THE CYTOTOXICITYACTIVITY OF ETHANOLIC EXTRACT OF *Acanthus ilicifolius L* LEAVES USING BRINE SHRIMP LETHALITY TEST (BSLT)METHOD

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* were 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,* Cytotoxicity, BSLT

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 very popular plants that can be used as raw materials for traditional medicine and herbal 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 rosa sinensis, 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 caused by the presence of secondary metabolites contained in the plants.Secondary metabolites are various chemical compounds produced by plant cells with various biological effects[6]. Different types of secondary metabolites found in the medicinal plants which play an importantrole in many kinds ofdiseases.

Acanthus ilicifolius also known asJeruju is one of the plants that is used as a medicinal plant. *A. ilicifolius* is a member of the Acanthaceae family and is a mangrove shrub grows 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 extractof *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 phenylethanoid glycosides in *A. ilicifolius* are isoacteoside and acteoside[11]. Pharmacology 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]. Previous studies have reported that methanolic extract of *A. ilicifolius* leaves have strong antioxidant (IC_{50} : 17.51 µg/ml) andantifungal activity against *Candida albicans*[9]. Zhang *et al*. reported the potential hepotoprotective activity of phenylethanoid glycosides from *A. ilicifolius*against carbon tetrachloride (CCl_4)-induced liver injury *in vivo* and *in vitro*[11]

The pharmacological effects of plants are due to the presence of secondary metabolites contained in them. The effectiveness of these active components as herbal medicines can be determinedthrough 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 test describes the level of toxicity of the extract against *Artemia salina* larvae. The results of this test can be used to identify a wider range of plant bioactivity. Therefore, the present study was carried out to determinecytotoxicity assayof the ethanol extract of *A. ilicifolius* leavesagainst larvae of *A. salina.*

MATERIALS AND METHOD

Solvents

96% ethanol, aquadest, Tween 80, sea water, H_2SO_4, $FeCl_3$, HCL,chloroform, ammonia

Sample Collection and Preparation

Fresh leaves of *A. ilicifolius*were collected from Gitgit Village, Sukasada District, Buleleng Regency, 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 water and dried at room temperature. The dried leaves were powdered by blender. Powder extracted with 96% ethanol for five days with stirring. The filtrate was collected and evaporated using rotary evaporator.

Phytochemical Screening

- **Test for Alkaloids**
  A quantity of 0.1 g of the extract added with 10 ml of chloroform and a few drops of ammonia. The chloroform fraction was separated and acidified with a few drops of concentrated sulfuric acid. The acid fraction was taken and divided into 2 tubes, then Dragendorf and Meyer reagents were added. The presence of alkaloids was indicated by the formation of a white precipitate in the Meyer reagent, and a red precipitate in the Dragendorf reagent.
- **Test for Flavonoids**
  2 ml of *A. ilicifolius* leaf extract was heated, then ethanol was added. Magnesium powder was added to the solution and then HCl was added. Red or orange coloration indicates the presence of flavonoid.
- **Test for Phenolic Compound**
  0.1 g of *A. ilicifolius* leaves extract was dissolved in methanol, then 2-3 drops of 5% FeCl3 solution were added. Dark green color indicates the presence of phenolic compounds
- **Test for Saponins**
  A. ilicifolius leaves extract was added 5 mL of water, shaken in a test tube, a stable foam was formed (1 cm high foam and stable for 30 minutes).
- **Test for Steroids**

Identification of steroids was carried out using Libermann-Burchard reagent. 5 grams of sample was extracted with ± 10 mL n-hexane, then filtered. The extract was dried on a test stain board, added three drops of acetic anhydride and then one drop of concentrated sulfuric acid (H_2SO_4), the presence of a steroid group compound was indicated by the formation of a brownish or violet ring at the solution boundary.

- **Test for Tannins**

  0.1 g of *A. ilicifolius* leaves extract was dissolved in methanol, then 2-3 drops of 1% $FeCl_3$ solution were added and observed forDark green or bluish black color.

The Cytotoxicity Assay

The cytotoxicity assay was carried out with the Brine Shrimp Lethality Test (BSLT) by using *Artemia 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 10 larvae of *A. salina* were added to each test 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.

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

LC_{50}assessment was analyzedwith Probit analysisby Microsoft excel. Determination of the toxicity category is carried out based on theTable 1.

Table 1 : LC_{50} Categories[14]

Categories	LC_{50} (ppm)
Non Toxic	>1000
Toxic	30-1000
Very Toxic	<30

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical group test were performed and the result are presented in Table 2. Result indicated that alkaloids, flavonoids, phenols, saponins, steroids, and tannins were detected in the ethanol extract of *A. ilicifolius* leaves.

Table 2 : Phytochemical Screening Result

Chemical Group Test	Observation	Inference
Alkaloids	(+)	Presence of alkaloid
Flavonoids	(+)	Presence of flavonoids
Phenols	(+)	Presence of phenols
Saponins	(+)	Presence of saponins
Steroids	(+)	Presence of steroids
Tannins	(+)	Presence of tannins

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 3, it showed thatin 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 103,6 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.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[15].

Table 3 : 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			

CONCLUSION

Ethanol extract of *A. ilicifolius* leavesis toxic based on Brine Shrimp Lethality Testand hascytotoxicity activity potential with a LC_{50} value of 103,6 ppm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR`S CONTRIBUTION

This research was designed and conducted in collaboration of all authors

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