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# The Effectiveness of Bay Leaf Extract (*Eugenia polyantha*) on the Stamina Enhancement of Male Mice (*Mus musculus*)

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**Abstract**

*Bay leaves are a common culinary spice used in Indonesian cuisine and are known to contain various chemical compounds including essential oils, flavonoids, tannins, antimicrobial compounds, and vitamin C. This study aims to investigate the effectiveness of bay leaf extract in enhancing the stamina of male mice. The research was conducted using a quantitative experimental method with a completely randomised design (CRD). A total of 20 male mice were used as samples and divided into five treatment groups, which included a control group (P0), a dosage of 3 mg/g body weight (P1), a dosage of 6 mg/g body weight (P2), a dosage of 12 mg/g body weight (P3), and a dosage of 18 mg/g body weight (P4), with each treatment repeated three times. The swimming test was employed to measure the duration of swimming in mice as an indicator of stamina. Data were analysed using ANOVA. The results indicated that the administration of bay leaf extract at various doses over a period of 7 days did not produce a significant (P=0,715) effect on the stamina enhancement of male mice. Therefore, bay leaf extract was not effective in improving the stamina of male mice at the tested dosages.*

## 1. INTRODUCTION

In contemporary times, human activities are increasingly on the rise, and maintaining good health and well-being is fundamental for daily activities. The use of products similar to dietary supplements is essential to enhance physical performance, improve post-exercise recovery, increase muscle mass, and reduce body fat. These dietary supplements are botanical products, which may include extracts, compositions, or herbal concentrates **[1].** The recommended raw materials for the production of supplements include active ingredients derived from herbal plants, in addition to synthetic chemical substances.

Plants exhibit a wide variety of species that are utilised by the Indonesian population for medicinal purposes or as dietary supplements **[2]**. One of the herbal plants utilised is bay leaf.

Bay leaf (*Eugenia polyantha*) is a popular type of spice used in traditional Indonesian cuisine **[3]**. In addition to imparting a distinctive aroma and flavour of food, bay leaves are also known to contain various active compounds, such as essential oils, flavonoids, tannins, antimicrobial substances, and vitamin C, which have the potential to provide health benefits **[4].** Various previous studies have demonstrated that these compounds possess biological activities, including antioxidant, antifungal, antibacterial, antimalarial, antidiarrheal, anti-inflammatory, antihyperlipidemic, and antimicrobial properties **[5].** However, studies examining the effects of bay leaves on physical stamina, particularly in the context of enhancing physical performance, remain limited. In a review article by Novira et al. (2019), the potential health benefits of bay leaves were highlighted; however, specific evidence supporting the role of bay leaves in improving stamina is not yet robust. Stamina is defined as the ability of an organism to sustain prolonged physical activity and resist fatigue over a specified duration, particularly when engaging in high-intensity activities **[6]**.

Activities that directly contribute to enhancing physical stamina include regular and structured physical exercise. The type of activity employed to aid in improving stamina is exercise **[7].**

This study aims to evaluate the effectiveness of bay leaf extract in enhancing stamina using male mice (*Mus musculus*) as test subjects. The swimming test method was employed in this study to measure the swimming duration of the mice and to assess the stamina values resulting from the administration of bay leaf extract at various doses **[8]**.

## 2. MATERIALS AND METHODS

### Materials

The materials used in this study include 15 male mice (*Mus musculus)*, bay leaves (*Eugenia polyantha)*, feed pellets, distilled water (aquadest), and 96% ethanol. The equipment utilized consists of a mice cage measuring 70 cm x 40 cm x 20 cm, a drinking bottle for mice, and aquarium (swimming chamber for mice) measuring 50 cm x 35 cm x 35 cm, porcelain dishes, an analytical balance, an oven, a stopwatch, a camera, syringes, a blender, a 100-mesh sieve, a spatula, measuring cylinders, a glass stirring rod, filter paper, a glass funnel, a volumetric flask, and a rotary evaporator with a water bath.

### Methods

The type of research method employed in this study is experimental research utilising quantitative techniques. This type of research is systematic, meticulous, and logical, allowing for control over specific conditions. In experimental research, the researcher can exercise control over the independent variables both before and during the study **[9].** This study employs a research method designed to investigate the presence of causal relationships and the extent of such relationships by administering specific treatments to several experimental groups while providing a control treatment for comparison **[10].** By utilising this type of method, the effectiveness of administering bay leaf extract (*Eugenia polyantha*) in enhancing the stamina of male mice (*Mus musculus)* can be determined.

