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Original Article

ANTI-INFLAMMATORY ACTIVITY AND IRRITATION TEST OF TOPICAL DOSAGE FORMS OF CLOVE LEAF ESSENTIAL OIL (*SYZYGIUM AROMATICUM*) IN ANIMALS

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ABSTRACT

Objective: Clove leaves (*Syzygium aromaticum*) contain compounds, namely eugenol, alpha-humulene, beta-caryophyllene, and caryophyllene oxide with the main compound eugenol, which has anti-inflammatory activity. One of dosage form to treat inflammation on the skin is a topical dosage form because it is quickly absorbed into the skin. This study aimed to formulate Clove Leaf Essential Oil (CLEO) and evaluate the physical properties, anti-inflammatory activity, and irritation effects of various topical dosage forms (emulgel, balsam, massage oil, and stick balm).

Methods: Research design was a pre-posttest control group. Essential oils were obtained using the steam distillation method. The four different concentrations of CLEO topical dosage form (emulgel, balsam, massage oil, and stick balm) are 5% and 10%. Then, test are conducted on the physical qualities (organoleptic, pH, displaced volume, spreadability, sticking power, moisture content and viscosity). Anti-inflammatory activity using the carrageenan-induced paw edema method in Wistar rats was divided into four groups are dosage form CLEO concentrations of 5% and 10%, base preparation, and positive control. Anti-inflammatory activity results were analyzed with Post-hoc statistical analysis with a 95% confidence level. The irritation method was used to measure the Primary Irritation Index (PII) followed procedure by Indonesian Food and Drug Authority about Guidelines for *in vivo* Preclinical Toxicity Testing.

Results: All these dosage forms met the physical requirements. Anti-inflammatory activity will rise as result of increasing the CLEO content in the formulae. All these dosage forms did not cause irritation in animal.

Conclusion: Among the four formulations, balsam CLEO 10% showed maximum inhibitory oedema (94.05%), at the end of 360 min followed by balsam CLEO 5%, emulgel CLEO 10%, stick balm CLEO 10% and massage oil CLEO 10%.

Keywords: Eugenol, Essential oils, Anti-inflammatory, Irritation test, Clove leaf, Emulgel, Stick balm, Balsam, Massage oil

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INTRODUCTION

Inflammation is a response to tissue injury and infection in the body's cells, resulting in cell damage [1]. Arachidonic acid will be released by cells so that it will be activated by several enzymes, including cyclooxygenase and lipoxygenase. Prostaglandins and leukotrienes are also responsible for inflammatory symptoms [2]. One of the natural ingredients that has anti-inflammatory potential is eugenol which comes from clove leaves. The potential of clove leaves is estimated to reach 2 million tons per year at a plant age of 6.5-8.5 y with an oil yield of 2-3%. The oil in clove leaves contains eugenol (90%), eugenol acetate (>8%), caryophyllene (2%), and other substances with an average content of below 0.5%. Clove leaf oil is clear to pale yellow in color, thick like oil, easily soluble in organic solvents, slightly soluble in water, and has a molecular weight of 164,201.

The mechanism of eugenol as an anti-inflammatory is by suppressing the action of NF-kB which is a receptor in the inflammatory pathway, however, the use of essential oils can directly irritate the skin surface so that using this dosage form can make it easier to use and provide a local anti-inflammatory effect. Another goal is to accelerate the anti-inflammatory effect because it works directly on the area of inflammation and does not go through the digestive system.

This research was focused to develop Clove Leaf Essential Oil (CLEO) into various forms, namely semisolid (balsam, emulgel, and stick balm) and liquid form (massage oil). Balsam, stick balm and emulgel are semisolid preparations which have the advantage of not causing irritation to the skin, having good adhesive power, being stable in use, easy to carry everywhere and having good distribute to the skin [3]. The liquid preparation is massage oil made from plant or animal oils. This topical preparation was tested for anti-inflammatory activity by observing the reduction in the percentage of edema in the dorsum of rats induced by carrageenan. The preparation was also tested for its irritating effect on rabbit test animals by applying it to the skin of the rabbit's back to observe the presence of erythema and edema. Based on the description above, research objectives can be formulated to determine the anti-inflammatory activity of semi-solid and liquid preparations of clove leaf essential oil (*Syzygium aromaticum*) in male Wistar rats and irritation tests in rabbits.

