INTERNATIONAL JOURNAL OF HEALTH & MEDICAL RESEARCH

ISSN(print): 2833-213X, ISSN(online): 2833-2148

Volume 02 Issue 10 October 2023

DOI : 10.58806/ijhmr.2023.v2i10n06

Page No. 371-380

Phytochemical Profile and Toxicity Testing of African Leaf Extract (Vernonia Amygdalina Delile) with Liquid Variation Scavenger Method Brine Shrimp Lethality Test (BSLT)

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ABSTRACT:

Background: The African leaf (Vernonia amygdalina Delile) is one of the plants used as a traditional medicine, one of which is anti-cancer. **Aim:** The study aims to identify compound groups, toxic effects and LC_{50} values of African leaf extracts with variations in the leaf fluid.

Method: In this study, African leaves were extracted using a maseration method with three fluids namely n-hexane, ethyl acetate and 70% ethanol. The extract was qualitatively studied by the group of chemical compounds and toxicity tests were conducted using the method of Brine Shrimp Lethality Test. (BSLT).

Result: The results of this study showed that n-hexane extract contains flavonoid and glycoside compounds. Ethyl acetate extract has a flavonoid compound, saponins and glucosides. The results of a linear regression analysis showed that 70% ethanol extract was an extract with the highest LC_{50}value with a LC_{50} value of 31.80 ppm, an ethyl acetate extracts with LC_{50} values of 51.70 ppm and n-hexane extracts with LC_{50}, with a 91.88 ppm value of LC_{50}.

Conclusion: This study showed that the three extracts included a strong toxic ketegori with a range of 0-100 ppm.

KEYWORDS: Vernonia amygdalina Delile, African Leaf, Toxicity, BSLT.

I. INTRODUCTION

Indonesia is a country on the continent of Asia that is famous for its diversity, both the diversity of the tribes and its natural diversity. One of the natural diversity that Indonesia has is biodiversity, especially plants. Besides, Indonesia also has an ethnic diversity that has a wide range of knowledge about traditional medicine using ingredients from plants [1]. Indonesia has a very complete biodiversity. This award makes Indonesia the best herbal medicine country in the world. Various kinds of medicinal plants can grow fertile in Indonesia. The medicinal plants are the main ingredients in the making of spices and herbal medicines [2].

One of the medicinal plants found in Indonesia, the African leaf (Vernonia amygdalina Delile) is known to have been empirically and scientifically tested. The use of African leaves empirically by the community is used for various diseases including as a cure for cancer, preventing heart disease, lowering cholesterol, prevention of stroke, reducing blood sugar, digestive disorders and weight loss [3] . According to research by Ijeh and Chukwunonso (2010), African leaves contain compounds such as saponins, flavonoids, sesquiterpen lactones, and steroid glycosides. Additionally, chemical analyses conducted by [3] show that simplisia and ethanol extracts contain flavanoids, polyphenolates, tannin, monoterpen, seskuiterpen, steroids/triterpenoids, ketonons and saponines. These compounds that are the main ingredients have effects and give benefits in herbal treatment using African leaves.

Literature study of probit toxicity analysis of African leaf extract with the method of brine shrimp lethality test (BSLT) showed that African leaves ethanol phase extract has toxic potential with a LC_{50} value of 123 ppm [4].The separation of active compounds or extraction is carried out using two or more fluids. Selection of the fluid is based on the polarity of the compounds that the African leaves possess. The terpenoid and alkaloid groups are generally soluble in semi-polar to nonpolar fluids such as ethyl acetate, n-hexane, while the flavonoid, saponin and glycoside groups are solute in polar fluid such as ethanol [5]. So the active compounds in the African leaves can be separated according to its polarity.

The BSLT method is a simple preliminary test for screening toxicity of plant extracts using shrimp larva Artemia salina

Leach [6]. This toxicity test with the BSLT method has a wide spectrum of pharmacological activity, the procedure is simple (without aseptic techniques), fast and does not require large costs (no animal serum required), and the results are representative and reliable [6] ; [7]. Based on the description above, a toxicity test of African leaf extract (Vernonia amygdalina Delile) with the method of brine shrimp lethality test has been carried out. (BSLT). The separation of active compounds is done by maseration method using different fluids such as n-hexane, ethyl acetate and ethanol that have radiation differences.

