The potential biopesticide toxicity test of *Ipomoea batatas* (L.) Lam (*Purple Sweet Potato leaf extract*) against *Artemia salina* Leach larvae using the *Brine Shrimp Lethality* Test Method

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Abstract- *Ipomoea batatas (L.)* Lam (Purple Sweet Potato) can grow and breed in various regions in Indonesia that can be used as food preparations and animal feed. The phytochemical content of *Ipomoea batatas (L.)* Lam (Purple Sweet Potato) includes flavonoids, tannins, saponins, and alkaloids. The content of phytochemical compounds can be potential as a biopesticide. The biopesticides are pesticides that are made by utilizing natural ingredients derived from plants. This study aims to determine the toxicity of LC*50* Ipomoea batatas (L.) Lam extract which can be potential as a biopesticide. *Ipomoea batatas* (L.) Lam was extracted using 70% ethanol solvent. The dilution was carried out with the concentrations used, namely 100 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm, and negative controls. This research is true experimental research using the Brine Shrimp Lethality Test method. The exposure of *Ipomoea batatas* (L.) Lam extract to *Artemia salina* Leach larvae was carried out for 24 hours. The results showed mortality at a concentration of 100 ppm is 63%, 250 ppm is 66%, 500 ppm is 73%, 750 ppm is 100%, and 1000 ppm is 100%. Then the data analysis uses EPA Probit Analysis program version 1.5 Used for calculating LC/EC values. The results showed that *Ipomoea batatas* (L.) Lam extract had an LC_{50} of 120.75 ppm which was categorized as moderate toxic and could potentially be a biopesticide.

Index Terms- Biopesticide, *Ipomoea batatas* (L.) Lam, *Artemia salina* Leach, *Brine Shrimp Lethality Test* method, Toxicity of LC_{50}, secondary metabolites

I. INTRODUCTION

The biodiversity in Indonesia is very abundant, one of which is purple sweet potato (*Ipomoea batatas* (L)). The content of purple sweet potato leaves includes calcium, vitamin B, beta-carotene, protein, zinc, and iron and secondary metabolite compounds are the flavonoids, tannins, alkaloids, and saponins. These secondary metabolites can function as anti-inflammatory, anti-bacterial, and anti-fungal antioxidants. Secondary metabolites produced by various types of plants can be potential as a substitute for synthetic pesticides. [(1)].

The content of secondary metabolites: Flavonoids have the ability to poison the insects through the inhibition of the respiratory system, Saponins are poisons toxin that can have antioxidant and antibacterial effects through the mechanism of increasing catalase formation, Alkaloids are stomach poisons that inhibit the digestive system chains, Tannins have the ability to bind protein, so it can be anti-bacterial..[(2)]. The cytotoxic properties of a metabolite compound in plants can be determined based on the results of the brine shrimp lethality test which is the number of *Artemia salina* Leach larvae mortality on exposure to the concentration of the test compound.[(3)] A plant extract can be said to be toxic or toxic if it has an LC_{50} value of less than 1000 µg / ml after 24 hours of exposure. [(4)]

Biopesticides are pesticides that are made by utilizing secondary metabolites produced by organisms. Biopesticides are generally used to control pests because they are insecticidal. [(5)]. Exploration of potential metabolites as biopesticides can be done by using the Brine Shrimp Lethality Test. the Brine Shrimp Lethality Test Method is a method as a preliminary test to determine the toxicity of a metabolite compound that has potential as a biopesticide.[(6).] The Brine Shrimp Lethality Test method is the method that is easy to apply, relatively inexpensive, does not require aseptic conditions and the results can be scientifically justified..[(7)]

II. METHODS

This research was conducted in the Laboratory of Environmental Health, Faculty of Health, Dian Nuswantoro University, Semarang, which was conducted from May to June 2020.

1. **Identification of Secondary Metabolites.** Identification of total metabolite compounds by UV-Vis spectrophotometry method in the range of wavelength 400 - 450 nm, continued by making a standard curve and determining the levels of the metabolite compounds.[(8)]
2. **Brine Shrimp Lethality Test.** This study is a true experimental study with the Brine Shrimp Lethality Test method. The independent variable in this study is the

