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ORIGINAL RESEARCH

TOXICITY TEST OF DURIAN LEAF EXTRACT (Durio Zibethinus) MANONTO ON ARTEMIA LARVA

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ABSTRACT

Introduction: *Periodontitis is a chronic inflammatory disease that occurs due to the interaction between subgingival microorganisms and the body's immune-inflammatory response in periodontal tissue. Durian (Durio zibethinus) Manonto leaves are known to contain secondary metabolites that have the potential to be used in dental and oral health, therefore, toxicity testing is necessary to determine their safety.* ***Aim:*** *The purpose of this study was to determine the toxicity of durian (Durio zibethinus) Manonto leaf extract against Artemia salina larvae using the Brine Shrimp Lethality Test (BSLT).* ***Methods:*** *This was a laboratory experimental study with varying extract concentrations of 25%, 50%, and 75%. The observed parameter was the percentage of larval mortality after 24 hours of exposure, which was analyzed to determine the LC_{50} value.* ***Result:*** *The results showed that durian (Durio zibethinus) Manonto leaf extract had a toxic effect on Artemia salina larvae, with mortality increasing with increasing extract concentration. Based on the criteria of the Brine Shrimp Lethality Test (BSLT) method, the Manonto durian leaf extract at all tested concentration variations exhibited LC_{50} values of less than 1000 ppm, indicating that the extract can be classified as toxic.* ***Conclusion:*** *The results of this study demonstrate that Manonto durian leaf extract possesses potential biological activity; however, its use must carefully consider dosage safety aspects.*

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INTRODUCTION

One plant that has the potential to be used as a periodontitis prevention agent is durian (Durio zibethinus), especially the local Manonto variety originating from certain regions in Indonesia. Several studies have shown that durian leaves contain bioactive compounds, such as flavonoids, tannins, and saponins, which have antibacterial, anti-inflammatory, and antioxidant properties. Leaves are an important part of the plant, and each plant generally has several leaves. Leaves are found only on the stem and are never found on other parts of the plant. The meaning of leaves is as one of the organs that is an important part of the plant. The thickness of the leaf, the color of the leaf surface, the shape of the base and tip of the leaf, the shape of the leaf edge.[1]

Periodontitis is a chronic inflammatory disease that affects the supporting tissues of the teeth, such as the periodontal ligament, cementum, and alveolar bone. This disease is caused by infection with pathogenic bacteria in dental plaque, and if left untreated, can cause permanent tissue damage and even tooth loss. Periodontitis has a high prevalence in the general population, making it a serious oral health problem, especially in developing countries. In the early stages, the gums appear red, swollen, and bleed easily.

The safety of using durian leaves in extract form must be determined by conducting the initial stages of developing natural ingredients as medicines. The most commonly used initial toxicity test is the toxicity test on Artemia larvae, making it suitable for use in the Brine Shrimp Lethality Test (BSLT) method.

This method is fast, simple, and inexpensive for testing the toxicity of bioactive compounds from plant extracts, including durian (Durio zibethinus) leaf extract. The Brine Shrimp Lethality Test (BSLT) uses Artemia salina Leach larvae as test animals. The Brine Shrimp Lethality Test (BSLT) is a test to determine the pharmacological activity of a natural extract.

In this method, newly hatched larvae are exposed to various concentrations of Manonto durian leaf extract, then their mortality rate is observed for 24 hours to calculate the LC_{50} value, which is the concentration that causes 50% of larvae to die. This LC_{50} value is an important indicator for assessing whether an extract is toxic or safe at a given exposure level. This research is expected to contribute to the development of local phytopharmaceuticals based on Indonesian natural resources that are safe and effective for oral health.

Previous studies have demonstrated the use of *Artemia salina* larvae as a preliminary model for evaluating the toxicity of natural extracts. Fajaryantie[16] evaluated crude extracts of the leaves, stems, and stem bark of *Durio zibethinus* Murray using the Brine Shrimp Lethality Test (BSLT) and reported an LC_{50} value of 45.26 ppm for the leaf extract, indicating toxic activity against *Artemia salina* larvae. The use of *Artemia salina* as an alternative model has also been reported in dental research for preliminary toxicity assessment due to its simple, rapid, and economical application. Therefore, the BSLT can provide useful preliminary information regarding the biological toxicity and safety of natural materials before further evaluation for potential applications in dental and oral health.