II. RESEARCH METHODE

A. Tools and materials

The tools used in the study are aerators, aquariums, glass tools, aluminum foil, mixer bars, porcelain cups, cylinder paper, 15-watt flashlights, micropipets, analytical balances, drop pipettes, a set of maseration tools, rough scales, vacum rotary evaporators and vials. The ingredients used are aquadest, CH_3COOH, CH_3COOH anhydrate, 2% dimethylsulfoxide (DMSO 2%), African leaf extract, ethyl acetate, ethanol 70%, $FeCl_3$, non-berodium salts, H_2SO_4 concentrated, HCl 2 N, concentrating HCl, choroform, egg Artemia salina Leach (Supreme Plus), n-hexane, Dragendroff peroxide, Mayer peroxides, Wagner peroxids, yeast and magnesium powder.

B. Plant Determination

The determination aims to establish the truth relating to the macroscopic morphological characteristics of the African leaf plant (Vernonia amygdalina Delile) to the library. Determination was carried out at the Herbarium Bogoriense, the Botanical Research and Development Hall of the Biological Development and Research Centre, LIPI Bogor.

C. Sample Processing

Samples of African leaves were taken from Dok IX district, North Jayapura district in the city of Jaipura, Papua. African leaf samples are disorted wetly to separate dirt or other foreign material, then washed with running water, sliced in small pieces and then dried by placing in an open place with good air circulation and not exposed to direct sunlight because drying directly to sunlight will damage the active components of African leaves. African leaves are dried and then dried [8].

D. Extraction of Active Compounds

The extraction of the active components is carried out by maseration or immersion with a fluid that has different levels of radiation, namely n-hexane, ethyl acetate, and 70% ethanol. African leaf powder weighs 250 grams, and is maserated with each of the fluids namely, n-heksane, ethylacetate and 70% ethanol as much as 2 L. The powder is inserted into a masseration vessel, then sufficient fluid is added for the washing process and then humidified for 15-30 minutes. The remaining fluid of the vessel is added until all the simplisia are perfectly immersed and then inhabited in a place protected from sunlight for 3 x 24 hours while occasionally mixed and filtered. The residues are remaserated with the same fluid. The filter is collected and applied using a vacuum rotary evaporator to obtain extract of n-hexane, ethyl acetate, and 70% thick ethanol [9].

$$\text{Yield } (\%) = \frac{\text{Extract weight}}{\text{Dry Simplicia Weight}} \text{ X } 100\ \%$$

E. Ethanol Free Testing

Identification is done by the extracts dissolved with H_2SO_4 concentrated in the reaction tube then added acetate acid and covered with cotton, then heated until boiling after which the ester odor on cotton is identified, if the extract does not contain ethanol then does not scratch the thick ester ring [10].

F. Phytochemical Screening

1. Alkaloid Testing

The extracts are inserted into the reaction tube and diluted with 70% ethanol, then added 5 drops of HCl 2 N, then heated and filtered. 1 ml is tricked and placed in a reaction pipe, where each reaction tubes are added dragendorf reaction, sawing recession and wagner reaction. The formation of the reservoir indicates that the sample contains alkaloids. The reaction with the Mayer reaction will form white reservoirs, with the Dragendorff reaction a red reserver and with the Wagner reaction brown reservances. [11].

2. Flavonoid Testing

The extract is mixed with 3 mL of 70% ethanol, then coated, heated, and coated again and then filtered. The obtained filter, then added 0.1 g Mg powder and 2 drops of concentrated HCl. The appearance of orange, red, or yellow color indicates the presence of flavonoids. [11].

3. Steroid/Triterpenoid Testing

The extract is added with 1 ml of chlorophorus and mixed. Each 2 anhydrate acetate and H_2SO_4 are added to the Steroid Test positive filter if it produces a blue or green color, while the triterpenoid produces the red or purple color [11].

4. Saponin Testing

The extract is boiled with 10 mL of water in a water bath. The filtrate was shaken and allowed to stand. A positive sample contains saponin if 1 cm of foam forms for no less than 10 minutes and when 1 drop of 2 N HCl is added the foam does not disappear (Harborne, 1996).

5. Tannin Testing

The extract is added a few drops (2-3 drops) $FeCl_3$, if the color is green, it means that the sample contains pyrogalol tannin, and when it is produced the color green means that it contains catechol tannin [11].

6. Glycoside Testing

The extract is dissolved in 5 mL of CH_3COOH anhydrate then added 10 drops of H_2SO_4 concentrated. The shape of blue or green indicates the presence of glycosides [11].

