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Toxicity test of Kedayan root infusion (*Aristolochia sp.*) using brine shrimp lethality test method

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ABSTRACT

Kedayan roots are still used by the Lundayeh Dayak people as traditional medicine. This plant is one of the *Aristolochia sp.* species whose production and distribution as traditional medicine or food supplements are prohibited by the Food and Drug Supervisory Agency of the Republic of Indonesia because it has side effects that are harmful to the body. The purpose of this study was to determine the toxicity of kedayan root infusion using the Brine Shrimp Lethality Test (BSLT) method based on the LC50 value. Toxicity test was carried out by varying the concentration of Kedayan root infusion, namely 100, 500, and 1000 ppm, as an intervention against *Artemia salina* Leach with a negative control in the form of 2.8% saline solution for 24 hours with 3 replications, then observing the presence or absence of movement of *Artemia salina* L. Larvae. Test results data in the form of % mortality obtained, then analyzed using the probit regression analysis method to determine the LC50 value. The results of this study indicate that the kedayan root infusion has very high toxicity, the LC50 value is 101.9278 ppm.

1. Introduction

Kedayan root is one of the plants belonging to the *Aristolochia sp.* species, which has been prohibited from being produced or distributed as traditional medicine or food supplements by the Food and Drug Supervisory Agency of the Republic of Indonesia. The ban was issued in 2001, due to reports based on scientific research results that traditional medicines containing *Aristolochia sp.* plants have side effects in the form of advanced kidney failure (BPOM, 2001). *Aristolochia sp.* is known to have a variety of chemical compounds, including aristolochic acids and esters, protoberberine, isoquinolines, biphenyl ethers, lignans, coumarins, aristolactam, aporphine, tetralone, benzyliso-quinoline, amides, terpenoids, steroids, and flavonoids (Jubaidah & Nurhasnawati, 2018; Kuo et al., 2012). Aristolochic acid compounds contained in the plant *Aristolochia sp.* are reported as the compounds that cause the most side effects, especially in Asia and the Balkans Currently, aristolochic acid is listed as a group I carcinogen by the IARC (IARC Monographs, 2012).

Currently, aristolochic acid is listed as a group I carcinogen by the IARC (IARC Monographs, 2012). Aristolochic acid content (AAs, I and II) in plants of the Aristolochiaceae family is thought to be responsible for the toxic effect. Among them are nephrotoxic if used at high doses for a long time, triggering tumor growth in the kidneys, bladder, stomach, and subcutaneous tissue and anemia in experimental animals (Wang et al., 2018).Traditional medicinal products containing plant species *Aristolochia sp.* until now are still not allowed to be produced in Indonesia. However, even though its use is prohibited by the government, the people of North Kalimantan, especially the Lundayeh Dayak tribe, still use boiled kedayan root as traditional medicine. The decoction of the kedayan root has been used for generations as an anti-venom, anti-snake venom, and a medicine to treat stomach pain (Setiawan et al., 2019). In addition, one of the plant species *Aristolochia sp.*, namely *Aristolochia longa* is also used by the Moroccan community as a traditional medicine which has been reported to

have efficacy as an anti-inflammatory agent, analgesic, preventing arthritis, treating cancer, and being used as a weight loss agent (El Omari et al., 2020).

Utilization of kedayan root decoction as traditional medicine is still carried out by the Dayak lundayeh tribe (Setiawan et al., 2019; Supriningrum et al., 2016), and based on one of the studies related to acute toxicity tests, the administration of *Aristolochia sp.* aqueous extract for rats was declared relatively safe (Aigbe et al., 2019), so the researchers were interested in conducting a toxicity test on kedayan root infusion using the Brine Shrimp Lethality Test (BSLT) method. Where in this study the kedayan root infusion will be made into 3 concentration series, namely 100, 500, and 1000 ppm, which is then intervened in *Artemia salina* L. to determine the level of toxicity of the infusion by looking at the LC50 value of the kedayan root infusion.The BSLT method is the earliest method to determine the toxicity of natural materials. This method is very effective, efficient, and the results can be trusted (Salam, 2021). The test animal used in this study was *Artemia salina* L., the test animal is generally known as brine shrimp, which is a halophilic invertebrate animal belonging to the Crustacean group, which has an important position in the marine ecosystem. Not only its use as a feed ingredient in aquaculture, *Artemia salina* L. is very useful as a toxicity detection organism (Zhang et al., 2012)

2. Materials and Methods

2.1. Tools and materials

The tools used in the research are aerator (Amara®), stirring rod, beaker (Iwaki®), measuring cup (Iwaki®), incandescent lamp (Philips®), loop, pipette, scale (Constant®), vial, and water bath. (HH6® water bath thermostat). The materials used were kedayan root obtained from Krayan District, Nunukan Regency, North Kalimantan Province, aquades (technical), *Artemia salina* L. seeds (Supreme plus®), NaCl (technical), yeast (Fermipan®).

