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TEST ANTINFLAMMATION CAPSULE COMBINATION OF MORINGA LEAVES (Moringa oleifera L) AND KARUK LEAVES (Piper sarmentosum Roxb. Ex. Hunter) EXTRACT TEST AGAINST MICE (Mus musculus)

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ABSTRACT

Empirically utilized medicinal plants include Moringa leaves (Moringa oleifera L) and Karuk leaves (Piper sarmentosum Roxb. Ex. Hunter). Moringa leaves contain active compounds, such as vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, and oxalates. The leaves of karuk contain polyphenols, flavonoids, saponins, and tannins. This study aimed to assess the anti-inflammatory activity of combination capsules of Moringa and Karuk leaf extracts in mice (Mus musculus). Anti-inflammatory agents are drugs that can suppress or reduce inflammation. The aim of the experimental method used in this study was to determine the optimal concentration for anti-inflammatory activity. Carrageenan as an inflammatory mediator in mouse paws. The anti-inflammatory test involved five groups of animals, each consisting of five mice. The negative control group received Na-CMC, the positive control group was administered diclofenac sodium, and the test samples involved a combination capsule of Moringa and Karuk leaf extract at doses of 350, 700, and 1,050 mg. Measurements were performed every hour for 6 hours after carrageenan induction. Data analysis employed the One-way ANOVA method to ascertain whether variations in the dose of the combination capsule of Moringa and Karuk leaf extracts affected anti-inflammatory activity in mice. The results indicate that the greatest inflammation inhibition occurs with the 1,050 mg dose, resulting in 23% inhibition, followed by 22% at 700 mg and 13% at 350 mg. Statistical analysis yielded a p-value of 0.048, which was less than 0.05. This suggests a significant difference in the anti-inflammatory activity among the test groups. The combination capsule of Moringa and Karuk leaf extract at a dose of 1,050 mg demonstrated more effective anti-inflammatory properties than the other combinations but remained below the effectiveness of diclofenac sodium.

Keywords: Anti-inflammatory, Capsule, Moringa leaves, Karuk leaves

INTRODUCTION

Before the discovery of chemical drugs, medicinal plants were used for centuries. When taken directly, medicinal plants are generally safe and almost always free of side effects. Karuk and Moringa plants are among the many medicinal plants available in Indonesia and are known for their significant benefits. According to people's experiences, both plants offer various advantages.

Moringa stands out as one of the plants with the highest nutritional content ever discovered by humans. Rizkayanti et al. (2017) identified several active compounds in the Moringa plant, including vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, and oxalates. This

comprehensive profile of active compounds shows the potential therapeutic properties of moringa in various health aspects.

Karuk leaves boast numerous health benefits, including their role in stimulating heart and blood circulation, lowering cholesterol, and acting as antitumor, antipyretic, antiepileptic, anti-inflammatory, diuretic, antihypertensive, antioxidant, antidiabetic, antibacterial, and antifungal properties (Pratama Putra, Dharmayudha and Sudimartini, 2017). These leaves are particularly rich in flavonoids, saponins, polyphenols, monoterpenes, and sesquiterpenes, which are traditionally used to treat gallstones, carminatives, coughs, and urinary laxatives (Septiani, Choirunnisa and Syam, 2017). Additionally, Karuk roots have been used in the treatment of pleural inflammation, fungal dermatitis on the feet, coughs, and asthma (Virgianti, 2017). Knowledge of these advantages stems from personal experiences, contributing to the potential utilization of these plants in traditional medicine.

Capsules are a form of medicinal preparations with significant potential. Capsules effectively mask the unpleasant taste and odor of medications. In light of the aforementioned context, further research is necessary to assess the anti-inflammatory potential of capsules containing Moringa and Karuk leaf extracts. This study aimed to determine whether the combination of Moringa and Karuk leaf extracts in capsule form possesses anti-inflammatory properties and is safe for public consumption.

RESEARCH METHODS

The research employed in this study was experimental. This type of research is designed to conduct experiments (experimental research) to identify symptoms resulting from specific treatments. The objective of this study was to assess the impact of a combination capsule of Moringa and Karuk leaf extracts on carrageenan-induced inflammation in mice at various doses.

