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Anti-inflammatory effectiveness of *Cymbopogon citratus* DC. Staff in Na CMC gel preparation

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ABSTRACT: *Cymbopogon citratus* DC. Stapf contains saponins, tannins, and flavonoids. Traditionally, tea from lemongrass leaves is useful as an anti-inflammatory, anti-malarial, anti-mutagenic, anti-mycobacterial, antioxidant, hypoglycemic, and neurobehavioral. A study has been conducted to formulate an anti-inflammatory gel preparation from *C. citratus* extract. The study was conducted by macerated a *C. citratus* leafs using 70% ethanol, then evaporated in a rotary evaporator at a temperature of 40 °C until viscouse extract obtained and evaluated. The anti-inflammatory gel was prepared using Na CMC 6%, with the concentration of *C. citratus* extract 6%, 8%, and 10%. The resulting gel preparation was evaluated for its physical and chemical properties and then tested for its anti-inflammatory effects using male mice (*Mus musculus* L.). Flavonoids, alkaloids, tannins, and saponins are found in this *C. citratus* extract. The characteristics of the C. citratus extract gel made were, brown and dark brown, had a distinctive *C. citratus* odor, semi-solid, and homogeneous, had a pH of 5.54–5.62, a viscosity value between 57000-73000 cP, had pseudoplastic flow properties, a specific gravity between 1.055-1.078 g/cm^3, had an adhesiveness of 231-252 seconds, and a spreadability of 3490.11-3021.20 mm^2. The gel contained 10% of C. *citratus* extract, indicating the highest anti-inflammatory effect, with a decrease in edema diameter of 67.79% in the legs of male mice.

KEYWORDS: Anti-inflammatory gel; lemongrass; topical formulation; Na CMC; rat paw oedema.

INTRODUCTION

Lemongrass, a member of the Gramineae family, is a type of grass with a lemon scent and is known as *Cymbopogon citratus* (*C. citratus*). This plant is widely cultivated in Indonesia. Traditionally, it is said that tea made from *C. citratus* leaves is commonly used as an anti-inflammatory for gastrointestinal disorders and fever[1],[2]. These infusions are commonly used to treat bronchitis, throats, and laryngitis [3],[4]. Studies indicate that *C. citratus* has many pharmacological effects, such as anti-inflammatory, antibacterial, free radical scavenging, antioxidant, antimalarial [5], Antinociceptive, Hypocholesterolemic, antifungal, anti-obesity, antimalarial, antihypertensive, dermatotoxic, anti-diarrheal, antimutagenic, anti-protozoan, anti-hepatotoxic, anthelmintic, acaricidal, antiglycation, hypoglycemic, and neurobehavioral [6]. *C. citratus* plants contain many secondary metabolites, as saponins, tannins, anthraquinones, flavonoids, phenols, alkaloids, terpenes, aldehydes, alcohols, and esters[7],[8]. The anti-inflammatory effect has been proven by research showing that polyphenol compounds, especially in *C. citratus* leaf extract, are a natural source of a new and safe anti-inflammatory drug [9],[10]. In a study on polyphenols from the leaves (*C. citratus* DC. Stapf), as an anti-inflammatory agent, with the method of edema induced by 1% carrageenan, it was found that a concentration of 1% could reduce edema in the feet of Wistar rats by 30% and at a concentration of 4% by 43%[2]. Citral, derived from *C. citratus*, significantly inhibits inflammatory mediators and can be used as an ingredient in lotions, creams, gels, and ointments to treat topical inflammation [4].

A system consisting of a suspension of small particles or large inorganic molecules in a liquid. It is a semi-solid preparation that has the advantage of a fairly high water content so that it provides moisture that is cooling and provides a comfortable feeling to the skin. It is not sticky, easy to apply, easy to wash, and does not leave an oily layer on the skin, thus reducing the risk of further inflammation owing to the accumulation of oil in the pores [11],[12]. One of the gelling agents that is widely used in research is carboxymethyl cellulose (CMC) because it is non-toxic, non-irritating, biocompatible with the skin, suitable for biomedicine, and has

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good spreadability even with the addition of extracts. Na CMC 3.0-6.6% is good as a gelling agent [11],[13]. *C. citratus* extract as an anti-inflammatory has great potential for developing into a gel pharmaceutical preparation. Therefore, a study was conducted on an anti-inflammatory gel formulation with the active substance of *C. citratus* leaf extract using the gelling agent CMC.