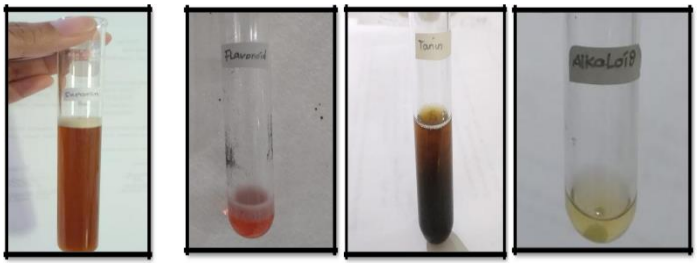
concentration of purple sweet potato leaf extract and the dependent variable is the number of mortality of *Artemia salina* Leach larvae (LC_{50}). The sample size used was 160 *Artemia salina* Leach larvae, with each test tube included 10 Artemia salina Leach larvae for the testing process. The *Artemia salina* Leach larvae used were 48 hours old and randomly taken. This research was conducted 3 times of replication. Extract concentrations used are 100 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm, and one as a negative control. Mortality data obtained were then further analyzed using microsoft office excel with a regression test to determine the LC_{50} Probit value. [9]

III. RESULT

Based on the research results obtained as follows:

Table 1. Phytochemical test results

Parameter	Reagent ingredients	Changes observed	Results
Saponin	Sampel + Aquadest	Formed foam that does not disappear for more than 1 minute	+
Flavonoids	Sampel + Mg + HCl	Red formed	+
Tannin	Sampel+ FeCl3 10%	Greenish black formed	+
Alkaloids	Sampel + Cloroform+ Ammonia + H_2SO4 + Mayer reagent	White sediment formed	+



(1) Saponin (2) Flavonoids (3) Tannin (4) Alkaloids

Table 2. Brine Shrimp Lethality Test Results

Concentration	Test 1		Test 2		Test 3		Total Dead	Average mortality ± standard deviation	% Dead	Log concentration	Probit Value
	+	-	+	-	+	-					
Control	10	0	10	0	10	0	0	-	0%	-	-
100 ppm	3	7	2	8	6	4	19	0,63	63%	2.00	5,33
250 ppm	3	7	3	7	4	6	20	0,66	66%	23,979.00	5,44
500 ppm	4	6	1	9	3	7	22	0,73	73%	26,990.00	5,58
750 ppm	0	10	0	10	0	10	30	0,10	100%	28,751.00	8,09
1000 ppm	0	10	0	10	0	10	30	0,10	100%	3.00	8,09

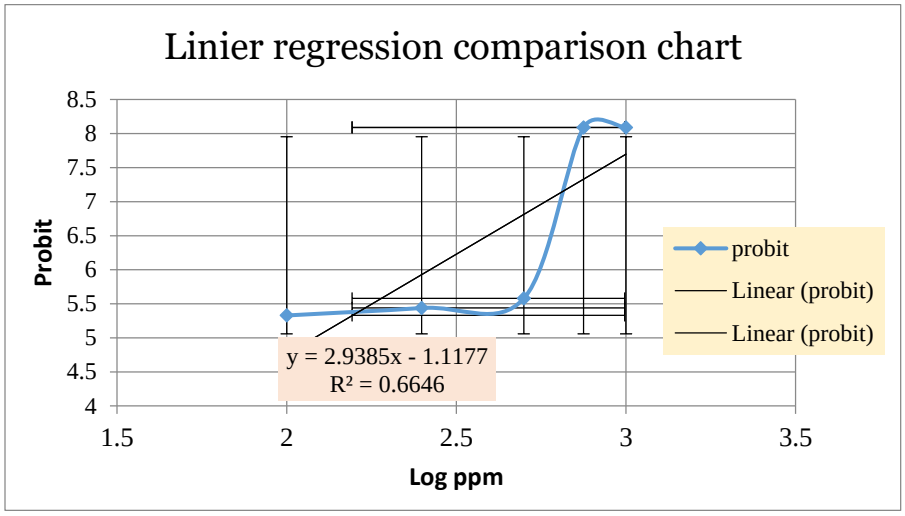


Figure 1. Linear regression comparison chart

Based on the results of the regression calculations obtained:
a = 2,938
b = -1,117
r = 0,664
Regression equation: LC50 Value:y = ax + b
log LC50 (y) = 5
y = ax + b
y = 2,938x + (- 1,117)
5 – (-1,117) = 2,938x
6,1177 = 2,938x
X = 2,0822
LC50 = Antilog 2,0822
LC50 = 120,75 ppm

IV. DISCUSSION

Ipomoea batatas (L.) Lam (Purple Sweet Potato) can grow in various regions in Indonesia, including in the Boja region of Central Java where most farmers plant purple sweet potato leaves. In addition to the fruit consumed, the leaves can also be used as processed vegetables but now people rarely use sweet potato leaves. Based on interviews with farmers that sweet potato leaves during the growth process produce fruit (purple yam) leaves that grow will be denser with creeping and usually only cut it to facilitate the process of taking cassava when it is harvest time. In addition, purple sweet potato leaves are used for livestock clothing. [10]. The existence of sweet potato leaves can make the idea of making biopesticides.

