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Acute Toxicity of Ethanol Extract and A Fraction Of *Sansevieria Trifasciata* Prain Using The Brine Shrimp Lethality Test (BSLT)

Siti Hamidatul Aliyah [a, 1, *]; Yessi Seftiara [b, 2,]; Yulianis [c, 3,]
[a,b,c] Pharmacy Program, Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, Jambi, Indonesia
[1,2,3] sitihamidatula@gmail.com*
*Corresponding author

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ABSTRACT

Sansevieria trifasciata Prain plants are simple to grow in Indonesia and have therapeutic potential. The potential toxicity effect present in medicinal plants is important to be identified for safety assurance. The objective of this study is to identify the potential acute toxicity of leaves ethanol extract and fraction of snake plants (*S.trifasciata*). This study used the Brine Shrimp Lethality Test (BSLT) method. *S. trifasciata* leaves are extracted with ethanol by maceration. The ethanol extract was then fractionated using n-hexane, ethyl acetate, and n-butanol solvents. Extract and fraction *S. trifasciata* leaves with concentrations of 1000, 500, 100, 50, and 10 µg/ml were exposed to *Artemia salina* (L.). larvae for 24 hours. The level of toxicity is determined based on LC50 obtained based on the number of dead larvae, through probit analysis. The ethanol extract and the fraction of *S.trifasciata* leaves are active cytotoxic characterized by LC_{50} of 251,187 ppm for the ethanol extract, 116,950 ppm for the n-hexane fraction, 76,033 ppm for the ethyl acetate fraction, 20,230 ppm for the n-butanol fraction and 34,751 for the remaining fraction. The ethanol extract, n-hexane fraction, ethyl acetate fraction, and the remaining fraction were categorized as toxic, while the n-butanol fraction was categorized as very toxic.

Introduction

Since the 19th century, *Sansevieria trisfasciata* Prain (*S.trifasciata*) has been known and grown as an ornamental plant. The leaves are used in traditional medicine to cure diarrhea,

coughing, irritation of the respiratory tract, and hair growth in addition to being used as an attractive plant[1,2]. The pharmacological activities of *S.trifasciata* have been investigated, including its antioxidant, anticancer, antidiabetic anaphylactic, and antibacterial properties[3].

There is still ~~a~~ limited ~~of~~ information about the safety test of *S. trifasciata's* potential as a therapeutic candidate. Before conducting a potential or benefit test, security must be addressed. An assessment of a preparation's safety can be made by performing a toxicity test. Acute toxicity testing is the first type of testing for toxicity [4]. An acute toxicity test is one type of toxicity test performed with how to administer the chemical being tested once or several times 24-hour period. This type of study aimed to establish the LC_{50} or LD_{50} of specific medications or preparations [5].

Several things to consider before doing the toxicity test are a species selection animal, method of administration, method of treatment, dosage, and the number of animals to be used as well as various other environmental factors. Several bioactive compounds that have been successfully isolated and their activity monitored with BSLT show a correlation against a specific anticancer test. Something A compound is said to be toxic if its LC50 is more small or equal to 1000µg/ml [6].

The Brine Shrimp (*Artemia salina* Leach) Lethality Test (BSLT) method is often used to detect the presence of compounds that have acute toxicity in the process of active compounds isolated from natural materials by determination of the lethal concentration 50% (LC_{50}) value[7]. Based on the preliminary studies, the researchers are interested in using the brine shrimp lethality to measure the acute toxicity of *S. trifasciata* ethanolic extract and fraction.

Method

A. Plant material

S.trifasciata plant is obtained from the area of Kota Baru, Jambi City, Jambi province, Indonesia. This plant has been identified in the Laboratory Herbarium Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University with the specimen code 081/K-ID/ANDA/IV/2016. This study used fresh leaf samples.

B. Extraction and fractionation

Approximately 2400 grams of fresh leaves sample that cut into small piece were firstly macerated with 1000 mL of 96% ethanol p.a for 72 hours. Afterwords, the filtrate was then separated from the residue. This protocol were repeated 3 (three) times and all of the filtrate was combined and evaporate in vacuo using rotary evaporator to obtain ethanol crude extract (58,08 g). The ethanol crude extract from *S. trifasciata* (40 g) was suspended in water. Then it was extracted successively with different organic solvents such as hexane, ethyl acetate and butanol to obtain hexane (4,88 g), ethyl acetate (3,97 g), and butanol (5,26 g) and residual methanol fractions (25,48 g), respectively.

C. Phytochemical Screening of Extract and Fraction

The phytochemical screening of *S. trifasciata* was carried out to determine the presence of following compounds: saponin, tannin, flavonoid, alkaloid, steroid, triterpenoid[8,9].

D. Thin-layer Chromatography (TLC)

Thin-layer chromatography was performed with silica gel as the stationary phase. The mobile phase used n-butanol: dichlorometane (DCM) 9:1 (v/v). Chromatograms were detected using ultraviolet light at wavelengths of 254 nm [10].