MATERIALS AND METHODS

Materials

The leaf and stem powders of *C. citratus* (DC.) Stapf was collected from the Research Institute for Spices and Medicinal Plants (BALITTRO), Bogor, West Java, Indonesia. Na CMC, propylene glycol, Methyl paraben, distilled water, 70% ethanol, magnesium, concentrated HCl, $FeCl_3$, 1% carrageenan, 0.9% NaCl, and Voltaren emulgel® as positive control.

Test animals

The test animals used in the study were 24 male mice (*Mus musculus* L.) of the Deutche Denken Yoken (DDY) strain aged 2-3 months, weighing 20-30 g and obtained ethical approval by the Health Research Ethics Commission of National Development University "Veteran" Jakarta with No. B/1396/V/2018/KEPK. The test animals were randomly divided into six groups, each consisting of four mice.

Methods

Preparation of *C. citratus* extract

Lemongrass extract was prepared by the maceration method using 70% ethanol, with a ratio of simplicia to solvent of 1:10. 1000 g of simplicia powder of *C. citratus* was put into the macerator, and 10 L of 70% ethanol was added. The leaf and stem powders of lemongras were macerated for 18 h and then filtered. The extraction process was repeated twice, and the extract results were combined and evaporated with a rotary evaporator at no more than 40 °C until a viscous extract was obtained [14].

Phytochemical screening

Flavonoid Test: A total of 0.5 g -of lemongrass (*C. citratus*) extract in a cup was added to 2 mL of 70% ethanol, stirred, then 0.5 g of magnesium powder and 3 drops of concentrated HCl[14]. The presence of flavones is indicated by the formation of an orange to red color, while flavanols if the color is red to dark red, and dark red to purplish red indicates the presence of flavanones; weak if the foam is <1 cm, strong if the foam is 1.2 cm, and very strong if the foam is more than 2 cm [14]. Saponin Test: 0.5 g of extract was put into a test tube containing 20 mL of distilled water, shaken vigorously, and then observed for 15-20 minutes to form foam. The presence of saponin in the extract is strongest if the saponin content is greater than 2 cm, strong if the foam is 1.2 cm, and weak if the foam is smaller than 1 cm [14]. Tannin Test: 0.5 g of extract was put into a cup containing 2 mL of 70% ethanol, stirred, and 3 drops of $FeCl_3$ were added; a distinctive blue color occurred. blue-black, green, or blue-green, and the sediment indicates the presence of tannin. Alkaloid Test: 10 mg of extract was added to 5 mL of 25% ammonia, and then 20 mL of chloroform was added. The mixture was filtered to obtain water and organic solvent layers. If Dragendorff's reagent is added, an orange color is formed, indicating the presence of alkaloids, or a white precipitate is formed if Mayer's reagent is added [14].

Gel extract of C. *citratus* preparation

All ingredients were weighed according to those listed in Table 1. Na CMC (gelling agent) is dissolved with hot distilled water 20 times in a mortar while stirring continuously until perfectly dispersed and a gel is formed. Methyl paraben and propylene glycol were dissolved in distilled water and added to the viscous extract of *C. citratus*. The two solutions were mixed, added to the gel, and stirred until homogeneous. The resulting gel was evaluated for its physical and chemical properties, including organoleptic examination (shape, odor, color), pH, viscosity and flow properties, homogeneity, specific gravity, spreadability, and adhesiveness [15], [16].

Table 1. ***C. citratus*** gel extract formulas.

Materials	Weight (%)			
	Blank	II	II	III
C. citratus extract	0.00	6.00	8.00	10.00
Na CMC	6.00	6.00	6.00	6.00
Propylene glycol	8.00	8.00	8.00	8.00
Methyl paraben	0.20	0.20	0.20	0.20
Distilled watert ad	100	100	100	100

Anti-inflammatory activity test

Anti-Inflammatory Activity Test with Carrageenan Induction Method on Rat Paws [17]. The protocol was approved by the Health Research Ethics Commission UPN Jakarta. Each mouse was weighed and marked on its left paw by using a marker up to the ankle. The initial diameter of the mouse paw was measured before treatment and expressed as the basic paw diameter (Do) using a digital caliper. The left mouse paw was cleaned with 70% ethanol before being injected intraplantar with 0.1 mL of 1% carrageenan suspension. One hour after injection with the carrageenan suspension, each group was given a topical treatment of 100 mg. The diameter of the left paw of the mouse was measured again using a digital caliper 60 min after administration of the anti-inflammatory gel. The changes in the level of swelling that occurred were recorded every hour for 8 h as the diameter of the rat's paw (Dt). The diameter of inflammation is the difference in the diameter of the mouse paw before and after injection with carrageenan [1], [18]–[20]. Calculate the percentage of edema and percentage of edema inhibitor:

$$\% \text{ Oedem} = \frac{\text{Dt} - \text{Do}}{\text{Do}} \text{ x } 100\%$$

Information:
Dt: Diameter of the mouse's paw in each group at time t
Do: Diameter of the mouse's paw in each group before any treatment
% Inflammation Reduction = (a - b)/a x 100%
a =% average inflammation in the control group
b =% Average inflammation in the test and comparison groups

