Optimization Of Nanogel Formulation Of *Piper Nigrum* And *Erythrina Subumbrans* Extract As Anti-Inflammatory Based On Lontar Usadha Tiwang: Effectiveness Test On *Mus Musculus*

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Abstract.

Public health in Bali has long been supported by traditional medicine based on local wisdom, one of which is usadha tiwang. In practice, usadha tiwang uses black pepper (Piper nigrum) and dadap leaves (Erythrina subumbrans) to treat mokan disease, which is characterized by swelling and pain, typical symptoms of inflammation. Scientifically, both have been proven to have anti-inflammatory activity in both extract and cream preparations, although their use in topical nanogel formulations has not been explored. This study aims to conduct phytochemical screening, develop a combination nanogel formulation of P. nigrum and E. subumbrans extracts, and evaluate anti-inflammatory activity in vivo. Extraction was performed by maceration, using 96% ethanol (1:5) for P. nigrum and 70% ethanol (1:5) for E. subumbrans. A total of six nanogel formulas were prepared with varying gel base concentrations. Activity testing was performed on mice (Mus musculus) with 1% carrageenan induction on the paw, divided into eight groups (F1–F6, negative control, and positive control). Edema evaluation was performed using a plethysmometer, and the data were analyzed using the Kruskal-Wallis test. Screening results showed that P. nigrum ethanol extract contained alkaloids, flavonoids, and steroids, while E. subumbrans extract contained alkaloids, flavonoids, saponins, and steroids. All formulations had transmittance values >90%. Statistical analysis showed a significant difference between treatment groups (p=0.002). The positive control (Voltaren® Emulgel) differed significantly from all test groups (p=0.000). Of the six formulas, F3 showed the most optimal effect with an inflammation inhibition percentage of 100% at the 60th minute of observation.

Keywords: *Nanogel; Piper nigrum; Erythrina subumbrans; Anti-inflammatory and Usadha Bali.*

I. INTRODUCTION

Health is defined as a person's state of well-being, both physically, mentally, and socially, and not merely the absence of disease to enable them to live productively. Health efforts implemented in an integrated and sustainable manner to maintain and improve the health of the community can be carried out through promotive, preventive, curative, rehabilitative, and/or palliative forms by the central government, local governments, and/or the community.[1] One of the health efforts established by the Bali Provincial Government is traditional Balinese medicine. Traditional Balinese medicine is carried out based on its benefits and safety which have been proven empirically or passed down from generation to generation.[2] As time progresses and is influenced by various aspects of life, traditional medicine is now increasingly practiced by the community.[3] When treatment with modern medicines causes various problems due to the chemical content, people become more aware of the detrimental effects of modern medicines and make them start turning back to traditional medicines.[4] Another factor that causes people to still be interested in traditional medicine is the economic factor that influences this paradigm. Modern medicine is expensive and medically incapable of being overcome, this is the main reason people will turn to traditional Balinese healing methods known as usadha.[3] Since ancient times, usadha has been very well known and popular among the Balinese people.

This is evidenced by the many manuscripts written on lontar using Balinese language and script. Basically, this usadha treatment is still a reference in treating various types of diseases. One of the collections of Balinese usadha treatments can be seen in the catalog owned by the Bali Provincial Language Center, such as usadha tiwang.[4] The treatments written in the usadha tiwang palm leaves discuss several diseases such as mokan, tiwang, cough, and others. Mokan is a disease with symptoms of swelling and pain or soreness.[5] Swelling (tumor) and pain (dolor) are one of the signs or symptoms of inflammation.

