



Cytotoxic Test of Ethanol Extract of Bintaro Fruit and Peel (*Cerbera odollam* Gaertn.) against *Artemia salina* Leach Larvae

Virsa Handayani¹, Rezki Amriati Syarif², A. Ainun Mutiara Rahma³

^{1,2,3} Pharmacognosy-Phytochemical Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia

ABSTRACT: Bintaro (*Cerbera odollam* Gaertn). Plants belonging to the Apocynaceae family have properties such as antifungal, insecticide, antioxidant and antitumor. Bintaro fruit contains alkaloids, flavonoids, terpenoids, tannins and saponins. Bintaro plants are poisonous because they contain alkaloid group compounds which are toxic. This study aims to obtain data and information regarding the toxicity ratio of bintaro rind and bintaro fruit (*Cerbera odollam* Gaertn.) to *Artemia salina* Leach larvae by determining the value of Lethal Concentration 50 (LC₅₀). This type of research was experimental with quantitative methods, using *Artemia salina* Leach larvae. Each extract was divided into 4 concentration groups. In the ethanol extract of bintaro fruit peel, namely at concentrations of 500, 1000, 1500 and 2500 ppm. Meanwhile, bintaro fruit extract at concentrations of 1000, 1500, 2000 and 2500 ppm, each of which was carried out 3 replications with 10 larvae. Data analysis using probit analysis showed that the ethanol extract of bintaro fruit peel (*Cerbera odollam* Gaertn.) had LC₅₀ = 701.455 µg/mL which was categorized as toxic. Meanwhile, the ethanol extract of bintaro fruit (*Cerbera odollam* Gaertn.) has LC₅₀ = 1199.5 µg/mL which is categorized as nontoxic.

KEYWORDS: Bintaro Fruit, *Brine Shrimp Lethality Test* (BSLT), Cytotoxic, Fruit Skin.

INTRODUCTION

Medicinal plants are all types of plants that can be used as medicinal ingredients, both singly and in mixtures. Medicinal plants are plants that can contain active substances that function to treat certain diseases or if they do not contain certain active substances but contain the resultant effect/synergy of various substances that play a role in the treatment of certain diseases (Supriat, H.S. *et al.*, 2023).

Cytotoxic test is an in vitro toxicity test using cell culture which is used to detect the presence of cytotoxic activity of a compound (Haryoto, *et al.*, 2013). Toxicity tests on plant extracts are usually carried out to determine the level of safety of an extract (Vitalia, N., *et al.* 2016). Toxicity tests are widely used in tracing toxic compounds derived from natural products (Supriat, H.S. *et al.*, 2023). One of the methods used for cytotoxic testing is the method *Brine Shrimp Lethality Test* (BSLT). BSLT is a test method using *Artemia salina* Leach larvae. as a test animal (Fatimah & Santoso, 2020). This test method is based on active plant compounds which are toxic and capable of killing *Artemia salina* Leach larvae (Vitalia, N., *et al.* 2016). The test results are expressed as LC₅₀ (Kurniawan & Ropiqa, 2021). In addition, this method is also easy to do, cheap, fast, and accurate (Maukar, *et al.*, 2013).

One of the natural ingredients that has medicinal properties is the Bintaro plant (*Cerbera odollam* Gaertn). Plants from the *Apocynaceae* family have properties such as antifungal, insecticide, antioxidant and antitumor (Supriat, H.S. *et al.*, 2023). Bintaro fruit has three layers, namely the outer skin (epikarp), the middle layer resembling fibers such as coconut fiber (mesokarp), and the inner layer in the form of fruit seeds (endocarp) (Ulung, 2014). Bintaro plant skin and leaves contain pseudondicaan.

Bintaro seeds contain 0.6-1% serberin, serberoside, teviridosid, fatty oil, odoline (bitter substance), and papagan poison. The sap contains a laxative (Utami, 2008). Empirically, Bintaro fruit sap is used as fish, rat, pig and mosquito repellent.

