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Research Article

# Anti-Inflammatory Activity Test of N-Hexane Fraction Cream (Rhaphidophora Pinnata (L.F) Schott.) on Carrageenan Induced Back Edema in Mice

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## A B S T R A C T

**Objective:** One of the medicinal plants used traditionally by Indonesians is Rhaphidophora pinnata (L.f.) Schott, Family Araceae. Based on hereditary experience, the use of herbal plants is considered quite effective in treating various diseases. The results of phytochemical screening showed that Rhaphidophora pinnata leaf extract contained triterpenoid/steroid compounds which were thought to be anti-inflammatory agents. Topical administration of drugs can increase bioavailability and drug efficacy by avoiding first-pass metabolism in the liver. The advantage of the desired local effect can also be achieved with the use of topical anti-inflammatory drugs.

**Methods:** The leaves were dried into simplicia and then the simplicia characterization was examined. Extracted by percolation method using 96% ethanol and concentrated by rotary evaporator, then fractionated with n-hexane and concentrated. Formulation and evaluation of creams with doses of n-hexane 2.5, 5 and 10% fractions were made. The method of measuring anti-inflammatory used in this study refers to the method of inflammation-associated edema.

**Results:** Evaluation of cream preparations F1, F2 and F3 stable during storage up to 12 weeks from organoleptic, homogeneity, pH (5.9-6.3), spreadability (2.42-2.80 cm2), good dispersibility and irritation test. The F2 formula is the best in terms of pH and cream spreadability tests. The comparative significance value of the anti-inflammatory activity test at F2 compared to hydrocortisone gel was not a significant difference (P> 0.05).

**Conclusion:** Cream formulation F3 of the 5% dose of Rhaphidophora pinnata leaf hexane n fraction in the cream preparation has the same effectiveness as the comparison of hydrocortisone cream in the form of cream.

***Keywords:*** anti-inflammatory activity, fractionation, cream, Rhaphidophora pinnata (L.f.).

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## INTRODUCTION

Currently, research and development of medicinal plants have become an important global topic, several studies, especially in the field of medicinal properties and chemical analysis, are based on indications of medicinal plants that have been used by the public with empirically tested properties. The success of this research has convinced the users of medicinal plants about its properties and uses and has contributed to the emergence of a new paradigm in the world of modern medicine, namely "back to nature"[1,2].

One of the medicinal plants used traditionally by Indonesians is Rhaphidophora pinnata (L.f.) Schott, Family Araceae. Based on hereditary experience, the use of herbal plants is considered quite effective in treating various diseases such as pain relief, cough, anti-anaemia and anti-rheumatism[3,4]. The results of phytochemical screening showed that Rhaphidophora pinnata leaf extract contained triterpenoids/steroids, alkaloids, tannins, and saponins. On examination of the n-hexane fraction, a triterpenoid/steroid content was using Liebermann-Burchard reagent. The two-way TLC results obtained were green, so it was concluded that they contained steroid compounds[5,6].

There are two main groups of drugs used to control inflammation: steroids and non-steroidal anti-inflammatory agents. Anti-inflammatory steroids work to inhibit the phospholipase enzyme so that arachidonic acid is not formed. Arachidonic acid is not formed meaning neither prostaglandins nor leukotrienes are formed[7–9]. The inflammatory response is closely related to the repair process. Inflammation is the body's reflective response to infection, antibody binding to antigens in the body, mechanical irritation, or injury and tissue damage. Some of the symptoms that can be recognized in the inflammatory process include rubor (redness), edema (swelling), color (heat), dolor (pain), and function laesa (loss of function). These symptoms arise due to the release of inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, and leukotrienes which cause inflammatory reactions[10,11].

Inflammatory treatment that works by inhibiting the release of prostaglandins into the injured tissue. However, the steroid class has side effects that can irritate the stomach. Based on the side effects, it is necessary to change the route of drug administration through the topical route. Topical administration of drugs can increase bioavailability and drug efficacy by avoiding first-pass metabolism in the liver. The advantage of the desired local effect can also be achieved with the use of topical anti-inflammatory drugs. The systemic effects of the drug from topical treatment depend on its ability to penetrate the drug into the skin as well as the ability to enter the circulation or be absorbed into deeper tissues to inhibit cyclooxygenase[12–15].