2.2. Research procedure

Sample collection and preparation

Sampling was carried out in Krayan District, Nunukan Regency, North Kalimantan. The samples obtained were washed thoroughly, then chopped and dried by aerating in a room for 3 days (El Omari et al., 2020).

Infusion Preparation

Making a kedayan root infusion is done by weighing 10 g of dried kedayan root, then wrapped in a cloth and then put into a measuring cup containing 50 ml of distilled water, then boiled at 90^0C for 15 minutes with occasional stirring (Bourhia et al., 2019).

2.3. Toxicity test method Brine Shrimp Lethality Test (BSLT)

Preparation of *Artemia salina* Leach Larvae

The hatching of *Artemia salina* L. was carried out by weighing 10 g of *Artemia salina* L. eggs, then put into a container filled with 500 ml artificial seawater with 2.8% salinity for 48 hours. During hatching, an aerator and lighting were provided as a tool for hatching *Artemia salina* L. larvae (Asaduzzaman et al., 2015).

Toxicity Test

The test was carried out by preparing a test solution by diluting the Kedayan root infusion fluid with a concentration of 200,000 ppm (10 g/50 ml) using artificial seawater (2.8% salinity) into a solution with a concentration series of 1000, 500, and 100 ppm while for negative control only given artificial sea water (salinity 2.8%). Then a calibrated vial was prepared, and 10 larvae of *Artemia salina* L. aged 48 hours were added to each vial. Then 2 ml of Kedayan root infusion of each concentration was put into the vial. Finally, artificial seawater (2.8% salinity) was added to the 10 ml mark. After that, it was given yeast as food for Artemia salina L. It was left for 24 hours and given light. Each intervention was replicated 3 times. The details of the intervention are listed in Table 1.

Table 1. Concentration group of kedayan root infusion intervention against *Arthemia salina* L. larvae

Concentration group (ppm)	Infusion Volume	The volume of artificial seawater with 10 larvae	Final volume
1000	2 mL (5000 ppm)	8 mL	10 mL
500	2 mL (2500 ppm)	8 mL	10 mL
100	2 mL (500 ppm)	8 mL	10 mL

After being left for 24 hours, observations were made using a loupe to see whether there was a movement of the *Artemia salina* L. larvae as a standard criterion for assessing the mortality of the larvae. The formula used to determine the percentage of larval mortality for the given intervention is as follows (Nguta & Mbaria, 2013):

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of test larvae}} \times 100\%$$

2.4. Data Analysis

The data obtained were analyzed using probit regression in the Microsoft excel® system to obtain a linear equation which would then be used to determine the LC50 (Lethal Concetration 50%) value of the kedayan root infusion (Meyer et al., 1982).

3. Results and Discussion

In this study, the toxicity test of the kedayan root infusion was carried out using the BSLT method. This method is a fairly simple method but has quite accurate test results. Where with the help of test animals in the form of *Artemia salina* L. which is known to be very sensitive to environmental changes and chemical contamination in the ecosystem in which it lives, so it is able to detect the toxicity of a compound (Ningdyah et al., 2015). The sensitivity of *Artemia salina* L. is caused by the structure of the skin layer, which is very thin and with fairly large pores, causing the active compounds to be very easy to enter the body of *Artemia salina* L. (Nur Zaki Hanifah, 2015). Another reason for using *Artemia salina* L. as a toxicity detection organism is that it has the same response as mammals (Gajardo & Beardmore, 2012). This is because Artemia salina Leach has the same type of DNA dependent and RNA polymerase and also has oubaine-sensitive Na^+ and K^+ dependent TPAase similar to mammals (Salam, 2021).

