

# Anti-inflammatory effect of ethyl acetate fraction of galing plant extract (*Cayratia trifolia*) on male wistar rats induced by carrageenan

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## 2 Anti-inflammatory effect of ethyl acetate fraction of galing plant extract (*Cayratia trifolia*) on male wistar rats induced by carrageenan

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**Abstract.** Inflammation (inflammation) is a local reaction from living tissue or cells to an excitatory or injury. This study aims to determine the anti-inflammatory activity of ethyl acetate fraction of galing plant extract (*Cayratia trifolia*) on one of the inflammatory parameters, namely swelling in the legs of rat with 1% carrageenan induction. In addition, this study also aims to determine the effective dose of ethyl acetate fraction of galing plant extract (*Cayratia trifolia*) as anti-inflammatory. This study used 25 male wistar rats divided into 5 groups. Group 1 (negative control) Na CMC 0.5%, group 2 (positive control) diclofenac sodium 0.0065 mg /gBW rats, and group 3, 4 and 5 suspension ethyl acetate fractions of galing plant extract 0.0065; 0.013 and 0.026 mg / gBB rats. Inflammation in rats by inducing 1% carrageenan as much as 0.10 ml. The volume of edema every hour is known from the difference in foot volume at certain hours with normal foot volume. The AUC value of the edema volume was calculated by trapezoid method every one hour and calculated for anti-inflammatory power (IP). The results showed that ethyl acetate fraction of galing plant extract (*Cayratia trifolia*) could reduce the volume of udema in the legs of male white wistar rats which was induced by caragenine with an effective dose of 0.0065 mg/gBW.

### 1. Introduction

Traditional medicine is a mixture of unrefined natural ingredients comes from plants or animals used for treatment traditional [1]. Traditional medicine is one of an alternative for the treatment of different diseases because the side effects are considered smaller and the price is cheaper than modern medicine. So that the use of traditional medicines can be accounted for, it needs to be done various studies both to look for active components and to assess its effectiveness and safety [1].

Inflammation is a local reaction of tissue or cells to a stimulus or injury. This study aims to determine the anti-inflammatory activity of the ethyl acetate fraction of galing plant extract (*Cayratia trifolia*) on one of the inflammatory parameters, namely swelling in the feet of wistar rat with 1% carrageenan induction. In addition, this study also aims to determine the effective dose of ethyl acetate fraction of galing plant extract as anti-inflammatory.



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## 2. Materials and Methods

This study used 25 male wistar rats divided into 5 groups. Group 1 (negative control) Na CMC 0.5%, group 2 (positive control) diclofenac sodium 0.0065 mg /gBW rats, and group 3, 4 and 5 suspension ethyl acetate fraction of galing plant extract 0.0065; 0.013 and 0.026 mg / gBB rats. Inflammation in rats by inducing 1% carrageenan as much as 0.10 ml. The volume of edema every hour is known from the difference in foot volume at certain hours with normal foot volume. The AUC value of the edema volume was calculated by trapezoid method every one hour and calculated for anti-inflammatory power (IP). The AUC value of the edema volume obtained was analyzed statistically by the Kruskal-Wallis and Mann-Whitney tests using SPSS release 16.

## 3. Results and Discussion

The galing plant obtained is processed into simplicia on all parts of plant. Simplicia is pollinated and sieved with No. 30/40 sieve. Simplicia what is used is simplicia which passes on No. 30 sieve and does not pass on the sieve no.40. The simplicia powder is then extracted using a maceration method in 96% ethanol solvent for 5 days. The filtrate obtained was concentrated with rotary evaporator. The viscous extract obtained was fractionated using a separating funnel successively with n-hexane, chloroform, diethyl ether and ethyl acetate. This process done until the liquid is clear. The amount of solvent used for fractionation proportional to the amount of water-ethanol added to the ethanol extract (ratio of 1: 1). Ethyl acetate fraction is collected and concentrated by rotary evaporator until a viscous ethyl acetate fraction is obtained. Preliminary testing begins with phytochemical screening to find out the compounds contained in the ethyl acetate fraction of galing plant extract are thought to be anti-inflammatory. Phytochemical screening includes testing phenolic, alkaloids, flavonoids, saponins, tannins, and triterpenoid. If the results are positive, then the confirmation test is continued using TLC to ensure that there is a positive substance in the phytochemical screening test.

