyl acetate
13	18,135	9,38	trans sabinene hydrate
14	18,473	5,05	cis-sabinenehydrate
15	20,317	11,57	3-cyclohexen-1-ol (terpinen-4-ol)
16	20,919	2,66	p-menth-2-en-1-ol
17	22,624	0,86	trans-piperitol
18	23,537	5,78	3-cyclohexene-1-methanol
19	25,095	3,02	bicyclogermacrene

Preliminary tests were also carried out to determine the lowest and highest components of the selected Smix (Surfactant and Co-surfactant) mixture. This preliminary test consisted of F1: MEO 1%, Polysorbate 80% 1%, PEG 400 15%, 100% aquadest and F2: MEO 1%, Polysorbate 80% 15%, PEG 400 1% and 100% ad aquadest. High transmittance (more than 90%) is an early indication that the size of the globules produced is nanometer in size. The measurement results of the nanoemulsion preparation in the preliminary test showed that the F2 preparation already had a transmittance of 99.50%.

Stable nanoemulsion preparations were obtained using the simplex lattice design method and the optimization process of polysorbate 80 and PEG 400 components as surfactants and cosurfactants. This simplex lattice design is operated with a software Design Expert 10. This research used 2 independent variables and produced 8 formulas. Each preparation is then tested for the physical characteristics of the preparation (Borges et al., 2018). The preparation with the optimum composition of Polysorbate 80 and PEG 400 will then be dispersed into a gelling agent to become a nanoemulgel preparation.

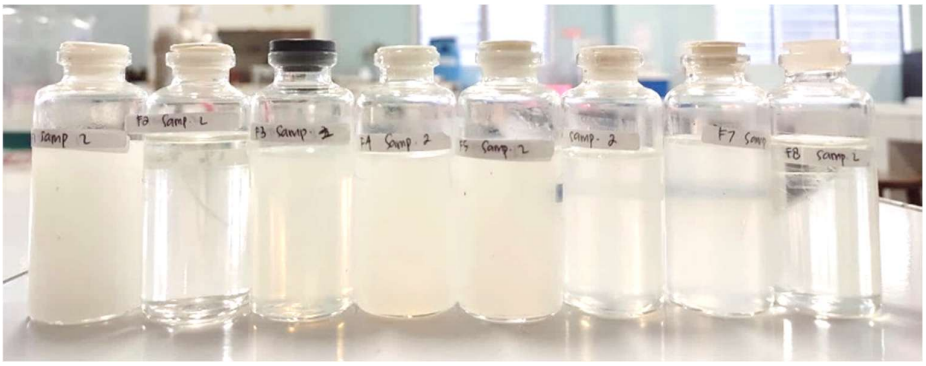

Figure 2. Formulas 1-8 are used to optimize the Smix (Polysorbate 80 and PEG 400) nanoemulsion of Marjoram Essential Oil (MEO).

Optimization of the Smix nanoemulsion MEO components have the composition: MEO 1%, Polysorbate 80, and PEG 400, each with a range of 1-15%, and distilled water ad 100%. Optimization was carried out using the Simplex Lattice Design method and the help of Design Expert 10 software. Eight running formulas will be created in this optimization (Table 2).

Table 2. Composition of optimized formulation materials for polysorbate 80 and peg 400 and test responses to MEO nanoemulsion preparations.

Formula	Ingredients (%)				Test Response			
	MEO	Polysorbate 80	PEG 400	Aq	pH	Viscosity (cps)	%T	Phase Separation
1	1	1	15	ad 100	5,91	9,66	3,1	0
2	1	15	1	ad 100	5,61	7,08	98,2	0
3	1	8	8	ad 100	5,42	48,3	98,9	0
4	1	4	12	ad 100	5,42	44,4	6,4	0
5	1	1	15	ad 100	5,53	48,3	3,1	0
6	1	12	4	ad 100	5,66	14,8	99	0
7	1	8	8	ad 100	5,55	7,92	99	0
8	1	15	1	ad 100	5,58	9,36	98,3	0

The nanoemulsion preparation (Figure 2) was evaluated for its physical characteristics, including pH, viscosity, % transmittance, and phase separation. All data from test results are entered as response values for each formula in the Design Expert software for statistical analysis (Ivanova & Karelson, 2022) and to determine the most optimal Smix composition. The response criteria were selected based on an objective within the range to obtain maximum viscosity and transmittance values, minimum pH values, and absence of phase separation. The optimum composition of Smix (Polysorbate 80: PEG 400) in MEO nanoemulsion consists of Polysorbate 80: PEG 400 9.958: 6.042 with a desirability of 0.710.