Inflammation is defined as a series of responses due to tissue damage, especially caused by trauma, infection, toxic chemicals, and environmental agents. Other symptoms of inflammation that usually appear are heat (*calor*), redness (*rubor*), and impaired function (*functio laesa*).[6] Empirical treatment of mokan disease in the usadha tiwang lontar can be done by using black pepper (*Piper nigrum*) and dadap leaves (*Erythrina subumbrans*) by applying them to the external part of the body that feels painful or swollen.[5] Black pepper (*Piper nigrum*) has a main bioactive compound of piperine. Other metabolite compounds are alkaloids and essential oils with components of dipentene, limonene, entoxylene, caryophyllene, phellandrene.[7] Dadap leaves (*Erythrina subumbrans*) have benefits as a reliever of postpartum fever, breast milk stimulant, stomach ache, internal bleeding.[8]

Dadap leaves contain flavonoids, tannins, alkaloids, and saponins which make these leaves have benefits as anti-inflammatory.[9] The active metabolites in these two plants are flavonoids which have benefits in reducing the inflammatory response through inhibition of cycloxygenase and lipoxygenase pathways, inhibiting the synthesis of arachidonic acid. Alkaloids have antihistamine effects that work by inhibiting the release of interleukin (IL) and suppressing the release of histamine by mast cells.[8,9] Tasleem et al.,[10] reported that the active substance piperine in black pepper has potential activity as an analgesic and anti-inflammatory in mice. In the study of Dhargawe et al.,[11] piperine has potential effects as an analgesic, anti-inflammatory, and antipyretic in mice. Jain et al.,[12] reported that the use of black pepper extract has potential as an analgesic therapy proven by tests on mice induced by pain. Research related to dadap serep leaves (*Erythrina subumbrans*) by According to Rangkuti et al. [8], the 70% ethanol extract of dadap serep leaves demonstrated significant anti-inflammatory effects when administered orally to mice (p-value = 0.000). Research by Wardani et al.,[9] using cream of dadap serep leaf extract also showed an anti-inflammatory effect in mice induced by carrageenan (p value <0.05).The potential synergistic benefits of combining these two plants have not been fully explored. According to the Lontar Usadha Tiwang manuscript, these plants were traditionally applied topically by blowing the preparation onto the affected body part. Previous studies have utilized cream formulations to assess their anti-inflammatory activity.[8]

However, further exploration of a combination of *Piper nigrum* and *Erythrina subumbrans* extracts formulated as a nanogel has yet to be reported. Topical nanogel formulations offer advantages such as enhanced penetration of active compounds, facilitated by nanoglobules that act as carriers capable of penetrating the stratum corneum.[13] The development of herbal treatments based on Balinese medicinal plants and advanced formulations is expected to contribute to the growth of Bali's local UMKMs and the preservation of traditional medicine rooted in local wisdom, known as Usadha Bali. Therefore, this study aims to perform phytochemical screening of secondary metabolites, optimize the nanogel formulation combining *Piper nigrum* and *Erythrina subumbrans* ethanol extracts, and evaluate the anti-inflammatory activity of the nanogel in carrageenan-induced mice.

II. METHODS

This study employed an experimental design using a Completely Randomized Design (CRD) approach. The research stages included the identification of secondary metabolites from the extracts of *Piper nigrum* and *Erythrina subumbrans*, formulation and optimization of the combined nanogel preparation, and *in vivo* evaluation of anti-inflammatory activity. The nanogel formulations consisted of six variations (F1–F6), along with a negative control (F0) and a positive control (Voltaren® emulgel). Anti-inflammatory activity was assessed in mice (*Mus musculus*) through induction of edema using 1% carrageenan (0.05 mL) administered intraplantarly, followed by measurement of paw edema volume with a plethysmometer at various time intervals. The observational data were statistically analyzed using SPSS.

The inclusion criteria for this study were male *Mus musculus* aged 2–3 months with a body weight of 20–40 g. The exclusion criteria included mice with physical deformities or limb defects. The number of mice per group was determined based on Federer's formula[14]:

$(t-1)(n-1) > 15$

$(t-1)(8-1) > 15$

$(t-1)(7) > 15$

$7t - 7 > 15$

$7t > 22$

$t > 22/7$

$t > 3,14$

`Therefore, the required number of samples for each group was 4 mice. To anticipate potential dropouts during the study, the number was increased to 5 mice per group. The total number of mice used in this study was 40. The independent variable in this research was the nanogel formulation containing *Piper nigrum* and *Erythrina subumbran*s extracts in various nanogel bases, while the dependent variables were the edema volume and the percentage of inflammation inhibition in the carrageenan-induced mouse paws. The materials used in this study included black pepper fruits (*Piper nigrum*), leaves of *Erythrina subumbrans*, PEG 400, Tween 80, Carbopol 940, Triethanolamine (TEA), Methylparaben, distilled water, 70% ethanol, 96% ethanol, water for injection, carrageenan, Liebermann–Burchard reagent, $FeCl_3$, Mayer's reagent, Dragendorff's reagent, 2N HCl, acetic acid, chloroform, and concentrated H_2SO_4. The instruments used in this study included an analytical balance, mortar, pestle, spatula, parchment paper, filter paper, beaker glass, watch glass, object glass, cover glass, stirring rod, metal spatula, horn spoon, glass bottles, glass funnels, 10 mL volumetric flasks, plastic pots, 5 L glass jars, aluminum foil, 1 cc syringes, blender, overhead stirrer, magnetic stirrer, rotary evaporator, UV-Vis spectrophotometer, and plethysmometer.

Data collection was carried out in several stages. First, plant determination was conducted. Second, ethical clearance documents were submitted to the Ethics Committee of STIKES Bina Usada Bali. Third, extraction simplicia of *Piper nigrum* fruits and *Erythrina subumbrans* leaves was performed. The simplicia of *Piper nigrum* and *Erythrina subumbrans* were obtained from the Pusat Pengolahan Pasca Panen Tanaman Obat (P4TO), Karangasem Regency, Bali. Extraction of *Piper nigrum* was carried out using the maceration method with 96% ethanol as the solvent at a ratio of 1:5. The extraction of *Erythrina subumbrans* leaves was conducted by maceration using 70% ethanol at the same ratio (1:5). Maceration was performed for 5 days with occasional stirring. The extracts were concentrated using a rotary evaporator, followed by fractionation of *Erythrina subumbrans* leaves with n-hexane to obtain thick extracts. Fourth, phytochemical screening of the thick extracts of *Piper nigrum* and *Erythrina subumbrans* was performed, including tests for flavonoids, alkaloids, saponins, tannins, and steroids.The fifth stage involved the formulation of nanogel preparations at various concentrations. The negative control consisted of a nanogel formulation without active ingredients (F0), while the positive control used Voltaren® emulgel. The formulation details are presented in Figure 1.

INGREDIENT	USE	FORMULA (%b/b)						
		F0	F1	F2	F3	F4	F5	F6
Piper nigrum Extract	Anti-inflammatory	0	0,24	0,24	0,24	0,24	0,24	0,24
Erythrina subumbrans Extract	Anti-inflammatory	0	0,01	0,01	0,01	0,01	0,01	0,01
PEG 400	Co-surfactant	2	2	2	3	4	3	4
Tween 80	Ionic surfactant	4	4	3	4	3	2	2
Methylparaben	Preservative	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Carbopol 940	Gelling agent	1	1	1	1	1	1	1
TEA	pH adjustment	0,6	0,6	0,6	0,6	0,6	0,6	0,6
Aquadest ad	Solvent	100	100	100	100	100	100	100

Fig 1. Formulation of Nanogel Containing *Piper nigrum* and *Erythrina subumbrans* Extracts

The preparation of nanogel formulations F1–F6 was carried out by weighing the required amounts of each ingredient. The nano phase was prepared by mixing Tween 80 and Methylparaben using a magnetic stirrer at 600 rpm. Subsequently, PEG 400 was added gradually and homogenized for 10 minutes. Distilled water (10 mL) was then added slowly and mixed using an overhead stirrer at 600 rpm for 30 minutes. The resulting nano mixture was evaluated for percent transmittance using a UV-Vis spectrophotometer, with distilled water as the blank, measured at a wavelength of 650 nm.[15] Next, the gel base was prepared by dispersing Carbopol 940 in hot water. TEA was incorporated into the Carbopol 940 mixture, followed by the addition of the remaining distilled water. The gel mixture was homogenized using an overhead stirrer at 600 rpm for 30 minutes. In the final stage, the gel base was combined with the nano mixture and homogenized using an overhead stirrer at 600 rpm for another 30 minutes. The negative control formulation (F0) was prepared using the same procedure, except without the addition of the extracts.