Based on the description above, the research will be carried out, namely the cream formulation and anti-inflammatory activity test of the n-hexane fraction of Rhaphidophora pinnata (L.f.) Schott leaves.

## MATERIAL AND METHODS

### Plant and Chemical Material

Rhaphidophora pinnata plants can be found in the city of Medan, North Sumatra. The part that is taken is the leaves. Rhaphidophora pinnata leaf has been determined by the Herbarium Bogoriense Indonesian Institute of Science, it is known that the species is (Rhaphidophora pinnata (L.f.) Schott.) With the Family Araceae[16].

### Extraction and fraction

Simplicia Rhaphidophora pinnata leaves are mashed using a blender. After becoming a powder soaked with 2.5-5 parts of solvent in a closed vessel. A percolator was prepared and the result of the simplicia immersion was put in to produce a dilute extract. The dilute extract is evaporated on a water bath until a thick extract is obtained. The ethanol extract of dragon tail leaves was fractionated using n-hexane and water as a solvent. Fractionation was carried out by dissolving the viscous extract in a mixture of 9 parts ethanol - 1 part water (1: 9) and 10 parts n-hexane. Then do the cornering and take the n-hexane fraction and then concentrate it using a rotary evaporator at a temperature of 45ºC to produce the n-hexane fraction of Rhaphidophora pinnata leaves[17–19].

### Formulation of Rhaphidophora pinnata leaf n-hexane fraction

The dosage formulation selected for this test is in the form of a cream for each dose of 5 grams. A cream base based on the Yadav 201420 formula, which uses a basic type of oil-in-water cream

| | | |
|---|---|---|
| R/ | Stearic Acid | 12 |
| | Cetyl alcohol | 1 |
| | Trieatanolamine | 1 |
| | Glycerin | 10 |
| | Distilled water Ad | 100 (*w/w*) |

The doses of n-hexane fraction used in this test were 2.5%, 5% and 10%, Hydrocortisone cream 2.5% as a positive control and cream base as a negative control.

### Evaluation of cream preparations

Evaluation of cream preparations carried out only on creams with doses of 2.5%, 5% and 10% including organoleptic examination, homogeneity test, pH examination, the examination of the washability of the cream, spreadability test, skin irritation test and examination of the physical stability of cream preparations.

1. **Organoleptic examination**

   Examination of preparations is carried out by observing the preparations including appearance, color, and smell, which is done visually [21].

2. **Homogeneity test**

   Examination of preparations is carried out in the following manner: 0.1 gram of the preparation is smeared on a piece of transparent glass, it must show a homogeneous arrangement and should not show any particle spots [21–25].

3. **pH test**

   PH determination is carried out using a pH meter. Measurements were made three times for each preparation when the preparation was finished [21–25].

4. **Check the washability of the cream**

   The preparation is weighed as much as 1 g, rubbed on the palms of the hands then washed with a volume of water while rinsing hands. Water is passed from the burette slowly, visually observed until no residual cream remains on the palms, then note the volume of water used [21–25].

5. **Scatter power test**

   The 0.5 g dosage is carefully placed on graph paper covered with transparent glass. The preparation is left (15 seconds), calculated the area given by the preparation, then covered again with a glass plate given a certain load (5 grams to 30 grams) and left for 60 seconds. Then calculate the area given by the preparation [21–25].

6. **Skin irritation test**

   A total of 0.1 g of cream is weighed, applied to the skin of the inner arm with a size of 2x2 cm, then covered with gauze and plaster. After that, look at the symptoms

after 24 hours of use. This irritation test was carried out for each formula on 6 panellists [21–25].

**7. Check the physical stability of the cream preparations**

The freshly made cream preparations were stored at room temperature for 12 weeks, then organoleptic observations were made for changes in color, odor, and shape. [21–25].

### Experimental Animals

Healthy adult mice (Mus musculus L.) (20-35 g BW). Mice were placed in polycarbonate cages. They were fed a standard pellet diet and water ad libitum. The tested animals were divided into 5 groups randomly totalling 25 mice, calculated based on the Federer formula. Groups I, II, and III were the treatment groups that were given cream of n-hexane fraction of dragon tail leaves with concentrations of 2.5%, 5%, and 10%. Group IV as a positive control for Hydrkortisone® cream 1% and group V as a negative control without drugs. The test preparation was a cream that was applied topically to the back skin of mice that had been induced by 3% carrageenan. Previously, the test animals had their dorsal hair shaved (1.25 cm2) with scissors and then applied with Veet® to shed their hair. Furthermore, it is left for 24 hours to avoid inflammation caused by shaving and shedding of hair.

