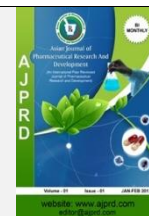


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Research Article

## Characterization and Anti-Inflammatory Activity of Ethanol Extract of Sikkam (*Bischofia Javanica* Blume) Stem Bark

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### ABSTRACT

One of the medicinal plants that have been known since ancient times is sikkam (*Bischofia javanica* Blume) which is used as a natural dye in rattan and bamboo matting. Lawang Agung Village Community Kab. Lahat South Sumatra uses sikkam as a spice in cooking which is believed to be anti-inflammatory. This study aimed to assess the anti-inflammatory activity of ethanol extract of sikkam stem bark (*Bischofia Javanica* Blume) to reduce the edema volume of male white rats induced by carrageenan 1% and also to determine the effective dose of extract to reduce the volume of rat foot edema.

Ethanol extract of sikkam bark (*Bischofia Javanica* Blume) was obtained by maceration. Anti-inflammatory activity test was divided into 5 groups. Group I (negative control) was given 0.5% CMC, Group II (positive control) was given diclofenac sodium 2.25 mg /kgBW, while Group III, IV and V were given ethanol extracts of sikkam bark with doses of 50,100 and 200 mg/kgBW respectively. Each rat was induced by 1% carrageenan subplantar injection. Examination of anti-inflammatory effects was measured using a digital plethysmometer at 30 to 360 minutes. Data were analyzed statistically using ANOVA (analysis of variance). The results showed that negative controls did not show an anti-inflammatory effect that had a significant difference with the other treatment groups. In conclusion, the ethanol extract of sikkam bark (*Bischofia Javanica* Blume) has effective anti-inflammatory activity at a dose of 200 mg/kgBW.

**Keywords:** Anti-inflammatory, *Bischofia javanica* Blume, flavonoids

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### INTRODUCTION

Anti-inflammatory drugs that are steroids and non-steroid have side effects. Some side effects include stomach ulcers, osteoporosis, aggravating diabetes mellitus, muscle weakness, anaemia and kidney injury<sup>1</sup>. Because it needs to be done research to get alternative drugs.

One of the medicinal plants that have been known since ancient times is sikkam (*Bischofia javanica* Blume) which is used as a natural dye in rattan and bamboo matting<sup>2</sup>. Lawang Agung Village Community Kab. Lahat South Sumatra uses sikkam as a spice in cooking which is believed to be anti-inflammatory<sup>3</sup>. Sikkam is a plant that has efficacy as an antipyretic, analgesic, anti-inflammatory, lowers blood pressure, expectorant, and antihelmintic. The content of several active compounds medicinal plants such as tannins, flavonoids, alkaloids, saponins and

steroids/triterpenoids are responsible for anti-inflammatory properties<sup>4</sup>. Tannins are antibacterial and shrink the intestinal wall<sup>5,6</sup>. Flavanoid compounds and terpenoids can also be efficacious as an antidiarrheal in addition, this plant can also be efficacious as an antileukemia and anti-inflammatory<sup>4</sup>. Besides that sikkam has antioxidants effects<sup>7</sup>.

Some parts of flavonoids, namely quercetin, apigenin and luteolin, reduce cytokine expression and secretion. In this case, flavonoids may have therapeutic potential in the treatment of inflammatory-related diseases as cytokine modulators. Flavonoid activity in the inflammatory response includes inhibition of inflammatory mediators such as reactive oxygen species (ROS) and nitric oxide (NO); Regulating the activity of inflammatory enzymes, such as cyclooxygenase (COXs) and inducible nitric oxide synthase (iNOS)<sup>8</sup>. Based on these things, it is necessary to

conduct research on the anti-inflammatory activity of ethanol extract of sikkam stem bark on white rats induced by carrageenan 1%.

## MATERIALS AND METHODS

### Plant and Chemical Materials

This research was conducted at the Phytochemical Laboratory of the Faculty of Pharmacy, University of North Sumatra. The stem bark of sikkam are collected from Jalan Sudirman Medan, North Sumatra.