The particle size of the resulting nanoemulsion is crucial because it will affect the drug's release rate to be absorbed. The size distribution of the nanoemulsion globules was analyzed using a Particle Size Analyzer. The average globule size of marjoram essential oil nanoemulsion at a loading dose of 1% MEO was 22.94 nm with a polydispersity index of 0.65 (Figure 3a). With the increase in the essential oil content in the nanoemulsion preparation, the average

particle size produced also increases. The average globule size of marjoram essential oil nanoemulsion at a loading dose of 2% MEO was 142.4 nm with a polydispersity index of 0.91 (Figure 3b). The polydispersity index value represents the homogeneity of the nanoemulsion particles' diameter to describe the nanoemulsion particles' size distribution. Suppose the polydispersity index value is closer to zero. In that case, the particles formed will be more homogeneous (Sinko, 2019), but the particle size distribution value is still considered uniform and homogeneous, namely <0.7 (Prihantini et al., 2020).

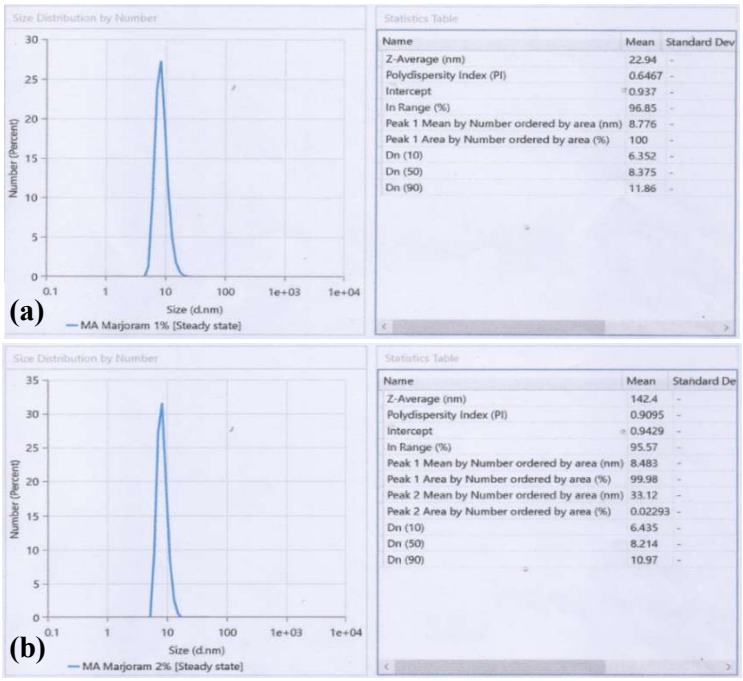

Figure 3. The average globule size of optimum MEO nanoemulsion. *Description*: Loading drug MEO 1%: 22.94 nm with a polydispersity index of 0.65 (a). Loading drug MEO 2%: 142.4 nm with a polydispersity index of 0.91 (b).