**Table: 1.** Treatment of Anti-Inflammatory Test Animals

| Group | Treatment | Concentration |
|---|---|---|
| I | Rhaphidophora pinnata leaf n-hexane fraction cream | 2,5% |
| II | Rhaphidophora pinnata leaf n-hexane fraction cream | 5% |
| III | Rhaphidophora pinnata leaf n-hexane fraction cream | 10% |
| IV | Hydrocortisone Cream (Positive Control) | 1 % |
| V | Placebo (Negative Control) | - |

### Anti-inflammatory test

The anti-inflammatory measurement method used in this study refers to the inflammation-associated edema method [26]. The anti-inflammatory test was performed by measuring the thickness of the back skin edema every hour for 6 hours using a calibrated calipers. The difference in skinfold thickness values that can then be calculated AUC and percent (%) inflammation inhibition of each treatment group.

$$\text{AUC}_{0\text{-}6} = \sum_{6}^{0} \left( \frac{(\text{Yn}-1 + \text{Yn})\,(\text{Xn} - \text{Xn}-1)}{2} \right)$$

Description :

$AUC_{0-6}$ = area under curve from 0 to 6 hour (mm.hour)

$y_{n-1}$ = difference in skin fold thickness at hour - (n-1) (mm)

$y_n$ = difference in skin fold thickness at nth hour (mm)

$x_{n-1}$ = hour - (n-1) (hour)

The percentage value of inflammation inhibition is calculated using the formula:

$$\text{Inhibition of Inflammation (\%)} = \frac{(\text{AUC o-6})\text{o} - (\text{AUC o-6})\text{n}}{(\text{AUC o-6})\text{o}} \times 100\ \%$$

Description :

$(AUC_{0-6})_0$= average AUC of negative control total (mm.hours)

$(AUC_{0-6})_n$= total AUC value in the nth treatment group (mm.hours)

The results of the calculation of the average AUC value in each treatment were used to calculate the percent inhibition of inflammation which was then analyzed statistically [27].

## RESULT AND DISCUSSION

### Extraction and Fractionation

Rhaphidophora pinnata leaf simplicia powder extracted using percolation obtained a thick extract of 124.35 grams. From the thick extract of Rhaphidophora pinnata leaves, the n-hexane fraction was 39.20 grams. In the n-hexane fraction of Rhaphidophora pinnata leaves, a qualitative test was carried out by dropping the Lieberman-Burchard reagent, which appeared green, indicating that the fraction contained steroids.

### Evaluate cream preparations

Evaluation of cream preparations carried out on creams with doses of 2.5%, 5% and 10% includes organoleptic examination, homogeneity, pH examination, an examination of the washability of the cream, spreadability test, skin irritation test, examination of the physical stability of the cream. Organoleptic examination results including appearance, color, and odor which are done visually can be seen in Table 2 below:

**Table: 2.** Organoleptic examination results for cream preparations

| Formula | Color | Odor | Form | Homogeneity |
|---|---|---|---|---|
| F1 | LG | Ty | SS | h |
| F2 | DG | Ty | SS | h |
| F3 | DG | Ty | SS | h |

Description :

LG = Light Green; DG = Dark Green; Ty = Typical; SS = Semi Solid; h = Homogeneous

F1 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 2,5%

F2 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves %

F3 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 10%

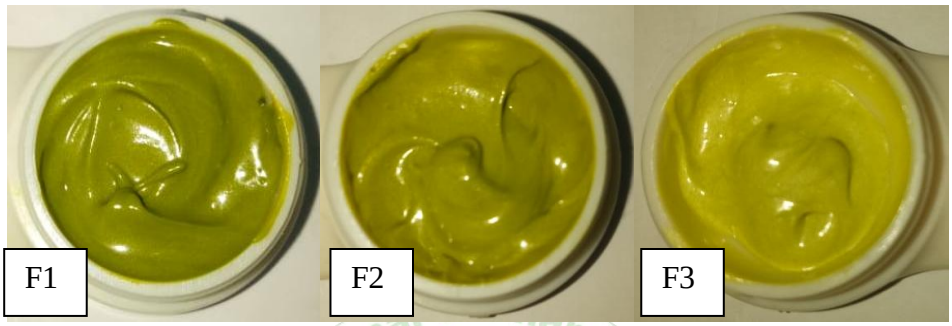


**Figure: 1** Photo of cream preparation containing n-hexane fraction of dragon tail leaves

Description :

F1 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 2,5%

F2 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 5 %

F3 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 10%

The homogeneity examination aims to determine whether the cream preparations are evenly distributed or not. The test is done visually by applying ointment on a piece of glass. The homogeneity test results on the cream preparations showed a homogeneous arrangement which was marked by no coarse grains on the preparations smeared on the glass. The results of the homogeneity test can be seen in Figure 2.