### Extraction Process of Bay Leaf (*Eugenia polyantha*)

The extraction process of bay leaves was carried out using the maceration method, which involves the immersion technique applied to the material to be extracted. Maceration is a technique used to extract or obtain the desired compounds from a solution

or solid employing immersion. This method allows the solvent to penetrate the material, facilitating the release of the active compounds into the liquid **[11].**

Bay leaves were harvested from tree branches in a quantity of 4 kilograms. Subsequently, the leaves were sorted to remove any impurities present. The leaves were then thoroughly washed under running water to eliminate any adhering dirt or contaminants **[3].** The bay leaves were then cut into small pieces and air-dried indoors for 7 days until completely dry. The dried leaves were ground into powder using a blender and then sieved through a No. 100 mesh sieve **[12].**

The extraction method employed was maceration for 5 x 24 hours with a ratio of 1:10. A total of 300 grams of bay leaf powder (*Eugenia polyantha*) was placed in a glass container, then 2250 ml of 96% ethanol was added to completely submerge the powder. The container was stored in a place protected from direct sunlight and left to stand for one week, occasionally being stirred **[13].** After one week, the mixture was filtered using filter paper. The filtrate was then concentrated using a rotary evaporator to obtain the bay leaf extract. Concentration was performed until a thick mass containing more than 50% total solids was achieved with the gold standard method of Maceration, under vacuum in an oven at 50°C. The yield obtained was weighed and calculated as a percentage by weight.

$$\text{Yield (\%)} : \frac{\text{Weight of extract}}{\text{Weight of simplicic}} \times 100\%$$

**Animal Preparation**

Preparation of Male mice (*Mus musculus*) was placed in plastic tubs covered with wire mesh. The tubs were lined with rice husks, which were replaced every 1 to 2 days. The mice underwent an acclimatisation period of one week (7 days). During this maintenance period, the mice were fed pellet feed twice daily and provided with drinking water **[14]**.

**Preparation of Test Animals**

The test animals used in this study were male mice (Mus musculus) obtained from the Animal House Abduh Ticus Centre. This experimental study has approval Ethical Clearance from the Public Health Faculty of Sriwijaya University with number xxxx. The selected mice were healthy males aged 2–3 months, weighing between 20 and 30 grams. Each mice was weighed prior to the experiment to record the initial body weight. At the conclusion of the study, the mice were weighed again to determine the final body weight and assess any weight gain [3].

**Subject Grouping**

The subjects were randomly divided into 5 groups, with each group consisting of 3 mice. Group I served as the control group and was only given pellet feed. Groups II, III, IV, and V received oral treatment with extract of bay leaves (*Eugenia polyantha*) at different dosages: Group II received 3 mg/g body weight, Group III received 6 mg/g body weight, Group IV received 12 mg/g body weight, and Group V received 18 mg/g body weight. The treatment was administered once daily for one week **[3].**

**Treatment of Animals**

The treatment to be tested on the mice involved the use of the swimming endurance test. The motor activity of the mice was assessed by placing them in an aquarium filled with water at a depth of 18 cm. The swimming procedure began with an acclimatisation period for the test animals and was conducted once daily for seven minutes over one week **[15].** On the seventh day, record the time when the mice exhibit signs of fatigue before treatment is administered. On the eighth day, the mice were given treatment according to the assigned dosage of bay leaf extract for each group.

After a week, the test animals were subjected to the swimming endurance test again until the mice showed signs of fatigue,

indicated by allowing their heads to remain submerged for 5 seconds **[16]**. At this point, the mice were removed from the water, and the duration of their swimming was recorded. The parameters measured included the inability of the mice to move their legs for swimming, their bodies being upright with a stationary tail, and their heads remaining submerged for 5 seconds. Following this, the mice were allowed to rest for 30 minutes before receiving the oral treatment according to their respective groups. After the 30-minute rest, the mice were placed back into the tank and allowed to swim until they became fatigued again. The Temperature was measure and control by Thermometer. The mice were tested in groups. The duration of swimming after treatment was recorded. The tonic effect data were obtained from the difference in swimming duration before and after treatment **[17].**. The collected data were then compiled and analyzed statistically.