Equipment and Materials

The materials employed in this research included the following: Moringa and Karuk leaf extracts, vivapur 101, avicel pH 102, lactose, aerosol, talc, magnesium, ethanol 70%, distilled water, carrageenan 1%, Na-CMC, NaCl 0.9%, and Diclofenac Sodium tablets. The tools utilized in this study included a sonde, 1mL syringe, stirring rod, gloves, cup, beaker, filter paper, analytical balance, thread, ruler, incubator, drying cabinet, water bath, electric stove, dropper, spatula, mortar and stamper, and sieve. The test subjects for this study were mice.

Data Analysis

Data analysis was conducted to establish the relationship between each independent and dependent variable. The following data analysis methods were used:

1. Percentage of Inflammation Inhibition

The data obtained in this study are quantitative, involving the calculation of the difference in values before and after administering capsules containing a combination of Moringa and Karuk leaves to mice every hour for 6 hours. Observations were performed at 0, 1, 2, 3, 4, 5, and 6 hours.

2. Statistical Analysis

Subsequently, the collected data were analyzed to assess the impact of variations in the dosage of the combination capsule of Moringa and Karuk leaves, utilizing statistical methods with a confidence level of 95%. Data were tested for normality and homogeneity to determine whether they were normally and homogeneously distributed. If the data met these criteria, One-way ANOVA was performed. Conversely, if the data were not normally and homogeneously distributed, the Kruskal-Wallis test was conducted.

RESULTS AND DISCUSSION

A. Plant Determination Results

Plant determination was performed at the Laboratory of the Faculty of Biology, Galuh Ciamis University. The plant parts assessed included leaves, stems, and roots. The results of this study confirm that the plants used in this study were Moringa and Karuk leaves.

B. Simplicia Moringa and Karuk leaves

The samples utilized in this research to produce simplicia were fresh Moringa and Karuk leaves obtained from the Cihaurbeuti area, Ciamis Regency, West Java, Indonesia. The collected Moringa and Karuk leaves underwent a thorough washing process using running water to remove any dirt. After draining, the leaves were subjected to a drying process aimed at reducing their water content. This involved covering them with a black cloth and allowing them to air dry in a well-ventilated area. To shield the simplicia from direct sunlight and to preserve its compounds, a black cloth cover was used during the drying process. The duration of the drying process was 14 days, which was significantly influenced by weather conditions. **Table I** presents the water content data for the simplica.

Table I. Simplicia Water Content

Simplicia	Wet weight	Dry weight
Moringa leaves	7000 g	750 g
Karuk leaves	3000 g	290 g

Karuk leaves exhibited a water content of 9.6%, while moringa leaves had a slightly higher content of 10.7%. The 10.7% water content in moringa leaves suggests that the reduction in water content achieved through drying is still unsatisfactory. To assess the quality of the simplicia, its yield value must first be determined; if it remains higher than 10%, the simplicia should undergo further drying. Exceeding the 10% yield limit can compromise the quality of simplicia. According to the Herbal Pharmacopoeia Edition II, simplicia, a medicinal substance, should ideally contain no more than 10% water. This served as the standard water content for medicinal simplicia. It can be concluded that the standards are not met by Moringa leaves simplicia owing to challenges in controlling the drying process temperature, resulting in seemingly dry simplicia with elevated water content. Elevated water content (>10%) encourages microbial growth, thereby compromising the stability of the extract (Utami *et al.*, 2017). Increasing the drying time is the most effective means of optimizing the drying of moringa leaves.

C. Ethanol Extract of Moringa and Karuk leaves

Simplicia underwent maceration by soaking in a glass container containing 70% ethanol. Soaking was conducted to assess the quality of the extract. The yield obtained from a sample is crucial because it determines the quantity of extract acquired during the extraction process. Additionally, the yield data were linked to the active compounds present in a sample. Thus, an increase in the yield corresponded to an increase in the number of active compounds within the sample.

The filtered results were subsequently subjected to evaporation in a bath maintained at a temperature of 50°C to prevent damage to the active substances. The evaporation process yielded 200 grams of concentrated moringa leaf ethanol extract and 40 g of karuk leaf ethanol extract. Detailed results of the yield of Moringa and Karuk leaf extracts are presented in **Table II**.