The sixth stage involved evaluating the anti-inflammatory effect using the 1% carrageenan-induced paw edema method. The carrageenan solution was prepared by weighing 20 mg of carrageenan, dissolving it in 20 mL of distilled water, and heating the mixture to 90°C.[16] Mice designated for treatment were quarantined and fasted for at least 18 hours prior to the experiment. Group allocation was performed randomly. The animals were divided into eight groups, each consisting of five mice, and a mark was made above the ankle joint using a marker for identification. Paw edema volume was measured using a plethysmometer.[9] The initial paw volume (t_0) was recorded before induction. Edema was induced by intraplantar injection of 0.05 mL of 1% carrageenan solution into the left hind paw of each mouse.[16] The edema response was observed for 30 minutes after carrageenan induction, followed by measurement and recording of edema volume. Subsequently, 100 mg of each nanogel formulation was evenly applied to the left paw of the mice in each group, and the response was observed for another 30 minutes. Edema volume measurements were recorded at 10, 30, 60, 90, 120, and 180 minutes. The collected data, representing paw edema volumes from the various treatment groups, were used to calculate the percentage increase in paw edema volume using the following formula:

$$\% \text{ Increase in Edema Volume} = ((V_t - V_0) / V_0) \times 100\%$$

Notes:

V_t = Paw volume of the mouse at time t

V_0 = Initial paw volume of the mouse before treatment (t_0)

Source: Wardani et al.,[9]

After calculating the percentage increase in edema volume, the percentage of inflammation inhibition was determined using the following formula:

$$\% \text{ Inhibition of Inflammation} = ((a - b) / a) \times 100\%$$

Notes:

a = Mean percentage increase in edema volume of the negative control group

b = Mean percentage increase in edema volume of the test group

Source: Wardani et al.,[9]

The observed paw edema volume data were analyzed using SPSS. Normality was tested using the Shapiro–Wilk test, followed by a homogeneity test using Levene's test. If the p-value was greater than 0.05, one-way ANOVA was performed; otherwise, the Kruskal–Wallis test was used as an alternative. Post hoc analysis was conducted using the LSD test. The schematic of the anti-inflammatory effect observation on mice paws treated with nanogel formulations is presented in Figure 2.

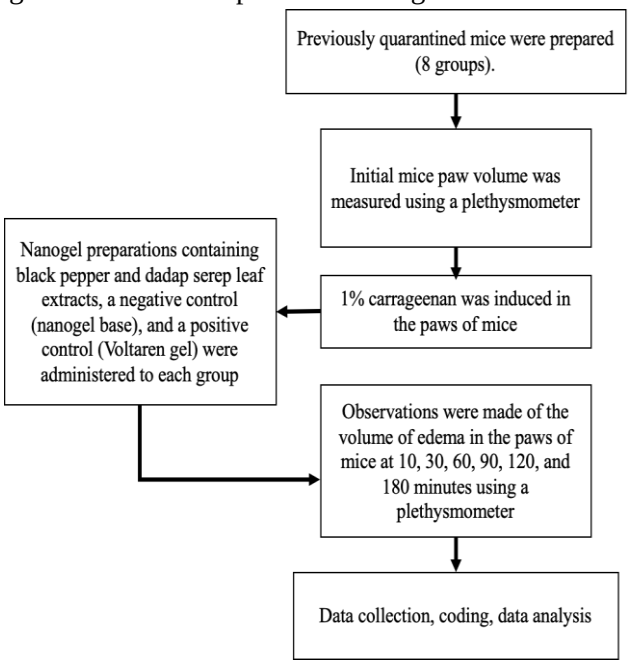

Fig 2. Schematic of the Observation of the Anti-inflammatory Effect on Mice Paws Administered with Nanogel Extract

