Ehanol extract from *Achantus Ilicifolius* L. leaves as anti-inflammatory ulcers in *Mus musculus* L.

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**Abstract.** This study aims to determine the effective concentration of *Acanthus ilicifolius* L. leaves to treat the number of ulcers and gastric ulcer severity in white mice (*Mus musculus* L.) which were induced 500 ppm of aspirin. The approach used was quantitative by using a completely randomized design (CRD) factorial pattern with two factors. The results of statistical analysis of the number of ulcers for factor A (source) show the value of $F_{\text{count}} > F_{\text{table}} = 15 > 3.16$, while factor B (concentration) shows the value of $F_{\text{count}} > F_{\text{table}} = 64 > 2.38$ at the test level $\alpha = 0.05$. While the results of statistical analysis of factor A ulcer severity (Source) shows the value of $F_{\text{count}} > F_{\text{table}} = 4 > 3.16$ and factor B (concentration) $F_{\text{count}} > F_{\text{table}} = 38 > 2.38$ at the test level $\alpha = 0.05$. The highest percentage of treatment was at the concentration of P2 (250,000 ppm) and P3 (500,000 ppm) at 17%. The ability of gastric ulcer healing in mice is because the parts of the *Achantus ilicifolius* L contain several phytochemical compounds including flavonoids, saponins, alkaloids, phenolics, and terpenoids. Therefore, it can be concluded that the *Achantus ilicifolius* L leaves with a concentration of 250,000 ppm are effective in treating the number of gastric ulcer and ulcer severity in mice.

1. **Introduction**

Inflammation is an attempt from the body to damage organisms that attack, eliminate and regulate repair in body tissues. Inflammation is caused by physical trauma, chemicals, and microbiological substances which are normal protective responses to injury to the tissue [1]. Currently, many drugs that can treat inflammation have been developed, but inflammatory synthesis drugs are known to have a variety of adverse side effects. Therefore, at present, the community is trying to choose an alternative by consuming natural medicines. One of the natural ingredients known to have an anti-inflammatory effect is *Acanthus ilicifolius* which has a very significant ability to inhibit the secretion of anti-inflammatory cytokinins in mononuclear cells [2].

*Acanthus ilicifolius* is a type of mangrove plant that contains a lot of glucoside compounds, alkaloids, flavonoids, fatty acid steroids, lignans, and components of phenols and terpenoids [3,4]. The chemical compound in the plant functions as neuralgia, analgesic, anti-inflammatory, antioxidant, antifertility, hepatoprotective, antitumor, antileukemia, anticancer, antimicrobial, antiviral and antifungal can also be a natural insecticide [5]. To treat various diseases, this plant has been used as a crude drug for the treatment of asthma, diabetes, dyspepsia, leprosy, hepatitis, paralysis, snake bites, rheumatoid arthritis and diuretics [6].
The people in Gampong Jawa, Banda Aceh often use Acanthus ilicifolius as a traditional medicine to treat burns and cure ulcer disease. However, they use traditional medicinal plants as suggested by others or based on their own experiences. This is what causes the lack of influence of traditional medicinal plants in curing due to inappropriate use and dosage. Often the community is wrong in determining the raw materials for making traditional medicine and does not know how to process these ingredients. This has most of the time causes the side effects rather than get the benefits from the plants.

This is what causes the need for information about the parts of plants that can be used in making traditional medicines with the right concentration so that it can have positive effects based on the results of the research.

The ethanol fraction of Achantus ilicifolius can inhibit tumor growth [6]. The presence of polyphenols and tannins showed significant results for anti-ulcer activity using cold water fractions [7]. The methanol fraction of the Achantus ilicifolius leaves significantly inhibited soft tissue swelling in mice [8]. Literature research shows that the use of Achantus ilicifolius still needs to be developed as medicinal plants. Based on the description, a study was conducted on the potential study of the use of Achantus ilicifolius leaves as an ulcer anti-inflammatory drug in white mice (Mus musculus).

2. Research Method
The study was conducted at the Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University. The research activity was conducted in February - June 2019.

2.1. Extract of Achantus ilicifolius plants
4 kg of Achantus ilicifolius leaves were cleaned and weighed, then they were dried in open air protected from direct sunlight. The dried leaves were then chopped into small pieces and grounded into powder and then weighed. Then the 9 kgs of weighed samples were put in a sample soaking bottle and 5 litres of 70% ethanol were added. The sample was left for 3 days while stirring. The results of soaked sample using filter paper, then evaporated with a rotary evaporator to obtain a pure extract which is considered to have a concentration of 10% [9].

2.2. The Making of Nanoemulsion from Acanthus ilicifolius Plant Extract
This process includes the oil phase and water phase. The oil phase consisted of a mixture of 20 grams of root extract, 20 grams of the stem, and 20 grams of leaves of jeruju plant. While the water phase consisted of 6 ml of maltodextrin, 6 ml of tween 80, and 6 ml of phosphate buffer solution. The homogenization of the oil phase in the water phase used centrifuge with a stirring speed of 15,000 rpm for 15 minutes [9].

2.3. Preparation of Experimental Animals
The experimental animals used were ± 72 white male mice weighing between 25-30 grams. Before being treated, the mice were acclimatized for 1 week and given enough food and drink. Mice were not fed for 36 hours and were only given drinks before the treatment [10].

2.4. Parameter Measurement
The abdomens of the mice were dissected along a larger curvature and examined for the number of ulcers in each stomach, then each ulcer was recorded. The average score of ulcer severity for each animal would be expressed as an ulcer index [11].