Table II. Extract Yield Results

Material	Simplicity Weight	Extract Weight
Moringa leaves	750 g	200 g
Karuk leaves	290 g	40 g

Table II indicates that the yield of the Karuk leaf extract was 13.7%, whereas that of the Moringa leaf extract was 26.6%. Given that both Moringa and Karuk leaf extracts

achieved yields of not less than 10%, positive outcomes were obtained. A satisfactory yield, as outlined in the Herbal Pharmacopoeia Edition II, should not be below 10%. The yield data confirmed that the extracts from Moringa and Karuk leaves met the required yield standards. Notably, higher yield values, as suggested by Wijaya et al. (Wijaya, Novitasari and Jubaidah, 2018), contribute to the production of superior extracts.

D. Phytochemical Screening of Ethanol Extract of Moringa and Karuk leaves

Phytochemical screening is a method for identifying the presence of secondary metabolite compounds in natural substances. In this study, qualitative phytomia screening was conducted involving a color reaction using specific reagents. The results of the phytochemical screening are presented in **Table III**.

Table III. Phytochemical Screening Results

Phytochemical Test	Reagent	Result
Flavonoids	Weighed 0.5 g of sample, put it in a test tube and added 2 mg of Mg powder, then added 3 drops of concentrated HCl	Orange colored
Saponin	Saponins can be detected by the foam test in hot water. The sample in the test tube was shaken vigorously for several minutes. Stable foam was visible for 5 minutes and did not disappear when adding 1 drop of 2 N HCl	Foaming for more than 5 minutes
Flavonoids	Weighed 0.5 g of sample, put it in a test tube and added 2 mg of Mg powder, then added 3 drops of concentrated HCl	Orange colored

Table III indicates the presence of flavonoids in the ethanol extracts of Moringa and Karuk leaves. A positive result is characterized by a color change from blackish-green to orange, which is attributed to the presence of phenolic compounds. Color formation occurs because of the conjugation of the aromatic group when phenol and acid react. The appearance of foam in this test signifies the presence of glycosides that are capable of forming foam in water. This foam test was employed to identify saponins in the ethanol extracts of Moringa and Karuk leaves (Supriyanto *et al.*, 2017).

E. Combination Capsules of Moringa and Karuk Leaves Extracts

1. Making Capsules

Thick extracts of Moringa and Karuk leaves were ground into dry extract powder using Vivapur 101 in a 1:1 ratio for capsule production. The weighing calculations for 300 capsules are included in attachment 6. Each ingredient was weighed, and a binder solution by mixing 10.5 g of Avicel PH 102 with warm water at a temperature below 50°C. Add 3.15 g of Aerosil, 42 g of powdered Moringa leaves extract, and 12 g of extra powdered Karuk leaves. After homogeneity was achieved, the binder solution (Avicel) was gradually

incorporated until the mixture reached a smooth consistency. After sifting through mesh number 18, it dry for an hour at a temperature below 50°C in an oven or drying cabinet. Subsequently, combine it with 2.1 g of talc, 1.05 g of magnesium stearate, and 43.2 g of lactose until homogenous. Finally, it was shifted once more through mesh number 20.

2. Capsule Shell Filling

Fill the capsule shell manually by placing each moringa leaf extract powder into the capsule shell one at a time. The capsule shell used had a size of 0, was transparent, and clear in color. Because the capsule weighs more than 300 mg, a shell number of 0 was used. When filling the capsules by hand without additional tools, divide the powder into an appropriate number of parts—300 in this case—and then insert each part into the capsule body before sealing it. After filling, the capsules were cleaned with a flannel cloth to obtain a shiny appearance.

3. Evaluation of Capsule Preparations

a. Weight Uniformity Test

The weight uniformity test aims to determine the weight deviation per capsule, which is related to the deviation in the dose per capsule. The capsule weight uniformity must comply with the applicable regulations; otherwise, significant deviations may result in varying dosages. The average data from the weight uniformity test results were as follows:

Table IV. Weight Uniformity Test Results

No	Capsule weight (mg)	7,5%	15%
1	352	√	√
2	353	√	√
3	351	√	√
4	353	√	√
5	354	√	√
6	354	√	√
7	352	√	√
8	354	√	√
9	352	√	√
10	356	√	√
11	353	√	√
12	354	√	√
13	354	√	√
14	355	√	√
15	352	√	√
16	354	√	√
17	353	√	√
18	354	√	√
19	353	√	√
20	353	√	√
X	**353.3**	**There are no deviations**	**There are no deviations**