The equipment used in this study are laboratory glassware, vaporizer cups, spatulas, blenders (Panasonic), excises, distillation sets, electric ovens (Storks), furnaces, electric heating mantles (EM 2000), hairdryers (Maspiion), analytical balance (Vibra AJ), rough balance (Saherand), water bath (Yenaco), rotary evaporator (Boeci 461), drying cabinet, microscope, object glass, deck glass, stopwatch, mercury pletismometer, mouse cages, masks, gloves, animal scales, sonde, Erlenmeyer, beaker, measuring cup, test tube, stir bar, spatula, watch glass, dropper, hot plate, mortar, label, and aluminium foil.

While the materials used in this study are; N-hexane, ethylacetic acid, ethanol, acetic acid anhydride, concentrated sulfuric acid, concentrated hydrochloric acid, potassium bromide, n-hexane methanol and distillate ethylacetic acid, distilled water, diclofenac sodium and carrageenan.

### Simplisia preparation

Sikkam stem bark (*Bischofia javanica blume*) has been collected cleaned from dirt by washing under running water until clean and drained. The sample is weighed as wet weight and dried on a drying rack at 40 C. The sample is considered dry when it is fragile. The dry sample is weighed and the next sample is pulverized using a blender.

### Preparation of ethanol extract of sikkam bark

As much as 300g of Simplicia powder was put into a closed vessel, 2250 ml of 80% ethanol (1: 7.5) was added to the closed container and then wrapped in aluminium foil for 5 days, protected from light and stirring occasionally. The macerate was filtered with filter paper into filtrate 1. The residual product is dried and extracted again by adding 750 ml of 80% ethanol (1: 2.5) solvent for 2 days protected from light and stirring occasionally. Then maserat is filtered into filtrate 2. After that, filtrate 1 and 2 are combined and evaporated using a rotary evaporator 400C, dried with a freeze dryer<sup>9</sup>.

### Alkaloid examination

Simplisia powder was weighed as much as 500 g and 2g ethanol extract of sikkam stem bark (EESSB) then added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated on a water bath for 2 minutes, cooled and then filtered. The filtrate was used for the following experiments:

- 3 drops of filtrate are taken, then 2 drops of Mayer reagent are added
- 3 drops of filtrate are taken, then 2 drops of Bouchardat reagent are added
- 3 drops of filtrate are taken, then 2 drops of Dragendorff reagent are added

Alkaloids are considered positive if sediment occurs or at least two or three of the above experiments<sup>10</sup>.

### Tannin examination

A total of 1 g of simplicia powder and 2g of ethanol extract of sikkam stem was filtered with 10 ml of distilled water, filtered then the filtrate was diluted with distilled water until it was colourless. 2 ml of solution is taken and then added 1 to 2 drops of reagent iron (III) chloride. A blue or blackish green color indicates tannin<sup>10</sup>

### Glycoside examination

Simplisia powder was weighed as much as 3 g and 2 g of ethanol extract of sikkam stem then filtered with 30 ml of a mixture of 7 parts by volume 96% ethanol and 3 parts by volume of distilled water (7: 3), refluxed for 10 minutes, cooled and filtered. To 20 ml of filtrate add 25 ml of water, and 25 ml of lead (II) acetate 0.4 M, beaten, allowed to stand for 5 minutes then filtered. The filtrate was extracted 3 times, each time with a 20 ml mixture of 3 parts by volume of chloroform (p) and 2 parts by volume of isopropanol (p).

To the juice, extract add sodium sulfate anhydride (p), filtered and evaporate at a temperature of no more than 500C. The rest is dissolved with 2 ml of methanol (p), then put 0.1 ml of solution into a test tube, and evaporated on a water bath. To the remaining 2 ml of water and 5 drops of Molish are added, then carefully added 2 ml of sulfuric acid (p), a purple ring is formed at the liquid limit, indicating the presence of a sugar bond (Molish reaction)<sup>9</sup>

### Saponin examination

A total of 500 mg of simplicia powder and 2g of ethanol extract of sikkam stem were put into a test tube and 10 ml of hot distilled water were added, cooled and then shaken vigorously for 10 seconds, formed foam or foam, not less than 10 minutes as high as 1-10 cm. The addition of 1 drop of 2 N hydrochloric acid solution, if the foam does not disappear indicates the presence of saponins<sup>9</sup>.