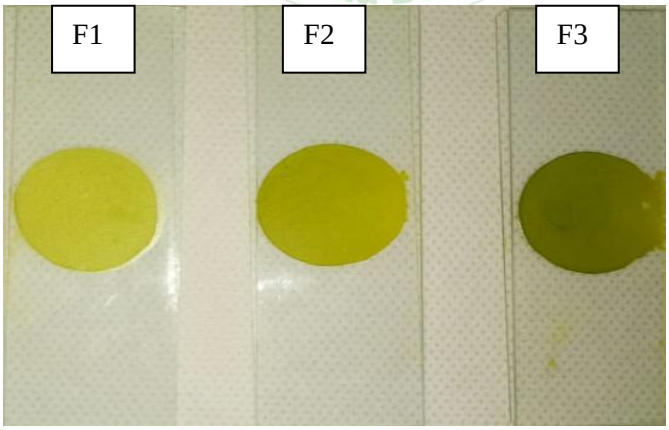


**Figure: 2.** Preparation of Homogeneity Test Results

PH examination to determine the safety of the preparation when used so that it does not irritate the skin. If there is an incompatibility with the pH of the skin, it can cause irritation which results in discomfort in use. The pH test results on the cream formulas of F1, F2 and F3 were 6.3, 6.2, 5.9, respectively. These results are following the criteria for skin pH, which is at susceptible 4.5-6.5. If the pH of the preparation is too acidic it can cause skin irritation, whereas if the pH of the preparation is too alkaline it will cause the skin to become scaly.

The examination of the washability of the cream aims to see whether the cream is easy to wash after use. The washability of the cream can be influenced by the physical and chemical properties of the nutritious substance, the type

and nature of the cream as a carrier, the area of use, the nature and condition of the user's skin. The results of washing checks are good so that they are good for use on the skin and are easy to wash

The spreadability test aims to determine the extent of the distribution of cream when applied to the skin so that it can make it easier to apply the cream on the skin. A good cream has a good spreadability ranging from 5-7 cm so that it can be applied to large skin surfaces without excessive emphasis. Measurement of the spreadability of the cream preparations showed quite good dispersion, namely 2-4 cm.

The cream irritation test aims to find out whether the cream irritates the skin. The test results showed no irritation to the skin so that the cream preparation can be used as a topical preparation. Physical stability checks carried out for 12 weeks at room temperature showed good and stable results. The results of pH, absorption, washability, irritation and stability tests can be seen in table 3.

**Table: 3.** Examination of evaluation of cream preparations

| Formula | pH | Dispersibility | Washability | Irritation Test | Stability |
|---|---|---|---|---|---|
| F1 | 6,3 | 2,42 $cm^2$ | Baik | Tidak Mengiritasi | Stabil |
| F2 | 6,2 | 2,56 $cm^2$ | Baik | Tidak Mengiritasi | Stabil |
| F3 | 5,9 | 2,80 $cm^2$ | Baik | Tidak Mengiritasi | Stabil |

**Description:**

F1 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 2,5%

F2 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 5 %

F3 = Cream with n-hexane fraction of Rhaphidophora pinnata leaves 10%

### Anti-inflammatory activity test of Rhaphidophora pinnata leaf fraction

The results of the anti-inflammatory activity test by measuring the thickness of the edema of each treatment were observed every 1 hour until the 6th hour, AUC and the percentage of inflammation inhibition can be seen in Figure 3 and Table 4.