**Table 1.** Average% volume of edema, total AUC, and% DAI of various treatment groups

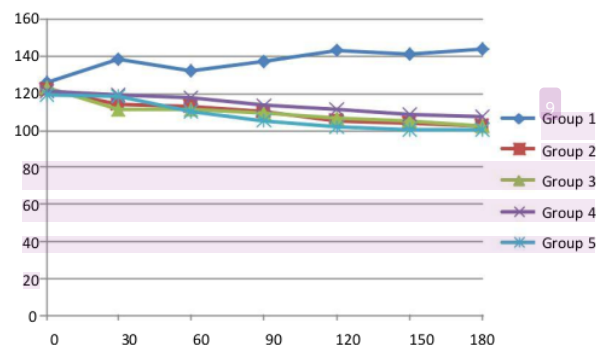
Group	% Volume of Edema							% Total of AUC (ml.hour)	% IP
	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes		
Negative Na-CMC 0.5%	126.31	138.65	132.42	137.24	143.15	141.53	144.36	88.75 <sup>b</sup>	0
Positive Na-diclofenac	122.18	114.28	113.17	110.42	105.44	103.67	102.35	67.08 <sup>a</sup>	21.5
Galing 0.0065mg per gram BB	123.05	111.64	111.25	109.32	106.52	105.44	102.25	68.43 <sup>a</sup>	22.6
Galing 0.013mg per gram BB	121.32	119.27	117.38	113.34	111.26	108.54	107.21	67.52 <sup>a</sup>	24.4
Galing 0.026mg per gram BB	119.25	118.27	110.32	105.29	101.63	100.45	100.33	66.84 <sup>a</sup>	23.8

### Notes

a: significant different ( $p < 0.05$ ) on positive control group with Mann-Whitney test  
b: significant different ( $p < 0.05$ ) on negative control group with Mann-Whitney test

The results obtained both phytochemical screening and assertion tests showed that the ethyl acetate fraction of galing plant extract contained secondary metabolites of alkaloids, flavonoids, saponins, and triterpenoids / steroids. Furthermore, the treatment of test compounds in male wistar rats at 2-3 months old to see the anti-inflammatory effect of galing plant ethyl acetate fraction. The inflammatory parameters observed were edema in the feet of rat with sub plantar 1% carrageenan administration. Carrageenan does not cause other tissue damage and does not cause scars, and provides a more sensitive response to anti-inflammatory drugs. Carrageenan latent time starts 1 hour and maximal edema formation occurs 3 hours after carrageenan administration [1]. The data observed were the percentage of edema volume, the Area Under Curve (AUC) value which illustrated the magnitude of the edema, and the percentage of anti-inflammatory power (IP). The results of this observation can be seen in Table 1.

Based on Table 1, the peak occurrence of edema caused by carrageenan occurs at 30 minutes. Furthermore, with the administration of galing both doses of 0.0065; 0.013; and 0.026 mg / gBW and diclofenac sodium, the percentage of edema volume decreases. This shows that galing can reduce the volume of edema or as anti-inflammatory. While the negative control group with the administration of ethyl acetate fraction carrier is CMC Na 0.5% the volume of edema remains even rising. Graph about the description of the percentage of edema volume of all treatment groups can be seen in Figure 1. Furthermore, % of the volume of the edema was used to calculate the AUC value. AUC describes the amount of inflammation that occurs. After that, % IP was also calculated to describe the percentage of anti-inflammatory power. The AUC value was inversely proportional to % IP. The smaller the AUC value means the amount of inflammation decreases so that the greater the percentage of anti-inflammatory power. Based on Table 1, the total AUC value of the negative control group was the largest compared to other groups. The positive control group of Diclofenac Na has a greater AUC value than the galing group of all doses. This is also shown % IP of galing greater than the drug Diclofenac Na. The higher the dose of galing, the higher than IP. This proves that galing can effect reducing edema which is one sign of inflammation or also called anti-inflammatory. A description of the total AUC value than the galing group of all doses. This is also shown % IP of galing greater than the drug Diclofenac Na. The higher the dose of galing, the higher than IP. This proves that galing can effect reducing edema which is one sign of inflammation or also called anti-inflammatory. A description of the total AUC value and % IP of all treatment groups can be seen in Figure 2.