In this study, it was found that the concentration of the extract had a correlation with the mortality of *Artemia salina* L. The correlation that occurred was a linear correlation between the logarithm of the concentration and the probit mortality percentage. The following are the results of the toxicity test of the BSLT method using the kedayan root infusion, which is presented in Table 2.

Table 2. Results of Toxicity Test of Kedayan Root Infusion (*Aristolochia sp.*) Against *Artemia salina* Using the BLST Method

Concentration (ppm)	log ppm	Probit	Death percentage	$\bar{x}$ Mortality	Total Artemia test
100	2.00	5.00	50%	5	10
500	2.70	5.84	80%	8	10
1000	3.00	6.28	90%	9	10

The data obtained in Table 2 shows that the concentration with the highest mortality percentage is 1000 ppm followed by a concentration of 500 and 100 ppm with a percentage of 90%, 80%, and 50%, respectively. Where the higher the concentration of the extract, the higher the percentage of mortality obtained. This is in accordance with what was stated by (Ningdyah et al., 2015) that the higher the concentration level of the extract, the more potential to cause death in *Artemia salina* L.

Furthermore, the results of the toxicity test in Table 2, are then analyzed using probit regression by making a graph of a straight line equation that describes the relationship between the probit value and the concentration logarithm. Where the value of 5 or probit 50% mortality of test animals is substituted into the regression equation obtained as the value of Y so that the resulting value of X is the logarithm of concentration. The results obtained from the antilog X in the straight-line equation are expressed as the value of LC50 (Salam, 2021). The regression equation obtained can be seen in Figure 1.

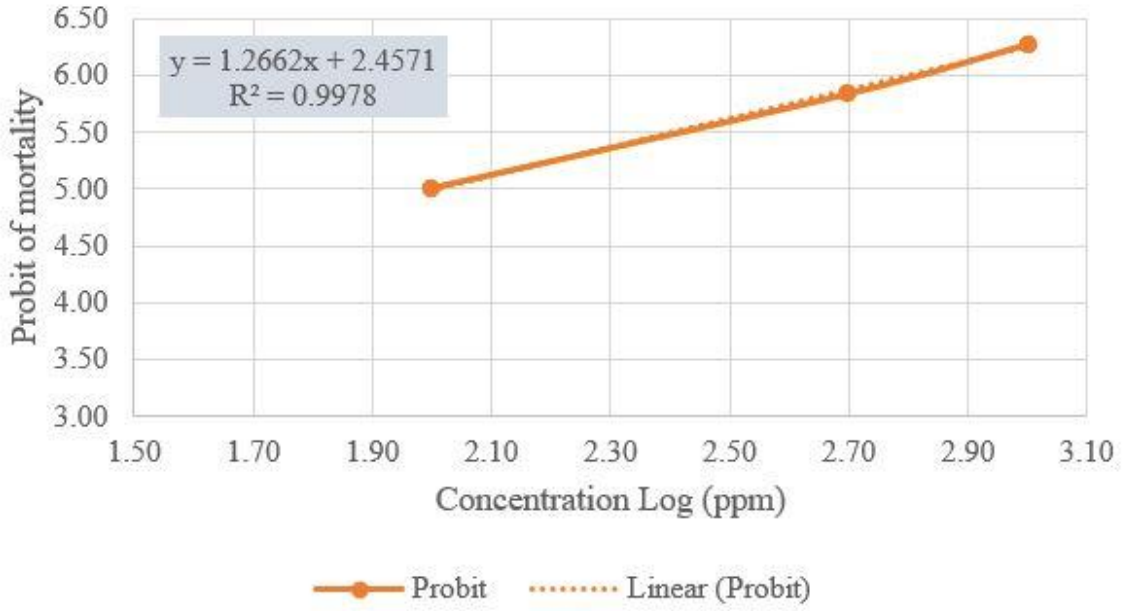

Fig. 1. Artemia salina Leach mortality probit regression graph. After being given the intervention of aqueous extract of kedayan root (Aristolochia sp.)

The value of the regression equation is declared linear if the R value obtained is close to 1. This is in accordance with the R value obtained from this study, which is 0.9978. Based on the R value, it can be concluded that the correlation between the logarithm of the Kedayan root infusion concentration and the mortality of *Artemia salina* L. is a positive correlation where the contribution of the logarithm of the concentration of the Kedayan root infusion to the mortality percentage probit is very strong with a percentage of 99.78%.

