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Anti-Inflammatory Activity of *Rhaphidophora pinnata* (L.F) Schott Leaf Extract

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Abstract

BACKGROUND: Cancer growth is influenced by many factors and in general it is an interaction between gene factors and environmental factors, especially the microenvironment that exists around cancer. The inflammatory response plays a decisive role in various stages of cancer growth.

AIM: The aim of this study was to determine the anti-inflammatory activity of ethanol extract of *Rhaphidophora pinnata* leaves.

METHODS: *R. pinnata* leaf extract was obtained by percolation method using 96% ethanol as the solvent at room temperature. Anti-inflammatory activity was determined based on the paw edema method. Thirty male albino mice were treated orally with sodium carboxyl cellulose suspension (as negative control group), *R. pinnata* leaf extract (35, 70, 140, and 280 mg/kgBW), and diclofenac (as positive control group), 60 min before 0.2 mL 1% carrageenan injection. The paw thickness was measured using plethysmometer before injecting the carrageenan and after 1, 2, 3, 4, 5, and 6 h.

RESULTS: The subplantar injection of carrageenan caused a time-dependent paw edema in the mice. Oral administration of *R. pinnata* leaf extract inhibited paw swelling at 1, 2, 3, 4, 5, and 6 h after carrageenan injection. *R. pinnata* leaf extracts doses of 35, 70, 140, and 280 mg/kgBW gave a percentage inhibition of 56.56%, 56.18%, 62.77%, and 49.30%, respectively. The effective dose of *R. pinnata* leaf extract as an anti-inflammatory was 140 mg/kgBW.

CONCLUSION: Ethanol extract of *R. pinnata* leaf has anti-inflammatory activity in male albino mice.

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Keywords: Anti-inflammatory; Ethanol extract; Paw edema, *Rhaphidophora pinnata* leaf

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Introduction

Inflammation is a reaction toward infections or injuries, involving many kinds of mediators. Inflammation happens in two phases, namely, acute phase and chronic phase. Acute phase is a type of body immune when an injury occurs or when foreign objects enter the body, characterized by the presence of local vasodilation, platelet activity, or the increase of vascular permeability which causes liquid accumulation in the certain area. If the inflammation reaction fails and antigen settles persistently, then inflammation will grow into chronic. This is indicated by the leukocyte infiltration and the phagocytic cells in the area of inflammation [1], [2], [3].

Inflammation can increase the mutation rate and mutating cell proliferation caused by phagocytic activity. This indicated that inflammation and cancer cell have a reciprocal correlation; the damage on DNA inside cancer cell will worsen the inflammation process and promote the cancer [4].

One method to examine the anti-inflammation activity in a compound is paw edema method, which

is induced by carrageenan. Paw edema method is traditionally used for search and development of new nonsteroidal anti-inflammatory drugs (NSAIDs) for assessing inflammatory responses until 6 h after carrageenan induction [5]. Carrageenan are a complex group of polysaccharides, with three types: Lambda, kappa, and iota. This compound will cause an acute inflammation model and non-immune, indicated by the formation of edema, hyperalgesia, and erythema as a result of pro-inflammation mediator such as bradykinin, histamine, and reactive oxygen [4], [6]. Clinically, inflammation will be applied using NSAID, which is proven to be effective but will cause some side effects such as stomach irritation, osteoporosis, and disruption in cardiovascular system; hence, the other source of safer herbal plants are necessary as alternative therapy.

Rhaphidophora pinnata is a plant which is empirically used as an anti-cancer. The previous research showed that the leaf performs cytotoxicity activity with *brine shrimp test* method and MTT assays. *R. pinnata* also possess antioxidant activity with 1,1-diphenyl-2-picrihidrazil, which is an active anti-cancer toward cell MCF-7 and is a antimutagenic [7], [8]. The previous study showed that *R. pinnata* had the

potential to be an anti-cancer and hence possess the anti-inflammatory and analgetic activity. From the description above, the purpose of this research was to determine the inflammatory activity from the ethanol extracts of *R. pinnata* leaves.

Materials and Methods

Carrageenan were purchased from Sigma-Aldrich, ethanol p.a (Merck), methanol p.a (Merck), sodium carboxyl methyl cellulose (CMC), and aquadest were obtained from local supplier (Brataco Chemika, Medan, Indonesia); sodium diclofenac tablet was obtained from local pharmacy (Dexa Medica, Indonesia).

R. pinnata leaves were obtained from Medan city, North Sumatera on February 2019 and authenticated by the Research Centre for Biology, LIPI, Bogor, Indonesia. The leaves were cleaned by tap water, dried in oven at 40°C for 5 days, coarsely powdered, and kept in airtight container till use. The extraction process of *R. pinnata* leaves was done using percolation method with 96% ethanol as solvent at room temperature. The dried powdered leaves were macerated with 96% ethanol for 3 h and then the mixture was transferred into percolator chamber. The solvent was added with a flow rate of 1 mL/min until the percolation was finished. The extract was concentrated in rotary evaporator and kept in desiccator.