METHODS

This is an experimental laboratory study aimed at determining the toxicity level of Manonto durian (Durio zibethinus) leaf extract against Artemia salina larvae using the Brine Shrimp Lethality Test (BSLT) method.

The study was conducted by administering Manonto durian leaf extract at three concentrations: 25%, 50%, and 75%, to hatched and actively moving Artemia salina larvae. Larvae were exposed to each concentration for 24 hours, then the number of dead and surviving larvae was observed. The parameter observed in this study was the percentage of larval mortality after 24 hours of exposure. The obtained data was then analyzed to determine the LC_{50} (Lethal Concentration 50%) value as an indicator of the

extract's toxicity level. The extract is considered toxic if the LC_{50} value is less than 1000 ppm according to the BSLT method criteria.

RESULTS

The toxicity test of durian (Durio zibethinus) leaf extract on Artemia salina larvae was conducted using the Brine Shrimp Lethality Test (BSLT) method. This test aims to determine the level of toxicity of the extract by observing the number and percentage of Artemia salina larvae deaths after 24 hours of exposure. The toxicity test was conducted on three extract concentration variations: 25%, 50%, and 75%, with each variation tested at solution concentrations of 1 ppm, 10 ppm, and 100 ppm.

The concentrations of 1 ppm, 10 ppm, and 100 ppm indicate different levels of extract exposure to Artemia salina larvae. The unit ppm (parts per million) represents the amount of the test substance in mg/L of solution. Using these concentration variations allows for the evaluation of the dose-response relationship, where larval mortality increases with increasing extract concentration. The BSLT test results showed that the higher the concentration, the higher the percentage of larval mortality. Therefore, the LC_{50} value can be calculated and used as an indicator of extract toxicity.[4] Each treatment used 10 Artemia salina larvae and was performed three times. In addition to the treatment group, a control group without extract was used to ensure that the larval mortality was caused by the toxic effects of the Manonto durian leaf extract.

The results of observations on the number and percentage of Artemia salina larval mortality after 24 hours of treatment are presented in Table 1. Based on Table 1, no larval mortality was found in the control group. Therefore, the percentage of larval mortality in the treatment group was not adjusted, and all larval mortality can be attributed to exposure to Manonto durian leaf extract. The data in Table 1 shows that increasing the concentration of the extract solution was followed by an increase in the number and percentage of Artemia salina larval mortality.

Table 1. Observation data on the mortality of brine shrimp larvae (Artemia salina Leach.) after 24 hours of treatment.

Kode Sampel	Jumlah larva mati tiap konsentrasi			Persentase kematian larva udang (%)			% kematian larva udang - % kematian kontrol		
	1 ppm	10 ppm	100 ppm	1 ppm	10 ppm	100 ppm	1 ppm	10 ppm	100 ppm
25%	1 3 2	3 5 7	6						
Total Kematian	4	9	18	13	30	60	13	30	60
50%	1 2 3	4 5 4	7 6 8						
Total Kematian	6	13	21	20	43	70	20	43	70
75%	3 3 2	6 5 7	10 9 9						
Total Kematian	8	18	28	27	60	93	27	60	93
Kontrol	0 0 0	0 0 0	0 0 0						
Total Kematian	0	0	0	0	0	0			

At a 25% extract concentration, the mortality rate of Artemia salina larvae at a concentration of 1 ppm was 13%, increasing to 30% at a concentration of 10 ppm, and reaching 60% at a concentration of 100 ppm (Table 5.1). This pattern indicates that at a 25% extract concentration, increasing the concentration of the extract solution leads to an increased mortality rate of Artemia salina larvae.

At a 50% extract concentration, the percentage of larval mortality at a concentration of 1 ppm was 20%, increasing to 43% at a concentration of 10 ppm, and reaching 70% at a concentration of 100 ppm (Table 1). These results indicate that Manonto durian leaf extract at a concentration of 50% has a stronger toxic effect than the 25% concentration, as indicated by the higher percentage of larval mortality at each solution concentration.

Meanwhile, the 75% extract concentration resulted in the highest percentage of larval mortality compared to the other concentrations. Larval mortality at 1 ppm was 27%, increasing to 60% at 10 ppm, and reaching 93% at 100 ppm (Table 1). These data indicate that the higher the concentration of Manonto durian leaf extract, the greater the toxic effect on Artemia salina larvae.

