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RESEARCH ARTICLE

THE CYTOTOXICITY ACTIVITY OF ETHANOLIC EXTRACT OF *ACANTHUS ILICIFOLIUS* L LEAVES USING BRINE SHRIMP LETHALITY TEST (BSLT) METHOD

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Abstract

Aim and objective: *Acanthus ilicifolius* also known as jeruju is a plant that has a lot of bioactivity that can be used as a potential medicinal plant development. This study aimed to determine the cytotoxicity activity of ethanol extract of *A. ilicifolius* leaves.
Methods: This study used Brine Shrimp Lethality Test (BSLT) method using *Artemia salina* larvae with test solution series concentration of 10, 50, 100, 500, 1000 ppm and control without extract. *A. salina* was added to each test tube. After 24 hours, the larvae mortality was observed. LC_{50} assessment was analyzedwith probit analysisby Microsoft excel.
Result: The largest mortality percentage was shown at a concentration of 1000 ppm ethanol extract of *A. ilicifolius* leaves with an average mortality value of 80%, while the concentrations of 10 ppm, 50 ppm, 100 ppm, and 500 ppm had an average mortality value of 20%, 40%, 57% and 63%.The ethanol extract of *A. ilicifolius* leaves categorized as toxic with a LC_{50} value of 103,6 ppm.
Conclusion: It was concluded that ethanol extract of *A. ilicifolius* leaves has cytotoxicity activity potential.
Keywords: *Acanthus ilicifolius*, *Artemia salina*, BSLT, cytotoxicity.

INTRODUCTION

Nowadays, the use of medicinal plants is very popular almost all over the world. They have been used traditionally as medicine for thousands of years in countries such as China, India, Thailand, Japan and Indonesia. Indonesia is a country that has abundant biodiversity. Indonesia has tropical forests with 3000 species of plants and 1,845 of them are medicinal plants[1]. Medicinal plants are well-known as the basic materials of herbal medicine and traditional medicine. Medicinal plants are plants that contain active substances in part or all of plant parts that can be used to treat or prevent disease[2]. Some medicinal plants that are widely used are *Artocarpus altilis, Centella asiatica, Piper betle Hibiscus rosasinensis, Blumea balsamifera, Alium sativum, Curcuma longa, Mimosa pudica, Carica papaya,* and *Acanthus ilicifolius*. The various kinds of pharmacological effects of these plants are as antioxidants, antibacterial, antihypertension, antidiabetic, anti-inflammatory, analgesic and many other effects[3,4,5]. This pharmacological activity is due to the presence of chemical constituents contained in the plants such as secondary metabolites. Secondary metabolites are various chemical compounds produced by plant cells with various biological effects[6]. Various types of secondary metabolites in plants play a role in providing pharmacological activity.

Acanthus ilicifolius also known as Jerujuis one of the plants that is used as a medicinal plant. *A. ilicifolius* is a member of the Acanthaceae family and is amangrove vegetation plants grow in tropical and subtropical intertidal habitats[7]. *A. ilicifolius* has bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoid, and steroids[8]. Andriani *et al.*, reported that methanolic extract of *A. ilicifolius* leaves contained alkaloids, flavonoids, polyphenols, tannins, steroids, and glycosides[9]. Phenylethanoid glycosides mostly found in *A. ilicifolius* leaf ethanolic extract[10]. The main type of phenylethanoid glycosides containedin *A. ilicifolius* are isoacteoside and acteoside[11]. Previous studies showed that *A. ilicifolius* leaves extract has antifungal, antioxidant, analgesic, antimicrobial, and hepatoprotective activities[11,12,13]. *A.*

ilicifolius leaves infusion at a concentration of 40% has potential effect as analgesic in mice[13]. Other studies have reported that methanolic extract of *A. ilicifolius* leaves have strong antioxidant (IC_{50}: 17.51 µg/ml) and antifungal activity against *Candida albicans*[9]. Zhang *et al*., reported that phenylethanoid glycosides content of *A. ilicifolius* has potential as hepotoprotective in liver injury induced by carbon tetrachloride (CCl_4) *in vivo* and *in vitro*[11].

The pharmacological effects of plants are due to the presence of secondary metabolites of the plants. The pharmacology activity of these active components as herbal medicines can be determined through a preliminary analysis in the form of a cytotoxicity analysis. The method that is often used in the analysis of cytotoxicity is the Brine Shrimp Lethality Test (BSLT).This assay can describes the toxicity effect of the extract against *Artemia salina* larvae. This test can be used as an initial step to identify more plant bioactivity. Therefore, this present study was conducted to determine the cytotoxic activity of ethanol extract of *A. ilicifolius* leaves against *A. salina.*

MATERIALS AND METHODS

Solvents

96% ethanol (Brataco®), aquadest (Brataco®), Tween 80 (Sigma aldrich®), H_2SO_4(Merck®), $FeCl_3$ (Brataco ®), HCL (Merck®), chloroform (Brataco®), ammonia (Brataco®)

Sample Preparation

Fresh leaves of *A. ilicifolius* were collected and picked in November 2021 from Gitgit Village, Sukasada District, Buleleng Regency, and Bali and were botanically identified by Balai Konservasi Tumbuhan Kebun Raya "Eka Karya" Bali (LIPI).

Extraction

The fresh leaves of *A. ilicifolius* were washed with flowing water and dried by aeration without exposure to direct sunlight. The dried leaves were powdered by blender. Leaf powder was extracted using 96% ethanol for five days with stirring. The filtrate was collected and evaporated using rotary evaporator and obtained 9.3 grams of the crude extract.

