



Anti-Inflammation Effectiveness of Ginger (*Kaempferia galanga* L.) and Shallots (*Allium ascalonicum* L.) Extract Combination on Sprague-Dawley Male Rat

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ABSTRACT

Inflammation can be caused by various stimuli which include physical injury, infection, heat and antigen antibody interactions. Ginger (*Kaempferia galanga* L.) and Shallots (*Allium ascalonicum* L.) are medicinal plants that are often used by Indonesians as an alternative to natural medicine and have been studied to have good anti-inflammatory properties because they contain quercetin. This study aims to determine the effective dose of the combination of Ginger ethanol extract and Shallots as anti-inflammatory with a smaller dose. This study was carried out using male rats in 5 treatment groups, consisting of negative control (CMC-Na 0.5 %), control positive (diclofenac Na tablets 0,1 mg/kg body weight rat), and 3 combination doses of Ginger extract: Shallots extract, dose combination I (3 mg/kg body weight Rat: 2 mg / kg body weight Rat), dose combination II (6 mg / kg body weight Rat : 1 mg/kg body weight Rat), and the dose combination III (6 mg/kg body weight Rat: 2 mg kg body weight Rat) using Carrageenan Method on Rat's Feet. The results of the anti-inflammatory activity test showed that all the dose combination treatment groups had anti-inflammatory. The dose combination III was significantly different from the negative control group and showed the most effective dose of combination. The formulated combination shows a synergistic effect between ginger and shallots as an anti-inflammatory. Average percentage (%) of edema inhibition combination III is 71,08 %.

Keywords: Anti-inflammatory; Combination dose; Ginger; Shallots; *Sprague-Dawley*

INTRODUCTION

Anti-inflammatories are drugs that have the activity of suppressing or reducing inflammation. Inflammation is a series of reactions that occur in injured or infected tissue. When tissue becomes infected, a vascular response occurs where fluid, blood components, white blood cells (leukocytes) and chemical mediators accumulate at the site of tissue damage or infection (Maulana, 2020)

Kaempferia galanga (*Kaempferia galanga* L.) or what is known as "kencur" in Indonesia is used as a

food ingredient. This plant is often made into a paste because it is believed to overcome fatigue. Based on the results of the review, traditionally this plant is often used to treat diarrhea, migraines and increase energy, and overcome fatigue (Cahyawati, 2020).

Anti-inflammatories are medicines that relieve inflammation, here among them are Ginger (*Kaempferia Galanga* L.) and Shallots (*Allium ascalonicum* L.). Many medicinal plants have been studied and shown to contain active ingredient that are efficacious for treatment, including Ginger and

Shallots plants which are known to have anti-inflammatory properties. Research that has been conducted to test the anti-inflammatory effect of the Ginger plant showed that Ginger has an anti-inflammatory effect at a dose of 250 mg/kg BW (Chotimah, 2014).

The main components contained in *K. galanga* include ethyl-p-methoxycinnamate (31.77 %), methylcinnamate (23.23 %), carvone (11.13 %), eucalyptol (9.59 %) and pentadecane (6.41 %). *K.galanga* extract is reported to have anti-inflammatory, analgesic, antidiarrheal, antibacterial, sedative, cytotoxic, insecticidal, antihelminth and antioxidant effects (Fauzia, 2017).

The dose of 300 mg/kg BW showed the best percentage of inhibition compared to doses of 100 mg/kg BW and 30 mg/kg BW. Ginger contains the compounds 6-gingerol and 6-shogaol which are effective as anti-inflammatory agents. The 6-Gingerol compound works by inhibiting the expression of TNF- α , downregulating the expression of COX 2 protein and inducible nitric oxide synthase (iNOS), reducing the protein levels of IL-6, IL-8, IL-1 β , PGE2, and mRNA in inflammatory cells. Meanwhile, the 6-Shogaol compound works by inhibiting the release of inflammatory mediators such as TNF- α , Nitric Oxide, and IL-1 β . inhibits activation of NF- κ B-mediated pathways and subsequent release of TNF- α , IL-1 β , IL-6, and Prostaglandin E2 (PGE2) in BV2 microglia cells exposed to liposaccharides (LPS) (Ozkur, 2022).

Shallots is a type of plant that is a member of the Liliaceae family. This plant contains quercetin compounds which have anti-inflammatory properties. Quercetin is one of the most powerful flavonoid class active substances. Flavonoids are a large group of antioxidants called polyphenols consisting of anthocyanidins, biflavones, catechins, flavanones, and flavanols. Mechanism of internal flavonoids hinder the process inflammation through 2 ways, by inhibiting permeability capillary and inhibit arachidonic acid metabolism and secretion of lysosomal enzymes from cells neutrophils and endothelial cells (Cantika, 2024).