In the Brine Shrimp Lethality Test (BSLT) toxicity test, the negative control showed no Artemia salina larval mortality after 24 hours of exposure. This indicates that the media and solvents used did not affect larval viability, so all deaths in the treatment group can be attributed to the toxic effects of the test extract. This is consistent with previous research that reported that the negative control showed no larval mortality after 24 hours of testing.[5]

Based on research by Satya et al. (2021), the negative control was included in the Brine Shrimp Lethality Test (BSLT) study as a comparison to ensure that any larval mortality was truly caused by the test sample. However, in reporting the results, the primary focus of the BSLT method is the number and percentage of larval deaths, not the number of live larvae. Therefore, when no mortality is found in the negative control, the data is conventionally reported as zero (0%) without including the number of live larvae in the results table, as the negative control did not show any larval mortality after 24 hours of observation.[6]

Log Regression Curve [sample] VS probit value

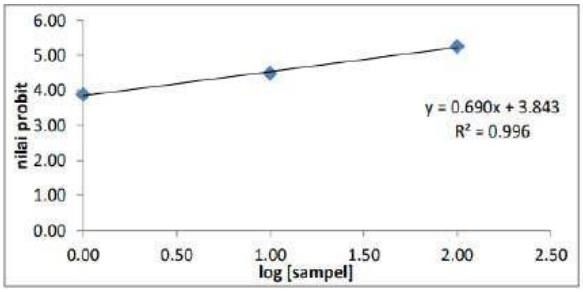

Figure 1. 25% Linear Regression Graph Curve

Figure 1 shows a linear regression curve between the log concentration and the probit value for Manonto durian leaf extract at a concentration of 25%, with the equation of the curve y = ax + b. To determine the LC50 value (x), the probit value is 5 (y), which is inserted into the regression equation y = 0.690x + 3.843.

x = (y − 3.843) / 0.690

x = (5 − 3.843) / 0.690

x = 1.6768

Therefore, log x = 1.6768, yielding:

x = antilog 1.6768

The LC50 value of Manonto durian leaf extract is 47.51 ppm.

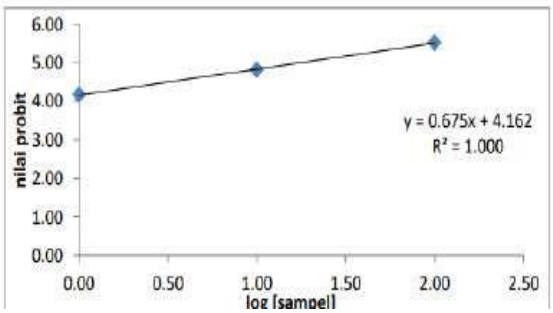

Figure 2. 50% Linear Regression Graph Curve

Figure 2 shows a linear regression curve between the log concentration and the probit value for Manonto durian leaf extract at a concentration of 50%, with the known curve equation y = ax + b.

To determine the LC50 value (x), the probit value is 5 (y), entered into the regression equation y = 0.675x + 4.162.

x = (y − 4.162) / 0.675

x = (5 − 4.162) / 0.675

x = 1.2415

Therefore, log x = 1.2415, yielding:

x = antilog 1.2415

The LC50 value of Manonto durian leaf extract is 17.44 ppm.

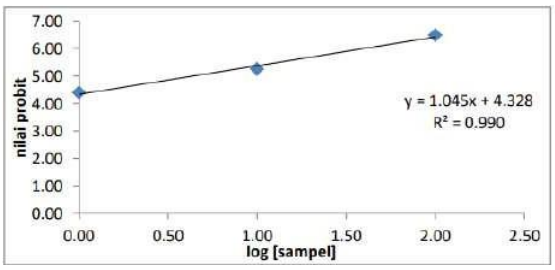

Figure 3. 75% Linear Regression Graph Curve

Figure 3 shows a linear regression curve between the log concentration and the probit value for Manonto durian leaf extract at a concentration of 75%, with the equation of the curve y = ax + b.

To determine the LC50 value (x), the probit value is 5 (y), which is inserted into the regression equation y = 1.045x + 4.328.

x = (y − 4.328) / 1.045

x = (5 − 4.328) / 1.045

x = 0.6431

Therefore, log x = 0.6431, yielding:

x = antilog 0.6431

The LC50 value of Manonto durian leaf extract is 4.40 ppm.