MATERIALS AND METHODS

Material (Style subheading)

Water vapor distillation, digital scales (Otsuka®), glassware (Pyrex®), basis, stirring rod, water bath, parchment paper, erlenmeyer, aluminum foil, cup, separating funnel, mortar, stamper, stopwatch, rat cage, rabbit cage, 2 ml injection syringe, 30 ml balsam pot. Clove leaf essential oil (CLEO), distilled water, anhydrous sodium sulfate (Na_2SO_4), carragenan, vaselinum album, liquid paraffin, cera alba, menthol. Research activities are carried out at the Universitas Negeri Semarang Biology Laboratory, Universitas Islam Indonesia Integrated Laboratory, and Universitas Islam Sultan Agung Integrated Pharmacy Laboratory. Animals are using Wistar rats weighing 150-210 g and 2-4 mo old and rabbit New Zealand weighing 1.5-2 kg and 3-4 mo old. In the cage room it is placed with good ventilation, sufficient light, temperature 22 ±3 °C and humidity 30-70%. It has a cage area of 150 cm^2 for mice and 300 cm^2 for rabbits. They were provided with food and water *ad libitum*. For the usage of animals and study protocol, approval from Komisi Bioetika Penelitian Kedokteran/Kesehatan Fakultas Kedokteran was obtained (No.60/II/2023/Komisi Bioetik).

Methods (Style subheading)

Preparation of clove leaf essential oil (*Syzygium aromaticum* (L.)

The extraction of essential oil from clove plant leaves by steam distillation. The process of evaporating the oil contained in clove leaves together with water vapor, which is then condensed again,

resulting in water and oil which are collected in the erlenmeyer flask. The water and oil that are still mixed are separated using a separating funnel due to the difference in volume weight of the water and oil. 200 gs of clove leaves were used with 2 l of distilled water; each steam distillation treatment was replaced with new leaves, accompanied by the same amount of new water [4].

Characterization of clove leaf essential oil

Clove leaves were collected at Curug Sewu Kendal. Determination of the levels of eugenol compounds contained in clove leaf essential oil using the GC-MS analysis method. Spesific gravity test. The specific gravity of CLEO was measured using a 25 ml pycnometer. For the first step, clean the pycnometer with ethanol and dry it until it is completely dry. Weigh the empty pycnometer and record the weight. Fill the pycnometer with water until it is full and open the capillary cap. Soak the pycnometer in a container filled with ice water until the temperature drops to 20 °C. After the temperature is reached, lift the pycnometer, then close the capillary tube, clean the water on the outside of the pycnometer and weigh it [5]. Refractive index test. Determination of the refractive index using a refractometer. The working principle of a refractometer is that if light passes from a less dense medium to a denser medium, the light will bend or refract from the normal line. The way it works is by inserting CLEO into a refractometer by maintaining a stable room temperature with the temperature of CLEO, then firing a laser. The laser beam that comes out will bend and be marked. The refractive index is a comparison between the speed of light in air and the speed of light in a substance at a certain temperature [6]. Optical Rotation. Determination of Optical Rotation using a polarimeter. The polarimeter tube was filled with a sample of clove leaf essential oil and made sure there were no air bubbles in the tube. The tool will automatically read the optical rotation value and observe the value read on the tool scale and record it [7]. The magnitude of the rotation direction of the polarized light field is expressed by the optical rotation value.

Table 1: Formulation design of balsam, emulgel, massage oil and stick balm

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8
CLEO	5	10	5	10	5	10	5	10
Liquid paraffin	8	8	15	1.25	-	-	2.4	2.4
Cera alba	6	6	-	-	-	-	11.69	11.69
Menthol	6	6	-	-	-	-	-	-
Vaselinum album	75	70	-	-	-	-	69.1	64.1
Carbopol 940	-	-	4	4	-	-	-	-
TEA	-	-	8	8	-	-	-	-
Sorbitol	-	-	2	2	-	-	-	-
Span 80	-	-	2.5	2.5	-	-	-	-
Tween 80	-	-	17.5	17.5	-	-	-	-
Methyl paraben	-	-	0.18	0.18	-	-	-	-
Propyl paraben	-	-	0.02	0.02	-	-	0.1	0.1
Propilen glycol	-	-	10	10	-	-	-	-
Aquadest	-	-	49.55	44.55	-	-	-	-
Menthol	-	-			4	4	-	-
VCO	-	-	-	-	40	40	-	-
Oleum arachidis	-	-	-	-	51	46	-	-
Lanolin	-	-	-	-	-	-	5.31	5.31
Isopropyl myrstate	-	-	-	-	-	-	6.4	6.4