No capsule exhibited a weight deviation from the average weight exceeding the value specified in column A (7.5%), and none surpassed the threshold listed in column B (15%). This conclusion was drawn from the weight uniformity test results table, which involved testing 20 capsules containing a combination of Moringa and Karuk leaf extracts.

b. Time Crush Test

The disintegration time test is crucial for assessing the disintegration time of the capsule preparation. For a therapeutic effect, the capsule must undergo fragmentation into smaller particles, enabling absorption of its contents in the digestive tract. The results of the disintegration time test are presented in **Table V**.

Table V. Time Crush Test

Capsuls	Crush Time
1	04.10
2	04.12
3	04.18
4	04.20
5	04.22
6	04.25
Averages	**04.17**

The average disintegration time results for the capsule combination of Moringa and Karuk leaf extracts satisfied the standard, as indicated by the results of the disintegration time test in Table V. The Indonesian Pharmacopoeia III Edition 2017 specifies that the disintegration time of a capsule must not exceed 15 minutes, and the obtained results are in accordance with this requirement.

F. Anti-Inflammatory Test Results

The anti-inflammatory test was conducted using a measuring thread and ruler. The purpose of this test was to observe the reduction in inflammation in the test animals. Different dose variations were employed in each treatment, including a 350 mg dose converted to 11.3 mg/10 ml, a 700 mg dose converted to 22.75 mg/10 ml, and a 1050 mg dose converted to 34.1 mg/10 ml. The positive control used diclofenac sodium solution at a dose of 0.325 mg, while the negative control involved Na CMC solution at a dose of 1 mg/10 mL, and carrageenan solution served as the inducer at a dose of 1 mg/10 mL. Data regarding the average reduction in inflammation every 1 hour for 6 hours are presented in **Table VI**.

Table VI. Average Decrease in Edema Volume

Sample	Average decrease in mouse edema volume (cm)							
	The beginning	Induction	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
Na-CMC	1,4	2,12	1,84	2,46	2,64	2,81	2,46	2,44
Diclofenac Sodium	1,32	2,0	2,22	2,02	1,9	1,7	1,56	1,46
Capsules Dosage 350 grams	1,26	1,76	2,12	2,0	2,06	1,66	1,96	1,52
Capsules Dosage 700grams	1,46	2,12	2,36	2,38	2,14	1,88	1,74	1,64
Capsules Dosage 1050grams	1,5	2,36	2,42	2,62	2,28	2,12	1,86	1,72

Based on **Table VI**, it is apparent that the anti-inflammatory value decreases each hour over a six-hour period. A graphical representation of the data concerning the average decrease in the anti-inflammatory effects is shown in **Figure 1**.

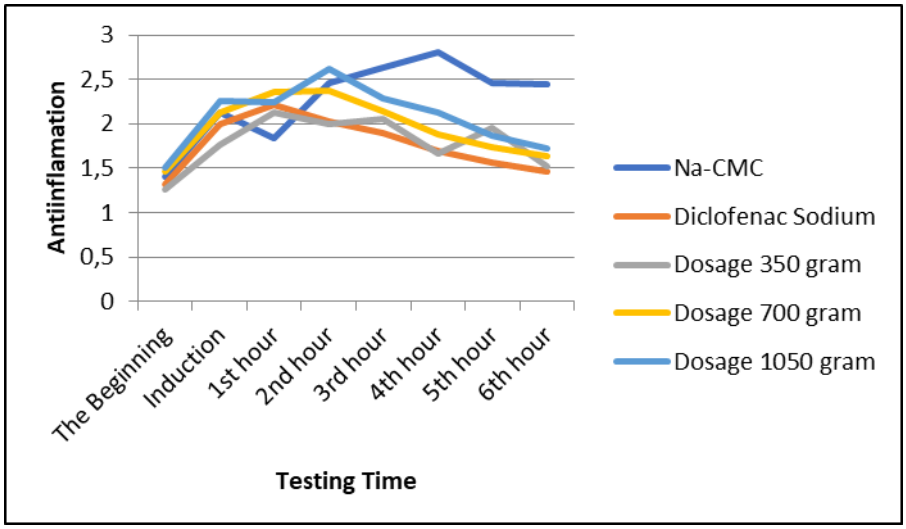

Figure 1. Decreased Inflammation Every Hour For 6 Hours

Table VI and Figure 1 illustrate a progressive reduction in inflammation each hour over the six-hour observation period. From the first to the second hour, inflammation experienced a further increase, followed by a decline from the third hour onwards until the sixth hour.