III. RESULT AND DISCUSSION

This study obtained ethical clearance from the Komisi Etik Penelitian Kesehatan (KEPK) of STIKES Bina Usada Bali under approval number 292/EA/KEPK-BUB-2025. Plant determination was conducted at the Herbal Materia Medica Laboratory Unit (UPT Laboratorium Herbal Materia Medica) with registration number 000.9.3/2971/102.20/2025 for *Erythrina subumbrans* (dadap serep) leaves and 000.9.3/2972/102.20/2025 for *Piper nigrum* (black pepper), confirming that both samples were correctly identified. The simplicia of black pepper and *E. subumbrans* leaves were each subjected to maceration (1:5 ratio) using ethanol as the solvent. The filtrates were concentrated using a rotary evaporator to obtain thick extracts. The concentrated extract yield of black pepper was 18.1724 g from 300 g of simplicia, resulting in a yield percentage of 6.057%. Meanwhile, the *E. subumbrans* leaf extract yielded 31.782 g from 1000 g of simplicia, corresponding to a yield of 3.1782%. The yields of both extracts were considered suboptimal, as they did not exceed 10%.[17] Phytochemical screening was subsequently performed on both the *Piper nigrum* and *Erythrina subumbrans* leaf extracts. The results are presented in Table 1.

Table 1. Phytochemical Screening Results of *Erythrina subumbrans* Leaves and *Piper nigrum* (Black Pepper)

Plant	Test Results			
	Alkaloid	**Flavonoid**	**Saponin**	**Steroid**
Piper nigrum (Black Pepper)	+	+	-	+
Erythrina subumbrans (Dadap Serep) leaves	+	+	+	+

The preparation process of the *Piper nigrum* and *Erythrina subumbrans* leaf nanogel formulations was carried out based on the formulation shown in Figure 1. The main difference among the nanogel formulations lies in the variation of Tween and PEG 400 concentrations. Formulations F1 to F6 represent increasing PEG:Tween ratios, from the lowest to the highest. The transmittance percentage of the nano formulations, measured using a UV–Vis spectrophotometer, is presented in Table 2. Based on the results, the highest transmittance value was obtained in formulation F6, with a percentage of 96.80%. All formulations (F0–F6) showed transmittance values greater than 90%, which indicates good nanoemulsion clarity.[15,18] This result demonstrates that the dispersed nano mixtures exhibited transparency, allowing light to pass through when illuminated.[15]

Table 2. Results of the Percentage Transmittance Measurement of Nano Formulations

	Formula						
	F0	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Transmittance Results (%)	96,50	96,00	96,00	96,00	96,80	96,80	96,90

The mixing of black pepper and *Erythrina subumbrans* leaf nano extracts with the gel base produced final nanogel formulations (F1–F6) that appeared transparent with a yellowish-green hue and exhibited a slightly pungent aroma characteristic of black pepper along with the distinct scent of *E. subumbrans* leaves. The resulting nanogel formulations (F1–F6) are shown in Figure 3. Based on visual observation, formulation F6 displayed the most transparent and lightest color.

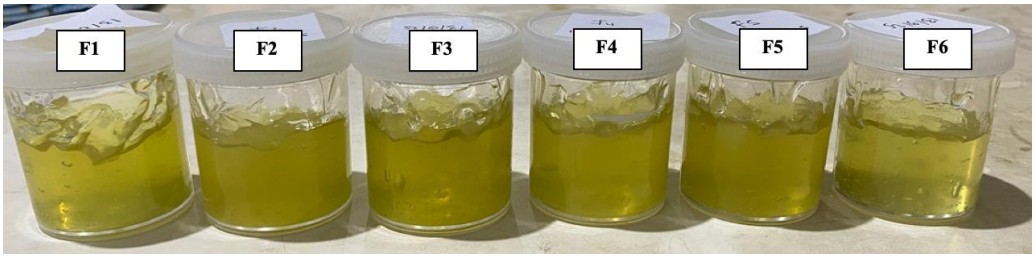

Fig 3. Final Nanogel Formulations (F1 to F6)

