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Anti-Inflammatory Activity of Kalanchoe Pinnata (B. Pinnatum) Stem Extract on Acetic Acid-Induced Inflammation in Mice (M. Musculus)

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ABSTRACT

The aim of this study was to test the anti-inflammatory activity of Kalanchoe Pinnata (Bryophyllum pinnatum) stem through feeding its infusion to mice (Mus musculus) injected intramuscularly with acetic acid to induce inflammations. The Complete Randomized Design (CDR) under the experimental method was employed wherein the five set-ups had been replicated three times: T0 (10 mg/kg aspirin in 10 ml/kg distilled water), T1 (10 ml/ kg distilled water), T2 (10 v/v Kalanchoe pinnata stem extract at 10 ml/kg), T3 (50 v/v Kalanchoe pinnata stem extract at 10 ml/kg), and T4 (80 v/v Kalanchoe pinnata stem extract at 10 ml/kg). Their anti-inflammatory activities were acquired through the mean percentage score. Meanwhile, Analysis of Variance (ANOVA) was used to validate the significant difference between the treatments administered. The findings showed that after 4 hours, the average gain in thickness due to inflammation in the mice's (Mus musculus) hind legs were minimized by the treatments: T0 = ~0.79 mm; T1 = ~1.51 mm; T2 = ~1.46 mm; T3 = ~1.36 mm; and, T4 = ~1.16 mm. Moreover, the ANOVA showed that the p-value calculated was lower than the significance level under which the data was tested (0.0000<.01). It was concluded that given at high concentrations, Kalanchoe pinnata (Bryophyllum pinnatum) stems are anti-inflammatory. This entailed that the plant part has considerable effectiveness against inflammations.

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1. INTRODUCTION

Inflammation is one of the body's defense mechanisms when foreign substances enter the body. But, in some cases, they can go out of hand. Discomfort, swelling, heat, redness, and reduced function are among the symptoms of inflammation. It can manifest itself practically everywhere in the body. Inflammation of the skin can be caused by several things, including immune system failure, allergic reactions, or infection. Rash, redness, skin heat, and blistering are all frequent signs of these infections. Skin inflammations can be acute, such as from a skin infection, or chronic, such as from skin illnesses (Lee *et al*., 2013).

To combat them, anti-inflammatory medicines, namely synthetic glucocorticoids, and non-steroids, are the way to go (Gunaydin & Bilge, 2018). Except, overprescription and misuse of these drugs may pose serious side effects on their users (Arnold, 2013). At times, they can get pricey, and not everyone can access them. Inflammations may occur to anyone anytime, and safer and cheaper alternatives to cure them exist - one of which is herbal medications (Dolan *et al*., 2019).

Kalanchoe pinnata *(Bryophyllum pinnatum)*, on the other hand, has exhibited cutaneous anti-inflammatory activities (Chibli *et al*., 2014). Locally known as "katakataka" in the Philippines, its use has been gaining popularity due to its leaves which are known to be valuable (Valdez & Canapi, 2015). Not much is heard about others of its parts. Hence, this study focused on the plant's stem and conducted experiments on acetic acid-induced inflammations in laboratory mice's *(Mus musculus)* hind legs to ascertain its anti-inflammatory property.

2. METHODS

2.1. Experimental design and treatments

This study employed Complete Randomized Design with five (5) different treatments, each replicated three (3) times to determine the anti-inflammatory activity of the Kalanchoe pinnata (*Bryophyllum pinnatum*) stem extract. The treatments were divided into five (5) groups with three (3) laboratory mice (*Mus musculus*) in each that randomly received the treatments (see **Figure 1**).

The treatments were as follows:

T0 - 10 mg/kg aspirin in 10 ml/kg distilled water.
T1 - 10 ml/kg distilled water.
T2 - 10 v/v K. pinnata (*Bryophyllum pinnatum)* stem extract at 10 ml/kg.
T3 - 50 v/v K. pinnata (*Bryophyllum pinnatum*) stem extract at 10 ml/kg.
T4 - 80 v/v K. pinnata (*Bryophyllum pinnatum*) stem extract at 10 ml/kg.

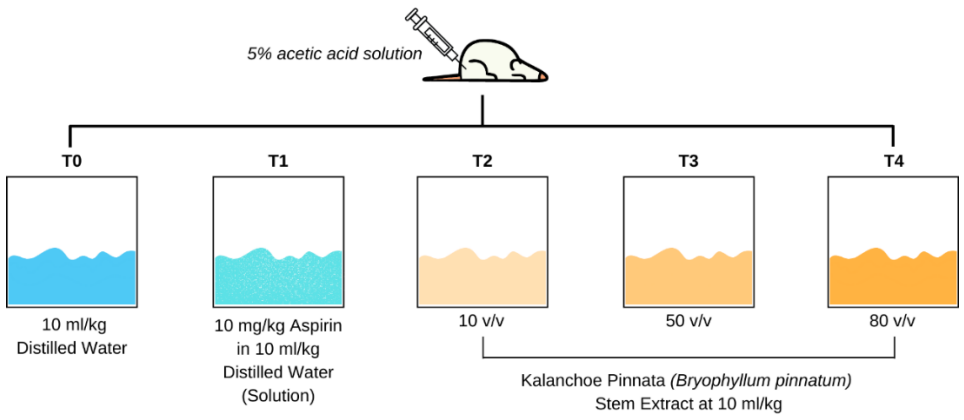

Figure 1. Dependent variables (treatments).