E. Preparation of *Artemia salina* larvae

As many as 0.5 grams of artemia eggs are input in a plastic bottle filled with 1 liter of seawater. Hatching container placed at room temperature and served aerators. Then leave it for 36 hours until the eggs hatch into larvae.

F. BSLT Acute Toxicity Test

Knop grass leaf ethanol extract *S.trifasciata* leaves extract and fraction each dissolved with Dimethylsulfoxide (DMSO) with a ratio of 1:5. Stock solution is made with a concentration of 5000 ppm. Furthermore, the stock solution is diluted to make a series of extract concentrations of 1000, 500, 100, 50 and 10 ppm. Each tube is filled with 10 ml seawater. A total of ten larvae that has aged 36 hours are put in the tube that has been filled with seawater, then each tube is given 5 ml of *S. trifasciata* leaf ethanol extract and fraction are a suitable concentration. Each test is always accompanied by control and each concentration is made in three replications There are also tubes negative control filled with seawater and 10 larvae each, as well as filled control tubes with larvae, seawater, and DMSO. Tube saved for 24 hours without cover and irradiation lamp. After 24 hours, the number of dead larvae calculated to determine the value of probit and analyzed to determine the LC50 value. The classification of the level of toxicity is based on the LC50 value, which is in the "very toxic" category if it can kill 50% of larvae at concentrations <30 ppm, "toxic" at concentrations of 30–1000 ppm, and "not toxic" at concentrations > 1000 ppm [11].

G. Data analysis

Toxicity test data in the form of a percentage larval mortality at each extract and fraction *S. trifasciata* leaves the concentration was analyzed with probit analysis using the MS Excel 2010 program. Phytochemical test data analyzed descriptive.

Results and Discussion

Extraction of 2400 g *S. trifasciata* fresh leaves with ethanol solvent obtained 58,08 g. Fractionation with hexane, ethyl acetate and butanol obtained by hexane fraction (4,88 g), ethyl acetate fraction (3,97 g), butanol fraction (5,26 g) and residual fraction (25,48 g) (Table 1-2). Fresh samples are used to avoid unintended chemical changes to the active ingredient caused by drying. After being completely cleaned, samples are next coarsely diced to increase their surface area, allowing the solvent to enter the cell more easily and maximizing the removal of chemical compounds from the sample. The use of ethanol as a solvent, namely because ethanol is a solvent that extracts intracellular components from plant materials more easily via cell membranes. Methanol is not suited for extraction since it has poisonous qualities, whereas ethanol is much safer [12].

Table 1. Weight of crude extract of *S. trifasciata* leaves

Sample	simplisia (g)	extract (g)	rendemen (%)
Ethanol extract	2400	58.08	2.42

Table 2. Weight of fraction and percentage yield of fraction of *S. trifasciata*

No	Sample	fraction (g)	rendemen fraction (%)
1	Hexane fraction	4.88	12.2
2	Ethyl Acetate fraction	3.97	9.925
3	Butanol fraction	5.26	13.15
4	Residual fraction	25.48	63.7

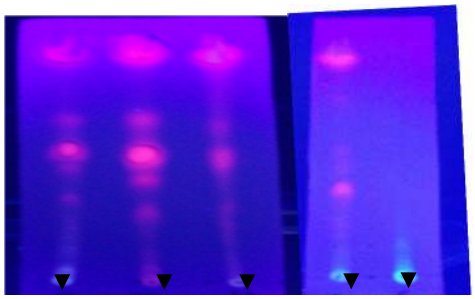
The test results for the content of secondary metabolites in the extract are known to contain saponins, tannins, flavonoids, and steroids. The hexane fraction contains steroid compounds, and the ethyl acetate fraction contains tannin and steroid compounds. The butanol fraction and the remaining fraction contained saponins, tannins, and flavonoids (table 3). The findings of this phytochemical study differed slightly from those of Komala, et al (2012)'s testing, which found that *S.trifasciata* prain includes saponins, flavonoids, steroids, and triterpenoids, and Febriani et al., (2019) findings that it also contains polifenol, steroids, and alkaloids[13]. Secondary metabolite concentrations in plants can vary depending on where and under what conditions they are grown, such as soil nutrients and temperature. Additionally, variations in the extraction techniques and utilized solvents can impact variations in the content of secondary metabolites [14,15].

Table 3. Phytochemical screening of extract and fraction of *S. trifasciata*

No	Chemical compound	Sample				
		Ethanol extract	Hexane fraction	Ethyl Acetate fraction	Butanol fraction	Residual fraction
1	Saponins	+	-	-	+	+
2	Tannins	+	-	+	+	+
3	Flavonoids	+	-	-	+	+
4	Alkaloids	-	-	-	-	-
5	Steroid	+	+	+	-	-
6	Triterpenoids	-	-	-	-	-

key : + = Present, - = Abscent

The ethanol extract, n-hexane fraction, ethyl acetate fraction, n-butane fraction, and the residual fraction have been separated, according to the results of the TLC test that was performed (Figure 1). It is clearly differentiated stains are there in each sample in the ratio of n-butanol: DCM (9:1) solvent.