Based on the larval mortality percentage data, a probit analysis was performed to determine the LC_{50} (Lethal Concentration 50%), which is the extract concentration that causes 50% mortality of Artemia salina larvae. The LC_{50} value was determined by analyzing the relationship between the log concentration of the extract solution and the probit value of larval mortality using linear regression.

The results of the probit regression analysis for the 25% extract concentration variation are shown in Figure 1. The graph in Figure 1 shows a linear relationship between the log concentration and the probit value with a regression equation of y = 0.690x + 3.843 and a coefficient of determination of R^2 = 0.996, resulting in an LC_{50} value of 47.51 ppm. The results of the probit regression analysis for the 50% extract concentration variation are presented in Figure 2. The graph shows a very strong linear relationship between the log concentration and the probit value, with a regression equation of y = 0.675x + 4.162 and an R^2 value of 1.000.

Based on this analysis, the LC_{50} value was 17.44 ppm, indicating that Manonto durian leaf extract at a 50% concentration had a higher toxicity level than the 25% concentration.

Furthermore, the results of the probit regression analysis for the 75% extract concentration variation are shown in Figure 3. The graph in Figure 3 shows a regression equation of y = 1.045x + 4.328 with a coefficient of determination of R^2 = 0.990, and an LC_{50} value of 4.40 ppm. The lowest LC_{50} value at the 75% concentration variation indicates that Manonto durian leaf extract at this concentration has the highest level of toxicity to Artemia salina larvae.

In the probit analysis of the Artemia salina toxicity test, the mortality percentage was converted to a probit value and plotted against the log of the concentration to form a linear

regression. The coefficient of determination (R^2) indicates the strength of the linear relationship between dose and response.

The differences in R^2 values at concentrations of 25%, 50%, and 75% are due to differences in the variation in the larvae's response to the extract. The higher the R^2 value, the stronger the dose-response relationship, while a lower R^2 value indicates greater variation in the data in the BSLT test.[7]

Overall, the toxicity test results showed that the Manonto durian (Durio zibethinus) leaf extract at all tested concentrations had an LC_{50} value of less than 1000 ppm. Based on the Brine Shrimp Lethality Test (BSLT) criteria, these results indicate that the Manonto durian leaf extract is toxic to Artemia salina larvae.

DISCUSSION

Based on the research results obtained in the previous subchapter, Manonto durian (Durio zibethinus) leaf extract showed a toxic effect on Artemia salina larvae at all concentrations tested. This toxic effect was demonstrated by an increase in the percentage of larval mortality with increasing extract concentration, and was confirmed by the LC_{50} values obtained from probit regression analysis.

The Brine Shrimp Lethality Test (BSLT) method was used in this study as an initial toxicity test because it is simple, rapid, and has a good correlation with the toxic activity of bioactive compounds in biological systems. The results showed that at a 25% concentration of Manonto durian leaf extract, the LC_{50} value was 47.51 ppm. This value decreased significantly at the 50% concentration variation, with an LC_{50} value of 17.44 ppm, and further decreased at the 75% concentration variation, with an LC_{50} value of 4.40 ppm.

This decrease in the LC_{50} value indicates that the higher the concentration of Manonto durian leaf extract, the lower the concentration required to cause 50% mortality of Artemia salina larvae. This indicates that increasing extract concentration correlates with increasing levels of toxicity. According to the Brine Shrimp Lethality Test (BSLT) criteria, an extract is categorized as toxic if its LC_{50} value is less than 1000 ppm.[8] All LC_{50} values obtained in this study were well below this limit, so Manonto durian leaf extract at concentrations of 25%, 50%, and 75% can be categorized as toxic to Artemia salina larvae.

These results align with the concept that BSLT is used as an initial screening method to identify the potential toxicity of a natural product before further testing in more complex biological systems. The toxic effects of Manonto durian leaf extract observed in this study are suspected to be related to the secondary metabolite compounds found in durian leaves. Durian leaves are known to contain flavonoids, tannins, and saponins, which have biological activities, including antibacterial and anti-inflammatory activity.[9] These compounds have also been reported to cause toxic effects on test organisms when used at certain concentrations. Flavonoids, for example, can disrupt cell membrane function and intracellular enzyme activity, while tannins can precipitate proteins and damage cell wall integrity. Saponins are known to lyse cell membranes through pore formation, thus causing cell death.[10]

The relationship between increasing extract concentration and increased mortality of Artemia salina larvae observed in this study demonstrated a dose-response relationship. This pattern is an important indicator in toxicity testing, as it demonstrates that the toxic effects are not random, but rather are influenced by the amount of active ingredient administered. The higher the concentration of Manonto durian leaf extract, the greater the likelihood that the active compounds will interact with the biological systems of Artemia salina larvae and cause mortality.