In the 350 mg dose test group, the reduction in inflammation commenced in the second hour, reaching its peak, and subsequently subsiding until a noticeable drop was observed in the fifth hour. Comparatively, the 700 mg test group exhibited the fastest onset time and the most effective reduction in inflammation. In this group, a decrease in inflammation was initiated in the second hour and continued steadily until the sixth hour. In the 1,050 mg test group, inflammation reduction commenced between the second and third hours, followed by an increase in the fourth hour, and a significant decrease in the fifth to sixth hours. The onset variation in the condition of each test animal, including body weight, enzyme production, digestive tract absorption processes, and unique biological and physiological factors, contributed to the observed differences.

Table VII. Average Decrease in Edema Volume

Sample	Before	After	Difference	% Decrease
Na-CMC	2,0	2,55	0	0
Diclofenac Sodium	2,0	1,8	0,2	11 %
Dosage 350 mg	1,76	1,75	0,01	0,57 %
Dosage 700 mg	2,12	2,0	0,12	5 %
Dosage 1050 mg	2,28	2,16	0,12	5,2 %

Table VII reveals that variations in the amount of inflammation reduced each hour for the six-hour duration before and after treatment. The diagram in Figure 2 visually presents the data depicting the changes in the inflammation reduction.

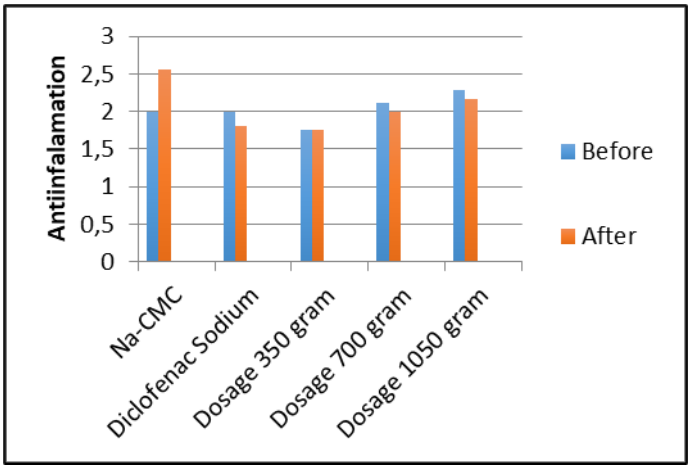

Figure 2. Average Reduction In Inflammation Before And After Being Given The Test Substance

The test substance used in this study was a combination of Moringa and Karuk leaf extract capsules. The table and diagram depicting the reduction in inflammation before and after treatment clearly show a noteworthy decrease in inflammation. This reduction is attributed to the presence of compounds in the extracts of both plants, specifically saponin and flavonoid compounds, which possess potential anti-inflammatory properties. Flavonoids, to exhibit anti-inflammatory and saponin-producing properties, and can inhibit cyclooxygenase or lipoxygenase, as well as the accumulation of leukocytes, by preventing exudate formation and vascular permeability (Al-Khayri *et al.*, 2022) In the 350 mg test group, there was a 0.57% reduction in inflammation before and after treatment, while the 700 mg group exhibited a 5% reduction, and the 1050 mg group demonstrated the most effective reduction at 5.2%. These results surpassed those of the other three doses of extracts from the leaves of Moringa and Karuk combined into capsules. The test group receiving 1,050 mg, with a higher concentration of flavonoids and saponins than the other test doses, showed the most effective reduction in inflammation. When compared to the diclofenac sodium-positive group, the test group demonstrated a more effective reduction in inflammation.