▪ DISCUSSION

The extract obtained in this study was 350 g (yield 35%). The results of the organoleptic examination of the *C. citratus* extract are in viscous form, blackish brown with a distinctive Lemongrass, *C. citratus,* odor and a slightly sour, bitter taste, and soluble in propylene glycol. The extract had an acidic pH of 5. Lemongrass (*C. citratus*) extract is proven not to contain ethanol, so it meets the requirements as an active substance for gel preparations. The physical and chemical properties of *the C. citratus* extract are shown in Table 2.

Table 2. Phytochemical screening of *C. citratus* extract.

No.	Compound group	Observation	Result
1.	Flavonoid	Reddish-orange color formed	+
2.	Saponins	stable foam formed	+
3.	Tanins	Blackish-blue-green color formed	+
4.	Alkaloids	Brownish-orange color formed,	+
		white sediment formed,	+
		red color formed	+

The results of the gel preparation evaluation are shown in Table 3. All formulas had a semi-solid gel form, brown color, and a distinctive odor of lemongrass, which increased with increasing concentration of *C. citratus* extract. The C. citratus gel preparation had a pH of 5.54-5.62, which was more acidic than that of the blank gel (6.07). These results indicate that more extract added to the preparation can increase its acidity, as the lemongrass extract has an acidic pH of 5.0. The average pH of the gel preparation falls within the acceptable range for skin pH, between 4.5 and 6.5. All gel formulations were homogeneous. Upon examination, the gel was evenly dispersed on the glass, exhibiting no clumping, a smooth surface, and an absence of coarse grains. This uniformity ensured that the active ingredients in the gel preparation were consistently distributed with

each application to the skin [21]. The average specific gravity of all gel preparations was greater than one, indicating that the gel had a specific gravity higher than that of water.

Table 3. Evaluation of the physical and chemical characteristics of *C. citratus* extracellular gel.

Formula	Organoleptic and homogeneity	pH	Specific gravity	Viscosity (cP) and flow properties	Adhesion time (s)	Spreadability (mm^2)
Blank	Semi-solid, creamy, odorless, and homogeneous	6.07±0.02	1.043±0.001	54000±1000 Pseudoplastic	220±2.5	3665.68±62.09
FI	Semi-solid, brown, citrus-like odor, and homogeneous	5.62±0.001	1.055±0.001	57000±1000 Pseudoplastic	231±4.0	3490.11±159.31
FII	Semi-solid gel, brown, strong citrus odor	5.57±0.001	1.065±0.001	69000±1000 Pseudoplastic	240±3.6	3149.94±152.46
FIII	Semi-solid, dark brown, very strong lemongrass flavor, and homogeneous	5.54±0.001	1.078±0.001	73000±1000 Pseudoplastic	252±5.1	3490.11±260.21

The average viscosities of the Blank, FI, FII, and FIII groups were 54000±1000, 57000±1000, 69000±1000, and 73000±1000 cP, respectively. The thicker the preparation, the greater its viscosity. Many factors influence viscosity, including stirring and mixing during the preparation process, addition of a gel base, presence of humectants, and particle size [22],[23]. The results of the flow property test show a consistency curve approaching a low rate of shear and does not have a yield value; therefore, it is interpreted as a pseudoplastic flow with a non-Newtonian system. The results of evaluating the flow properties on the blanks, F I, F II, and F III, are shown in Figure 1.

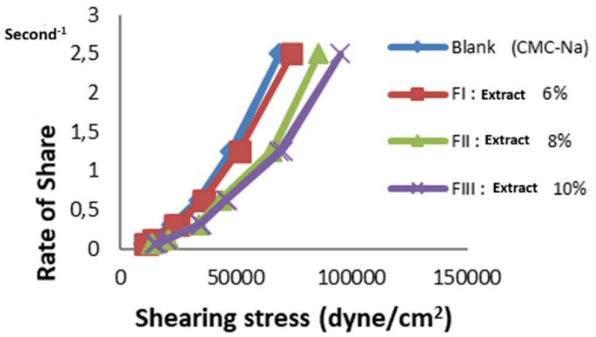

Figure 1. Rheogram of flow properties of gel preparations.