The anti-inflammatory activity test was conducted by inducing inflammation in the mice's hind paws using a 1% carrageenan solution.[11] This induction method is widely used to investigate the effects of new anti-inflammatory agents and to study the mechanisms involved in the inflammatory process. The commonly used concentration of carrageenan ranges from 1–3%, with a volume of 50–150 µL administered via

intraplantar injection.[16] Carrageenan induction in the mice's paw produces an inflammatory response that develops in two distinct phases. The first phase occurs within 1–2 hours after carrageenan injection and is associated with the onset of acute inflammatory trauma. During this phase, serotonin and histamine are released at the site of inflammation, accompanied by an increase in prostaglandin synthesis within the damaged tissue. The second phase involves the release of prostaglandins and is mediated by leukotrienes and bradykinin.[19] The inflammatory response resulting from carrageenan injection is characterized by the release of TNF-α and IL-1β. TNF-α subsequently stimulates the secretion of IL-1β and neutrophil chemoattractant-1, both of which play crucial roles in activating COX-2, thereby enhancing prostaglandin synthesis.[20] The anti-inflammatory effect of the nanogel formulation containing black pepper and *Erythrina subumbrans* leaf extracts was evaluated by observing the edema volume in the mice's paws [11,16]. The anti-inflammatory activity was assessed based on the percentage of inflammation inhibition [11]. The observations of paw edema volume in mice induced with 1% carrageenan at 10, 30, 60, 90, 120, and 180 minutes are presented in Table 3 and Figure 4.

Table 3. Mean Edema Volume (mL) Observed at Different Time Intervals

Formula	Results of Average Edema Volume (mL) at various times (minutes)					
	10	30	60	90	120	180
F0 (Negative Control)	0,010	0,010	0,009	0,009	0,009	0,009
F1	0,010	0,009	0,008	0,008	0,005	0,005
F2	0,010	0,006	0,006	0,006	0,006	0,005
F3	0,011	0,009	0,005	0,005	0,005	0,005
F4	0,013	0,010	0,010	0,008	0,005	0,005
F5	0,010	0,010	0,010	0,010	0,005	0,005
F6	0,010	0,010	0,009	0,005	0,005	0,005
Positive Control (Voltaren® Emulgel)	0,010	0,006	0,005	0,005	0,005	0,005

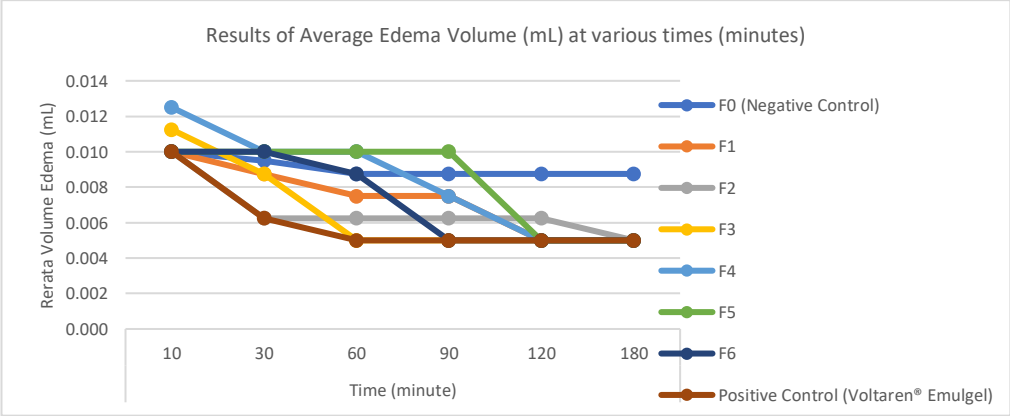

Fig 4. Results of Average Edema Volume (mL) at various times (minutes)

Based on the statistical analysis, a normality test was performed on the edema volume data at various time intervals for each treatment group using the Shapiro–Wilk test, which yielded a p-value of 0.000. Subsequently, the Levene test was conducted to assess data homogeneity, resulting in a significance value of p = 0.000, indicating unequal variances among the groups. Therefore, the assumption of homogeneity of variance was not met, and a non-parametric Kruskal–Wallis test was applied. The obtained p-value of 0.002 indicated a significant difference among groups F1–F6, the negative control, and the positive control. A post hoc analysis using Tukey's HSD test was then performed to identify groups exhibiting statistically different effects, as presented in Table 4.