F1 and F2 are balsam, F3 and F4 are emulgel, F5 and F6 are massage oil, F7 and F8 are stick balm, respectively

Preparation of balsam

Paraffin liquid, cera alba, menthol, vaselinum album, and clove oil, and then Vaseline album, cera alba, menthol, and paraffin liquid were melted using a cup over a water bath at a temperature of 70-75 °C then stirring occasionally until the ingredients melted, and the cup was removed from the water bath. That mixture waited until warm and then mixed in the CLEO gradually while stirring until it was homogeneous. Mixing was performed at a cool room temperature, and then the homogeneous balsam preparation was placed into the balsam pot.

Preparation of emulgel

A gel phase was made by mixing Carbopol 940 with warm water and leaving it for 1×24 h so that it swells completely and is stable. After the gel was stable, TEA was mixed until homogeneous. The water phase was prepared by dissolving Tween 80 in distilled water, methylparaben, propylparaben, and sorbitol in propylene glycol, and then mixed until homogeneity. The oil phase was prepared by mixing Span 80 into liquid paraffin. The emulsion phase was prepared by mixing the water and oil phases using a melting technique. After the emulsion phase had cooled, CLEO was added to the gel phase and stirred until homogeneous [8].

Preparation of massage oil

Menthol was dissolved with arachidis oil in a mortar (M1). VCO mixed with CLEO in a mortar (M2). Stirred mixtures (M1) and (M2) were added to some of the arachidis oleum. The mixture was placed in a bottle, and the remaining oleum arachidis were added and mixed until homogeneous [9].

Preparation of stick balm

Cera alba, propylparaben, lanolin, alba Vaseline, liquid paraffin, and isopropyl myristate were melted in a water bath at a temperature of 85 °C. CLEO was added to the warm mixture, stirred, cooled, placed in a balm-stick container, and refrigerated for 24 h [10].

Evaluation parameters

Phsyical appearance

Organoleptic tests were carried out by observing the aroma, color, and shape using the five human senses. Good organoleptics must be in accordance with the ingredients used, such as the aroma, odor, and color [11]. A homogeneity test was carried out by dripping 3-4 drops of massage oil into a glass object and covering it with another glass object. Visually observe the homogeneity and absence of coarse grains in the massage oil [11].

pH determination

The pH was checked using a pH meter by pouring the sequential oil preparation into a glass beaker and testing with a pH meter. The pH parameters are adjusted to the skin's pH, namely 4.5-7. If the preparation is alkaline, it will cause the skin to feel slippery dry quickly and can affect skin elasticity, whereas if the preparation is acidic it will cause the skin to become easily irritated [12].

Spreadability

Balsam was weighed as much as 0.5 g and placed in the center of a round glass scale. Another round of glass was placed on top of the balsam, left for 1 min, and the diameter of the spread preparation

was measured. A load of 50 g was added to the top of the glass and it was left to sit for 1 min. The diameter of the spread preparation was noted, and additional loads of 100 g and 150 g were added. The diameter of the spread was recorded after 1 min [13].

Displaced volume test

The transferred volume test was carried out by pouring the massage oil from the package into a dry measuring cup with a volume no more than two and a half times the volume being measured. The sample was allowed to sit for 30 min until no air bubbles were formed. Ten measuring cups were used to measure the volume. The transferred volume parameters must correspond to the volume stated on the label or brochure, which is not more than 100% or not less than 95% [11].

Viscosity

Viscosity tests were performed to determine the viscosity of the massage oil preparation using a Brookfield viscometer. Viscosity in preparations was measured using a spindle size of 61 µm [14]. The oil viscosity parameter was between 2.3-6.0 cps [11].