These results indicate that gel adhesion increases with higher specific gravity and viscosity. The greatest adhesion was exhibited by the formula containing the largest *C. citratus* extract (252 s), whereas the formula containing the smallest extract had the greatest adhesion (231 s). The decrease in gel spreadability was closely related to the gel viscosity value. The greater the gel viscosity value, the smaller the resistance or obstacle for gel preparation to spread; thus, the spreadability value decreased [24]. If the gel is too thin, it will be difficult to stick to the skin; however, if it is too thick, it will be difficult to apply it to the skin surface. Although there was a decrease in spreadability, the results still showed that all gel preparations met the gel spreadability requirements.

The results of the calculation of the percentage of inflammation reduction (edema) in the feet of male mice (*Mus musculus* L.) are shown in Table 5 and the inflammation reduction curve in Figure 2. The results of the reduction in edema in the normal controls and negative controls did not show any decrease in edema at 8 h; the decrease was only 8.32%. An anti-inflammatory effect was demonstrated when test animals induced with 1% carrageenan exhibited a reduction in edema of 50% or more. These results indicated that the CMC Na gel base did not demonstrate anti-inflammatory effects [12]. However, there is a possibility of decreased edema due to the cold sensation of the gel preparation applied to the feet of rats. The positive control (administration of voltaren® Emulgel with the active substance sodium diclofenac, which works by stabilizing the lysosomal

membrane, inhibiting the release and activity of inflammatory mediators (histamine, serotonin, prostaglandins), inhibiting cell migration to the site of inflammation, and suppressing pain) decreased edema by 53.06%. The reduction in edema observed 8 h after the animals were treated with FI, FII, and FIII indicated a decreasing edema diameter of 50.30%, 59.98%, and 67.79%, respectively. All three gel preparations exhibited anti-inflammatory effects that intensified with increasing concentrations of *C. citratus* extract.

Table 3. Results of Percentage Reduction in Inflammation (Oedema) in the Feet of Male Mice (*Mus musculus* L.)

Groups	Reduction of inflammation (Oedema) (%)							
	1h	2h	3h	4h	5h	6h	7h	8h
Normal control	0	0	0	0	0	0	0	0
Negative control	2.95	4.93	6.78	7.19	7.60	8.06	8.38	8.32
Positive control	6.38	7.08	21.55	26.01	26.67	43.37	47.52	53.06
FI	22.79	14.72	14.12	22.70	33.34	40.06	44.20	50.30
FII	14.05	10.45	23.21	30.24	40.20	50.77	54.58	59.98
FIII	16.57	13.72	27.35	35.58	45.89	56.53	61.67	67.79

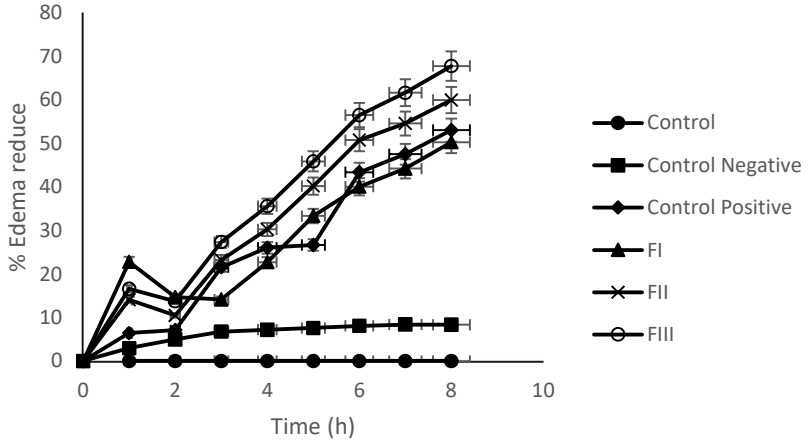

Figure 2. Rheogram of the anti-inflammatory effect of *C. citratus* extract gel.