G. Toxicity Test with Artemia salina Leach Shrimp Larvae

1. Hatching of Artemia salina Leach larvae

The Artemia salina Leach larvae were scrapped for 48 hours by soaking the eggs in artificial seawater (50 grams of non-berodium salt in 1.2 l aquadest) in the aquarium. Previously, the aquarium installed an aerator, which is useful forining oxygen levels in the Aquarium. The aquarium that has been filled with eggs and has been installed the next aerator is placed in a room that is sufficiently light. This light works for the growth of the Artemia salina Leach larva [12].

2. Preparation of stock solution

African leaf extract (n-hexane extract, ethyl acetate extract 70%) to be tested, each weighed as much as 20 mg then dissolved with 2% DMSO 1-3 drops (50-150 µL) to dissolve the extract then added 20 mL of seawater to obtain a concentration of 1000 ppm. Each extract was then diluted so that it obtained concentrations of 10, 50, 90, 130, and 170 ppm for n-hexaene extract.

3. Toxicity Testing

Ten larvae were inserted into each test vial that had been given a little sea water and added one drop of yeast solution as the larva nutrition, then the volume of sea water was supplemented to 10 mL, whereas the control solution contained only sea water, one dropless yeast solutions and shrimp larvaes. The test vial is then kept at room temperature for 24 hours. Each treatment is repeated three times. (triplo). The number of living larvae is calculated and the percentage of larva mortality is determined to determine the LC50 value. Extracts with LC50 values < 1000 ppm are said to be toxic. (Meyer et al., 1982). The percentage of larva mortality can be calculated by the following formula [12] :

$$\% \text{ Mortality} = \frac{\text{number of dead larvae}}{\text{number of early larvaes}} \text{ x } 100\%$$

The data obtained will be further analyzed with probit analysis to determine the price of LC_{50}.

4. Data analysis

The type of data in this research is primary data and secondary data. Primary data is obtained directly from the results of the research, whereas secondary Data is data from the literature that supports the research. Data presentation death larva Artemia salina Leach made linear regression equations:

$y = bx + a$

Where : y = death presentation; and x = concentration

By using the analysis method in Microsoft Office Excel by creating a straight line equation that connects between the concentration log values of the death presentation. Once the equation is obtained, then enter the y value as the value of 50% of the animal deaths of the test which will subsequently produce the value x as the concentration log. Antilog x is the LC_{50} value.

III. RESULT AND DISSCUSION

A. Plant Determination Results

The plant that has been identified at the Indonesian Institute of Science Research Center for Biology, Bogor is Vernonia amygdalina Delile of the Compositae tribe.

B. Extraction Results

The test sample in this study used leaves from African plants extracted by maseration method. Maseration is an easy extraction method and in its stages no heating process is carried out so as to avoid damage to the active substance contained by simplisia [13].

The maseration process is based on the use of different polarising fluids based on polarisation levels such as n-hexane, ethyl acetate and 70% ethanol. Selection of polarised fluids is based upon the polarity of the compounds that are present in the African leaf. n-heksane polarisating fluids are non-polar, where n-hexane has specific properties that will attract only nonpolar compound only, and then ethyl-acetate polarisant fluids where ethyl acid has a low level of polarity so that it is expected to attract compounts with a low degree of polarization, followed by 70% ethanol with a high rate of polarisation. It is expected that by using different polarising fluids based on polarisation levels, the active compounds contained in the African leaves can be attracted according to polarised levels [14].

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Table 1. Data on the yield of n-Hexane, Ethyl Acetate and Ethanol Extract 70% of African Leaves

Sample	Simplicia Weight (g)	Extract Weigh (g)	% Yield
n-hexan Extract	250	10,45	4,18%
etil asetat Extract	250	14,84	5,94%
Ethanol 70% Extract	250	20,86	8,34%

The fertilizer obtained for each solvent is 4.18% n-hexane extract, 5.94% ethyl acetate extract and 8.34% ethanol extract. The small size of the yield indicates the efficiency of the extraction process. The extraction efficiency can be influenced by the type of solvent, sample particle size, extraction time and extraction method used. Most yields are obtained from 70% ethanol extract [13].

C. Ethanol Free Test Results

Ethanol-free tests were conducted to free the extract from ethanol so that a pure extract was obtained without any contamination, besides that the etanol itself is toxic so that it will result in a false positive on the treatment of the sample [10]. The test results of ethanol-free 70% African leaf etanol extract showed that the extract is ethanole-free so it can be used for later stages.