***In vivo* anti-inflammatory activity**

This research have ethical permission from Komisi Bioetika Penelitian Kedokteran/Kesehatan Fakultas Kedokteran Universitas Islam Sultan Agung Number60/II/2023/Komisi Bioetik. Animals were tested for adaptation to the surrounding environment for 3 days, by providing regular food and keeping the cage clean. The test animals used were 24 divided into 4 groups, each group consisting of 6 mice there are positive control, negative control, formulation (balsam, emulgel, massage oil and stick balm) with a concentration of 5% and 10%) and each group contains 4 animals. In the initial test, each mouse was weighed and recorded, then the test animals were anesthetized first using ether, all mice had their fur shaved 2x2 cm until smooth and hair removal cream was applied to remove the hair and left for one day to avoid inflammation after shaving and administering hair removal cream. The back of each rat to be treated was marked to distinguish one rat from another on the tail, then the volume was measured first and recorded as initial thickness (T0) then 0.15 ml of 1% carrageenan was induced on the rat's back subcutaneously with angle 10-30 ℃. After being given the carrageenan induction, wait 3 h to see the effect of the carrageenan on the rats' backs. Then, 0.5 g of the test sample was applied to the back of the rat for each treatment, namely:

Group I: negative control (basis of each dosage form)

Group II: positive control

Group III: dosage form of 5% clove leaf essential oil

Group IV: dosage form of 10% clove leaf essential oil

The skinfold thickness on the back of the rat was measured using a digital slide timer and the skinfold thickness was recorded as the initial thickness before carrageenan induction (T0). Next, wait three hours after being induced by carrageenan, then topically treat it and measure the skinfold thickness again using a caliper as the back skinfold thickness after initial treatment (Tt). Measurements were carried out for 6 h every 1 hour. The percentage reduction in oedema is calculated using the formula:

Percent oedema= (Tt-T0)/T0 x 100%

Information:

Tt = back skinfold thickness for each group at time t

T0 = back skin fold thickness of rats in each group before any treatment

Then, the percent reduction in edema is calculated to determine the amount of edema inhibition using the formula:

Percent reduction in oedema = (a-b)/a x 100%

Information:

a = % edema in negative control group

b = % edema in the preparation treatment group

For the results obtained, the greater the percent reduction in oedema, the better the anti-inflammatory effect of the sample [15].

Irritations testing in animal

Data collection for the irritation test begins with the acclimatization process for 5-7 days and is placed in individual drums. Shaving the rabbit's back with a size of 2x2 cm and applying Veet cream to remove the fur on the rabbit's back is done at least 24 h before testing. After ensuring that it is clean, massage oil is applied to the rabbit's back with a dose of 0.5 ml. The area being treated is covered with gauze and plastered using non-irritant plaster. The observation process is calculated after removing the plaster at intervals of 24, 48, 72 h. Observe the erythema and edema that forms and record a score. Primary Irritation Index Category based on BPOM [16].

Data analysis

The results were expressed as mean values ±standard deviation (SD) using SPSS version 20. The statistical differences between groups through one-way analysis of variance (ANOVA) with significance level 95% (α=0.05) followed by Post Hoc test.

RESULTS AND DISCUSSION

Result (Style subheading)

Characterization of clove leaf essential oil

The characteristics of CLEO in this study include organoleptic determination, determination of eugenol compounds, determination of specific gravity, determination of refractive index, and determination of optical rotation that meets the requirements [17]. The yield obtained from the steam distillation process of clove leaves was 2.42%. In Nirwana's research, it was explained that using the water vapor distillation method on clove leaves obtained a yield of 3.44%; the yield range for clove leaf essential oil obtained using water vapor distillation was 1-4% [18]. Characteristics were carried out to determine the compound content in CLEO (table 2).