METHODS

A. Extract Manufacturing

Bintaro fruit simplicia and bintaro fruit rind (*Cerbera odollam* Gaertn.) obtained were weighed at 200 grams. The simplicia extraction process uses the maceration method with 96% ethanol solvent until it is submerged in a tightly closed container for 24 hours. The extraction process was carried out until the maceration solution was close to colorless. The extract is obtained by filtering the residue with the extract using filter paper. The extract solution was evaporated using a rotary vacuum evaporator at a temperature of 55-57°C to obtain a thick extract (Syarif, *et al.*, 2022).



B. Cytotoxic Test

a. Hatching of *Artemia salina* Leach larvae

Artemia salina Leach eggs were weighed as much as ± 50 mg and then put into an incubator vessel which was provided with a partition so that it had two sides of the chamber, namely the open side and the closed (dark) side. The incubator vessel is filled with sea water which has been filtered with whatman paper number 42. Then the aerator is placed in the vessel and illuminated with an incandescent lamp. After 48 hours, the eggs that have hatched become nauplii. Then the nauplii are given yeast suspension as a food ingredient and can be used as test animals (Handayani, *et al.*, 2022).

b. Preparation of Sample and Control Solutions

The stock solution was prepared by weighing 2,500 mg each of the ethanol extract of bintaro fruit rind and the ethanol extract of bintaro fruit dissolved in 2 mL of sea water. If the sample is insoluble or poorly soluble, 0.1-50 μ g or 2 drops of 1% dimethyl sulfoxide (DMSO) is added and seawater is added until the volume reaches 250 mL to obtain a stock solution concentration of 10,000 ppm. Test solutions of fruit peel ethanol extract were made in concentrations of 500 ppm, 1000 ppm, 1500 ppm and 2500 ppm. For the control (0 ppm) was done without the addition of extracts. The fruit ethanol extract test solutions were made in concentrations of 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm. For the control (0 ppm) was done without the addition of extracts (Handayani, *et al.*, 2022).

c. Cytotoxic Test *Brine Shrimp Lethality Test* (BSLT) Method

Each sample extract concentration was made in 3 replications. Then, at each solution concentration, 10 *Artemia salina* Leach larvae were put into the vial. The control was added with 10 mL of seawater without the test solution. Furthermore, observations were made after 24 hours and then counted the number of dead larvae from each vial then followed by probit analysis to determine the LC_{50} value. (Handayani, *et al.*, 2022).

The toxicity test was carried out in each sample extract group which was divided into 5 test groups, namely 4 treatment groups and 1 control or comparison group (seawater).

	Bintaro fruit skin ethanol extract (<i>Cerbera odollam</i> Gaertn.)	Bintaro fruit ethanol extract (<i>Cerbera odollam</i> Gaertn.)
Group 1	Concentration 500 + 10 larvae	Concentration 500 + 10 larvae
Group 2	Concentration 1000 + 10 larvae	Concentration 1000 + 10 larvae
Group 3	Concentration 1500 + 10 larvae	Concentration 1500 + 10 larvae
Group 4	Concentration 2500 + 10 larvae	Concentration 2000 + 10 larvae

RESULTS AND DISCUSSION

The cytotoxic test using the BSLT can be determined from the number of deaths of *Artemia salina* Leach due to the influence of extracts or natural compounds. The test results are expressed as LC_{50} (Kurniawan & Ropiqa, 2021). Good results and actively tested BSLT with the aim of knowing the LC_{50} (Wahyu Ningdyah, *et al.*, 2015). Mortality values were determined using probit analysis to determine toxicity values using Lethal Concentration (LC_{05}). The percentage of mortality of *Artemia salina* larvae can be calculated using the equation (Sumihe, *et al.* 2014). Using the linear regression equation is $y = bx + a$; $y = 50\%$ mortality in probit percentage, $a =$ intercept or value obtained from the linear regression equation, $x =$ log concentration, so that the LC_{50} value is obtained (Rahmi, *et al.*, 2022). If $LC_{50} < 30$ ppm it is said to be very toxic, $LC_{50} < 1000$ ppm is said to be toxic, while $LC_{50} > 1000$ ppm then the test preparation is not toxic (Dona, *et al.*, 2019).