Ipomoea batatas (L.) Lam (Purple Sweet Potato leaf extract) in maintaining its survival is to do primary metabolism, the results of primary metabolism in the form of primary metabolites such as carbohydrates, proteins, fats, vitamins, and minerals, but in addition to the existence of primary metabolism, also do Secondary metabolism results from primary metabolite precursors. The secondary metabolism produced by Ipomoea batatas (L.) Lam (Purple Sweet Potato leaf extract) is used in maintaining its life from the disturbance of the external environment. Secondary metabolism results in the form of Saponins, Alkaloids, Tannins, and Flavonoids. Secondary metabolites that have pharmacological bioactivity called bioactive compounds that can be used as anti-bacterial and biopesticide precursors[11]

Extraction of Ipomoea batatas (L) Lam (Purple Sweet Potato Leaf)

This study used purple sweet potato (*Ipomoea batatas* (L) Lam) leaves obtained from a garden in the Boja area, Semarang. Central Java. Test sample through the extraction process with the maceration method so that it is obtained *Ipomoea batatas* (L) Lam (Purple Sweet Potato Leaf). The extract is used for identification of secondary metabolites and brain shrimp lethality test [12]. The phytochemical test of *Ipomoea batatas* (L) Lam (Purple Sweet Potato Leaf) aims to test the content of chemical compounds present in the extract of *Ipomoea batatas* (L) Lam (Purple Sweet Potato Leaf). Based on phytochemical tests that have been carried out, it is obtained the results of the content of flavonoid, alkaloids, saponins, and tannins. [13]

Phytochemical test results show that the leaves of purple sweet potato (*Ipomoea batatas* L) contain positive flavanoid, this is characterized by the occurrence of a blackish red color after the test extract is added with concentrated HCl. (Kohyama et al., 2017) Blackish red color in the flavanoid test due to the formation of flavilium salts. [14].

The tannin content in the phytochemical test is characterized by a change in color to green when adding 1% FeCl3 solution, this

is because 1% FeCl3 reacts with one of the hydroxyl groups present in the Tannin compound. FeCl31% reagent was used to identify Tannin compounds with indicators of the formation of blackish green (Muthukumaran et al., 2016). [15].

Detection of the presence of alkaloid compounds is characterized by the formation of a yellowish-white precipitate after the extract is added with a major reagent, it is estimated that nitrogen in the alkaloids will react with K + metal ions from potassium tetraiodomerkuat (II) to form a precipitating potassium-alkaloid complex [16]. The presence of Saponin content is characterized by the formation of foam/froth for ± 10 minutes after adding aquadest. Compounds that have polar and nonpolar groups will be active on the surface so that when a saponin is shaken with water it can form micelles, the polar group faces outward while the nonpolar group faces inward so that it doesn't look like foam. [17].

Flavonoids are a group of phenol compounds that are mostly found in nature. These compounds are responsible for red, purple, blue, and yellow dyes in plants. Most of the flavonoids found in plants are bound to sugar molecules as glycosides and in mixed compounds. Flavonoid chemical compounds can be anti-bacterial because they can inhibit the synthesis of nucleic acids, inhibit cell membrane function and inhibit energy metabolism [18]

Tannin is a secondary metabolite compound found in purple sweet potato plants. Tannins are able to bind to proteins and are protective of proteins from the degradation of microbial enzymes and protease enzymes so tannins can be antibacterial [19]

Saponins are steroidal glycoside compounds or triterpenes found in Ipomoea batatas (L) Lam (Purple Sweet Potato Leaf) which can act as a defense mechanism for plant bodies. Saponins are easily soluble in water, insoluble in ether, have a bitter taste, and cause irritation to the mucous membranes of organisms. Saponin is a poison that can destroy blood vessels or hemolysis in the blood, therefore saponins can be anti-bacterial and anti-fungal..[20]

Alkaloids are secondary metabolites that are found in Ipomoea batatas (L) Lam (Purple Sweet Potato Leaf) tissue. Almost all alkaloids come from plants that are widespread and most are included in higher plants. Alkaloids are alkaline organic compounds found in some classes of plants, are bitter, and are insect repellents. Alkaloid compounds as biopesticide or anti-insects play a role by damaging the insect's protein structure, disrupting the nervous system's work and being toxic to insects [21][22]