The coefficient of determination (R^2) values for the three probit regression curves, which approached 1, indicate that the relationship between log extract concentration and the probit value of larval mortality is linear and strong. This indicates that the research data has a good level of reliability and that the analytical method used is appropriate for determining the LC_{50} value. Therefore, the obtained LC_{50} value can be used as an initial indicator of the toxicity level of Manonto durian leaf extract.

In the context of developing natural ingredients as candidate therapeutic agents, the results of this toxicity test have important implications.

Although Manonto durian leaf extract exhibits potential biological activity, the low LC_{50} value indicates that its use requires careful assessment.[10]

This toxicity test is not intended to directly conclude the safety or danger of the extract to humans, but rather serves as an initial step in determining a safe and appropriate dose range for further research. Therefore, the results of this study can serve as a basis for conducting further toxicity tests, such as subchronic toxicity tests, cytotoxicity tests in cell cultures, or in vivo tests using more complex animal models.[11]

Several studies have shown that BSLT can be used as an initial screening method, but it cannot replace in vitro cytotoxicity tests due to differences in biological response mechanisms between Artemia salina larvae and mammalian cells.

Research by Niksic et al. showed a correlation between BSLT test results and cytotoxicity tests on several human cancer cell lines, thus BSLT is considered effective as an initial stage of toxicity screening before further testing using cell cultures.[12] Furthermore, other studies have confirmed that in vitro cytotoxicity tests in cell cultures are necessary to confirm whether a compound is truly toxic at the cellular level, and can therefore be used as a comparison and validation of BSLT test results.[13] Therefore, cell culture-based cytotoxicity testing can complement BSLT results in determining a more comprehensive safety profile of the extract before further development.

Cytotoxicity testing on cultured extracts therefore requires further research to compare with other methods conducted in the laboratory or in vivo.

The results of this study are relevant to the potential use of Manonto durian leaf extract in dental and oral health, particularly as a supporting agent in the prevention or control of periodontal disease. The bioactive compounds contained in durian leaves have antibacterial activity against periodontal pathogens, but safety remains a key factor that must be considered. By

determining the initial toxicity level using the BSLT method, the development of durian leaf extract-based preparations can be carried out in a more targeted and responsible manner. Phytochemical results of crude Durio zibethinus leaf extracts showed the presence of flavonoids and alkaloids, which are secondary metabolites with high biological activity and are suspected to play a major role in the toxic effect on Artemia salina larvae in the Brine Shrimp Lethality Test (BSLT).[14] Based on the research results of Mailu et al. (2021), the toxicity of plant extracts to Artemia salina larvae is strongly suspected to be related to the content of alkaloid and flavonoid compounds. These compounds are known to have cytotoxic activity, thereby causing larval death in the brine shrimp lethality test.[15]

Overall, the results of this discussion indicate that Manonto durian (Durio zibethinus) leaf extract has potential biological activity accompanied by toxic effects on Artemia salina larvae. Therefore, this extract requires further research to determine the safety limits for its use, especially if it is to be developed as an alternative ingredient in the field of dental and oral health.

CONCLUSION

Based on the results of a toxicity test of Manonto durian (Durio zibethinus) leaf extract on Artemia salina larvae using the Brine Shrimp Lethality Test (BSLT), the Manonto durian leaf extract at a concentration of 25% showed an LC_{50} value of 47.51 ppm, at a concentration of 50% it was 17.44 ppm, and at a concentration of 75% it was 4.40 ppm. All LC_{50} values obtained were below 1000 ppm, so Manonto durian leaf extract at all concentrations can be categorized as toxic. The decrease in LC_{50} values with increasing extract concentration indicates that the higher the concentration of Manonto durian leaf extract, the greater the toxicity to Artemia salina larvae. These results indicate that Manonto durian leaf extract has potential biological activity, but its use requires attention to dosage safety.

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