The animal used in the experiment was a male albino mice of 2–3 months old and weighed around 20–30 g, obtained from Pharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Before the mice were put under experiment, they were kept for approximately a week for adaptation to environment, for health and weight control, for uniformity of food, and also for the approval from the Ethical Committee of Biology Department FMIPA USU.

The anti-inflammatory activity from the ethanol extract of *R. pinnata* leaves was determined according to the swelling of the feet of the mice after carrageenan induction [1]. There were 30 mice, divided into six groups of treatment, with each group consists of five mice. Before examined, all of the animals were fasted for 18 h, except for water. Food was not given at all during the observation. Group I, as the negative control group, was given 1% suspension of sodium carboxymethyl cellulose, Group II was given sodium diclofenac as positive control, while Groups III, IV, V, and VI were given the ethanol extract of *R. pinnata* leaves orally at various doses 35 mg/kgBW, 70 mg/kgBW, 140 mg/kgBW, and 280 mg/kgBW. Paw edema was induced by injecting 0.2 mL of 1% carrageenan into subplantar tissue of each mice paw. Mice paw was cleaned with ethanol 70% before injected. The paw thickness was measured

using plethysmometer before injecting the carrageenan and after 1, 2, 3, 4, 5, and 6 h [5], [6]. The percentage of inflammation inhibition was determined based on the following formula:

$$\% \text{ inflammation inhibition} = \frac{a - b}{a} \times 100\%$$

Where, a=Edema volume of control group.

b=Edema volume of treatment group.

The data were expressed as Mean \pm SEM and analyzed using one-way ANOVA followed by *post hoc* Tukey to find the different variables [8]. Differences were considered as statistically significant at $p < 0.05$ when compared with control. In this study, data were presented in tables and graphs.

Results and Discussion

Bioactive plant extract is resources for the development of anti-inflammatory agent with less side effect. Several studies have been conducted to prove the effect of flavonoids as anti-inflammatory. Flavonoids or polyphenol is good choice to use as anti-inflammation drugs because it could inhibit the production of free radical and lead to impede COX and LOX stimulation like *Coptosapelta flavescens*. Catechin and epigallocatechin gallate from green tea may inhibit COX2 expression induced by 12-O-tetradecanoylphorbol-13-acetate, tumor promoters in rat skin, and COX activity in macrophages induced by lipopolysaccharides [9], [10]. Quercetin-3-methoxy-40-glucosyl-7-glucoside from *Maytenus heterophylla* was effective as anti-inflammatory against carrageenan-induced paw edema in rats [11]. The previous study showed that *R. pinnata* contain flavonoids, so it is possible to develop it as anti-inflammatory.

The anti-inflammatory activity of ethanol extract of *R. pinnata* leaves was done by the induction of 0.2 mL of 1% carrageenan at mice paw. The inflammation which was induced carrageenan was described as a biphasic occurrence as various mediators were released to produce the inflammation responses. The first mediators were detected on early phase (1 h) including histamine, serotonin, and cyclooxygenase. On the other hand, final phase (more than 1 h) was detected by the production of prostaglandin E2 (PGE2), mediated by bradykinin and leukotriene [9].

The anti-inflammatory activity of ethanol extract of *R. pinnata* leaves was showed by the gradually increased percentage of inflammation except on negative control group. The curve on negative control group continuously increased started from the 1st to 6th h with significant statistics ($p < 0.05$) toward positive control on the 1th-6th h. This happened on the

group with the treatment of 1% sodium CMC due to the absence of medicine to ease the edema, causing the edema continued to grow. According to the statistic data, the negative control group indicated some significant differences toward the positive control group and treatment group ($p < 0.05$). These data showed that the induced carrageenan successfully formed an inflammation on the mice's feet. The lowest percentage of inflammation was seen from the extract activity with 140 mg/kgBW dose compared to the positive control group with sodium diclofenac and other treatment groups (Figure 1).

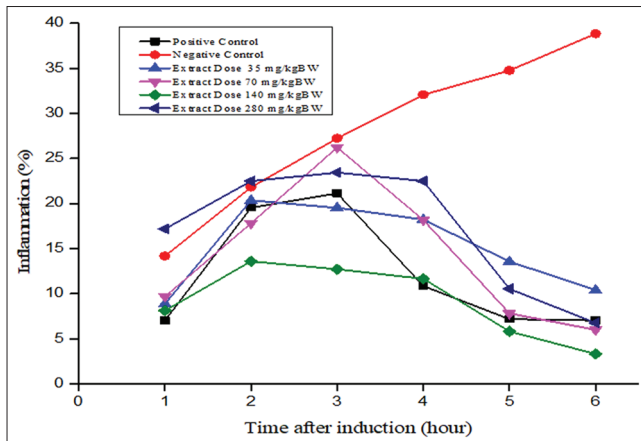


Figure 1: The percentage of inflammation by oral administration of diclofenac and ethanolic extract of *Rhaphidophora pinnata* leaves at 1, 2, 3, 4, 5, and 6 h after the induction of edema ($n = 5$)

The effects of inflammation inhibition were seen from the percentage in Figure 2. The negative control group did not show any inhibitions due to the absence of treatment. The bigger the inhibition percentage, the bigger the substance potential as an anti-inflammation. In contrast to the inflammation percentage, where smaller inflammation indicated the better potential for the substance as an anti-inflammatory. The 140 mg/kgBW EERP activity showed the biggest inhibition percentage compared to positive control group and other treatment groups.