### Steroid/steroid examination

A total of 1 g of simplicia powder and 2g of ethanol extract of sikkam stem was macerated with 20 ml of n-hexane for 2 hours, then filtered. The filtrate is evaporated in a vaporizer cup. To the remaining 2 drops of acetic acid anhydride and 1 drop of concentrated sulfuric acid are added. Embossed purple or red then turn green blue indicating the presence of steroids/ triterpenoids<sup>11</sup>

### Anti-inflammatory activity test on ethanol extract

The number of male white rats in each group is 6 and taken by random, i.e.

- Negative control, given a 0.5% Na CMC suspension
- Positive control was given with diclofenac sodium suspension at a dose of 2.25 mg/kg
- Group dose 100 mg/kg, given a suspension of ethanol extract of sikkam stem dosage  
100mg / kg
- Dose group 200 mg/kg, given a suspension of ethanol extract sikkam stem dose  
200mg / kg

Test preparation is given orally. Put a mark on the rat's leg as the measurement limit at plethysmometer tool, measure the normal foot volume at 30 minutes after administration of the preparation. Each animal was given 0.05 ml carrageenan solution with a concentration of 1% on the sole of the foot. Furthermore, the volume of the rat's feet was measured at 30 minutes, 60, 90, 120, 150 and 180 after carrageenan induced. Based on the measurement results obtained, edema value was reduced by the volume of edema formed at each time of measurement, normal foot volume, and percent edema inhibition by calculating the percentage of the edema ratio formed at each measurement time<sup>12, 13</sup>.

The formulas for measuring edema values and percent inhibition of edema are as follows<sup>13</sup>:

$$\% R = \frac{V_t - V_o}{V_o} \times 100\%$$

$V_o$

$\% R$  = percentage of inflammation

$V_o$  = initial paw volume

$V_t$  = volume of edema paw at time T

The percentage of inflammatory inhibition ( $\% IR$ ) can be calculated using the following formula.

$$\% IR = \frac{a - b}{A} \times 100\%$$

A

$\% IR$  = Percentage of inflammation inhibition

Data obtained from the results of the study were analyzed by one way analysis of variance (ANOVA) and continued with Tukey's further tests on the product and statistics program service solutions (SPSS).

## RESULTS AND DISCUSSION

### Extraction

In this study, the maceration method of maceration bark extract was used. A total of 300g of simplicia powder was

obtained from 82.13 g with a solvent percentage of 27.38% and the extracted color is blackish red.

### Phytochemical Screening

Characterization of simplicia powder and EESB were carried out before phytochemical screening test. These characterizations include determining of water content, water soluble extract content, soluble ethanol extract content, total ash content, and acid insoluble ash content. Its purpose for ensuring the uniformity of Simplicia quality as good as standard requirements.

Based on the general requirements of Indonesian Materia Medika, the water content of simplicia not exceed 10%. Determination of water soluble extract and ethanol soluble extract content were carried out to provide an initial description of the amount of compounds that can be extracted with water and ethanol. Determination of total ash content was carried out for providing an overview of internal and external mineral content that originated from the initial process to the formation of simplicia associated with organic and inorganic compounds obtained internally and externally. Determination of acid insoluble ash content aims to examine the amount of ash obtained from external factors such as sand or silicate soil<sup>12</sup>.

Phytochemical screening is carried out on simplicia powder and ethanol extract of sikkam bark (EESB) where the results are showing that the sikkam bark contains flavonoids chemical compounds, glycosides, tannins and triterpenoids. Flavonoids are screened with the addition of Mg, concentrated hydrochloric acid give red color. Glycoside screening is indicated by the formation of purple rings with the addition of Molish and concentrated sulfuric acid. Addition of  $FeCl_3$  1% gives blackish blue color which indicates the presence of tannins namely 3 hydroxyl groups. Color pink or purple on the addition of a few drops of Liebermann-Burchard reagent shows the presence of triterpenoids. Characterization of simplicia powder and EESB shown in Table 1 and its phytochemical screening shown in Table 2.