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2.2. Procedure

The procedures done in this study were according to the experiment layout. The Kalanchoe pinnata (*Bryophyllum pinnatum*) stems were cut from the plant and were air-dried for at least 2 weeks. The dried stems were pulverized and cooked in distilled water at a ratio of 1:10. The filtrates were strained using a perforated cloth. During the laboratory experiments, the extracts were diluted according to the different volume percentages of the experimental treatments.

On the other hand, mice (*Mus musculus*) of both sexes were bought from an animal breeder. They were kept in a large enough vivarium and were given free access to dry pellets and distilled water for at least 14 days before the experimentation. They fasted for 12-18 hours before the experiments.

During those long processes, the materials needed were collected one at a time. They were kept in a single place and cleaned free from unnecessary substances. On the day of the laboratory experiments, they were grouped according to what treatment they were in use.

In the experiments, the treatments were administered to the mice (*Mus musculus*) orally via a gavage needle. After exactly 30 minutes, the 5% acetic acid shots were injected intramuscularly into their hind legs, which served as the inflammation sites. The inflammatory growth was recorded using a caliper scaled at 0.05 mm every 30 minutes for a total of 4 hours. The measurements served as the data to establish the anti-inflammatory activity of the Kalanchoe pinnata (*Bryophyllum pinnatum*) STEM.

2.3. Data gathered

The following were the data gathered, tabulated, and calculated to attain the objectives of the study:

(i) Normal sizes of the laboratory mice's hind legs - before the actual experimentation of the laboratory mice (*Mus musculus*) in all treatment groups, the normal sizes of the laboratory mice's (*Mus musculus*) hind legs were measured to establish inflammatory growth.

(ii) Thickness measurements of inflammations - for every 30 minutes, the thickness measurement of the inflammation site in the laboratory mice's (Mus musculus) hind legs was measured, and it was done in repetition in 4 hours.

(iii) Average thickness gain of inflammations - the means of the measurements were calculated using the mean percentage score. The same formula was used to calculate the group mean for each of the treatments administered.

(iv) The significant difference between all the anti-inflammatory treatments - ANOVA was applied to the varying mean thickness gains of the groups of treatments to establish a significant difference between them in terms of their anti-inflammatory activity.

(v) The significant difference between all the experimental treatments - ANOVA was applied to the varying mean thickness gains of the experimental groups of treatments to establish a significant difference between them in terms of their anti-inflammatory activity.

2.4. Statistical analysis

The mean gain in thickness of the acetic acid-induced inflammations in the mice's (*Mus musculus*) hind legs were determined using the mean percentage score. It was done for the individual mouse and consequently for their group. Meanwhile, the Analysis of Variance (ANOVA) determines the significant difference between the anti-inflammatory activity of the treatments.

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3. RESULTS AND DISCUSSION

T0 or treatment zero had the lowest mean thickness gain of all the treatments. It garnered an average of ~0.79 mm (*SD* = 0.07). This only means that it was the treatment with the best anti-inflammatory activity among the treatments administered to the mice (*Mus musculus*). T0 was followed by T4. The average thickness gain of all the replications was ~1.16 mm (*SD* = 0.04). Among the experimental treatments, this treatment with the highest concentration of the Kalanchoe pinnata (*Bryophyllum pinnatum*) stem extract had the fastest minimization of the inflammatory growth of the mice's (*Mus musculus*) hind legs. It was trailed by T3 and T2, respectively. Both treatments are also experimental groups. T3 had an average gain in thickness of ~1.36 mm (*SD* = 0.05) while T2 had ~1.46 mm (*SD* = 0.07). They only differed by 0.1 mm. Finally, the experimental treatments were higher than the negative control T1, which had ~1.46 (*SD* = 0.07) (see **Table 1**).

Table 1. Mean thickness gain of inflamed mice's (*mus musculus*) hind legs after 4 hours based on the anti-inflammatory activity of treatments received.

Replications	Thickness gain of mice's hind legs due to acetic acid-induced inflammations minimized by treatments received orally in millimeters (mm)				
	T0	**T1**	**T2**	**T3**	**T4**
1	0.84	1.59	1.51	1.41	1.16
2	0.71	1.57	1.38	1.35	1.19
3	0.81	1.38	1.48	1.32	1.12
Overall mean/sd	0.79 (*sd* = 0.07)	1.51 (*sd* = 0.12)	1.46 (*sd* = 0.07)	1.36 (*sd* = 0.05)	1.16 (*sd* = 0.04)

Table 2 shows the analysis of variance between all of the treatments administered to the mice (*Mus musculus*) to inhibit the inflammatory growth in their hind legs induced by acetic acid injections. The three replications in the aspirin treatment (T0) had an average gain in thickness of ~0.79 mm (*SD* = 0.07); the ones in the distilled water treatment (T1) gave ~1.51 mm (*SD* = 0.12); and, the experimental groups gave ~1.46 mm (*SD* = 0.07) for the 10 v/v stem extract (T2), ~1.36 mm (*SD* = 0.05) for the 50 v/v stem extract (T3), and ~1.16 mm (*SD* = 0.04) for the 80 v/v stem extract (T4). Thus, their relationship, in terms of their anti-inflammatory activity, is significant, $F(2, 4) = 18.54$, $p = 0.0000$.