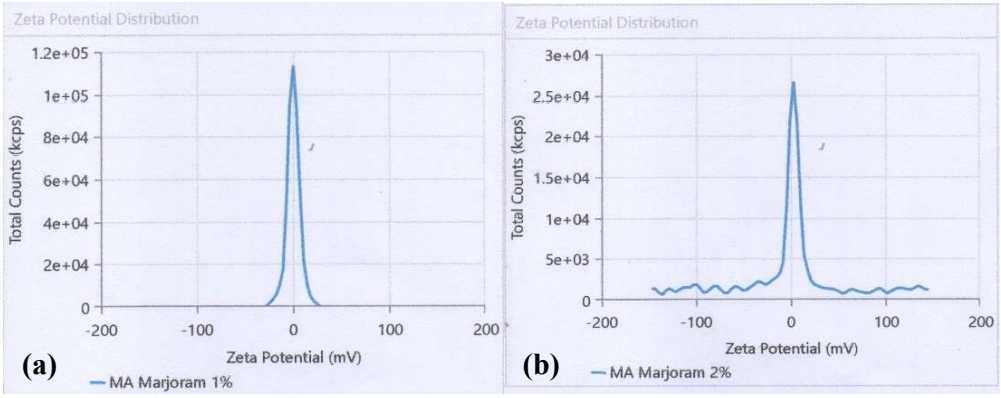

Figure 4. The zeta potensial of optimum MEO nanoemulsion. *Description*: Loading drug MEO 1%: 22.94 nm with a potential zeta of 0.1533 mV (a). Loading drug MEO 2%: 142.4 nm with and potential zeta of -1.022 mV (b).

The zeta potential of the nanoemulsion was measured to determine the nanoemulsion's surface charge properties related to the nanoparticles' electrostatic interactions. The particle composition and the particle dispersion medium affect the zeta potential. The zeta potential value describes the optimization of the repulsion or attraction of the particles. A good zeta potential value for nanoemulsion preparations is more than +30mV (Maurya et al., 2021). However, the zeta potential measurement results for the nanoemulsion (Figure 4) showed a value of 0.1533 mV for 1% MEO loading dose (Figure 4a) and -1.022 mV for 2% MEO loading dose (Figure 4b). This may be influenced by the surfactant used in the nanoemulsion formula, namely Polysorbate 80, because Polysorbate 80 is a nonionic surfactant.

3.3 Physical characteristics MEO nanoemulsion and nanoemulgel

Previous research showed that terpinene-4-ol and carvacrol, compounds in MEO, can cross the skin membrane after topical administration. However, the amount that can cross the membrane is still very limited, with a Tmax of 39 minutes and an AUC of 5.31 µg.hour/ml for the terpinene-4-ol and tmax components. 33 minutes and AUC 0.230 µg.jam/ml for the carvacrol component (Chooluck et al., 2012; Mason et al., 2017).

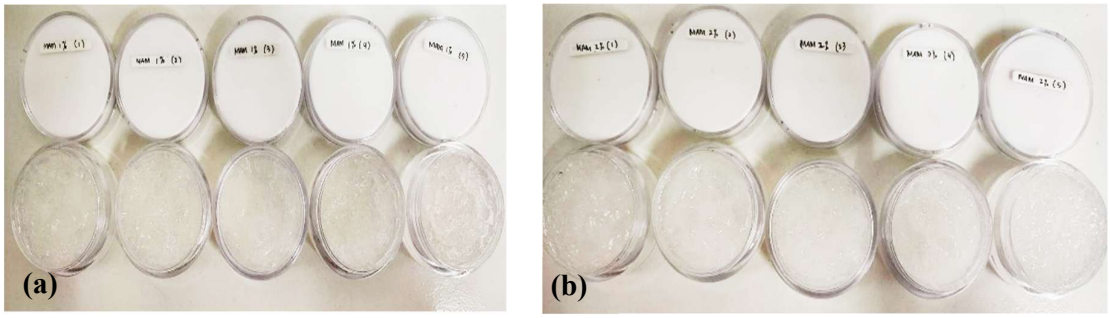

Figure 5. Nanoemulgel formulations with marjoram essential oil (MEO). *Description*: (a) Nanoemulgel containing 1% MEO, and (b) Nanoemulgel containing 2% MEO. These formulations demonstrate varying concentrations of marjoram essential oil as the active ingredient, highlighting potential differences in physical properties or performance based on essential oil content.

The development of nanoemulgel is a solution to this problem. Nanoemulsions are known to increase the penetration of drugs administered percutaneously (Yang et al., 2017). The presence of surfactants and co-surfactants helps increase penetration through the skin (Shaker et al., 2019). The nanoemulsion preparation has a low viscosity, so a gelling agent is added to form a nanoemulgel during its development. Nanoemulgel has a higher viscosity. Therefore, it can increase the product contact time when applied to the skin (Sengupta & Chatterjee, 2017).