The BSLT method cytotoxic test used *Artemia salina* larvae that were 48 hours old because at this time the larvae already had a high level of sensitivity due to their complete organs (Rahmi, *et al.*, 2022). Based on its morphology, *Artemia salina* larvae that are 48 hours old have started to have mouths and digestive tracts but their food reserves have started to run out so they start looking for food (Fadhli & Hasanah, 2019). *Artemia salina* begins to eat in instar III, that is, after the digestive tract is formed. In instar III or 48 hours old, the larval body will metamorphose and increase its body resistance (Reskianingsih, A. 2014).



The BSLT test was carried out by observing the mortality rate of the larvae after being incubated for 1x24 hours with the extract. The results obtained were then calculated as the LC₅₀ (Lethal Concentration) value of the extract (Rani, Z., *et al.* 2022). The LC₅₀ value was obtained from the percentage of death of *Artemia salina* larvae due to the extract (Alfath, T., 2018).

Based on the results of observations using the ethanol extract of bintaro fruit peels, it was shown that all concentration series groups caused death in larvae except for the control group which contained seawater. Concentrations of 500, 1000, 1500 and 2500 were used because these concentrations had a toxic effect on *Artemia salina* larvae. At a concentration of 2500 ppm it caused the highest average mortality of larvae, while at a concentration of 500 ppm it caused the lowest mortality of larvae. After probit analysis, it can be seen that the graph of the straight line equation $Y = 5.5261X + 10.713$. With the correlation coefficient obtained, namely $R^2 = 0.9973$, which means that 99.7% change in the probit value is influenced by the concentration of the extract. The graph shows the log concentration on the probit value obtained from the percentage of larval mortality, then enter the Y value, namely the probit value of 50% of the test animals and obtain an X value = 5.52, so the LC₅₀ antilog value is 5.52, which is 701.455 µg/mL. The LC₅₀ results obtained were less than 1000 µg/mL. Where, if the LC₅₀ ≤ 1000 ppm it can be concluded that the ethanol extract of bintaro fruit is toxic.

Based on the results of observations, it was shown that all concentration series groups caused death in the larvae except for the control group which contained seawater. Concentrations of 1000, 1500, 2000 and 2500 were used because these concentrations had a toxic effect on *Artemia salina* larvae. At a concentration of 2500 ppm it caused the highest average mortality of larvae, while at a concentration of 1000 ppm it caused the lowest mortality of larvae. Different concentrations in each test vial have different numbers of larval deaths, this shows that each concentration level has a different effect on larval mortality. The higher the concentration of the extract used, the higher the total number of larvae deaths. The graph shows the log concentration on the probit value obtained from the percentage of larval mortality, then enter the Y value, namely the probit value of 50% of the test animals and obtain an X value = 3.07, so the LC₅₀ antilog value is 3.07, which is 1199.5 µg/mL. The LC₅₀ results obtained were greater than 1000 µg/mL. Where, if the LC₅₀ ≥ 1000 ppm it can be concluded that the ethanol extract of bintaro fruit is not toxic.

The mechanism of larval death is thought to be related to the function of flavonoids and alkaloids. Flavonoids that inhibit the eating power of the larvae. The way toxic compounds work that cause the death of *Artemia* larvae is by acting as stomach poisoning (Rani, Z., *et al.* 2022). Alkaloid compounds have a toxin character, temporarily or permanently stopping appetite in insects so that it interferes with the growth and development of larvae and will only cause death in some time due to starvation. But in large quantities the alkaloids work as contact poisons and digestive poisons which will immediately kill the larvae and cause death because they attack vital organs such as the nervous system and affect heart activity (Kurniawan & Ropiqa, 2021).

The data obtained was measured using the LC₅₀ parameter (Lethal Concentration 50%) (Jannah, D. *et al.*, 2020). LC₅₀ (Lethal Concentration 50) is the concentration of a substance that causes death in 50% of experimental animals, namely *Artemia salina* Leach larvae (Kurniawan & Ropiqa, 2021). Mortality in 50% of the experimental animals was calculated at a defined 24hour exposure period (Syahdana, *et al.*, 2017). By entering the value (50% probit of death of the test animals) as y, the results of x are obtained as the log concentration value. The antilog of the x value is the LC₅₀ value (Rani, Z., *et al.* 2022).