Table 2. Ethanol Free Testing

Extract	Procedur	Result	literature	Refference	Information
Ethanol Extract	Extract + H_2SO_4 P + CH_3COOH (Heat)	Can't smell it ester smell	Can't smell it ester smell	niawati, 2015	(+)

D. Phytochemical Screening Results

After obtaining the thick weight of the extract and conducting an ethanol-free test, a phytochemical screening test is carried out to determine the presence of a phytochemical component in the extracts to be tested. Qualitative determination can be seen from color changes or the formation of foam or sediment if the sample reacts with certain chemicals.

Table 3. Phytochemical Screening of n-Hexane Extracts of African Leaves

Extract	Reagen Spesific	The color generated	Literature	References	Information
n- heksan extract	**Alkaloid**			]	(-)
	Dragendorf	No precipitate formed, green color	Red-orange precipitate		(-)
	Mayer	white precipitate formed	White precipitate		(+)
	Wagner	No brown precipitate is formed, brown in color	rown precipitate		(-)
	Flavonoid				
	Mg 0,1 g powder + HCl P 2 drops	Yellow	Red, yellow or orange		(+)
	Steroid/Triterpenoid				

	Kloroform 1 ml + asetat anhidrat +H_2SO_4	Blackish brown	Blue-Green (Steroid), Red-Purple (Triterpenoids)		(-)
	Saponin				
	hot water + HCl 2 N	Not formed foam	Formed foam	]	(-)
	Tanin				
	$FeCl_3$	Dark chocolate	Blackish green (Tannin pyrogallol), Green (Tannin catechol)		(-)
	Glikosida				
	Asetat anhidrat + H_2SO_4 P	Green	Blue-Green		(+)

can be done by observing the death of animals and this death response is considered to be the influence of the tested compound. The toxicity test is intended to determine the potential safety level of African leaf extract by looking at the number of deaths of Artemia salina and determining the LC_{50} value. (Lethal Concentration 50).

Table 4. Phytochemical Screening of African Leaf Ethyl Acetate Extract

Extract	Reagen Spesific	The color generated	Literature	References	Information
Etil asetat extract	**Alkaloid**			]	(-)
	Dragendorf	No precipitate or color is formed green	Red-orange precipitate		(-)
	Mayer	Formed white precipitate	White precipitate		(+)
	Wagner	Not formed precipitate, brown color	rown precipitate		(-)
	Flavonoid				(+)
	Mg 0,1 g Powder + HCl P 2 drops	Yellow	Red, yellow or orange		
	Steroid/Triterpenoid			]	(-)
	Kloroform 1 ml + asetat anhidrat + H_2SO_4	Blackish brown	Blue-Green (Steroid), Red-Purple (Triterpenoids)		
	Saponin				(+)
	Hot Water + HCl 2 N	Foam forms	Formed foam		
	Tanin				

	$FeCl_3$	Dark chocolate	Blackish green (Tannin pyrogallol), Green (Tannin catechol)		(-)
	Glikosida				
	Asetat anhidrat + H_2SO_4 P	**Green**	Blue-Green		(+)

The larva used in this study is a 48-hour-old larva because the larva is in the most sensitive state at the age of 48 hours. This is because at 48 hours the organs of Artemia salina have been fully formed (Meyer, et al., 1982). With the formation of the mouth, Artemia Salina has been able to drink artificial seawater that has been given African leaf extract with various concentrations, so the death of Artema salina is actually due to African leaves extract in these different concentrations.

Table 5. Phytochemical Screening of 70% Ethanol Extract of African Leaves

Extract	Reagen Spesific	The color generated	Literature	References	Information
Ethanol Extract	**Alkaloid**				(-)
	Dragendorf	No precipitate or color is formed dark green	Red-orange precipitate		(-)
	Mayer	Not formed precipitate, dark green color	White precipitate		(-)
	Wagner	Formed brown precipitate	rown precipitate	]	(+)
	Flavonoid				
	Serbuk Mg 0,1 g + HCl P 2 tetes	Yellow	Red, yellow or orange		(+)
	Steroid/Triterpenoid				
	Kloroform 1 ml + asetat anhidrat +H_2SO_4	Green (Steroid)	Blue-Green (Steroid), Red-Purple (Triterpenoids)		(+)
	Saponin				
	Air panas + HCl 2 N	Formed Foam	Formed Foam	]	(+)
	Tanin				
	$FeCl_3$	Dark chocolate	Blackish green (Tannin pyrogallol), Green (Tannin catechol)		(+)
	Glikosida				
	Asetat anhidrat + H_2SO_4 P	Green	Blue-Green		(+)

