TESTING THE TOXICITY OF BIRD'S NEST FERN LEAF EXTRACT (ASPLENIUM NIDUS) ON BRINE SHRIMP LETHALITY TEST BSLT LARVA USING BRINE SHRIMP LETHALITY TEST (BSLT)

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Abstract

Background: Bird's nest fern is a plant that lives on other trees and grows a lot in Indonesia. The leaves are the part that is usually used as a mixture of herbal medicines, such as in the treatment of stomach ulcers. This plant contains alkaloids, flavonoids, and steroid terpenoids, saponins. **Objectives**: The aim of this research was to determine the potential toxicity and LC50 value of bird's nest fern (Asplenium nidus) leaf extract against Artemia salina leach larvae. **Method:** The research design was experimental using the Brine Shrimp Lethality Test (BSLT) method. Using 150 artemia salina leach larvae and divided into 5 groups with a concentration series of 800 µg/mL, 600 µg/mL, 400 µg/mL, 200 µg/mL. Each group consisted of 10 larvae and 3 replications of treatment. **Results**: of the research calculated the death of Artemia salina leach larvae for 24 hours and were analyzed using probit analysis to calculate the LC50 value. Namely, the toxicity test shows the LC50 value is 90,99 µg/mL (toxic). It was concluded that the ethanol extract of bird's nest fern leaves has the potential to be toxic to Artemia salina leach larvae with an LC50 value of 106.346 µg/mL.

Keywords*: Bird's nest fern leaf extract, toxicity, Artemia Salina leach, BSLT.*

BACKGROUND

Cancer is a disorder characterized by uncontrolled cell growth with the destruction of a number of surrounding tissue cells and can cause fatalities such as loss of life (death). Cancer is known to be a malignant disease because the nature of cancer appears uncontrollably and can result in loss of life. Cancer cells grow rapidly and uncontrollably, constantly dividing, invading surrounding tissue, spreading into the bloodstream, and attacking major organs and the spinal cord (Puspitasari et al, 2022)

Medicinal plants that are often used by people include the bird's nest fern (Aaplenium nidus) which is used to treat fever and colds. This plant grows with other trees and lives in abundance in Indonesia. The part that is used as a mixture in herbal medicine is the leaves in the ulcer treatment process (Brahmana et al, 2022) The leaves of this bird's nest fern contain alkaloids, flavonoids and terpenoids (Wibowo et al., 2022)

Flavonoids are often said to have antitumor/anticancer potential (Nasution Nurul Hidayah, et al. 2022) Flavonoids are compounds that have been proven to be able to cause cell apoptosis and inhibit the proliferation of a number of cancer cells (Muti et al, 2020) Flavonoids are several polyphenolic compounds which are secondary metabolites from plants and have anticancer properties. Flavonoids include quercetin, which belongs to the flavonol subclass. Quercetin, genistein, and flavopiridol can be used as anticancer drugs (Sari Sirait et al., 2019)

Toxicity tests include tests to observe the pharmacological metabolism of compounds that occur over a certain duration, toxicity tests are used to identify the effects of toxins formed in a single dose of a combination of chemicals on pre-screening test animals for bioactive anticancer compounds (Jelita et al, 2020) Toxicity testing with BSLT is the easiest and most conventional method for researching the acute toxicity of certain compounds determination (lethal concentration) LC50 of active components from simplicia or certain plant extract dosage forms. If certain plant extracts have toxic properties based on the LC50 (lethal concentration) through the BSLT technique, the plant can be developed into a medicine. However, if it is not toxic, further research will be carried out on the plant to identify its properties (Putri et al, 2022).

METHODS

The research is experimental using the Brine Shrimp Lethality Test (BSLT) method. Using 150 artemia salina leach larvae and divided into 5 groups with a concentration series of 800 µg/mL, 600 µg/mL, 400 µg/mL, 200 µg/mL. Each group consisted of 10 larvae and 3 replications of treatment.

From the results of the toxicity test, it was processed and presented in the form of a graphic table, the toxicity test data was analyzed using probit analysis and using Microsoft Office Excel to look for linear regression of the relationship between probit and log concentration (Sampoerna et al. 2022).