The MEO nanoemulsion was then dispersed into a base gel (Figure 5) with the composition carbophol 940 1%, triethanolamine 0.1%, DMDM-Hydantoin 0.03%. Nanoemulgel preparations with drug loading MEO 1% and MEO 2% were replicated five times each (Figure 4). MEO 1% nanoemulgel has a pH of 5,49±0,45, spreadability of 4,81±0,37 cm,

and adhesion power of 1,76±0,32 seconds. While MEO 2% nanoemulgel have pH of 4,85±0,21; spreadability of 4,76±0,52 cm; adhesion power of 2,44±0,30 seconds The MEO nanoemulgel was then tested for its anti-hyperuricemia activity in vitro and anti-inflammatory activity in vivo.

3.4 Anti-hyperuricemia in vitro testing

Testing of antihyperuricemia activity in vitro refers to previous studies by (Wahyuni, 2016). Testing of xanthine oxidase inhibitory activity was carried out to determine the inhibitory power of MEO nanoemulgel on the xanthine oxidase enzyme, which can convert xanthine into uric acid. Variations in the concentration of the nanoemulgel used range from 20-50 ppm. Pirofel gel 0.5® (piroxicam 0.5%) and Sumifun Arthritis Herba Cream® were used as comparison samples. The selection of Pirofel gel 0.5® (piroxicam 0.5%) and Sumifun Arthritis Herba Cream® as positive controls in our study was based on their efficacy in treating inflammatory conditions, particularly arthritis-related. Piroxicam, a nonsteroidal anti-inflammatory drug (NSAID), functions by inhibiting cyclooxygenase (COX) enzymes, thus reducing the production of pro-inflammatory mediators in hyperuricemia. This mechanism is similar to our experimental formulation, which aims to reduce inflammation and pain through comparable pathways.

Table 3. Test results for determining xanthine oxidase inhibitory activity.

Sample	IC50 (ppm)
Nanoemulgel MEO 1%	67,72 ± 9,89
Nanoemulgel MEO 2%	25,30 ± 2,57
Sumifun Arthritis Cream	54,98 ± 3,21
Pirofel Gel 0,5%	15,18 ± 1,42

Similarly, Sumifun Arthritis Herba Cream® incorporates natural ingredients known for their anti-inflammatory properties in hyperuricemia therapy, providing a dual mechanism of action that complements the effects of our formulation. By utilizing these positive controls, we aim to benchmark the effectiveness of our MEO nanoemulsion against recognized treatments, ensuring that our results can be contextualized within the existing therapeutic landscape for inflammatory conditions.

The absorbance of each solution in the xanthine oxidase inhibition activity test was measured at a wave of 277.5 nm using a UV-Vis spectrophotometer. The in vitro anti-hyperuricemia testing results of preparations (Table 3) show that the preparation Pirofel Gel 0.5® (containing piroxicam 0.5%) has the most significant xanthine oxidase inhibitory power. Piroxicam gel has been known to be used to treat joint pain due to gouty arthritis (arthritis caused by the buildup of uric acid crystals in the joints). Nanoemulgel MEO 1% also has the lowest xanthine oxidase enzyme inhibitory power (indicated by the highest IC50 level). However, nanoemulgel MEO 2% has greater inhibitory power and can surpass the marketed preparation, namely Sumifun Arthritis Herba Cream®.

3.5 Anti-inflammation in vivo testing

The anti-inflammatory activity test of the MAM nanoemulgel preparation aimed to determine the ability of the preparation as an anti-inflammatory agent, which was described in the decrease in the volume of edema of the paws of white rats induced by 1% carrageenan. 100 mg of the preparation was applied to the feet of rats. The volume of leg edema was measured with a plestismometer.

The anti-inflammatory activity test was carried out on 4 groups, namely 2 dose groups (MAM 1% and MAM 2%) compared with a negative control in the form of a gel base and a positive control in the form of an anti-inflammatory gel preparation that is already on the market, namely Voltaren Gel. Based on preliminary testing, it was found that 2 variants of MAM nanoemulgel doses showed anti-inflammatory activity. The measurement results (Figure 5) show that 2% MAM nenoemulgel produces a better anti-inflammatory effect than the 1% MAM variant, which is indicated by a faster reduction in inflammatory volume.