CONCLUSION

1. Bintaro fruit peel ethanol extract (*Cerbera odollam* Gaertn.) has an effect on *Artemia salina* Leach larvae. Where, the samples used have the potential to have toxic effects.
2. The Lethal Concentration 50 (LC₅₀) value of the ethanol extract of bintaro fruit peel (*Cerbera odollam* Gaertn.) was 701.455 µg/mL.
3. The Lethal Concentration 50 (LC₅₀) value of the ethanol extract of bintaro fruit peel (*Cerbera odollam* Gaertn.) was 1199.5 µg/mL.
4. The difference in the results of the two extracts can be influenced by the morphology of the Bintaro fruit, which has a middle layer resembling a fiber like coconut fiber (mesocarp) which has more toxic compounds.



ACKNOWLEDGEMENTS

Thank you to the Pharmacognosy-Phytochemical Laboratory, Faculty of Pharmacy, Indonesian Muslim University, Institute for Research and Development of Resources, Indonesian Muslim University, which has provided the facilities so that this research can be completed properly.

REFERENCES

1. Alfath, T., (2018). Toxicity Test of Papaya Leaf Extract Against *Artemia salina* Leach Larvae. With the *Brine Shrimp Lethality Test* (BSLT). Thesis. Semarang State University.
2. Indonesian Ministry of Health. (2017). *Indonesian Herbal Pharmacopoeia Edition II*. Jakarta: Indonesian Ministry of Health.
3. Dona, R., Frimayanti, N., Ikhtiarudin, I., Iskandar, B., Maulana, F., & Silalahi, NT (2019). In Silico Study, Synthesis, and Cytotoxic Test of P-Methoxy Chalcone Compound Against MCF7 Breast Cancer Cells. *Journal of Science & Clinical Pharmacy*, 6(3), 243-249.
4. Fadhli, H., & Hasanah, SBU (2019). Cytotoxic Test of Kangkang Valve Stem Bark Extract (*Bauhinia Semibifida* Roxb) Using the *Brine Shrimp Lethality Test* (BSLT) Method. *Scientia: Journal of Pharmacy and Health*, 9(2), 141-145.
5. Fatimah, R., & Santoso, BSA, (2020). Acute Toxicity of Dekok Kersen Leaves (*Muntingia calabura*) Using the BSLT (*Brine Shrimp Lethality Test*) Method. *Pharmacy Medical Journal*, 65, 229-233.
6. Handayani, V., Rahman, R., & Amaliah, A. (2022). Toxicity Test of Wole Woe Stem Ethanol Extract Against *Artemia salina* Leach Larvae Using the *Brine Shrimp Lethality Test* (BSLT) Method. *As-Syifaa Pharmaceutical Journal*, 14(2), 131–138.
7. Haryoto, Muhtadi, Indrayudha, P., Azizah, T., Suhendi, A., & Haryoto, Muhtadi, Peni Indrayudha, Tanti Azizah, AS (2013). Cytotoxic Activity of Sala (*Cynometra ramiflora* Linn) Ethanol Extract Against HeLa, T47D and WiDR Cells. *Journal of Saintek Research*, 18, 21–28.
8. Jannah, DW, Maunatin, A., & Jannah, A. (2020). Identification and Toxicity Test of Shrimp Larvae (*Artemia salina* L.) Extract of Rice Bran Using Various Types of Solvents and Extraction Time. *Alchemy: Journal of Chemistry*, 8(2), 16-23.
9. Kurniawan, H., & Ropiqa, M. (2021). Toxicity Test of Cattail Leaf Ethanol Extract (*Acalypha hispida* Burm.f.) Using the *Brine Shrimp Lethality Test* (BSLT) Method. *Journal of Syifa Sciences and Clinical Research*, 3(2), 52–62.
10. Maukar, MA, Runtuwene, MRJ, & Pontoh, J. (2013). Analysis of the Phytochemical Content of the Soyogik (*Sauraula bracteosa* DC) Leaf Methanol Extract Toxicity Test Using the Maceration Method. *Scientific Journal of Science*, 13(2), 98.
11. Nuralifah, Parawansah, & Hasniana. (2021). Acute Toxicity Test of Water Extract and Ethanol Extract of *Gardenia jasminoides* Ellis Leaves Against *Artemia Salina* Leach Larvae Using the *Brine Shrimp Lethality Test* (BSLT) Method. *Indonesian Journal of Pharmaceuticals*, 1(1), 98-106.
12. Rahmi, A., Afriani, T., & Aini. (2022) Cytotoxic test of extracts and fractions of sambung leaves (*Blumea balsamifera*) using the *Brine Shrimp Lethality Test* (BSLT) method. *Scientific Journal of Pharmacy* 18(1), 26-33
13. Rani, Z., Ridwanto, Miswanda, D., Yuniarti, R., Sutiani, A., Syahputra, RA, & Irma, R. (2022). Cytotoxicity Test of Cocoa Leaf Ethanol Extract (*Theobroma Cacao* L.) With *Brine Shrimp Lethality Test* (BSLT) Method. *Indonesian Journal of Chemical Science and Technology*, 05(1), 31–41.
14. Reskianingsih, A. (2014). Acute Toxicity Test of Methanol Extract of *Phaleria macrocarpa* (Scheff) Boerl Fruit. Against *Artemia salina* Leach larvae. With the *Brine Shirimp Lethality Test* (BSLT) method. Thesis. Syarif Hidayatullah State Islamic University, Jakarta.
15. Sumihe, G., Runtuwene, MRJ, & Rorong, JA (2014). Phytochemical Analysis and Determination of LC₅₀ Value of Liwas Leaf Methanol Extract. *Scientific Journal of Science*, 14(2), 125-128.
16. Supriat, HS, Hidayat, M., Lewa, S., & Simbal, MY, (2023). Toxicity Test of Ethanol Extract of Bintaro Fruit Seeds (*Cerbera manghas* L) Against *Artemia salina* Leach Larvae. With the *Brine Shrimp Lethality Test* (BSLT) Method. *PHARMACY Scientific Journal & Research Report*. (2023). 1(1).