The linear regression equation obtained is Y = 1.2662x + 2.4571, and can be seen in Figure 1. This equation is used to calculate the LC50 value in this study. The LC50 value obtained can be seen in the calculation below.

Regression equation: $Y = 1.2662x + 2.4571$

$$X = \frac{(5 - 2.4571)}{1.2662}$$

$$= 2.008293$$

LC_{50} (antilog X) = antilog 2.008293

= 101.9278 ppm

The LC50 value is the value used as the basis for determining the toxicity category of a compound. Based on the results of the probit regression analysis that has been carried out, the LC50 value of the Kedayan root infusion is 101.9278 ppm. With a relatively small LC50 value, it is capable of causing death by 50% of the total *Artemia salina* L. used. This is because according to (Aras, 2013) that is, if a compound has an LC50 value of 0-250 ppm, it can be categorized as a very toxic compound.

The results of the toxicity test obtained in this study are in accordance with the results of the study (Bourhia et al., 2019), namely, the test animals given the infusion of *Aristolochia sp*. for a long time will show high toxicity. (Luciano & Perazella, 2015) also tested the toxicity of aristolochic acid in mice, in which the mice were given aristolochic acid for three months at a dose of 1 and 10 mg/kg once a week, resulting in severe gastric papillomatosis (wart growth) and progressing to cell carcinoma metastatic squamous. In addition, the study (El Omari et al., 2020) also concluded that albino rats tested with aristolochia infusion experienced liver, kidney, and intestinal damage after the test.

Similar to what was stated by (Aigbe et al., 2019) that the infusion of *Aristolochia sp*. given interperitoneally to rats was quite toxic. The adverse effects of infusion of *Aristolochia sp*. that occur include weight loss in female rats, oxidative stress on the kidneys and liver of rats, and potentially antispermatogenic (suppressing or inhibiting sperm production) if carried out for a long time.Based on the results of this study and considering several previous studies, the kedayan root plant is a plant that has very high toxicity. For this reason, it is necessary to conduct a more in-depth study related to the use of kedayan root as traditional medicine, especially in determining the concentration that is safe for consumption.

4. Conclusion

The conclusion that can be drawn based on the results of this study is that kedayan root infusion has very high toxicity with an LC50 value of 101.9278 ppm. For this reason, it is necessary to conduct an assessment regarding the determination of a safe concentration or dose so that this plant can be used as a medicinal plant.

Supplementary Materials

The authors can provide supplementary files, such as figures or tables. Supplementary data can be written in Figure S1: title and Table S1: title.

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Competing Interests

The authors declare that we have no conflict of interest in this study

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References

Aigbe, F. R., Sofidiya, O. M., James, A. B., Sowemimo, A. A., Akindere, O. K., Aliu, M. O., Dosunmu, A. A., Chijioke, M. C., & Adeyemi, O. O. (2019). Evaluation of The Toxicity Potential of Acute and Sub-Acute Exposure to The Aqueous Root Extract of *Aristolochia ringens* Vahl. (Aristolochiaceae). *Journal of Ethnopharmacology*, *244*, 112150. https://doi.org/10.1016/j.jep.2019.112150

Aras, T. R. (2013). *Uji toksisitas ekstrak teripang holothuria scabra terhadap Artemia salina*. (Skripsi, Vol. 1, Issue 1). Fakutas Ilmu Kelautan Dan Perikanan, Universitas Hasanuddin, Makassar.

Asaduzzaman, M., Rana, Md. S., Hasan, S. M. R., Hossain, Md. M., & Das, N. (2015). Cytotoxic (Brine Shrimp Lethality Bioassay) And Antioxidant Investigation of Barringtonia Acutangula (L.)". *International Journal of Pharma Sciences and Research (IJPSR)*, *6*(8), 7.