Phytochemical Screening

Test for alkaloids

Total 0.1 gm of extract was added with 10 ml of chloroform and a few drops of ammonia. The chloroform fraction was separated and acidified with the addition of a few drops of concentrated sulfuric acid. The acid fraction was divided into 2 tubes, and each was added with Meyer and Dragendroff reagent. The formation of a white precipitate on Meyer's reagent and a red precipitate on Dragendroff's indicates the presence of alkaloids[14].

Test for flavonoids

Total 2 ml of extract was heated and then added with ethanol. The solution is added with magnesium powder and HCl. The presence of flavonoids is indicated by the formation of a red or orange color[14].

Test for phenolic compound

Total 0.1g of the extract was dissolved in methanol and then 2-3 drops of 5 % $FeCl_3$ were added. The presence of phenolic compounds in the extract was indicated by the formation of a dark green color[15].

Test for saponins

Total 1g extracts added 5 mL of aquadest, shaken vertically for 10 seconds. Formation of foam 1-10 cm high which is stable for not less than 10 minutes indicates the presence of saponins[16].

Test for steroids

Total 0.5g of extract was added 2-3 drops of anhydrous acetic acid then added 3 drops of sulfuric acid, left for a few minutes. The color changes that occur are observed. A blue or green discoloration indicates the presence of steroids[17].

Test for tannins

Total 0.1g of *A. ilicifolius* leaves extract was dissolved in methanol, and then 2-3 drops of 1% $FeCl_3$ solution were added. The formation of a dark green or bluish black color indicates the presence of tannins[15].

The cytotoxicity assay

The cytotoxicity assay was carried out with the Brine Shrimp Lethality Test (BSLT) by using *A. salina*. Preparation of *A. salina* larvae is carried out by incubating the eggs for 48 hours in an aquarium filled with seawater. The test solution of *A. ilicifolius* leaves ethanol extract was made in a series of concentrations of 10, 50, 100, 500, 1000 ppm. After the solvent evaporated, 50 L of tween, 1 ml of seawater, and added 10 ml A. *salina* larvae into each tube. Then the sea water is added again up to 5 ml. Normal control was also made without the addition of extract. After 24 hours, the larvae mortality was observed[18-20].

$$\%\text{Mortality} = \frac{\text{Total Larvae Mortality}}{\text{Total Larvae}} x\ 100\%$$

LC_{50} assessment was analyzed with Probit analysis by Microsoft excel. The determination of the toxicity category is determined based on the LC_{50} value divided into three categories, that is non-toxic with an LC_{50} value of > 1000 ppm, toxic with an LC_{50} value of 30-1000 and ppm very toxic with an LC_{50} value of <30 ppm[20].

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical group test was performed and the result indicated that alkaloids, flavonoids, phenols, saponins, steroids, and tannins were detected in the ethanol extract of *A. ilicifolius* leaves.

Cytotoxicity activity

Cytotoxic activity assay was carried out by Brine Shrimp Lethality Test (BSLT). This method is a simple method that is often used for cytotoxic assay. Based on the Table 1, it showed that in the control group there was no larvae mortality. The largest mortality percentage was shown at a concentration of 1000 ppm ethanol extract of *A. ilicifolius* leaves with an average mortality value of 80%, while the concentrations of 10 ppm, 50 ppm, 100 ppm, and 500 ppm had an average mortality value of 20%, 40%, 57% and 63%. It shows that larvae mortality is not affected by seawater but by *A. ilicifolius* leaf extract. The higher the concentration of the extract, the higher the mortality of *Artemia salina* larvae. Based on the result, it showed that the

ethanol extract of *A. ilicifolius* leaves categorized as toxic with a LC_{50} value of 103.66 ppm. So that it has cytotoxicity potential, this is related to secondary metabolite compounds such as phenolics, flavonoids and tannins, which are contained in the extract which at certain levels has the potential for cytotoxicity and caused larvae mortality.

Table 1: Percentage of larvae mortality and LC_{50} of ethanol extract of *A. ilicifolius* leaves.

Concentration (ppm)	Log 10	Replication	Total larvae	Mortality	% Mortality	Probit	LC 50 (ppm)
Control	-	1	10	0	0%	0	0
		2	10	0	0%	0	
		3	10	0	0 %	0	
10	5.00	1	10	2	20%	4.16	103,6
		2	10	2			
		3	10	2			
50	5.70	1	10	3	40%	4.75	
		2	10	5			
		3	10	4			
100	6.00	1	10	6	57%	5.18	
		2	10	4			
		3	10	7			
500	6.70	1	10	7	63%	5.33	
		2	10	4			
		3	10	8			
1000	7.00	1	10	8	80%	5.84	
		2	10	6			
		3	10	10			

The mechanism of larvae mortality is related to the function of phenolic compounds, flavonoids and tannins in *A. ilicifolius* leaves which can inhibit larval feeding power (antifedant). The way these compounds work is by acting as stomach poisoning. Therefore, when these compounds enter the larva's body, the digestive system will be disturbed. This causes the larvae to fail to get a taste stimulus so they are unable to recognize the food so that the larvae starve to death[21].

CONCLUSION

Ethanol extract of *A. ilicifolius* leaves is toxic based on Brine Shrimp Lethality Test and has cytotoxicity activity potential with a LC_{50} value of 103.6 ppm.

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AUTHOR`S CONTRIBUTION

Sari DRAP: writing original draft, methodology. **Ugrasena PY:** research design, data collection. **Ekayani NWR:** statistical analysis, conceptualization. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

None to declare.

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