**Table: 1** Characterization of simplicia powder and EESB

No	Characterization	Result	
		Simplicia powder (%)	EESB (%)
1	Water content	5,99	7,32
2	Water soluble extract content	17,49	32,97
3	Ethanol soluble extract content	11,84	23,26
4	Total ash content	4,07	0,48
5	Acid insoluble ash content	6,06	0,01

**Table: 2** Phytochemical screening results of simplicia powder and ethanol extract of sikkam bark

No	Compounds	Result	
		Simplicia powder	EESB
1	Alkaloids	-	-
2	Flavanoids	+	+
3	Glycosides	+	+
4	Saponins	-	-
5	Tannins	+	+
6	Steroids/Triterpenoids	+	+

Information: (+) positive: Contains a class of compounds, (-) negative: Does not contain any class of compounds



### Anti-Inflammatory Activity Test

Anti-inflammatory activity test using a digital pletismometer with the principle of measurement based on Archimedes' law, that is, an object inserted into a liquid will exert force or pressure on the volume transferred. The method was chosen because it has advantages in terms of faster implementation, more accurate mouse volume examination results because the volume of mouse feet required on a digital recorder, the sensitivity of the tool is higher than that of a mercury pletismometer. The data obtained were analyzed by analysis of variance (ANOVA) using the SPSS 22 program.

Carrageenan-induced hind limb edema is the standard model of an acute inflammatory trial. Carrageenan is a

polymer linear composed of about 25,000 galactose derivatives whose structure depends on the source and extraction conditions. The measurement of anti-inflammatory power was done by looking at the ability of the sikkam rod in reducing the swelling of experimental animal feet due to injection of 1% carrageenan solution. After being injected with carrageenan, the mice showed swelling and redness of the feet and the mice were unable to walk as aggressively as before injection. The principle in this method is to measure the swollen foot volume of a test animal that has been induced by an inflammatory agent.

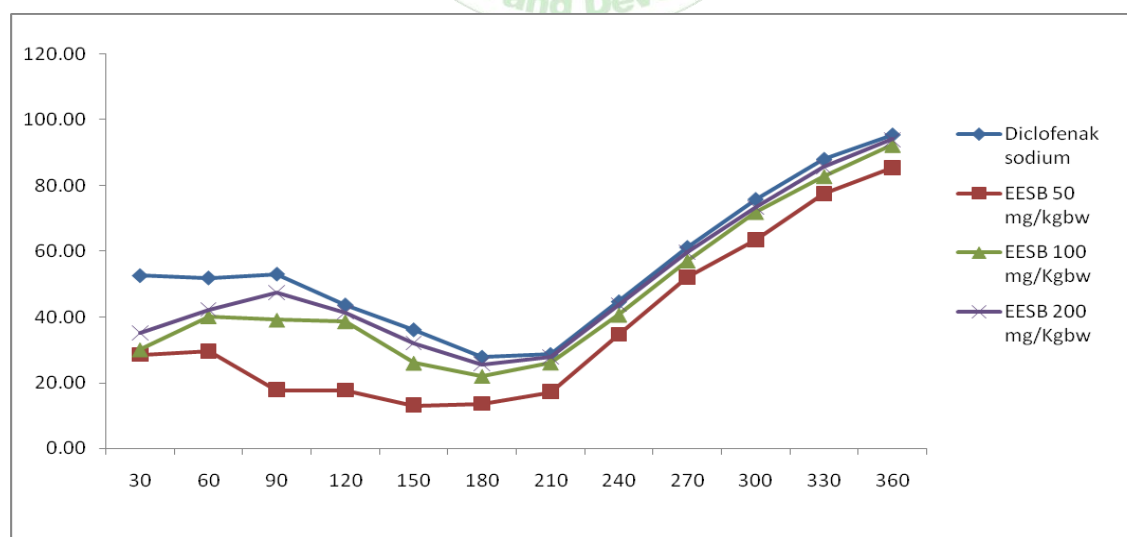
The percentage of inflammation inhibition to describe the effectiveness of sikkam stem ethanol extract to inhibit inflammation made with carrageenan can be shown in table 3