Table 2: Characteristics of clove leaf essential oils

Karakteristik	Result	SNI	Keterangan
Color	Yellow	yellow-brown color	Appropriate
Smell	Smell of cloves	Smell of cloves	Appropriate
Texture	Liquid	Liquid	Appropriate
Specific Gravity	1.04	1.025-1.049	Appropriate
Refractive index	1.533	1.5280-1.5350	Appropriate
Optical rotation	-1.421°± 0,037°	-1.3°	Appropriate

GC-MS analysis in table 3 was carried out at the Integrated Laboratory of the Islamic University of Indonesia Yogyakarta; the results of the analysis were obtained in the form of a chromatogram showing 4 peaks, then each peak was analyzed based on the mass or molecular weight of the compound. From the results of the analysis, 4 component compounds were obtained, namely the eugenol, a 2,3-Dimethylamphetamine ($C_{11}H_{17}N$), Caryophyllene ($C_{15}H_{24}$), alpha Caryophyllene ($C_{15}H_{24}$).

Characterization of physical parameters clove leaf essential oil formulation

Evaluation physical parameters from all dosage forms met the physical requirements from Indonesian Pharmacope. The results obtained in table 4 for each preparation are in accordance with the requirements for topical preparations [11].

Table 3: Result analysis clove leaf essential oil from GC-MS

No	RT	Area	Substance	RM	Mr (g/mol)	% Area
1	10.220	160383	Eugenol	$C_{10}H_{12}O_2$	164	28.07
2	10.330	81597	2,3-Dimethylamphetamine	$C_{15}H_{24}$	163	14.28
3	10.750	319334	Caryophyliene	$C_{15}H_{24}$	204	55.88
4	11.212	10136	Alpha Caryophyllene	$C_{15}H_{24}$	204	1.77

Table 4: Evaluation of physical parameters of different formulation batches (n=3)

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8
Color	Yellow	Yellow	Yellow	White	Yellow	Yellow	Yellow	Yellow
Homogeinity	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen
pH	6.025±0.053	5.648± 0.098	5.9±0.377	6.5±0.141	5.06±0.015	5.25±0.010	5.44±0.300	4.91±0.155
Displaced volume	-	-	-	-	99.45%±0.577	98.33%±1.000	-	-
Viscosity	10.72±0.640	7.28±0.288	2.934±341.666	2.481±155.754	5.20±0.069	5.32±0.080	-	-
Spreadability (gm cm/s)	4.43±0.152	4.9±0.1	5.6±0.449	5.2±0.047	-	-	-	-
Moisture test	-	-	-	-	-	-	44. 33 °C±1.527	44 °C±1
Sticking power	-	-	2.14±0.617	2.62±1.116	-	-	-	-

F1 and F2 are balsam, F3 and F4 are emulgel, F5 and F6 are massage oil, F7 and F8 are stick balm, respectively

Table 5: *In vivo* anti-inflammatory activity in formulation

Formulation code	% inhibition of oedema					
	At 60 min	At 120 min	At 180 min	At 240 min	At 300 min	At 360 min
Positive control balsam (Geliga®)	9.28%	24.41	43.21	54.83	67.80	96.69
Basis balsam	111.34	87.94	78.72	71.63	60.28	53.19
F1	27.81	30.19	45.33	66.42	79.01	91.67
F2	44.31	54.66	70.26	79.68	82.16	94.05
Positive control emulgel (Voltaren®)	47.2	53	54.68	64.78	71.65	79.45
Basis emulgel	144.52	114.38	102.05	95.2	85.61	78.76
F3	16.53	17.43	22.23	34.14	49.94	65.74
F4	15.67	6.56	18.54	30.4	43.09	72.24
Positive control massage oil (GPU®)	14.86	20.97	31.84	35.24	57.7	69.08
Basis massage oil	175.81	133.98	102.61	79.73	67.97	35.94
F5	24.04	22.95	26.43	27.17	36.41	51.6
F6	24.97	45.51	46.19	52.3	54.87	62.46
Positive control stick balm (Geliga®)	15.69	17.37	32.90	34.66	45.20	83.36
Basis stick balm	119.25	95.65	80.12	63.35	49.06	32.29
F7	15.08	22.58	35.23	37.06	50.97	54.93
F8	29.55	29.60	33.43	44.23	51.08	69.03

CP are positive control, formulations F1 and F2 are balsam, F3 and F4 are emulgel, F5 and F6 are massage oil, F7 and F8 are stick balm, respectively

