TOXICITY TEST (LD-50) of HERBAL PREPARATIONS Nepenthes ampularia AS The STANDARD of SECURITY of JAMU HYPERTENSION

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Abstract. The excavation of the potential utilization of herbs as a plant biopharma or as a material to manufacture. There is empirical evidence that Nepenthes has been used as a lowering of hypertension by taking a decoction of its pitchers. For the use and utilization as a medicinal plant need to be done research that refers to the scientifically certified Jamu. The research aims to detect acute toxic effects so that can be obtained the water safety overview Nepenthes pitchers. The toxicity test was carried out by the Brine Shrimp Lethality Test (BSLT), Artemia salina larva with four extract concentrations (0, 10, 500, 1000 ppm). Based on the results of toxic tests showed that the death rate of A. salina at all levels of concentration is 0 deaths. It is evidence that Nepenthes ampularia is not toxic to A. salina.

1. Introduction
The use of traditional medicine or herbal medicine has been known and can be used by the community since ancient times. Based on data from Basic Health Research (Riskesdas) in 2010, 50% of the Indonesian population uses herbal medicine both for maintaining health and for treatment due to illness. This Riskesdas data shows that herbal medicine as part of traditional medicine has been accepted by the Indonesian people [1]. The lower, middle, and upper classes are 58% and are classified as high in the use of herbal medicine as an alternative to the use of modern medicine to maintain health and cure diseases [2].

Developed countries whose health care systems are dominated by conventional medicine are now receiving traditional medicine, although they mention complementary/alternative medicine (complementary and alternative medicine), for example, the United States and European countries. In Asia, countries that use a lot of traditional medicine are China, Korea, India, and including Indonesia. Several studies related to the use of herbs have been published [3], [4], [5], [6], [7], [8].

Exploring the potential for the use of plants as biopharmaceutical plants or as materials for making herbal medicine, needs to be further developed, including Nepenthes spp. There is empirical evidence that the Tarakan community uses medicinal plants, nepenthes is used to reduce hypertension by drinking Nepenthes pitcher stew [9]. Nepenthes has potential as a medicinal plant to be investigated for anti-malaria [10]. Nepenthes contains antibacterial properties [11]. It has also been that bacteria that are symbiotic to the roots of Nepenthes have physiological and antagonistic characters as biological controllers of Fusarium oxysforum [12].

For the use and utilization of Nepenthes as a medicinal plant more broadly, it is necessary to conduct research that refers to herbal medicine so that the benefits of Nepenthes can be accepted and scientifically proven. A toxicity test needs to be done as an effort to determine the safety of using nepenthes pitcher decoction. The toxicity test is divided into acute, subchronic, and chronic toxicity tests. The acute toxicity test is intended to obtain information about the symptoms of poisoning, the cause of death, the sequence of
the death process, and the lethal dose range for the test animal (Lethal dose 50% or abbreviated as LD50) of a substance [13]. The acute toxicity parameter used to determine the safety of the nepenthes pitcher of boiled water in treatment was the LD50 value. Acute toxicity testing is very important for measuring and evaluating the toxic characteristics of a chemical. This test can provide information about the human health hazards that come from chemicals exposed to the body for a short time via the oral route. Acute test data can also form the basis of classification and labeling of a chemical. In this study, the LD50 test was carried out.

The BSLT method is believed to be effective in detecting the level of toxicity of a substance. Cheap, easy, fast, and effective make this method quite widely used, as has been reported in research, on the toxicity test of soyogic leaves (Saurauia bracteosa DC)[14], acute toxicity test of kb root ethanol extract(Coptosapelta tomentosa Valeton ex K .Heyne)[15], tiwai bulb (Eleutherine bulbosa (mill.) Urb.)[16], and acute toxicity test of soursop probiotic drink(Annona montana Macf.)[17].

This study aims to detect acute toxic effects so that an overview of the safety of nepenthes pitcher boiled water can be obtained. The general objective of this study was to determine the acute toxicity value (LD50%) of nepenthes pitcher extract in A. salina, while the specific objective of this research was to obtain information on the potential of Nepenthes spp to be developed as an herbal plant safe for human consumption.

The results of this study are expected to provide information about the value of acute toxicity (lethal dose 50%) of Nepenthes pitcher extract in A. salina. If the safety level of using Nepenthes is proven to be safe, it can be the basis for continuing research on the chemical content of Nepenthes secondary metabolites which have the potential to reduce hypertension and can be an effort to develop Nepenthes herbal medicine.

2. Materials and methods

2.1. Time and place

The study was conducted in 2014. The study of nepenthes plant pitcher sampling was conducted in the Research Forest of the University of Borneo Tarakan, while the sample test was conducted at the University of Mulawarman.

2.2. Research Materials and Equipment

Research materials and tools used in research include:

- The materials used were Nepenthes (Nepenthes ampullaria) leaf pitchers and distilled water. The type of bioindicator used is Artemia salina which is used for activity testing. And the materials used in this research consist of various types of organic solvents, various reagents commonly used for toxicity tests, and distilled water. Various kinds of glassware, electronic balances, as vacuum tops and ovens, and writing instruments.