## 3. RESULTS and DISCUSSION

### Body Weight of Mice

The recorded data during the observation of mice body weight before and after treatment over a period of 7 days during the research process. The following table presents the body weight data of the mice

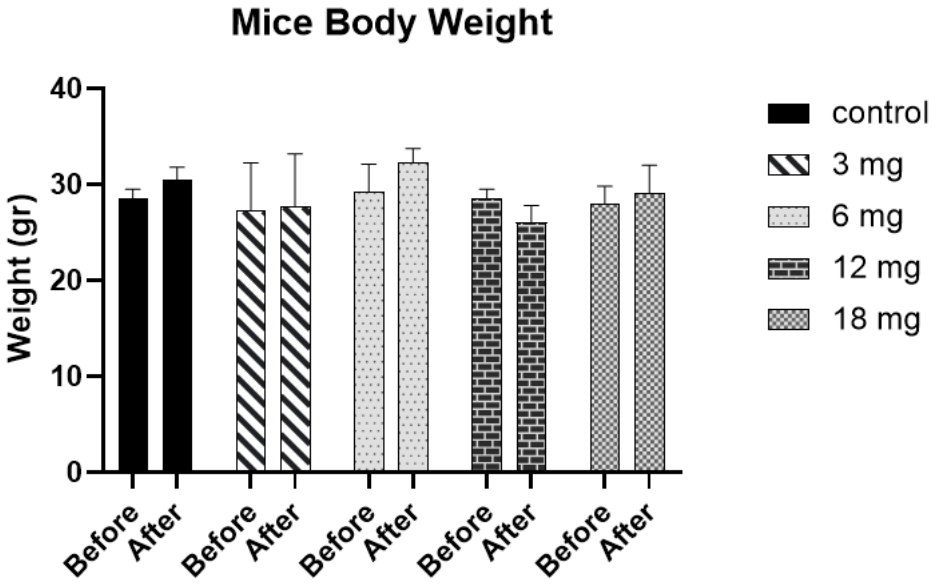
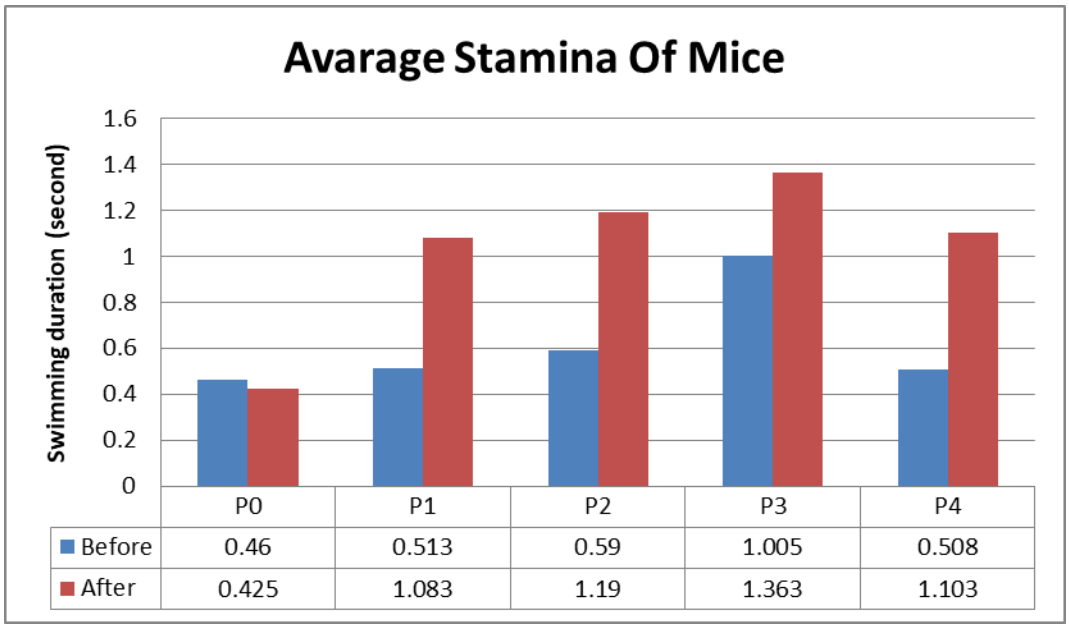



**Figure 1.** Mice body weight before and after treatment (mean ±SD).

Figure 1 presents the body weight data of mice before and after treatment, the graph indicating differences in the average body weight across the various treatment groups. In the control group (P0), the mean body weight before treatment was 28.5 grams, increasing to 30.5 grams following treatment. In treatment group P1, the average body weight decreased from 27.25 grams before treatment to 20.75 grams after treatment. For group P2, the mean body weight increased from 29.5 grams to 32.5 grams post-treatment. In group P3, the average weight decreased from 28.5 grams to 26 grams. Lastly, in group P4, the mean body weight decreased from 28 grams before treatment to 21.75 grams after treatment

**Improvement of Mice Stamina**

Following the observation, the results indicate that there is an effect of bay leaf extract on the increase of stamina in male mice. The following presents the average increase in stamina of male mice before and after treatment (administered with bay leaf extract), illustrated in the form of a bar chart.