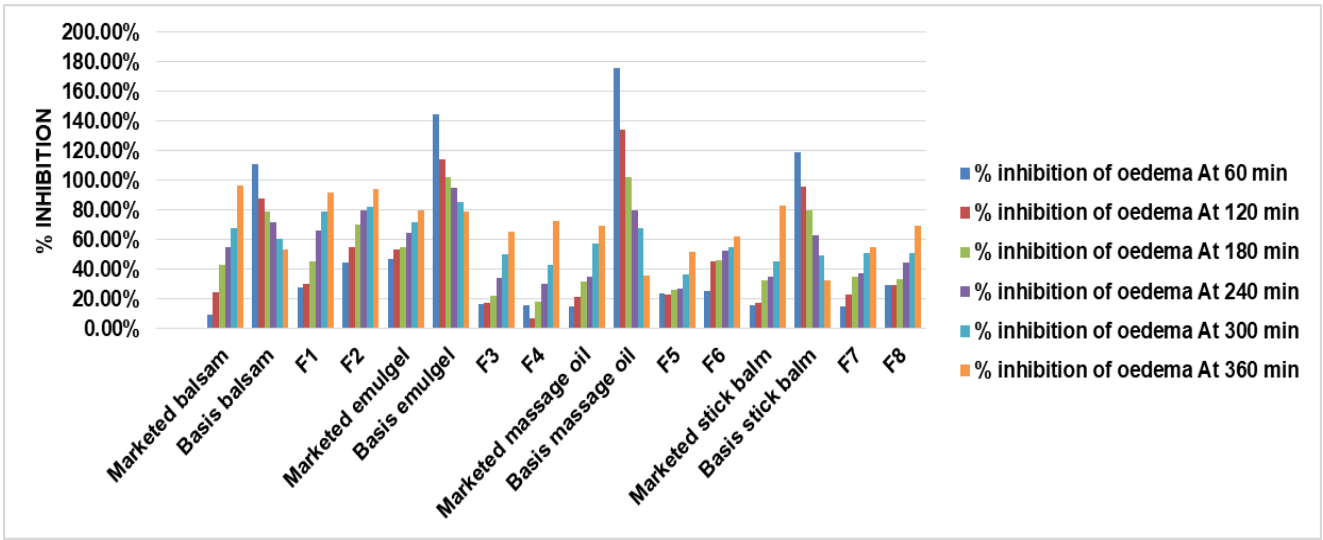

Fig. 1: Percentage inhibition profile of *in vivo* anti-inflammatory studies

Table 6: Data of statistical analysis anti-inflammatory activity at 360 min. p>0.05 not significant

Comparison formulation	P value	Significance
Positive control Vs F2	0.380	No
Positive control Vs F4	0.115	No
Positive control Vs F6	0.151	No
Positive control Vs F8	0.820	No
Negative control Vs F4	0.113	No

Irritations testing in animal

The irritation test aims to determine the irritating effect of the joint that has been made after being applied to the skin, which ensures the safety of the joint. The classification of the irritation value based on rule from indonesian food and drug authority about guidelines for *in vivo* preclinical Toxicity Testing. Massage oil obtained results of 0.0; balsam 0.1; stick balm 0.2; emulgel 0.3.

Discussion (Style subheading)

The GC (Gas Chromatography) instrument was used to separate the eugenol compound from other compounds and the MS method was used to detect the presence of the eugenol compound, which was indicated by the presence of wave 164. The GC-MS (Gas Chromatography-Mass Spectrometry) test obtained results indicating the eugenol compound at a retention time of 10.220 min and an area of 160383 or 28.07%. In [19]'s research, GC-MS testing resulted in eugenol compounds with a retention time of 7.605 min and an area of 762327 or 65.39%. The eugenol content can be influenced by the length of the drying process, if it is too long the oil content will evaporate, the origin of the clove leaf-producing area, climate, season, location, geography and geology. The eugenol content will be maximum if clove plants grow in a hot climate with sufficient rainfall at an altitude of 300-600 meters above sea level with a temperature of 22 °-30 °C. From the table 2 state that specific gravity, refractive index value and optical rotation of CLEO was in accordance with clove oil standards [17]. Quality and purity of the essential oil in accordance with Refractive index value and Optical rotation [6].