3. Results and Discussion
The results of statistical analysis of factor A (Source) with analysis of variance (ANOVA) show the value of $F_{count} > F_{table} = 15 > 3.16$, while concentration shows the value of $F_{count} > F_{table} = 64 > 2.38$ at the test level $\alpha = 0.05$. This shows that the use of ethanol extract of the parts of Achantus ilicifolius has a very real effect as an anti-inflammatory on the number of ulcers in white mice (Mus musculus).
result of the calculation of diversity coefficients (DC) was 8.3% so the further test used was the smallest real difference (SRD) (Figure 1).

The result of factor A (Source) statistical analysis with analysis of variance (ANOVA) shows the value of $F_{\text{count}} > F_{\text{table}} = 4 > 3.16$, while concentration shows the value of $F_{\text{count}} > F_{\text{table}} = 38 > 2.38$ at the test level $\alpha = 0.05$. The calculation of the diversity coefficient (DC) was 5% so the further test used was the smallest real difference (SRD) (Figure 2). While the ulcer index and percentage of treatment for gastric ulcers in mice can be seen in Table 1.

![Figure 1. Number of gastric ulcers in mice](image1.png)

Based on Figure 1 and Figure 2, the lowest number of ulcers was P0 (control) with an average of 1.22 and the highest at P1 (aspirin 500 ppm) with an average of 1.87 and 1.58. The concentration of P1 (500 ppm aspirin) with an average of 1.87 and 1.58 was significantly different from the treatment of administration of Achanthus ilicifolius leaf extract to the number of ulcers and the severity of ulcers in mice. This shows that the administration of jeruju leaf extract can reduce the number of ulcers and their severity levels.

The content of the biochemical compounds found in the Achanthus ilicifolius leaves is alkaloids, saponins, and terpenoids which function as antibiotics and anti-inflammatory which can reduce pain as well as improve blood circulation. Water from the leaf extract can also be used to cure fever, allergies to the skin, relieve pain and stop bleeding. Achanthus ilicifolius leaves contain saponins, flavonoids, and terpenoids which function as anti-inflammatory [12]. The role of flavonoids is to promote blood circulation throughout the body and prevent blockages in blood vessels, contain anti-inflammatory properties, as antioxidants and reduce analgesic pain [13].

The data obtained showed that the administration of extracts of jeruju leaves repaired ulcers in the stomach of 500 ppm aspirin-induced mice for 3 days. At roots, stems, and leaves with concentrations of P2 (250,000 ppm), P3 (500,000 ppm), P4 (750,000 ppm), and P5 (1,000,000 ppm) (Figure 1). This shows that the stomach condition of the mice was normal after the administration of Achanthus ilicifolius leaf extract for 14 days. While the treatment with 500 ppm of aspirin showed bleeding spots on the stomach of mice.

Aspirin is one of the NSAID drugs (non-steroidal anti-inflammatory drugs). Aspirin can cause ulcers or damage to the stomach because NSAIDs can cause disruption of gastric mucosal defenses and systemic inhibition of gastric mucosal protection through inhibition of COX activity in the gastric mucosa [14]. This is because aspirin is very selective in inhibiting COX-1 compared to COX-2 so aspirin has a relatively high tendency to cause gastric damage [15].
Figure 2. The severity of gastric ulcers in mice

Table 1 Gastric Ulcer Index and Percentage of Treatment in tested animals which were induced 500 ppm of Aspirin

| Treatment | Dosage (ppm) | Ulcer Index ± SD | Protection Ratio ± SD (%) |
|-----------|--------------|------------------|--------------------------|
| Control   | -            | 1.22 ± 0.00      | -                        |
| Aspirin   | 500          | 2.00 ± 0.00      | 5 ± 0.00                 |
| P2        | 250,000      | 1.32 ± 0.10      | 17 ± 0.10                |
| P3        | 500,000      | 1.32 ± 0.10      | 17 ± 0.10                |
| P4        | 750,000      | 1.27 ± 0.09      | 9 ± 0.09                 |
| P5        | 1,000,000    | 1.29 ± 0.09      | 11 ± 0.09                |

There was no damage found in normal treatment, whereas in the treatment where 500 ppm aspirin given, there was damage in the form of inflammation, lesions, and damage to the wall of the stomach. It can be concluded that the higher the ulcer index value indicates that the greater the damage to the stomach is experienced and the higher the percentage of treatment. This shows that the greater the ability of a sample to cure and reduce the level of gastric damage that occurred.

The treatment with Achantus ilicifolius leaf extract with a concentration of P2 (250,000 ppm) and P3 (500,000 ppm) showed a percentage of healing of 17%. The ulcer healing ability of the Achantus ilicifolius leaf extract is due to the several phytochemical compounds it contained including flavonoids, saponins, alkaloids, phenolics, and terpenoids. The mechanism of action of flavonoids can increase prostaglandin in the gastric mucosa, reduce histamine secretion from mast cells by inhibiting the mechanism of action of histidine decarboxylase enzymes, and has an antidote to free radical activities that have an important role in ulcer formation in the stomach. Saponin works by activating the protective factor of the gastric mucous membrane [16]. Flavonoids can increase prostaglandin, inhibit histamine secretion and secrete prostaglandin-like compounds [17]. Flavonoids are also known to inhibit the cyclooxygenase enzyme so that the formation of prostaglandins from arachidonic acid does not occur thereby reducing inflammation that occurs in gastric cells [18].

Phenolic compounds have the potential as antioxidants by countering free radicals in biological systems [19]. In peptic ulcer disease, this complex layer of tannins protects the stomach by increasing
greater defense against irritants, tannins can function as antioxidants, and increase tissue repair activities due to their anti-inflammatory activity [20].

4. Conclusion
Based on the results of research, it can be concluded that Achantus ilicifolius leaves with a concentration of 250,000 ppm are effective for treating the number of gastric ulcers and reduce its severity in white mice.

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