The combination of ginger and shallot extracts aims to study the pharmacological synergistic effect between ginger and shallot as anti-inflammatory agent by reducing the dose of both in hope will decrease the side effects of its use. Combination products consist of two or more drug elements in one dosage unit. Ethanol extract of Ginger rhizome in vitro has been shown to have an anti-inflammatory effect. Hakiem

(2014) has tested the 70 % ethanol extract of Shallots (*Allium ascalonicum* L.) as a wound healer in male Sprague-Dawley rats, showing that the ethanolic extract of Shallots with a concentration of 20 % has an effect on wound healing (Aronrer, 2009).

Administration of shallots (*Allium cepa*) methanol extract at doses of 100, 200, 400, and 800 mg/kg BW significantly ($p < 0.05$) was able to reduce the frequency of edema in the legs of white rats. The anti-inflammatory activity of shallots (*Allium cepa*) is due to the activity of the quercetin compound. Quercetin shows its anti-inflammatory activity by activating cyclic guanosine monophosphate (cGMP), adenosine triphosphate (ATP), Protein kinase G (PKG), sensitive potassium channels pathway which leads to hyperpolarization of nociceptive neurons (Oyewusi, 2021). In addition, quercetin also reduces protein kinase C epsilon type (PKC ϵ) and transient receptor potential cation channel subfamily V1 (TRPV1) in the spinal cord and DRG of peripheral neuropathy. Quercetin is similar to dipyrone and morphine which are used in pain reduction (Al-Khairi, 2022).

METHODS

Equipments and Materials

The tools used in this study were a water bath, rotary evaporator, analytical balance, animal scales, glassware, oral probe, oven, magnetic stirrer, clamp, test tube, capillary tube, plethysmometer, intraplantar syringe.

The ingredients for this study were Ginger rhizome, Shallots, 70 % ethanol (technical), CMC-Na (Sodium-Carboxymethyl Cellulose), 1 % carrageenan, 0.9 % physiological NaCl, 50 mg diclofenac sodium, Lieberman Burchard reagent, 1 % gelatin, FeCl₃ solution, filter paper, distilled water.

Procedure For Preparing Ginger and Shallot Extracts

Ginger and shallot extracts was made using maceration methods. A total of 500 g of ginger dry powder was put into a dark bottle, followed by 500 mL of 70 % ethanol. The mixture was macerated for 3 x 24 hours. Every 24 hours the extract was filtrated to separated the dreg. The dregs were added with solvent again and left for 24 hours. This procedure was repeated 3 times The extract obtained was concentrated using a rotary vacuum evaporator at a temperature of 60-70 °C to obtain ethanolic extract.

A total of 1 kg of shallots bulbs were cleaned and peeled then mashed using a blender to form shallots juice or juice. The shallots juice was then macerated repeatedly with 3000 mL 70 % ethanol for 3 x 24 hours. The maceration results were filtered using a Buchner funnel. The extract obtained was then evaporated and concentrated using a rotary vacuum evaporator at a temperature of 65 °C (Hakiem, 2014).

Characterization of Ginger and Shallot Extracts

Determination of Water Content

Determination of the water content of the viscous extract was carried out using the gravimetric method. The calculation of water content is based on the reduction of weight before and after drying process.

Determination of Ash Content

A total of 2 g of the ethanol extract was weighed carefully, put into a crucible that had been incandescent and tara, slowly incandescent until the charcoal ran out, the crucible was cooled and then weighed to a constant weight. The total ash content is calculated expressed in % b/b (Kementrian Kesehatan RI, 2013).

Phytochemical Test

Phytochemical tests were carried out on dry powder and extracts of ginger (*Kaenferia Galanga* L.). Shallots (*Allium ascalonicum* L.) phytochemical tests were intended to determine the presence of secondary metabolites including flavonoid compounds, tannins and saponins in the plant sample and plant extracts quantitatively. The sample was dried using an oven at 40 °C until the moisture content reached 10 % or less. After drying, the sample was mashed using a 100 mesh.

Anti-inflammatory Effect Test

This research was previously approved by the animal ethics committee. Testing the anti-inflammatory effect was carried out on hind paw rats inflammation induced by carrageenan. Carrageenan induce cell injury which lead to the release of mediators such as Prostaglandin E1 and Prostaglandin E2 and promote plasma proteins to accumulate in wound tissue to form edema that initiate the inflammatory. The edema can occurs for six hours and then gradually decreases until twenty-four hours after injection.