B EA H E S

Figure 1. TLC result of crude extract and fraction of *S.trifasciata* leaves using eluen n butanol: DCM (9:1). (E= crude extract, B= butanol fraction, ET= ethyl acetate fraction,H= hexane fraction, S= residual fraction)

Based on the LC50 value obtained from the ethanol extract and each fraction, it shows that *S.trifasciata* Prain has cytotoxic activity because it gives an LC50 value <1000 ppm. Cytotoxicity is the ability of a compound to inhibit cell growth, where an extract is declared active cytotoxic if the LC50 value is <1000 ppm [16]. The ethanol extract and the fraction of *S.trifasciata* leaves are active cytotoxic characterized by an LC50 of 251,187 ppm for the ethanol extract, 116,950 ppm for the n-hexane fraction, 76,033 ppm for the ethyl acetate

fraction, 20,230 for the n-butanol fraction and 34,751 for the remaining fraction. The ethanol extract, n-hexane fraction, ethyl acetate fraction, and the remaining fraction were categorized as toxic, while the n-butanol fraction was categorized as very toxic.

The cytotoxic ability of the ethanol extract and the fraction of *S.trifasciata* leaf is thought to be due to the influence of the secondary metabolites contained in these plants. According to Cahyadi (2009), the way these compounds work by acting as stomach poisoning. Therefore, if these compounds enter the body of the larvae, the digestive organs will be disrupted. In addition, this compound inhibits taste receptors in the mouth area of the larvae. This causes the larvae to fail to get a taste stimulus, so they are unable to recognize their food and starve to death [17].

Table 4. % Mortality of shrimp after treating with extract and fraction of *S. trifasciata*

Sample	concentration (µg/ml)	Log concentration	Number of mortality	Total of shrimp	% mortality	Probits	LC50 (µg/ml)
Crude ethanol extract	1000	3,00	23	10	76,67	5,613	251.189
	500	2,70	17	10	56,67	5,075	
	100	2,00	11	10	36,67	4,532	
	50	1,70	9	10	30,00	4,532	
	10	1,00	7	10	23,33	4,194	
Ethyl Acetate fraction	1000	3,00	25	10	83,33	5,954	76.033
	500	2,70	23	10	76,67	5,613	
	100	2,00	19	10	63,33	5,305	
	50	1,70	14	10	46,67	4,798	
	10	1,00	8	10	26,67	4,194	
Heksan fraction	1000	3,00	22	10	73,33	5,613	116.950
	500	2,70	18	10	60,00	5,332	
	100	2,00	14	10	46,67	4,798	
	50	1,70	12	10	40,00	4,798	
	10	1,00	10	10	33,33	4,504	
Butanol fraction	1000	3,00	28	10	93,33	6,476	20,230
	500	2,70	25	10	83,33	5,954	
	100	2,00	21	10	70,00	5,583	
	50	1,70	18	10	60,00	5,305	
	10	1,00	14	10	46,67	4,773	
Residual fraction	1000	3,00	27	10	90,00	6,476	34.751
	500	2,70	25	10	83,33	5,954	
	100	2,00	22	10	73,33	5,583	
	50	1,70	17	10	56,67	5,05	
	10	1,00	11	10	36,67	4,504	

According to Chahar et al. (2011), the mechanism of flavonoids can be cytotoxic, namely, flavonoids can promote apoptosis through inhibition of DNA topoisomerase I and II activity [18]. Meanwhile, terpenoid compounds can damage DNA by breaking bonds in DNA into single-strand DNA and can inhibit the process of cell mitosis. Saponin compounds have the property of being able to hemolyze erythrocytes and are very toxic when injected into the bloodstream and tannin compounds can increase p27 protein, which inhibits the cell cycle [19].

Conclusion

The ethanol extract and the fraction of *S. trifasciata* leaves are actively cytotoxic, characterized by an LC50 of 251,187 ppm for the ethanol extract, 116,950 ppm for the n-hexane fraction, 76,033 ppm for the ethyl acetate fraction, 20,230 ppm for the n-butanol fraction, and 34,751 ppm for the remaining fraction. The ethanol extract, hexane fraction, ethyl acetate fraction, and the remaining fraction were categorized as toxic, while the n-butanol fraction was categorized as very toxic. The test results for the content of secondary metabolites in the extract are known to contain saponins, tannins, flavonoids, and steroids. The hexane fraction contains steroid compounds, and the ethyl acetate fraction contains tannin and steroid compounds. The butanol fraction and the remaining fraction contained saponins, tannins, and flavonoids. Each of these compounds has cytotoxic activity.

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