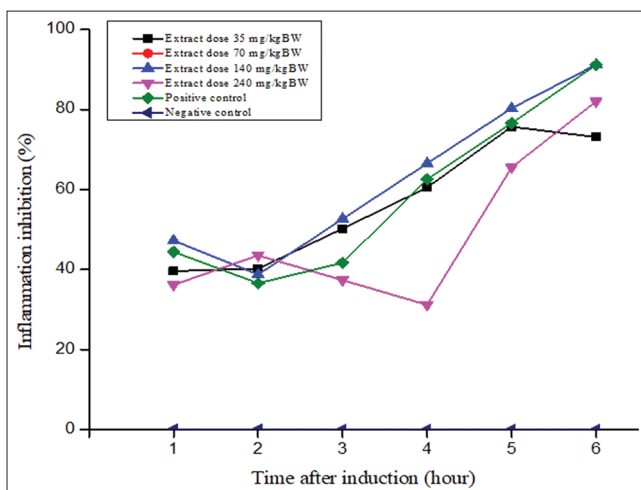


Figure 2: The percentage of inflammation inhibition by oral administration of diclofenac and ethanolic extract of *Rhaphidophora pinnata* leaves at 1, 2, 3, 4, 5, and 6 h after the induction of edema ($n = 5$)

The results of statistical analysis on inflammation percentage indicated that the dose treatment group did not possess any significant differences ($p > 0.05$) compared to positive control group. It also did not possess any significant differences among dose treatment groups ($p > 0.05$), which means that the dose treatment groups produce equal anti-inflammatory activity to positive control group toward the other similar groups with dose treatment.

Table 1: The average inhibition percentage of ethanol extract from *Rhaphidophora pinnata* leaves extract

Dose (mg/kgBW)	Percentage of inhibition
35	56.56
70	56.18
140	62.77
280	49.30

The effective dose 50% (ED_{50}) will cause therapeutic effects on 50% of population (median therapeutic dose). A medicine will produce effects if the concentration was on therapeutic range. ED_{50} from the extract was determined to ensure the dose of ethanol extract of *R. pinnata* leaves reaching 50% of the inhibition. The value was summed using the linear equation between inhibition dose and percentage (Table 1 and Figure 3).

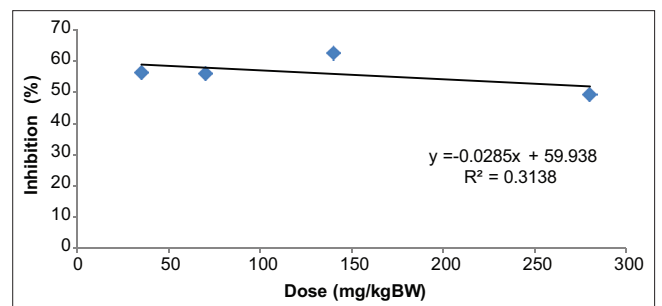


Figure 3: Linear regression of inflammation inhibition percentage

According to the linear regression that resulted from the equation, $y = -0.028x + 59.93$ with $R^2 = 0.3138$. From the equation, ED_{50} was summed from ethanol extract of *R. pinnata* leaves, where Y is an effective dose percentage (50%) and x is the ethanol extract of *R. pinnata* leaves which inhibited the inflammation for 50%. The calculation of ED_{50} ethanol extract according to the equation was as follows:

$$y = -0.028x + 59.93$$

$$50 = -0.028x + 59.93$$

$$x = 354.64 \text{ mg/kgBW}$$

Based on the calculation, the effective dose by 50% or ED_{50} from the ethanol extract of *R. pinnata* leaves was 354.64 mg/kgBW. The effective dose by 50% from the leaf extract was deemed as less potent compared to the dose of sodium diclofenac by 25–50 mg, but the anti-inflammatory effect produced was 35 mg/kgBW or almost the same as sodium diclofenac. This shows that the smallest dose on ethanol extract of *R. pinnata* leaves was able to inhibit the formation of edema, the same as sodium diclofenac, proving that high dose was unnecessary in the inhibition of edema.

Conclusion

It can be concluded that ethanol extract of *R. pinnata* leaves produces anti-inflammatory activity on male albino mice.

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