Tabel 4. Analisa Hasil Uji Tukey's HSD

Groups Compared	Mean Difference	Sig. (p-value)	Keterangan
K+ vs F1	0,00375	0,000	Significantly different
K+ vs F2	0,00375	0,000	Significantly different
K+ vs F3	0,00375	0,000	Significantly different
K+ vs F4	0,00375	0,000	Significantly different

K+ vs F5	0,00375	0,000	Significantly different
K+ vs F6	0,00375	0,000	Significantly different
K+ vs K-	0,00375	0,000	Significantly different
Description: K+: Positive control group (Voltaren® Emulgel) K-: Negative control group (F0)			

The results of Tukey's test showed that the positive control group differed significantly from all treatment groups (F1, F2, F3, F4, F5, and F6), with a significance value of p = 0.000 for each comparison. Additionally, the positive control group also exhibited a significant difference compared to the negative control group. These findings indicate that the effects produced by the positive control group were not comparable to those of the treatment or negative control groups, suggesting that the responses observed in groups F1–F6 were significantly different from the positive control. The percentage increase in edema volume over time and the mean percentage of inflammation inhibition obtained in this study are presented in Tables 5 and 6.

Tabel 5. Average of Edema Volume Increase At Various Times

Formula	% Average of Edema Volume Increase At Various Times (minutes)					
	10	30	60	90	120	180
F0 (Negative Control)	100	90	75	75	75	75
F1	100	75	50	50	0	0
F2	100	25	25	25	25	0
F3	125	75	0	0	0	0
F4	169	113	113	56	6	6
F5	100	100	100	100	0	0
F6	113	113	88	6	6	6
Positive Control (Voltaren® Emulgel)	100	25	0	0	0	0

Tabel 6. Average of Inflammation Inhibition Percentage at Various Times

Formula	% Average of Inflammation Inhibition at Various Times (minutes)					
	10	30	60	90	120	180
F0 (Negative Control)	0,00	0,00	0,00	0,00	0,00	0,00
F1	0,00	16,67	33,33	33,33	100,00	100,00
F2	0,00	72,22	66,67	66,67	66,67	100,00
F3	-25,00	16,67	100,00	100,00	100,00	100,00
F4	-68,75	-25,00	-50,00	25,00	91,67	91,67
F5	0,00	-11,11	-33,33	-33,33	100,00	100,00
F6	-12,50	-25,00	-16,67	91,67	91,67	91,67
Positive Control (Voltaren® Emulgel)	0,00	72,22	100,00	100,00	100,00	100,00

Based on Tables 5 and 6, after the administration of the nanogel formulations to each treatment group, the greatest increase in edema volume at the 10-minute observation was observed in group F4, with a mean percentage of 169%. The administration of F4 at the 10-minute mark did not yet produce an anti-inflammatory effect, as indicated by a negative mean percentage of inflammation inhibition (-68.75%). At the 30-minute observation, varying results were obtained for the mean percentage of inflammation inhibition, with the highest inhibition observed in groups F2 (72.22%) and the positive control (72.22%). Groups F4, F5, and F6 did not show improvement in inflammatory symptoms, as reflected by their negative mean percentage values. The highest inhibition (100%) was observed between 60 and 180 minutes in groups F3 and the positive control (Voltaren® Emulgel). Other groups, including F1 and F5, achieved maximum inflammation inhibition (100%) at 120 minutes, while group F2 reached this level at 180 minutes. Groups F4 and F6 did not reach 100% inhibition during the 180-minute observation period. No inflammation inhibition effect was observed in the negative control group (F0) throughout the study period. An example of the anti-inflammatory test results for the F3 nanogel formulation is presented in Figure 5.