| | P0 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|---|
| Before | 0.46 | 0.513 | 0.59 | 1.005 | 0.508 |
| After | 0.425 | 1.083 | 1.19 | 1.363 | 1.103 |

**Figure 1.** Bar Chart of the Average Increase in Stamina of Male Mice Before and After Treatment

Figure 1 indicates the differences in the average duration of stamina improvement in mice across the various treatments administered. The increase in stamina prior to treatment was observed within a time range of 45 to 60 seconds. Following treatment with bay leaf extract, there was a notable enhancement in stamina, with the time range extending to 68 to 96 seconds for treatments P1 (3 mg/g body weight), P2 (6 mg/g body weight), P3 (12 mg/g body weight), and P4 (18 mg/g body weight).

**Hypothesis Testing in Research**

This study performed data analysis using SPSS version 23 for Windows. The initial analysis involved assessing whether the data were normally distributed. The data distribution was evaluated using the Shapiro-Wilk normality test, as the total sample size was relatively small ($n < 50$). The results of the Shapiro-Wilk normality test indicate that the significance value (p) is above the established significance level ($p > 0.05$). The significance of the average value of the stamina group before and after treatment is also above the established significance level ($p > 0.05$). The data were not significant for P2 before treatment and P0 after treatment.

Subsequently, a homogeneity test was conducted to determine whether the data originated from populations with the same variance (homogeneous). The results of the homogeneity test on the data stamina values before and after treatment show significance values above the established significance level ($p > 0.05$). The data indicate that both groups originate from populations with similar variances (homogeneous). Subsequently, a statistical comparison analysis of the two data groups was conducted using the parametric One-Way ANOVA, as the data were found to be homogeneous. The results of the ANOVA test are presented in Table 1

**Table 1.** One Way Anova Stamina Test Results Before Treatment and After Treatment

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Before Treatment | Between Groups | .213 | 4 | .053 | .531 | .715 |
| | Within Groups | 1.505 | 15 | .100 | | |
| | Total | 1.718 | 19 | | | |
| After Treatment | Between Groups | 2.518 | 4 | .629 | 2.695 | .071 |
| | Within Groups | 3.503 | 15 | .234 | | |
| | Total | 6.020 | 19 | | | |

**ANOVA[a]**

| Model | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| 1 | Regression | 5.502 | 2 | 2.751 | 1.356 | .284[b] |
| | Residual | 34.498 | 17 | 2.029 | | |
| | Total | 40.000 | 19 | | | |

a. Dependent Variable: mice group

b. Predictors: (Constant), Before treatment, After treatment

Referring to Table 4 the results of the One-Way ANOVA test indicate a value of 0.715, which is greater than the significance level of 0.05. This suggests that there is no difference in the effectiveness of stamina improvement due to bay leaf extract (*Eugenia polyantha*), leading to the acceptance of the null hypothesis (H0). The calculated F-value is 1.356, while the critical F-value, determined manually from statistical data at a 5% significance level, is 3.59. Since the calculated F-value is less than the critical F-value (F-calculated < F-table) at a significance level of 0.05, it can be concluded that there is no significant relationship between the measurements taken before and after treatment in each group. Based on the decision-making process comparing the F-table and F-calculated values, where F-calculated < F-table for both the dependent and independent variables, it can be concluded that there is no partial effect on each treatment group of mice. The function of the One-Way ANOVA test is to assess the differences in means among groups in experiments with more than two samples. The results of the One-Way ANOVA presented in Table 4 indicate that there are no significant values produced in the tests, as the significance level is greater than 0.05 (sig > 0.05).

## 4. Discussion

The testing involved several aspects, including the evaluation of mice body weight data, assessment of stamina improvement in mice presented in graphical form, normality testing, homogeneity testing, and ANOVA analysis. The purpose of these tests was to determine whether there is an improvement in stamina resulting from the administration of bay leaf extract within the mice.

Based on the research, it was found that the study utilized 20 Swiss Webster mice (*Mus musculus*) weighing between 20-30 grams, which were divided into 5 treatment groups: the control group (P0), Treatment Group 1 received bay leaf extract at a dosage of 3 mg/g body weight (P1), Treatment Group 2 received

bay leaf extract at a dosage of 6 mg/g body weight (P2), Treatment Group 3 received bay leaf extract at a dosage of 12 mg/g body weight (P3), and Treatment Group 4 received bay leaf extract at a dosage of 18 mg/g body weight (P4).