The formula for calculating the percentage of larval death is calculated using the following formula

% mortality of shrimp larvae (Artemia salina leach)

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

RESULTS AND DISCUSSION

Table 1. Results of extraction of bird's nest fern leaves (*Asplenium nidus*)

Sample	Type of solvent	Sample weight Dry (g)	Thick extract weight (g)	Rendement (%)
Bird's nest fern (*Asplenium nidus*) leaves	Ethanol 96%	300	30,52	10.17%

Chart 1. Bird's Nest Fern Leaf Extrac

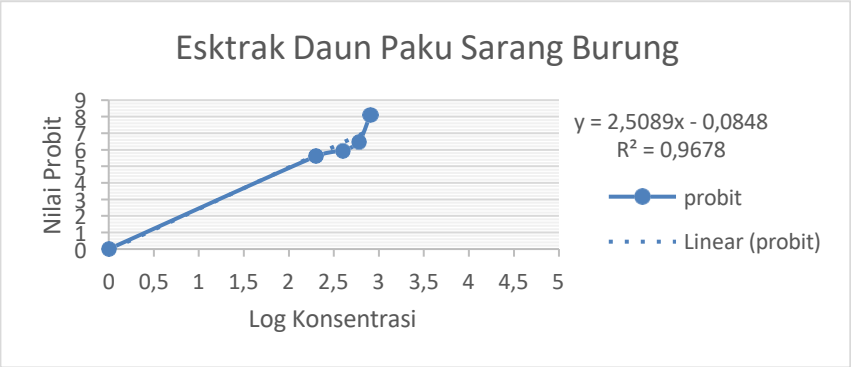

Table. 2 Toxicity Test Results of Bird's Nest Fern Leaf Extract

Number of Larval Deaths for Each Concentration							
Number of replications	800 µg/mL	400 ug/mL	300 ug/mL	200 ug/mL	(-)LC50		Ket
1	10	9	9	8	0		
2	10	10	8	7	0		
3	10	9	8	7	0	90,99 µg/mL	Toxic
Average	30	28	25	22	0		
Death presentation (%)	100	93	83	73	0		

Table 3. Toxicity Category Table

Category	LC50 (µg/ml)
Very toxic	<30
Toxsic	30-1000
Not toxsic	>1000

(Abriyani, 2022)

The % yield results show the amount of compound content in a sample or simplicia. In this study, the % yield of bird's nest fern leaf extract (*Asplenium nidus*) obtained as a thick extract was 10.17% so that it met the requirements for the % yield, the requirement for the yield of thick extract was that the value was not less than 10% (Rosa et al. 2023)

In this study, the toxicity of bird's nest fern (*Asplenium nidus*) leaf extract was tested. This test was carried out on artemia salina leach larvae aged 24 hours to 48 hours in sea water. Seawater was used as a diluent for the extract, which was used as a stock solution. Extract made with concentration of 10,000 µg/mL as a stock solution, then the stock solution that has been made is diluted into several concentrations, namely 800 µg/mL, 600 µg/mL, 400 µg/mL, 200 µg/mL, and 0 µg/mL as a negative control, with 3 replications/repetitions (triplo). Standard criteria for assessing shrimp larval mortality is if the shrimp larvae do not show movement after observation (Tambupolon et al. 2023)

Based on the table, the results of the larval toxicity test show the number and presentation of shrimp larvae deaths. At a concentration of 800 µg/mL it was 100%, at a concentration of 600 µg/mL it was 93%, at a concentration of 400 µg/mL it was 83%, at a concentration of 200 µg/mL it was 73%, and in the negative control the mortality rate was 0%. Based on these results, it can be concluded that the higher the extract concentration, the higher the larval mortality.

There is a positive correlation between the BSLT method and toxicity tests with cancer cell cultures, so this method is often used to screen for anticancer compounds. based on the BSLT method, LC50 values below 1000 µg/mL have stronger toxic activity, namely the lower the resulting concentration value, the higher the toxic effect on larvae. If the LC50 value is greater than 1000 µg/mL, then there is no toxic activity (Dalimunthe et al. 2022)

A compound is declared to have potential toxicity if it has an LC50 value of less than 1000 µg/mL. LC50 (Lethal Concentration 50) is the concentration of a substance that causes death in 50% of experimental animals, namely Artemia salina Leach larvae. As shown by the test table for the ethanol extract of bird's nest fern (Asplenium nidus) leaves shows an LC50 value of 90,99 µg/mL so it is toxic according to the level of toxicity according to the BSLT method with a range of LC50 values of 30-100 µg/mL in the experimental animal treatment of Artemia salina Leach using Microsoft Office Excel (Abriyani et al. 2022)

CONCLUSION

The ethanol extract of bird's nest fern (Asplenium nidus) leaves has the potential to be toxic to shrimp larvae (Artemia salina leach). And the BSLT test (Brine shrimp lethality test) of bird's nest fern (Asplenium nidus) leaf extract has an LC50 value of 90,99 µg/mL so it is included in the toxic category because it has an LC50 value <1000 µg/mL.

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