Table VIII. Standard Deviation of Reducing Inflammation

Group	Mean±SD	P-Value
Na-CMC	2.27±0.46	0.34
Diclofenac Sodium	1.77±0.31	0.82
Dosage 350 gram	1.79±0.29	0.48
Dosage 700 gram	1.96±0.33	0.61
Dosage 1050 gram	2.07±0.35	0.79

The levels of inflammation in the test animals exhibited significant variation across each treatment, as indicated by the results of the statistical tests on the average reduction in inflammation. No noticeable discrepancy was observed in the outcomes of inflammation among the mice across all the test treatments. All average reductions in inflammation data presented a normally distributed P-value > 0.05. The null hypothesis (H0) is rejected given that the One-way ANOVA results yielded a p-value of 0.048, which is less than 0.05. The levels of inflammation in the test animals showed significant variations depending on the test treatment. **Table IX** provides information on the average inhibition of inflammation every hour for six hours.

Table IX. Mean Percent Inflammation Inhibition

Treatment	Mean Percent Inflammation Inhibition (cm)						
	The Begining	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
Na-CMC	0	0	0	0	0	0	0
Diclofenac Sodium	0	-11%	-1%	5%	15%	22%	27%
Dosage 350 grams	0	-20%	-13%	-17%	5%	11%	13%
Dosage 700 grams	0	-11%	-11%	-0.9%	11%	17%	22%
Dosage 1050 grams	0	-7%	-15%	-0.8%	6%	16%	23%

Table IX indicates the percentage inhibition of inflammation each hour over a six-hour period. Figure 3 shows a diagram illustrating the percentage of inhibition of inflammation.

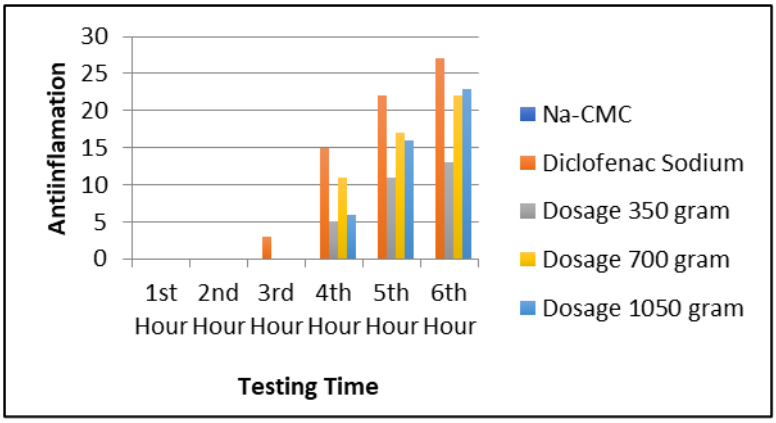

Figure 3. Mean Percent Inflammation Inhibition

It is evident from the table and inflammation inhibition diagram (Figure 3) that administering Na-CMC colloidal solution had no effect on the inhibition of inflammation in the feet of mice. Of all the dosage groups, capsules containing a combination of 1,050 mg Moringa and Karuk leaf extracts had the best ability to inhibit inflammation. With 27% in the edema volume of the comparison group (diclofenac sodium), the percentage of inflammation inhibition was higher than that of the test solution, indicating that sodium diclofenac had a higher inhibitory potential.

Table X. Standard Deviation of Inflammatory Inhibition

Group	Mean±SD	P-Value
Na-CMC	0,00±0,00	0,00
Diclofenac Sodium	1,77±0,31	0, 87
Dosage 350 gram	1,79±0,29	0, 17
Dosage 700 gram	1,96±0,33	0,34
Dosage 1050 gram	2,07±0,35	0,99

The inhibition values of the test animals exhibited significant variation across each treatment, as indicated by the results of the statistical tests on the average inhibition of inflammation. A One-way ANOVA was applied because the average inhibition of

inflammation in mice for all data demonstrated a normal distribution (P > 0.05). The alternative hypothesis (Ha) was accepted given that the One-way ANOVA results showed a p-value of < 0.05, specifically 0.048, signifying a notable difference in the values of inflammation inhibition among the test animals in each treatment group.

CONCLUSION

The research findings led to the conclusion that the combination of Moringa and Karuk leaf extracts in capsule form exhibits anti-inflammatory effects. These anti-inflammatory properties were evident in capsules containing 350, 700, and 1,050 mg of the combined Moringa and Karuk leaf extracts.

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