Toxicity Test of Ipomoea batatas (L) Lam (Purple Sweet Potato) with the Brine Shrimp Lethality Test method

An acute toxicity test can be performed using the Brine Shrimp Lethality Test method. The Brine Shrimp Lethality Test method was chosen because it is relatively inexpensive and relatively easy to work with. Brine Shrimp Lethality Test Method is a method in biological tests for screening in determining the toxicity of an active plant that is by using the Artemia salina Leach test The Brine Shrimp Lethality Test test is an LC_{50} test method using the Artemia salina Leach larvae as a test animal for 24 hours of exposure to the seawater environment, to determine the toxicity and biological tests of biocative compounds from secondary metabolites produced by of *Ipomoea batatas (L*) Lam (Purple Sweet Potato) [23].

Based on the results of the mortality test extract of Ipomoea batatas (L) Lam with a concentration variation of 100 ppm with the results of the percentage of deaths is 63%, the concentration of 250 ppm with the results of the percentage of deaths is 66%. %, the concentration with the result of the death percentage of 500 ppm is 73%, the concentration of 750 ppm with the mortality rate is 100%, and the concentration of 1000 ppm with the mortality rate is 100%, after knowing the percentage of deaths for each concentration, LC_{50} was searched using Microsoft Office Excel. LC50 results of 120.75 ppm which are included in the category of moderate toxic. (Used to calculate LC / EC values)

Based on the phytochemical test results of purple sweet potato leaves (Ipomoea batatas (L)) have secondary metabolite compounds namely alkaloids, flavonoids, saponins, and tannins. The compound is thought to function as an insecticide. (LC_{50}) values of secondary metabolites obtained Lethal Concentration 50 (LC_{50}) which is 120.75 ppm which is included in the category of moderate toxic. According to the major category of toxicity, bioactive compounds based on (LC_{50}) values are category values (LC_{50}) (ppm) very toxic <30, category values (LC_{50}) (ppm) between 30-1000 are toxic and category values (LC_{50}) (ppm)> 1000 are categories not toxic [24]

In the toxicity test, an appropriate test animal is needed, the test animal used in the study is *Artemia salina* leach. *Artemia salina* leach is one of the widely used and relevant test animals in several scientific fields such as ecology, physiology, ecotoxicology, and genetics because *Artemia salina* has a short life cycle, the ability to adapt to a wide range of salinity and extreme temperatures, short life cycle, high adaptability to adverse environmental conditions, reproductive parthenogenesis, small body size, and simple body organs. *Artemia salina* has a cell wall formed from the exoskeleton of very simple chitin so that secondary metabolite compounds can destroy the walls of *Artemia salina* and cause the death of *Artemia salina*. Death of *Artemia salina* can also occur through an osmoregulation mechanism. Artemia Salina will swallow the medium with a filter-feeding mechanism. So that it will swallow the surrounding media both toxic and non-toxic, including secondary metabolites (bioactive) produced by *Ipomoea batatas* (L) Lam can easily enter the body of *Artemia salina* and cause death.[25].

Secondary metabolites that damage the cell wall and cell membrane by inhibiting enzyme synthesis or inactivation of the enzyme, thereby causing loss of cell wall viability and cell membrane causing cell lysis. The secondary metabolite compounds inhibit cell wall synthesis mainly by interfering with the synthesis of peptidoglycan, this causes the cell walls in *Artemia salina* to become lysis and cause death. [26]. Secondary metabolites in small doses can have pharmacological effects and have toxic effects on Test animals *(Artemia salina*). Secondary metabolites have the potential for acute toxicity and can cause larval death of *Artemia salina* Leach. The mechanism of larval death is related to the function of secondary metabolite compounds which can inhibit the feeding power of larvae/antifeedants which act as stomach poisoning, causing the death of *Artemia salina* Leach [27]

V. CONCLUSION

***Ipomoea batatas* (L.) Lam** or Purple sweet potato leaf extract is medium toxic with LC_{50} value of 120.75 ppm as an antibacterial, not yet toxic enough to be an alternative biopesticide. Further research is needed to determine the effectiveness of secondary metabolites of flavonoids, tannins, alkaloids, and saponins quantitatively.

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AUTHORS' CONTRIBUTION

This research was conducted in the environmental health microbiology laboratory, Dian Nuswantoro University in collaboration between two authors, OAS and SI. OAS writers conduct research designs, analyze research results, write drafts of initial scripts. Authors SI and S manage the research analysis. The SI author manages the search literature and manages the final research report draft corrections. Both authors have read and agreed to the final draft of this article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AVAILABILITY OF DATA

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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