17. Syahdana, NL, Taufiqurrahman, I., & Wydiamala, E. (2017). Effectiveness Test of Binjai Leaf (*Mangifera caesia*) Ethanol Extract on *Artemia salina* Leach Larvae Mortality. *Journal Dentin* .1(1).
18. Syarif, RA, Handayani, V., & Angraeni, A. (2022). Standardization of Bintaro Fruit Ethanol Extract (*Cerbera odollam* Gaertn.) as Traditional Medicine. *Indonesian Fitofarmaka Journal*, 9(2).
19. Ulung, G. (2014). *Natural Health With 250 Medicinal Plants*. Jakarta: Gramedia Pustaka Utama.
20. Utami, P., (2008). *Medicinal plants*. Agromedia Editor.
21. Vitalia, N., Ahmad, N., & Ahmad, AR (2020). Toxicity Test of Pletekan Leaf Extract (*Ruellia tuberosa* L) with Ethanol and N - Hexane Solvents Using the *Brine Shrimp Lethality Test* (BSLT) Method, *Sandi Karsa Pharmaceutical Journal*.
22. Wahyu Ningdyah, A., Hairil Alimuddin, A., & Jayuska, A. (2015). Toxicity Test Using the BSLT (*Brine Shrimp Lethality Test*) Method on the Results of Fractionation of Tampoi Fruit Peel Extract (*Baccaurea macrocarpa*). 4(1).

Cite this Article: Virsa Handayani, Rezki Amriati Syarif, A. Ainun Mutiara Rahma (2023). Cytotoxic Test of Ethanol Extract of Bintaro Fruit and Peel (Cerbera odollam Gaertn.) against Artemia salina Leach Larvae. International Journal of Current Science Research and Review, 6(7), 4555-4559