**Table: 3** The Percentage of inflammation inhibition

Group	Percent inhibition of inflammation (minutes)					
	30	60	90	120	150	180
Diclofenac Sodium	52,64±11, 80*	51,94± 9,74*	53,11± 8,25*	43,74± 6,68*	36,20 ± 4,32*	27,89± 3,96*
EESB 50 mg/kgbw	28,55±9,01*#	29,57±8,26*	17,87±5,75*#	17,62±6,19*	13,06±3,70*	13,56±3,08*
EESB 100 mg/kgbw	29,97±9,30*#	40,09±8,12*	39,02±9,73*	38,51±6,30*	25,92±1,80*#	21,90±3,65*
EESB 200 mg/kgbw	35,15±7,66*#	42,20±5,46*#	47,34±4,16*#	41,38±5,06*	31,94±6,40*#	25,64±6,41*#

**Table: 3** The Percentage of inflammation inhibition

Group	Percent inhibition of inflammation (minutes)					
	210	240	270	300	330	360
Diclofenac Sodium	28,70±5,37*	44,71±4,28*	61,23±3,63*	75,81±2,49*	88,00±2,27*	95,39±1,04*
EESB 50 mg/kgbw	17,21±3,23*#	34,68±3,70*	52,08±2,08*#	63,53±3,88*#	77,60±2,69*	85,51±1,47*
EESB 100 mg/kgbw	25,94±2,41*#	40,55±3,00*#	57,05±2,16*#	71,80±1,33*#	82,75±1,61*#	92,27±1,60*#
EESB 200 mg/kgbw	27,83±4,36*	43,72±3,68*#	59,76±2,46*#	73,37±1,15*#	85,63±1,78*#	94,04±1,24*



**Figure 1:** Effect of treatment on percent inflammation inhibition

The negative control group provided 0.5% Na CMC suspension in the absence of active compounds, there was a significant increase in edema volume with much greater volume than the other treatment groups. This negative group becomes a reference in comparing the results achieved by other groups. Test groups with significantly

different results from negative control groups showed anti-inflammatory effects of extracts or drug ingredients that were given to test animals.

The positive control group was given a Diclofenac Sodium suspension at a dose of 2.25mg/kgBW. The results obtained

were significantly different from negative controls in each measurement time in minutes 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360. The treatment group which was given ethanol extract of sikkam bark in a dose of 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW had different results.

Based on Table 3, the percentage of diclofenac sodium inhibition edema 2.25 mg /BW as a positive control and become a standard reference in finding potential drug compounds that suppress the inflammation of animals induced by keragenan. Statistical test results showed that Group III receiving ethanol extract of sikkam bark at a dose of 50 mg/kg BW, in inhibition of edema 360 minutes percentage value of 85.51%. Group IV who were given extracts of 100 mg/kg BW had a value of 92.37% and Group V who were given extracts of 200 mg/kg BW had a value of 94.04%. All extract treatments showed activity in suppressing rat leg edema, but Group V which was given extract at a dose of 200 mg/kg BW showed the most effective results, this group was able to approach the value of 2.25 mg/kg body weight of diclofenac sodium which has a percentage of inflammatory inhibition 95.39%. The presence of anti-inflammatory effects is based on experimental tests due to the activity of secondary metabolites in the ethanol extract of sikkam bark, namely flavonoids, steroids/triterpenoids, and tannins. This is supported by the results of phytochemical screening tests that indicate the presence of their metabolites<sup>5</sup>. The anti-inflammatory mechanism of flavonoids through several pathways. Flavonoids directly inhibit the cyclooxygenase and lipoxygenase pathways also cause inhibition of eicosanoids and leukotriene biosynthesis, which is the final product of COX and the lipoxygenase pathway that leads to inducing inflammation. Inhibition of the cyclooxygenase pathway may have broader effects because the cyclooxygenase reaction is the first step on the path to eicosanoid hormones such as prostaglandins and thromboxanes<sup>13</sup>. Flavonoids also inhibit leukocyte accumulation by reducing the adhesion of leukocytes to the endothelium and resulting in decreased inflammation.

## CONCLUSION

Ethanol extract of sikkam bark 50 mg, 100 mg and 200 mg/kg BW has anti-inflammatory effect with the most effective dose is 200 mg / kg BW.

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