The test results were analyzed statistically using one-way ANOVA using SPSS version 16.0. ANOVA was used to determine whether there was a difference between each group. In this ANOVA test, the requirements for normality and homogeneity of the data were met. The normality test was carried out using the Kolmogorov-Smirnov method to determine the distribution of data on the percentage of inhibition of mouse paw edema at hours 1, 2, 3, 4, 5, 6, 7, and 8, where the results showed that the data for all treatment groups were normally distributed. The homogeneity test of the percentage of inhibition of rat paw edema was performed using the Levene method. The results showed that the percentage of inhibition of mouse paw edema varied homogeneously ($\rho \geq 0.05$) only at 1 h, whereas at 2, 3, 4, 5, 6, 7, and 8 h, it did not vary homogeneously ($\rho \leq 0.05$). Normally distributed and homogeneous data were analyzed statistically using one-way Analysis of Variance (ANOVA). Those that did not continue were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney U test.

At the eighth hour, the normal control group demonstrated a significant difference compared to the positive control group, as well as all low, medium, and high concentration test groups at the 0.05 significance level ($p \leq 0.05$). Similarly, the negative control group showed a significant difference from the positive control group and all low, medium, and high concentration test groups at the same significance level ($p \leq 0.05$). The positive control group demonstrated a significant difference when compared to the normal control group and the negative control group at the 0.05 significance level ($p \leq 0.05$). Additionally, the low concentration test group demonstrated a significant difference from the normal and negative control groups, as well as the high concentration test group, at the 0.05 significance level ($p \leq 0.05$).

The significance level of 0.05 ($p \leq 0.05$) is indicated by the differences observed between the medium concentration test group, the normal control group, and the negative control group. Additionally, the high

concentration test group showed a significant difference from the normal control group, the negative control group, and the low concentration test group at the 0.05 significance level ($p \leq 0.05$). The anti-inflammatory activity is due to the presence of compounds contained in *the C. citratus* extract, indicated by a decrease in edema diameter. Based on the results of phytochemical screening tests, lemongrass extracts contain flavonoids, saponins, tannins, and alkaloids, which are likely to have anti-inflammatory effects [25]. According to a previous study, flavonoid compounds in *C. citratus* can specifically inhibit the formation and release of substances that cause inflammation due to allergic reactions [26]. Compounds included in the flavonoid group have different effects on overcoming inflammation. The anti-inflammatory mechanism of flavonoids can occur through several pathways, one of which is by inhibiting the activity of COX and lipoxygenase enzymes, thereby inhibiting the biosynthesis of prostaglandins and leukotrienes.

This causes inhibition of leukocyte accumulation and neutrophil degranulation, so that it directly reduces the release of arachidonic acid by neutrophils and inhibits the release of histamine. Leukosit bergerak bebas di sepanjang dinding endotel pada kondisi normal. During inflammation, various endothelial-derived mediators and complement factors cause leukocyte adhesion to the endothelial wall. Flavonoids can reduce the number of leukocytes and leukocyte adhesion to the endothelium. As a result, the inflammatory response in the body decreases. Flavonoids can reduce the number of leukocytes and leukocyte adhesion to the endothelium. As a result, the inflammatory response in the body decreases. The anti-inflammatory mechanism of saponins is by inhibiting the formation of exudates and inhibiting increased vascular permeability based on oleanic acid, with a mechanism as an antioxidant [8]. Anti-inflammatory substances such as tannins work as antioxidants, can inhibit the release of free radicals such as cyclooxygenase (COX), lipooxygenase, and inducible Nitric Oxide Synthase (iNOS), and change intracellular pathways in immune cells [27]. These substances trigger various inflammatory and immune responses. All functions act as signaling mechanisms for redox regulation. Antioxidants can also recognize minimal oxidative stress so that they trigger protective mechanisms that are important in repairing tissue structure and integrity and reducing edema in body tissues [28].

▪ CONCLUSION

C. citratus extract 6%, 8%, and 10% can be made into an anti-inflammatory gel. The characteristics of the gel are semi-solid, brown and dark brown, have a distinctive smell of lemongrass, are homogeneous, have a pH of 5.54–5.62 suitable for skin pH, have a viscosity value between 57000-73000 cP, have pseudoplastic flow properties, have a specific gravity between 1.055-1.078 g/cm^3, have an adhesive power of 231-252 seconds, and have a spreadability in the range of 3490.11-3021.20 mm^2. Lemongrass extract gel (*Cymbopogon citratus* (DC.) Stapf 6%, 8%, and 10% showed increasing anti-inflammatory effects. *C. citratus* extract 10% in gel has the highest anti-inflammatory effect; the edema decreases in the feet of male mice (*Mus musculus* L.) by 67.79%.

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