Bourhia, M., Said, A. A. H., Chaanoun, A., El Gueddari, F., Naamane, A., Benbacer, L., & Khlil, N. (2019). Phytochemical screening and toxicological study of *Aristolochia baetica* Linn Roots: Histopathological and Biochemical Evidence. *Journal of Toxicology*, *2019*, 7. https://doi.org/10.1155/2019/8203832

BPOM, R. (2001). *HK.-00.05.4.03960*. https://janaaha.com/wp-content/uploads/2012/05/HK.-00.05.4.03960-larangan-aristolochia_sp.pdf

El Omari, N., El Blidi, O., Bouyahya, A., Sayah, K., Bakrim, S., Fettach, S., Tahri, R., Taghzouti, K., Chokairi, O., & Barkiyou, M. (2020). Toxicological investigations of Aristolochia longa Root extracts. *Journal of Toxicology*, *2020*, 1–11. https://doi.org/10.1155/2020/7643573

Gajardo, G., & Beardmore, J. (2012). The Brine Shrimp Artemia: Adapted to Critical Life Conditions. *Frontiers in Physiology*, *3*. https://www.frontiersin.org/articles/10.3389/fphys.2012.00185

IARC Monographs. (2012). *Volume 100 A: A review of human carcinogens* (Vol. 100). International Agency for Research on Cancer. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100A.pdf

Jubaidah, S. J., & Nurhasnawati, H. (2018). Analisis flavonoid total akar Tabar Kedayan (*Aristolochia foveolata* Merr). *Al-Kimia*, *6*(2), 135–140. https://doi.org/10.24252/al-kimia.v6i2.4996

Kuo, P.-C., Li, Y.-C., & Wu, T.-S. (2012). Chemical constituents and pharmacology of the Aristolochia species. *Journal of Traditional and Complementary Medicine*, *2*(4), 249–266. https://doi.org/10.1016/S2225-4110(16)30111-0

Luciano, R. L., & Perazella, M. A. (2015). Aristolochic acid nephropathy: epidemiology, clinical presentation, and treatment. *Drug Safety*, 55–64.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, *45*(05), 31–34. https://doi.org/10.1055/s-2007-971236

Nguta, J. M., & Mbaria, J. M. (2013). Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of Malaria in Msambweni district of Kenya. *Journal of Ethnopharmacology*, *148*(3), 988–992. https://doi.org/10.1016/j.jep.2013.05.053

Ningdyah, A. W., Alimuddin, A. H., & Jayuska, A. (2015). Uji toksisitas dengan metode BSLT (Brine Shrimp Lethality Test) terhadap hasil fraksinasi ekstrak kulit buah Tampoi (Baccaurea Macrocarpa). *JKK*, *4*(1), 75–83.

Nur Zaki Hanifah. (2015). *Uji toksisitas akut ekstrak metanol daun Sirsak (Annona muricata L) terhadap Larva Artemia salina Leach dengan metode Brine Shrimp Lethality Test (BSLT)* (Skripsi).

Salam A.A. (2021). *Uji toksisitas ekstrak metanol daun ficus elastica Roxb. Ex Hornem dan Ficus Benjamina terhadap Karva Artemia salina Leach dengan menggunakan metode Brine Shrimp Lethality Test (BSLT)* (Skripsi). Universitas Islam Negeri Sunan Ampel, Surabaya.

Setiawan, A., Listiani, L., & Abrori, F. M. (2019). Kajian etnobotani tumbuhan obat suku Dayak Lundayeh di desa Kaliamok Kecamatan Malinau Utara Kabupaten Malinau sebagai booklet untuk masyarakat. *Borneo Journal of Biology Education*, *1*(1), 51–67. https://doi.org/10.52222/bjbe.v1i1.961

Supriningrum, R., Warnida, H., & Sapri. (2016). Uji daya analgetik ekstrak etanol akar tabar Kedayan (*Aristolochia foveolata* Merr.) pada Mencit Putih Jantan dengan. *Media Sains*, *9* (April), 22–26.

Wang, L., Ding, X., Li, C., Zhao, Y., Yu, C., Yi, Y., Zhang, Y., Gao, Y., Pan, C., Liu, S., Han, J., Tian, J., Liu, J., Deng, N., Li, G., & Liang, A. (2018). Oral administration of *Aristolochia Manshuriensis* Kom In Rats induces tumors in multiple organs. *Journal of Ethnopharmacology*, *225*, 81–89. https://doi.org/10.1016/j.jep.2018.07.001

Zhang, Y., Mu, J., Han, J., & Gu, X. (2012). An Improved Brine Shrimp Larvae Lethality Microwell Test Method. *Toxicology Mechanisms and Methods*, *22*(1), 23–30.