Table 2. Mean Thickness Gain of Inflamed Mice's (*Mus musculus*) Hind Legs After 4 Hours Based on the Anti-Inflammatory Activity of Treatments Received.

Source	DF	Sum of Square	Mean Square	F	p
Replication	2	0.01	0.01	0.05	0.9506
Treatment	4	8.32	2.08	18.54	0.0000
Replication: Treatment	8	0.41	0.05	0.45	0.8873
Pooled Error	105	11.78	0.11		
Total	119	20.51			
at .01 level of significance					

Treatment	n	Means	LSD*
A (10 mg/kg aspirin in 10 ml/kg distilled water)	24	0.79	c
B (10 ml/kg distilled water)	24	1.51	a
C (10 v/v Kalanchoe pinnata stem at 10 ml/kg)	24	1.46	a
D (50 v/v Kalanchoe pinnata stem at 10 ml/kg)	24	1.36	a
E (80 v/v Kalanchoe pinnata stem at 10 ml/kg)	24	1.16	b

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Table 3 shows the post hoc analysis of the treatments since there was a significant difference between them. It synthesizes how they differ from each other and determines the significant difference between and among the treatments given to the move (*Mus musculus*). Treatments with the same letters are not significantly different but are comparable to each other. Meanwhile, treatments with different letters are not comparable but are significantly different from each other.

Table 3. Mean Thickness Gain of Inflamed Mice's (*Mus musculus*) Hind Legs After 4 Hours Based on the Anti-Inflammatory Activity of Treatments Received.

Source	DF	Sum of Square	Mean Square	F	p
Replication	2	0.05	0.02	0.24	0.7843
Treatment	4	1.11	0.02	5.72	0.0052
Replication: Treatment	8	0.08	0.02	0.21	0.9335
Pooled Error	63	6.09	0.10		
Total	71	7.33			

at .01 level of significance

Table 4 shows the analysis of variance (ANOVA) between the experimental treatments of the study. The average gain in thickness of the inflammations in the mice's (*Mus musculus*) suppressed by the experimental groups are as follows: ~1.46 mm (*SD* = 0.07) for the 10 v/v stem extract; ~1.36 mm (*SD* = 0.05) for the 50 v/v stem extract; and ~1.16 mm (*SD* = 0.04) for the 80 v/v stem extract. Hence, there is a significant difference between their effectiveness in minimizing the inflammations, F (2,4) = 5.72, p = 0.0052.

Table 4. Analysis from ANOVA.

Treatment	n	Means	LSD*
C (10 v/v Kalanchoe pinnata stem at 10 ml/kg)	24	1.46	a
D (50 v/v Kalanchoe pinnata stem at 10 ml/kg)	24	1.36	a
E (80 v/v Kalanchoe pinnata stem at 10 ml/kg)	24	1.16	b

This post hoc analysis was established for **Table 4** since there was a significant difference between the experimental treatments in terms of their anti-inflammatory effect on the inflammatory growth of the injected sites in the mice (*Mus musculus*). Treatments with the same letters do not differ considerably, but they are comparable. Treatments with various letters, on the other hand, are not comparable and are vastly different in terms of their anti-inflammatory activity.

4. CONCLUSION

The ANOVA analysis for all treatments found that aspirin ranked first and was significantly different from all other treatments. Looking at the means, it was remarkably successful in reducing inflammatory growth. It was followed by the experimental treatment of 80 v/v stem extract, which was the sole experimental treatment that was higher and significantly different from the distilled water. This rejects the initial null hypothesis. Kalanchoe pinnata (Bryophyllum pinnatum) stem has a significant anti-inflammatory effect.

Furthermore, the results demonstrate that Kalanchoe (Bryophyllum pinnatum) pinnata stem at high concentrations can have a considerable anti-inflammatory impact against inflammations. The first null hypothesis, that the plant stem has no substantial anti-inflammatory action, is thus rejected. The findings indicate that, while the 80 v/v stem extract was only second to aspirin, an already-tested and -proven anti-inflammatory medicine, it was

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the closest to it in the ANOVA. It was also greater than distilled water, which had no significant effect on the inflammations, supporting the notion.

Lastly, when the amounts of Kalanchoe pinnata (Bryophyllum pinnatum) stem extracts increased, the average gain in thickness of the inflammation at the inflammation sites decreased, indicating increased anti-inflammatory action. We can observe an inverse proportionality between concentration and inflammatory growth at both ends of the experimental groups. Meanwhile, the ANOVA for the experimental treatments indicates that there is a statistically significant difference between them. The third null hypothesis, that there is no significant difference between the experimental groups given, is thus rejected.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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