An analysis of Table 1 reveals fluctuations in the body weight of the mice, with the average weights recorded as follows: for the control group (P0), the average weight before treatment was 28.5 grams, which increased to 30.5 grams after treatment; for Treatment Group 1 (P1), the average weight before treatment was 27.25 grams, decreasing to 20.75 grams post-treatment; for Treatment Group 2 (P2), the average weight before treatment was 29.5 grams, which increased to 32.25 grams after treatment; for Treatment Group 3 (P3), the average weight before treatment was 28.5 grams, decreasing to 26 grams after treatment; and for Treatment Group 4 (P4), the average weight before treatment was 28 grams, which decreased to 21.75 grams after treatment. These results indicate that Treatment Groups P1, P3, and P4 experienced weight loss, attributed to the death of one mice in both P1 and P4; this phenomenon may be caused by the toxicity of bay leaf, as the body weight was one of the toxicity indicators **[17]**. The decrease in mice body weight was due to various external and internal factors, such as hormones, enzymes, genetic factors, and the bioactive compound Tanin in bay leaf that easily binds to intestinal cells' protein, leading to reduction of food absorption **[18].** This toxicity causes weight loss in P3. Despite this Tanin toxicity, the bay leaf has another active compound that may have a positive effect on the mice body weight. This Showed by the control group (P0) and Treatment Group 2 (P2) that exhibited an increase in body weight. It is noteworthy that the normal average weight for male mice at 8 weeks or 2 months of age ranges from 20 to 40 grams, while for female mice, it ranges from 22 to 63 grams **19].**

According to Irwadi (2014), endurance capacity is influenced by several factors, including the central nervous system, perseverance, aerobic capacity, anaerobic capacity, and speed reserves. Additionally, various factors affect the level of endurance, such as physical activity, muscle quality, muscle contractions, muscle strength, glycogen reserves, and nutrition. Parmaesih identifies several elements that impact muscle endurance, including genetics, age, sex, physical activity, and nutritional intake [20].

Based on the results, it was observed that the highest average of stamina in mice occurred in Group P2 (6), with a post-treatment duration of 02:01 minutes, ranging from 00:47 to 02:01 minutes (Figure 1). The average stamina of the mice in this group was higher compared to Group P1 (3), which recorded an average of 01:46 minutes, Group P3 (12) with 01:40 minutes, and Group P4 (18) with 01:31 minutes. This indicates that the bay leaf extract could increased the mice stamina as shown in the experienced group P2.

The method for testing stamina improvement using the swimming test, commonly known as the endurance swimming test, has several advantages compared to other methods. These advantages include the ability to maintain and enhance physical fitness, increase activity levels, and provide immediate stimulatory effects that can be observed spontaneously through the improvement in work capacity **[21].** The effect measured by the swimming test method involves mice maintaining an upright head position while continuously moving their legs to stay afloat on the water surface. This activity represents the motor function performed by the mice. The swimming duration is recorded from the moment the mice are placed in the aquarium until signs of fatigue are observed, indicated by the mice's head remaining submerged beneath the water surface for more than 7 seconds **[22].** Another sign of fatigue is when the mice stands upright without moving its tail. An increase in swimming duration indicates an improvement in stamina endurance among the mice **[23].**

Table 2 analyzes the normality test data using the Shapiro-Wilk method for stamina before and after treatment. The results indicate that both groups exhibit a normal distribution, while two other groups do not. To achieve a normal distribution, the significance value (p) must exceed the predetermined level of significance ($p > 0.05$). The lack of normal distribution in the latter groups is attributed to the fact that this study utilized only seconds as the measurement unit and the treatment was administered over a period of just 7 days **[22].**

Subsequently, a homogeneity test was conducted (Table 3), leading to the conclusion that the populations of the data exhibit similar variations. This finding is a prerequisite for proceeding with the parametric statistical analysis using One Way ANOVA**.**

The results obtained from the One-Way ANOVA test (Table 4) did not yield a significant value, as the comparison was made between F-calculated (F-hitung) and F-table (F-tabel). It was observed that F-hitung < F-tabel, indicating that there was no significant effect of the extract administered to the mice. Additionally, this study utilized seconds as the measurement unit, which is a consideration based on the research methodology [13] To determine the onset of fatigue before and after treatment, as well as to assess the extension of fatigue duration in the experimental animals [24]

## 5. Conclusion

The extract of bay leaves (*Eugenia polyantha*) administered to male mice (*Mus musculus*) at doses of 3 mg/g body weight, 6 mg/g body weight, 12 mg/g body weight, and 18 mg/g body weight for of 7 days did not result in any significant impact on the improvement of stamina in the mice. Thus, Bay leaf extract was ineffective at all tested doses for improving stamina in male mice (*Mus musculus*).

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