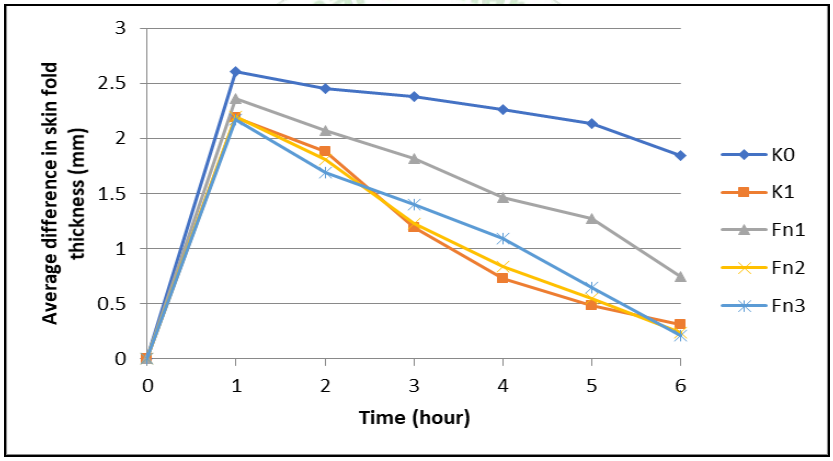


**Figure: 3.** The mean difference in the thickness of the back skin of mice in each group after 3% carrageenan application until the 6th hour

The difference in the thickness of the dorsal folds of the mice is the thickness of the edema used to calculate the AUC value and then proceed to calculate the percentage of anti-inflammatory power of each treatment. By using edema as an evaluation parameter, it can be analyzed the anti-inflammatory potential of synthetic compounds, plant extracts and natural products, both topically and systemically.[28].

AUC values in this study is used to determine the ability of test compounds in reducing edema. The AUC value data processing using statistics shows that the data distribution is normal (p> 0.05) and homogeneous. Continued data processing using the One Way Anova method with the result that there were significant differences in each treatment group (p <0.05). With the Tukey HSD post hoc test, conclusions were obtained as shown in Table 4

**Table: 4** Data on Average Thickness of Edema, AUC and (%) Percent Inhibition of Inflammation in Each Group After 3% Carrageenan Administration

| Treatment | Tebal Edema (mm) | | | | | | AUC 0-6 mm. hour | % PI |
|---|---|---|---|---|---|---|---|---|
| | 1 hour | 2 hour | 3 hour | 4 hour | 5 hour | 6 hour | | |
| **K0** | 2,61 | 2,45 | 2,38 | 2,26 | 2,13 | 1,84 | 12,73[b] | 0,00 |
| **K1** | 2,19 | 1,88 | 1,19 | 0,73 | 0,48 | 0,31 | 6,60[a] | 48,15 |
| **F1** | 2,36 | 2,07 | 1,82 | 1,46 | 1,27 | 0,75 | 9,37[ab] | 26,39 |
| **F2** | 2,20 | 1,81 | 1,23 | 0,84 | 0,55 | 0,24 | 6,73[a] | 47,13 |
| **F3** | 2,17 | 1,69 | 1,40 | 1,09 | 0,65 | 0,21 | 7,09[a] | 44.3 |

a = significantly different to negative control, b = significantly different from positive control

Based on the statistical analysis above, it was concluded that the K0 group was not significantly different from the Fn1 group. While the K1 group was not significantly different from F2, F3, and F1. The anti-inflammatory effect produced with a 2.5% dragon tail leaf fraction still showed no significant difference with the negative control group.

The same anti-inflammatory effect with 2.5% hydrocortisone is the dragon tail leaf fraction 5% and 10% and 2.5%.

Hydrocortisone cream 2.5% as a positive control showed good inflammation inhibition value, indicating that the topical anti-inflammatory test model was running well. Increasing the concentration of dragon tail leaf fraction from 2.5% to 5% provides greater inhibition of inflammation. Data on the percent inhibition of inflammation for each group every 1 hour to 6 hours can be seen in Table 5.The steroid compounds contained in the dragon tail leaf fraction work by inhibiting the enzyme phospholipase A2, an enzyme that plays a role in the synthesis of arachidonic acid which then produces inflammatory mediators[29,30].

## CONCLUSIONS

The cream formulation (F3) of Rhaphidophora pinnata leaf hexane fraction of 5% in the cream preparation has the same effectiveness as the comparison of hydrocortisone cream in the form of cream, showing no significant difference (p> 0.05) after statistical analysis of the Tukey HSD post hoc test paired with the AUC value. 6.73 mm.hours and percent inhibition of inflammation 43.17%

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