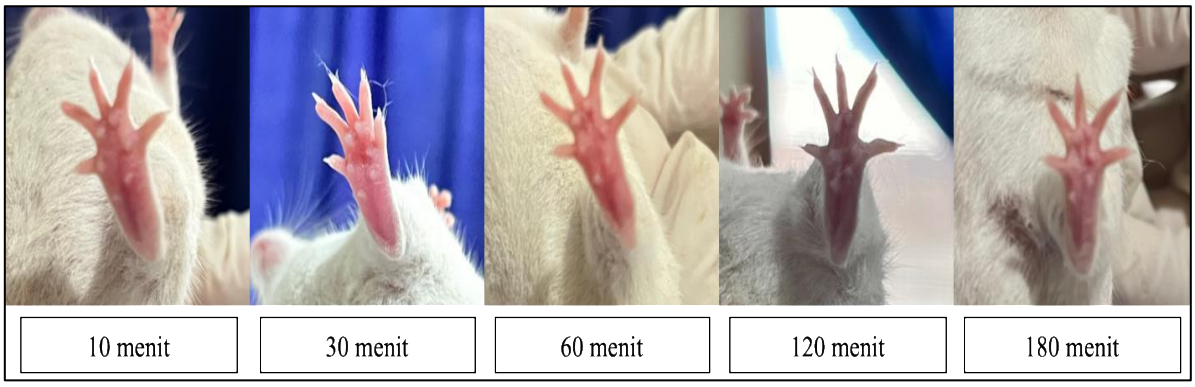

Fig 5. Contoh Hasil Uji Sediaan Nanogel Kelompok F3

Based on several studies, black pepper (*Piper nigrum*) is known to contain secondary metabolites such as flavonoids, alkaloids, and tannins.[7,21] Another beneficial compound found in black pepper is piperine.[7] According to Table 1, the phytochemical screening results showed that black pepper tested positive for alkaloids, flavonoids, and steroids. *Erythrina subumbrans* leaves are known to contain secondary metabolites including flavonoids, alkaloids, and saponins.[22] These compounds exhibit anti-inflammatory and antipyretic effects. Based on phytochemical testing, this plant tested positive for alkaloids, flavonoids, saponins, and steroids. The compounds responsible for the anti-inflammatory effect are flavonoids, which act by inhibiting the cyclooxygenase or lipoxygenase pathways and preventing leukocyte accumulation at the site of inflammation.[19] Other studies have shown that piperine, the main alkaloid compound in black pepper, exhibits potential anti-inflammatory effects, as demonstrated by carrageenan-induced paw edema assays.[10,11,23] The inflammatory mechanism is associated with the rupture of lysosomal membranes, leading to the release of hydrolytic enzymes that trigger a cascade of reactions promoting the production of key inflammatory mediators such as thromboxanes, prostaglandins, and leukotrienes.[24]

Piperine acts by inhibiting the activation of NF-κB and the phosphorylation of MAPK, thereby reducing the transcription of pro-inflammatory cytokines and enzymes such as COX-2 and iNOS.[25] The significant involvement of lysosomal enzymes in the inflammatory process has been widely documented. Other studies have also reported that piperine inhibits the production of nitric oxide and tumor necrosis factor-α, as well as the expression of several pro-inflammatory cytokines and matrix metalloproteinases.[26] Similarly to *Piper nigrum*, *Erythrina subumbrans* leaves have also been shown to exhibit anti-inflammatory effects. The leaves contain flavonoids, alkaloids, and tannin.[27] Phytochemical screening in this study revealed that the extract of *Erythrina subumbrans* leaves contains alkaloids, flavonoids, saponins, and steroids. Similar to black pepper, the flavonoid compounds are known to possess anti-inflammatory properties. Flavonoids, as members of the phenolic compound group, play a role in inhibiting COX-2 activity, thereby reducing prostaglandin formation and the release of arachidonic acid.[19,27] Another important class of secondary metabolites with anti-inflammatory activity is saponins. Saponins exert their anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), suppressing the expression of COX-2 and iNOS to reduce PGE_2 and NO levels, inhibiting the activation of the NF-κB and PI3K/Akt pathways, and decreasing the formation of reactive oxygen species (ROS).[28]

IV. CONCLUSION

The 96% ethanol extract of *Piper nigrum* contained alkaloids, flavonoids, and steroids, while the 70% ethanol extract of *Erythrina subumbrans* leaves contained alkaloids, flavonoids, saponins, and steroids. Significant differences were observed among the F1–F6 groups, negative control, and positive control ($p = 0.002$). The positive control (Voltaren® Emulgel) showed significant differences compared to all treatment groups ($p = 0.000$). The F3 group exhibited the most rapid and complete inhibition of inflammation (100%) at 60 minutes, indicating strong potential for further study.

V. ACKNOWLEDGMENTS

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