Experimental animals in this study used male rats of the Sprague-Dawley strain 2-3 months old with a body weight of 150-300 grams. Male white rats were weighed and the coefficient of variation calculated to ensure that the animals used were homogeneous so that the data obtained had high validity. The experimental rats were grouped into 5 groups and each group contained 5 rats as replicates, then the experimental rats were acclimatized for approximately 1 week. Acclimatization was carried out in order to familiarize the rats with the new cage environment. The grouping of experimental rats is divided as in Table 1.

Carrageenan Suspension 1 %

Carrageenan suspension was used with the aim of being an inductor to make the experimental animal's legs become inflamed (swollen), making carrageenan by weighing 100 mg of carrageenan, then putting it in a 10.0 mL flask and then filling it with 0.9 % NaCl solution, up to the mark line. Then incubated at 37 °C for 24 hours.

Table 1. Grouping of Animals Test

Group	Treatment
Negative Control	Na CMC 0.5 %
Positive Control	Suspension Na Diklofenak
Single Dose	Ginger extract 6 mg dose for Rat
Dose Combination I	combination dose of Ginger extract: Shallots extract, dose I (3 mg dose for Rat: 2 mg dose for Rat)
Dose Combination II	combination dose of Ginger extract: Shallots extract, dose II (6 mg dose for Rat: 1 mg dose for Rat)
Dose Combination III	combination dose of Ginger extract: Shallots extract, dose III (6 mg dose for Rat: 2 mg dose for Rat)

Anti-Inflammatory Activity Test with Carrageenan Method

Before testing each animal was weighed and marked on its left leg, then the volume of the rat's left leg was measured using a plestimometer and expressed as the initial volume (V_0). Each Rat paw was injected subplantarily with 0.3 mL of 1 % carrageenan. 3 hours after injection of 1 % carrageenan, each rat was treated topically on the induced leg with test preparation in accordance with treatment group Table 1.

After 60 minutes of treatment, the volume of the rat's left foot was measured using a plestimometer and expressed as the volume of the rat's paw (V_t). Measurements were made every 60 minutes for 360 minutes. Changes in the level of swelling that occurred were recorded as the volume of the rat's paws (V_t). The volume of inflammation is the difference in the volume of the rat's paws after and before the 1 % carrageenan injection.

RESULTS AND DISCUSSION

Yield of Ginger and Shallots Extracts

Ginger and shallots extracts were obtain by maceration method using 70 % ethanol polar solvent in order to extract the active to compounds contained in ginger and shallots effectively. The result of maceration of 500 g of powdered ginger extract in 5000 mL of solvent obtained 110.9 g of ethanol extract with an extract yield of 22.18 %. The result of maceration of shallots is a pink filtrate and after being concentrated a ethanol extract is formed, the color changes to dark brown. From 3000 mL of filtrate obtained 92.5 g of ethanol extract with a yield of 30.8 %.

Determination of Water and Ash Content

Determination of water content aims to

determine the amount of water content extract so that the stability and storage time of the sample can be known. Terms of water content in the extract is less than 10 % aims to avoid the rapid growth of fungi in the extract. Determination of water content was carried out in duplo with the average water content of Ginger extract 3.21 % and shallots extract 3.42 %. The results of both extracts met the requirements.

Determination of ash content aims to determine the amount of minerals contained in the extract. The requirement for ash content in the extract is less than 5 % (BPOM, 2022). Determination of ash content was carried out in duplicate with the results of determining the ash content of ginger extract obtained an average of 3.18 % and shallots extract obtained 3.35 %. The results of both extracts met the requirements.

Phytochemical Test

Phytochemical tests were carried out qualitatively on the extracts of ginger and shallots to determine the presence of flavonoids, tannins and saponins. Phytochemical test results can be seen in Table 2. The results of phytochemical testing show the presence of secondary metabolite compounds which are thought to have pharmacological activity, including anti-inflammatory.

The results of phytochemical testing show the presence of secondary metabolite compounds which are thought to have pharmacological activity, including anti-inflammatory. Ginger rhizomes contain the compounds 6-gingerol and 6-shogaol which are effective as anti-inflammatory agents. The quercetin compound in shallots is able to downregulate the expression of monocyte chemoattractant protein-1 (MCP-1), interleukin 6 (IL-6), and IL-8 through the p38 and ERK1/2 pathways in human retinal pigment epithelial cells (ARPE-19 cells). (Hytti et al., 2015).

Table 2. Phytochemical Test Results

Test compounds	Test results	
	Ginger	Shallots
Flavonoids	+	+
Tannins	+	+
Saponins	+	+

Description: sign (+) of the compound contained, sign (-) of the absence of the compound contained.