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The stock solution is made at a concentration of 1000 ppm using 2% DMSO 1-3 drops (50-150 µL) to help solubility of the extract that is insoluble in seawater. Testing the toxicity of African leaf extract was done with 5 concentrations of 10, 50, 90, 130, and 170 ppm for n-hexane extract, 10, 40, 70, 100 and 130 ppm concentrations for ethyl acetate extract and 10, 30, 50, 70, and 90 ppm concentrations for 70% ethanol extract. This is meant to see variations in the given response. The yeast solution is used as a source of food for Artemia salina [15]. The feeding was done at the time of the test. (larva usia 48 jam). Each treatment is repeated three times (triple) in order to obtain accurate or accurate data.

In this study used negative control of sea water and larvae Artemia salina Leach without the addition of raw solution/extracts. It's done to find out if the test animal's death response really comes from the sample and not due to the treatment's technical factors.

Table 6. Data on Death Results of Shrimp Larvae from African Leaf n-Hexane Extract After 24 hours

Observation	**Replication**	**Control**	**African leaf n-hexane extract**				
			10 ppm	**50 Ppm**	**90 ppm**	**130 ppm**	**170 ppm**
l number of larvae	I	10	10	10	10	10	10
	II	10	10	10	10	10	10
	III	10	10	10	10	10	10
	Total	**30**	**30**	**30**	**30**	**30**	**30**
Total mortality	I	0	0	2	7	7	6
	II	0	1	3	4	6	8
	III	0	1	2	4	6	7
	Total	**0**	**2**	**7**	**15**	**19**	**21**
% Mortality		**0%**	**6,7%**	**23,3%**	**50%**	**63,3%**	**70%**

The death data of the shrimp larva extract n-hexane leaf africa after 24 hours can be seen in the table above, see the number of larvae dying at a concentration of 10 ppm, for replication 1-3 the total of the dead larvaes is 2 and the percentage of mortality is 6.7%. At the 50 ppm concentration, for the replication-1-3 the total dead larvas is 7 and the percent mortality of 23.3%. At a 90 ppm concentrated, for Replication-1-3, the total dying larvas are 15 and the mortality percentages are 50%.

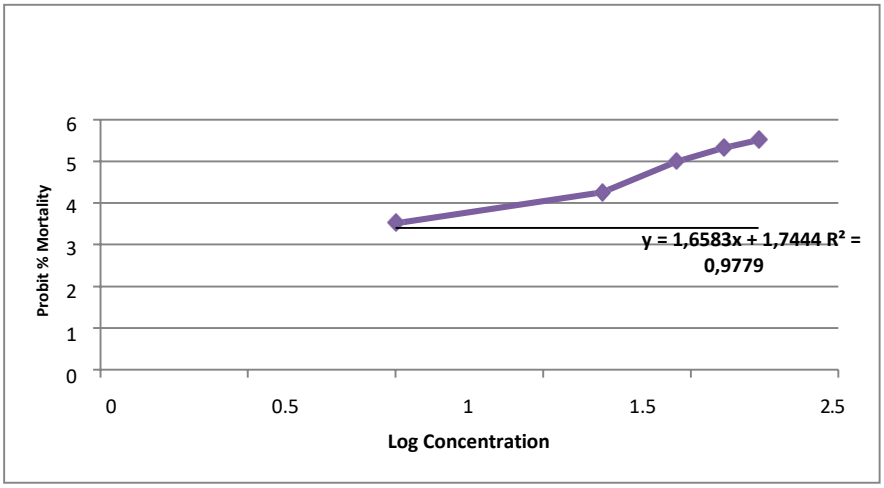

Figure 1. Regression Equation Curve of African Leaf n-Hexane Extract

Table 7. Data on the death results of shrimp larvae from African leaf extract ethyl acetate after 24 hours