2.3. Research methods

Nepenthes pitcher material that has been cleaned of dirt and small animals, in dry wind conditions is weighed according to the calculation of the dose. Toxicity test using the Brine Shrimp Lethality Test (BSLT) method. A. salina larvae are obtained by hatching from Artemia eggs by making artificial seawater media (sodium chloride salt solution 2% w / v) to boil for 15 minutes, then cooled to room temperature. A total of approximately 15 mg of A.salina eggs are sprinkled into 100 ml of the artificial water medium and the air flows through the aerator. The eggs will hatch into larvae after 24-36 hours of aeration. Then the larvae are separated from the eggs that do not hatch by transferring them to a new artificial seawater medium ready for use as bioindicators.

The steps for the toxicity bio-test were carried out by making the extract of Nepenthes sp. those to be tested for their toxic effects are grouped into three kinds of concentrations, namely 10, 100, and 1000 ppm. How to classify extracts/fractions into three kinds of concentrations. A total of 20 mg of extract/fraction tested were dissolved in 2 ml of the original solvent from the extract/fraction, to obtain a test solution with a concentration of 20 mg / 2 ml. Then the test solution is pipette 500 µl and placed into the test vial (vial). This method is done three times by placing it in a different vial. Thus, there are three vials of the test solution 500 µl each of which is the test group for a concentration of 1000 ppm.
Then the remaining test solution was pipette back to 50 μl and placed in a vial. This method is done three times by placing them in three different vials. Thus obtained three vials containing the test solution each 50 μl and are for the test group at a concentration of 500 ppm.

The remaining test solution after pipetting 500 μl, 50 μl every three times, then the test solution remains 350 μl. Then from the remaining test solution, 5 μl of pipetting was carried out by placing it in three different vials. This resulted in three vials containing the test solution, each 5 μl, which is the 10 ppm concentration test group. Each concentration group is then accompanied by a blank as a comparison.

Blank is the same solvent as the solvent from which the extract/fraction is concerned and pipettes of 500 μl, 50 μl, and 5 μl, according to the concentration group to be compared. Each vial containing the test solution has been grouped into three different concentrations of 10, 500 and 1000 ppm along with the blank, then evaporated until all the solvent has completely evaporated. The next stage is the observation of the toxic effects. Each vial of the test solution and vial blank which has been evaporated by the solvent then added approximately 2 ml of artificial seawater, 10 A. salina larvae, and followed by adding seawater until the volume is exactly 5 ml per vial. Then the test solution vials and blanks, each of which have been filled with 10 A. Salina larvae and 5 ml of seawater, are placed under a 40-watt fluorescent lamp with a distance of approximately 20 cm. Observation of the toxic effect was carried out by counting the number of A. salina larvae that died at intervals of three, six, and twelve hours after treatment.

3. Results and discussion

3.1. Characteristic of Nepenthes ampullaria

Nepenthes ampullaria was the dominant Nepenthes species that grows in the research forests of the University of Borneo Tarakan. The characteristics of N. ampullaria obtained in the field are as follows; Information: stem: vines, <15 m long, <8 mm diameter, <15 cm leaf internodes, brown cylindrical shape; leaf: thick spatula to lanceolate, <25 cm long, <6 cm wide, number of leaf veins longitudinal 3-5 on each side of the middle vein (midrib), short petiole sometimes absent, tendrils <15 cm long; pouch: rosette bag in the shape of a jar, green with reddish streaks, or sometimes red with brown streaks, <10 cm high, <7 cm wide, with two wings that are wide enough, a mouth that is oval and horizontal, a small peg-shaped cover or elliptical, the top pitcher is rarely seen, smaller than the bottom pitcher or rosette pitcher; inflorescences: panicle, <35 cm long. Female inflorescences are shorter than male inflorescences, the young parts of the plant are often covered with short brown fine hairs. The part used as the object in this study was the pitcher of Nepenthes ampullaria (Figure 1)

![Pitcher of Nepenthes ampullaria](image)

**Figure 1.** Pitcher of Nepenthes ampullaria

3.2 Extraction and Fractionation

A total of 10.8 g of thick ethanol extract was dissolved with 200 mL of ethanol: water (3:7), then partitioned with n-hexane repeatedly 5 x 50 ml. The n-hexane fraction was separated and evaporated to produce 3.13 g n-hexane extract which was dark yellow. The ethanol-water extract was evaporated by ethanol, then partitioned with 5x50 ml of chloroform and separated to produce an extract of 1.63 grams of chloroform and 9.25 grams of water extract. Based on the weight of the extract, it can be seen that the
compounds contained are mostly bound to the polar solvent. The concentrated extracts of n-hexane, chloroform, and water were then tested for their toxicity against larvae of Artemia salina L.

Observation of the toxic effect of N. ampullaria extract with n-hexane, chloroform, and water extract solvents on A. salina after three hours of treatment, there was no death of A. salina. In the observation six hours after treatment there was no death of A. salina, and twelve hours after treatment there was also no death of A. salina. Based on the toxic test results of N. ampullaria extract with n-hexane, chloroform, and water solvents in various treatments with concentrations of 0, 10, 500, and 1000 ppm did not show toxicity to Artemia salina because of 0 deaths. The extract showed toxic activity in a toxicity test if an extract could cause the death of 50% of the tested animals at a concentration of <1000 ppm[18].

Based on the results of the toxicity test of N. ampullaria extract, it did not cause the death of A. salina larvae in the 0 to 1000 ppm treatment, it was stated that the N. ampullaria extract with n-hexane, chloroform, and water solvents was classified as non-toxic.

4. Conclusions

Based on the results obtained, it can be concluded that the N. ampullaria extract is non-toxic to the larvae of A. salina L.

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