Result of physical parameters each dosage forms accordance with BPOM requirement. Homogeneity test to see that the ingredients in the dosage formulation are evenly mixed between the active substance and excipients. The physiological pH of the skin ranges from 4.5 to 6.5; hence, the greater the difference between the pH of the topical treatment and the physiological pH (which might be higher or lower), the more likely a negative reaction. Negative responses might occur when the skin struggles to neutralize the pH of the semisolid dosage form, causing fatigue. Negative responses lead skin to become dry, cracked, sensitive, and easily infected [20]. The pH testing of balsam, stick balm and emulgel accordance with BPOM (Badan Pengawas Obat dan Makanan) so that it does not cause irritation to the skin. Transferred volume testing on massage oil preparations aims to provide assurance that if transferred from the original container it will provide a constant volume of the preparation and in accordance with the label. The difference in CLEO concentration in each formula causes differences in the viscosity of the resulting preparation. The viscosity of balsam is inversely proportional to its spreadability, but it is different from emulgel because emulgel contains Carbopol 940, which functions as a gelling agent and the addition of CLEO, which affects viscosity. The higher the CLEO concentration used, the greater the resistance of the gel to flow and spread [20]. The viscosity value describes the ability to spread on the skin which affects the absorption of the active substance. Test the melting point on the stick balm preparation to determine the optimal temperature for storage in the container so that it does not melt.

In vivo anti-inflammatory activity state in table 5. Among the four formulations, the preparation showed the maximum inhibitory oedema (94.05%), namely CLEO balsam 10% at the end of 360 min followed by the formulation CLEO balsam 5%, emulgel 10%, stick balm 10% and massage oil CLEO 10%. Percentage inhibition profile of *in vivo* anti-inflammatory studies state in fig. 1. From this fig. it can conclude that as the observation time increases, the reduction in edema an increase, meaning the anti-inflammatory effect gets better. The anti-inflammatory activity test was carried out on mice that were induced with 1% carrageenan and observed every hour for 6 h. Based on research by [21] stated that the mechanism of eugenol and beta-caryophyllene as an anti-inflammatory is by inhibiting prostaglandins and neutrophil chemotaxis in white mice. The anti-inflammatory effect of eugenol is the same as a COX antagonist (indomethacin) and a selective COX-2 antagonist (celexocib). The performance of beta-caryophyllene is the same as eugenol by inhibiting pain with blocking the cyclooxygenase enzyme pathway so that prostaglandin production decreases [22]. The magnitude of the reduction in edema shows the great ability of the compound to provide anti-inflammatory activity by reducing inflammation by 50% or more.

The results of irritations testing in animal obtained for each dosage forms can be categorized as very mild irritation or non-irritant. We were not analysis the body weight of rabbits because in research by [23] these factors was no discernible difference in the weight of the rabbits during the test. (Sig-value>0.05). In research, [24] explained that the groups that have the potential to cause high irritation are phenol, alcohol, ketone and ether, respectively. Eugenol is one of the main compounds in CLEO which has a phenolic group that has low irritating potential. To reduce the level of irritation caused by CLEO by combining it with appropriate excipients to make a dosage forms.

CONCLUSION

All these dosage forms (massage oil, balsam, stick balm and emulgel) meet physical requirements anti-inflammatory activity and did not cause irritation in animal. Among the four formulations, the preparation showed the maximum inhibitory oedema (94.05%), namely CLEO balm 10% at the end of 360 min followed by the formulation CLEO balm 5%, emulgel 10%, stick balm 10% and massage oil CLEO 10%,

Suggestions for further research include rabbit skin histopathology and skin tissue imaging to determine the anti-inflammatory mechanisms of all topical dosage forms from clove essential oil. Suggestions for further research include rabbit skin histopathology and skin tissue imaging to determine the anti-inflammatory mechanisms of various preparations from clove essential oil.

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AUTHORS CONTRIBUTIONS

Data gathering and idea owner of this study: Ika Buana Januarti, Study design: Ika Buana Januarti, Fadzil Latifah, Data gathering: Ricky Firmansyah, Aulya D. Pertiwi, Niken Agustya, Hesti Widyawati, Writing and submitting manuscript: Ika Buana Januarti, Editing and approval of final draft: Ika Buana Januarti, Fadzil Latifah.

CONFLICT OF INTERESTS

Declared none

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