Observation	**Replication**	**Control**	**African leaf n-hexane extract**				
			10 ppm	**40 Ppm**	**70 ppm**	**100 ppm**	**130 ppm**
l nu[16]mber of larvae	I	10	10	10	10	10	10
	II	10	10	10	10	10	10
	III	10	10	10	10	10	10
	Total	**30**	**30**	**30**	**30**	**30**	**30**
Total mortality	I	0	1	3	6	7	7
	II	0	2	3	4	7	8
	III	0	1	5	7	7	8
	Total	**0**	**4**	**11**	**17**	**21**	**23**
% Mortality		**0%**	**13,3%**	**36,7%**	**56,7%**	**70%**	**76,7%**

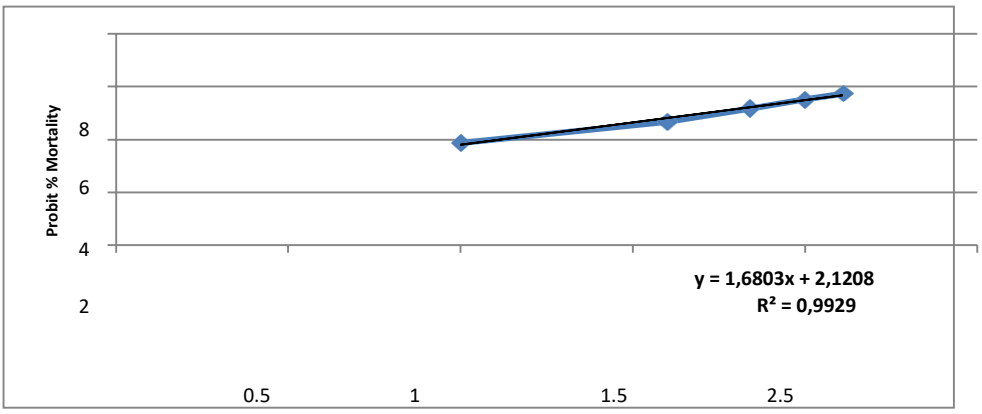

Figure 2. Regression Equation Curve of African Leaf Extract Ethyl Acetate

The death data of the larvae of shrimp extract ethyl acetate leaf africa after 24 hours can be seen in the table above, see the number of larvaes dying at a concentration of 10 ppm, for replication 1-3 the total of the dead larva is 4 and the percentage of mortality is 13.3%. At a 40 ppm concentration, for the replication-1-3 the total deceased larva was 11 and the percent of the mortality was 36.7%. At the 70 ppm concentration, for replication-13- the total dead larvas were 17 and their percentages of mortality were 56.7%. At a concentration of 100 ppm, for replication 1-3 the total dead larvae is 21 and the mortality percentage is 70%. At a concentration of 130 ppm, for 1-3 replications the total dead larvae was 23 and the mortality percentage was 76.7%.

Table 8. Data on Death Results of Shrimp Larvae from 70% Ethanol Extract of African Leaves After 24 hours

Observation	**Replication**	**Control**	**African leaf n-hexane extract**				
			10 ppm	**30 ppm**	**50 ppm**	**70 ppm**	**90 ppm**
l number of larvae	I	10	10	10	10	10	10
	II	10	10	10	10	10	10
	III	10	10	10	10	10	10

	Total	**30**	**30**	**30**	**30**	**30**	**30**
Total mortality	I	0	3	3	5	6	9
	II	0	1	5	6	7	7
	III	0	3	4	6	8	9
	Total	**0**	**7**	**12**	**17**	**21**	**25**
% Mortality		**0%**	**23,3%**	**40%**	**56,7%**	**70%**	**83,3%**