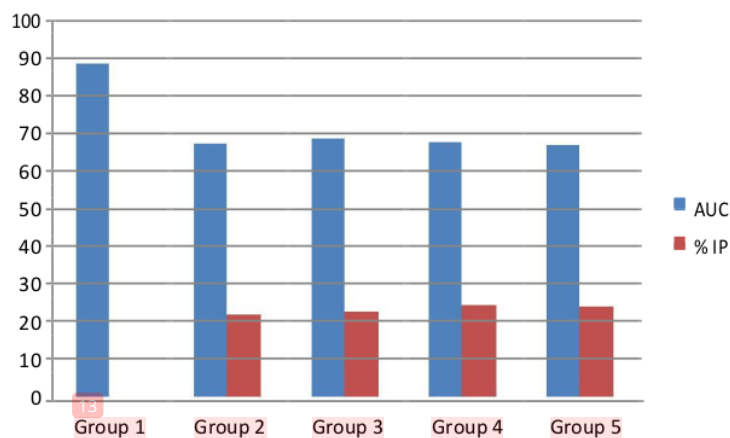


**Figure 1.** Graph of relation between % volume of edema to time of various treatment groups

Statistical tests with SPSS 16.00 were also carried out to reinforce galing's anti-inflammatory effects. The results obtained were significant differences in AUC values between the negative control groups and the galing group of all doses. This proves that the galing plant extract ethyl acetate fraction can have anti-inflammatory effects. In addition the results of statistics obtained there was no significant difference in AUC values between groups of Diclofenac Na with the galing group all doses.

This shows that the ethyl acetate fraction has an anti-inflammatory effect comparable to Diclofenac Na. Based on the results of statistical tests, the effective dose of galing ethyl acetate fraction was 0.0065 mg / gBW.

Galing ethyl acetate fraction can have anti-inflammatory effects allegedly caused by secondary metabolites contained therein, namely flavonoids, saponins and steroids. Some flavonoids play a role in inhibiting lipoxygenase while other flavonoids play a role in inhibiting prostaglandin synthesis [3]. Flavonoids also have an influence on collagen metabolism in several ways, among others by crosslinking with collagen fibers so that the crosslinking of collagen becomes strong, and is able to stop the damage to collagen structure due to the presence of enzymes from white blood cells this process arises during inflammation [4]. The effects of flavonoids as an indirect antioxidant also support the anti-inflammatory effects of flavonoids. The presence of free radicals can attract various inflammatory mediators [5]. Flavonoids can stabilize Reactive Oxygen Species (ROS) by reacting with reactive compounds from radicals so that radicals become inactive [5]. Many saponins have been reported to have an anti-inflammatory effect, but the mechanism is not yet clearly known. Saponins consist of steroids or triterpenes (aglycones) which have actions such as detergents. The anti-inflammatory mechanism most likely is suspected saponins capable of interacting with many lipid membranes [6] such as phospholipids which are precursors of prostaglandins and other inflammatory mediators. Steroids in the body can inhibit the enzyme phospholipase A2 which is the enzyme responsible for the release of arachidonic acid which is then metabolized by the enzyme cyclooxygenase and lipo-oxygenase which will then release inflammatory mediators [7].



**Figure 2.** Total AUC (ml.hour) and % IP diagrams of all treatment groups

#### 4. Conclusion

Ethyl acetate fraction of Galing (*Cayratia trifolia*) extract can have anti-inflammatory effects on male white rats induced by caragenine with an effective dose of 0.0065 mg / gBW.

#### Acknowledgements

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