Table 3. Percentage of Edema Inhibition at Different Dose and Time

Treatment	Edema inhibition (%) at different dose and time (minute)						
	60	120	180	240	300	360	X±SD
Control negative	0	0	0	0	0	0	0 ^a ±0
Control positive	47.13	65.56	74.54	87.71	88.60	94.18	76.28 ⁿ ±8,73
Single Dose (ginger extract)	32.60	49.57	60.70	62.42	73.64	85.30	60.70 ^{klm} ±3,93
Combination Dose 1	27.25	35.33	47.44	50.21	63.63	77.43	50.21 ^b ±7,08
Combination Dose 2	40.98	52.18	64.82	76.39	80.15	84.42	66.49 ^{lm} ±8,15
Combination Dose 3	42.67	59.24	71.08	75.57	85.52	92.42	71.08 ⁿ ±8,43

Anti-Inflammatory Effect Test

The measurement of the volume of edema was carried out for 6 hours at the 60, 120, 180, 240, 300 and 360 minutes respectively to see a decrease in the edema in experimental rat's feet after being treated. The transformation data on the average volume measurement results can be seen in Figure 1.

Based on the percentage average volume of rat foot inflammation, it can be seen that the negative control shows a percentage of edema that continues to increase from the 60 minutes to the 360 minutes. The negative control is used as a comparison parameter which does not have certain efficacy in treating a disease so it has no stimulation to reduce edema. In all test groups, it was found that the onset was from the 60 to the 360 minutes, the effect of the drug was still

visible and was significantly different from the negative control group. Flavonoid compounds are specifically able to inhibit the enzymes cyclooxygenase and lipooxygenase which play a role in treating symptoms of inflammation and allergies.

The results of the average percentage of inhibition (Table 3) show that the test group data is significantly different from the negative control, meaning that all test groups have anti-inflammatory activity. The positive control had the highest percentage value, namely 76.28 %, followed by combination dose III, the ratio of Ginger extract to Shallots extract of (6 mg: 2 mg dose for Rat). The formulated combination shows a synergistic effect between ginger and shallots as an anti-inflammatory.

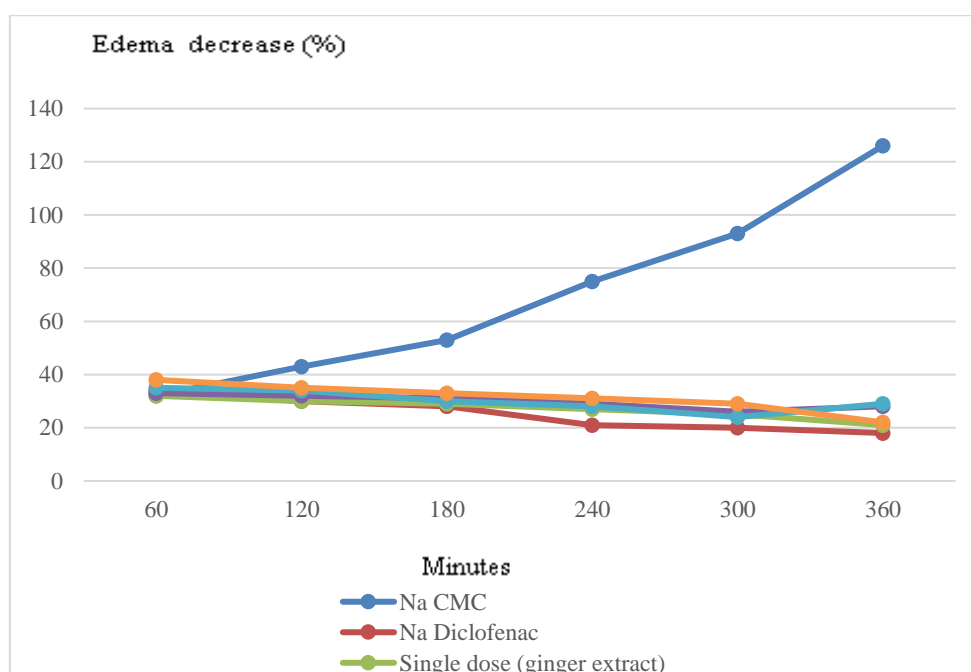


Figure 1. The percentage of edema decrease in Sprague Dawley rat.

CONCLUSION

Based on the results of the anti-inflammatory effectiveness test of the combination of Ginger extract (*Kaempferia Galanga L.*) and shallots extract (*Allium ascalonicum L.*) there are two conclusions that can be drawn. The effective dose of the combination of ginger extract and shallots extract as an anti-inflammatory against inflammation in Sprague-Dawley male white rats was a combination of dose III, the ratio of ginger extract to shallots extract of (6 mg: 2 mg dose for Rat) is the most effective combination dose and there is synergistic effect between Ginger extract to Shallots extract.

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