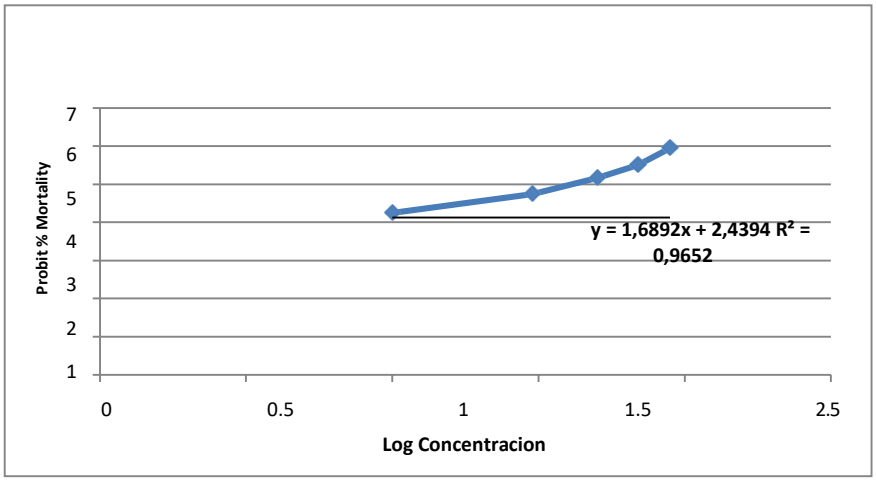

Figure 3. Regression Equation Curve of 70% Ethanol Extract of African Leaves

The death data of 70% of African leaves after 24 hours can be seen above, the number of larvae dying at a concentration of 10 ppm, for replication 1-3 the total number of dying larvaes is 7 and the mortality percentage is 23.3%. At a 30 ppm concentration, for the replication-1-3 the total death rate is 12 and the death rate percentages are 40%. At the 50 ppm concentration, for Replication-13- the total mortality rate is 17 and the Mortality Percentage of 56.7%. At concentration 70 ppm the total Mortality rate for Replication-1-3 is 21 and the Death Percent for 90 ppm. At the concentration 1 - 3, the total Death rate is 25 and mortality is 83.3%. Whereas for seawater control there are no dying Larvae of Artemia salina.

Seeing from the death gain of the Artemia salina larva, the extract with the highest mortality of the three extracts tested is a 70% ethanol extract at a concentration of 90 ppm.The price calculation data of LC_{50} with probit analysis can be seen in appendices 6, 7 and 8. Based on the results of probit analyses obtained price LC_{50} for n-hexane extract, ethyl acetate and 70% ethanol in succession - 82.62 ppm, 51.56 ppm and 31.29 ppm. Based upon the results obtaining it can be concluded that the lower the LC50 value, the greater the level of toxicity so that the ethanole extract 70% is the extract with the highest LC_{50} rating. It suggests that the group of secondary metabolite compounds in ethanol extract is 70% more than in ethyl acetate extract and n-hexane. n-hexane extract, ethyl acetate extract and 70% ethanol extract belong to the category of strong toxicity as they are in the 0-100 ppm range [17].

Glycosides have a mechanism as anti-cancer because flavonoids are an antioxidant through the mechanism of activation of the apoptosis pathway of cancer cells. The mechanism of cell apoptosis in this theory is the fragmentation of DNA. This fragmentation begins with the removal of the DNA's proximal chain by reactive oxygen compounds such as hydroxyl radicals. Flavonoids inhibit tumor/cancer proliferation, one of which is by inhibiting protein kinase activity, thus blocking the signal transduction pathway from the membrane to the nucleus cell, by inhibing the activity of the tyrosine kinase receptor. Flavonoids also work to reduce tumor resistance to chemotherapy agents[18]. Saponins and glycosides form complex compounds with cell membranes through hydrogen bonds that destroy the permeability properties of cell walls, cause release of cell content and cause cell death. Saponines can also decrease the activity of digestive enzymes and food absorption [19].

The mechanism of larva death is also related to the function of steroid and tannin compounds that can inhibit larva nutrition. (antifeedant). The way these compounds work is by acting as stomach poisoning or stomach venom. Therefore, when these compounds enter the body of the larva and its digestive system will be disrupted. In addition, the compound inhibits the sensory receptors in the larva mouth area. This results in larvae failing to obtain flavor stimuli, so they can't recognize their food so the larvaes die of starvation [20].

IV. CONCLUSIONS

Based on the observation and analysis of the data, the conclusion can be drawn is:

1. The identification of chemical compounds in n-hexane extract indicates the presence of flavonoid and glycoside compound, in ethyl acetate extract shows the existence of flawonoid, saponin, and glycoside, and in ethanol extract 70% indicates flavanoid, steroid, saponine, tannin and glycoside.
2. Three extracts (n-heksane, ethylacetate, and ethanol 70%) have toxic effects on the larvae of Artemia salina Leach. 70% ethanole extract is the highest LC50 extract with a LC_{50} value of 31.80 ppm, ethyl acetat extract of LC50 with a value of 51.70 ppm and n-hexan extract by LC50 values of 91.88 ppm. It suggests that the three extracts include a strong toxic ketegori with a range of 0-100 ppm.

ACKNOWLEDGMENT

We sincerely thank the Almarisah Madani University laboratory, Makassar, South Sulawesi for providing us with all the reagents to carry out this research.

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