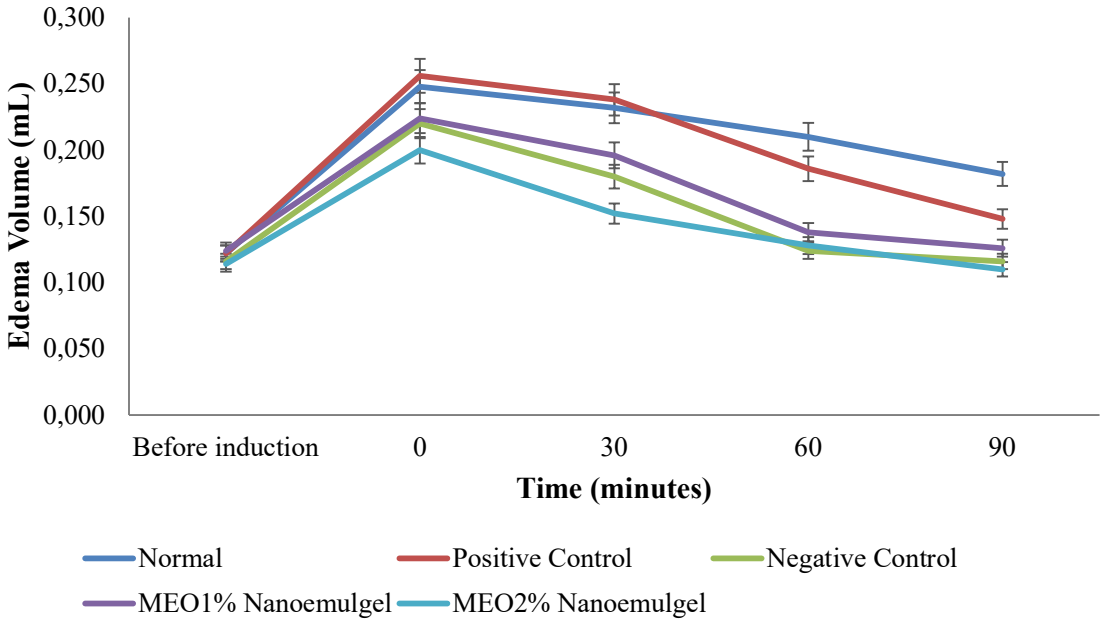

Figure 5. The graph of rat edema volume in anti-inflammatory activity test.

The variable 'before induction' refers to the average paw volume of the rats before they were induced with carrageenan. Following this, each group received its respective treatment. The time point labeled '0 minutes' represents the paw volume immediately after the carrageenan injection, marking the onset of edema. The subsequent measurements of edema volume were taken at 30 and 60 minutes post-injection to monitor the progression and response to the treatments over time.

The analysis showed that the anti-inflammatory effect of MEO nanoemulgel was dose-dependent. The larger the dose MEO was used, the inhibition of edema in the feet was also more significant. Based on statistical results, each group had a significant difference ($p<0.05$) from the negative control, indicating that MOE nanoemulgel has anti-inflammatory activity.

4. CONCLUSION

A novel nanoemulsion gel incorporating marjoram essential oil has been effectively created. The choice of the best formulation depended on each component's physical and chemical attributes. The optimal ratio of Polysorbate 80 surfactant to PEG 400 co-surfactant was 9.958:6.042. The study found that the most effective formulation for 1% MEO nanoemulsion had an average particle size of 22.94 nm with a polydispersity index (PDI) of 0.65. The 2% MEO nanoemulsion had a particle size of 142.4 nm with a PDI of 0.91. When dispersed into a gel base, the MEO nanoemulsion forms a nanoemulgel, demonstrating xanthine oxidase inhibition. The 2% MEO nanoemulgel showed an IC50 of 25.30±2.57 ppm and exhibited stronger anti-inflammatory activity than the 1% MEO nanoemulgel.

Additional research, such as an irritation test, stability test, and clinical assessments, including trials involving both animals and humans, are required to ascertain the efficacy of this preparation for treating inflammation and hyperuricemia via topical application.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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