Toxicity Test Of Ethanol Extract Of Gagatan Harimau Leaves (*Vitis Gracilis* BL.) On Artemia Salina Leach Larvae Using Brine Shrimp Lethal Test (BSLT) Method

Chemayanti Surbakti[1]*, Lisda Rimayani Nasution[2], Singgar Ni Rudang[3], Henni Cintya[4], I. Vany[5], P.A.T. Agnes[6], S.E.S. Elsa[7]

[1] Department of Pharmaceutical Biology, Faculty of Pharmacy
[2,4] Department of Pharmaceutical Chemistry, Faculty of Pharmacy
[3]Department of Pharmacology, Faculty of Pharmacy
[5,6,7] Pharmacy Faculty Students, Universitas Sumatera Utara, Medan, North Sumatra 20155, Indonesia.
*Corresponding Author:
Email: chemayantisurbakti@usu.ac.id

Abstract.

Gagatan harimau leaves (Vitis gracilis BL) is a plant contains several secondary metabolite compounds, namely alkaloids, glycosides, tannins, saponins and flavonoids and has been used traditionally to treat diarrhea, infectious diseases, inflammation and diabetes. The aim of this study was to to determine data on the percentage of deaths and the LC50 value of the ethanol extract of the Gagatan harimau (Vitis gracilis BL) against Artemia Salina Leach using the Brine Shrimp Lethality Test (BSLT) method. The method used is the Brine Shrimp Lethality Test (BSLT) with concentrations of 180 ppm; 200 ppm; 220ppm; 240ppm; 260ppm; 280ppm; 300 ppm and 0 ppm as negative controls each replicated three times. At each concentration, 10 Artemia salina Leach larvae were used as test animals, each concentration was 48 hours old. The toxic effect of the extract was determined by the percentage mortality of Artemia salina Leach larvae using probit analysis (LC50). The results of this research gave the linear regression equation Y = 11.674x-68.227. Therefore, the LC50 value is 6.275 and the LC50 gets a value is 187.068 ppm. Research on the toxicity of Gagatan harimau (Vitis gracilis BL.) ethanol extract using the BSLT (Brine Shrimp Lethality Test) method in this study is toxic because the LC50 is < 1000 ppm so it has the potential to be anticancer.

***Keywords**: Vitis gracilis BL, Toxicity Test and BSLT (Brine Shrimp Lethality Test).*

I. INTRODUCTION

Indonesia has approximately 30,000 plant species, of which 940 are used as medicinal plants. The use of medicinal plants for traditional healing has become an increasingly popular choice of treatment, largely due to the growing trend for a return to nature, and also because it is relatively safe and affordable. Even in the face of current developments, traditional medicine has gained attention as an alternative healthcare option. Various studies have recognised the presence of traditional remedies in society, thus promoting the health benefits of plants and fostering an environment conducive to the development of traditional medicines [1].Traditional medicine is composed of materials or concoctions that consist of plant materials, animal substances, mineral substances, galenic preparations, or mixtures of these components, which have been used for generations for medicinal purposes. Currently, an increasing number of people are resorting to natural substances as remedies, thus necessitating further research regarding the safety evaluation of traditional medicines [2].The utilization of traditional medicine for promotive, preventive, curative, and rehabilitative healthcare efforts is on the rise. This trend can be attributed to the "back to nature" movement and the public's belief in the advantages of traditional medicine over modern medicine. Some of these advantages include. relatively minimal side effects when used correctly and appropriately, the presence of complementary and/or synergistic effects within traditional medicine formulations/bioactive components of medicinal plants, where a single plant can exhibit multiple pharmacological effects, and traditional medicine being better suited for metabolic and degenerative diseases.

Prior to being documented in ancient texts, this knowledge was passed down through generations via oral traditions [2].Gagatan Harimau leaves (*Vitis gracilis* BL.) is a climbing shrub, up to 10 m long, round leaves with serrated edges and sharp ends. Flowers are arranged in panicles. Fruit round or slightly oval measuring ± 2 cm, smooth skin, various colors, sweet and sour flesh containing 2-4 seeds. The culture of the Karo people who live in the area around the Mount Sibuatan area usually uses it to treat several types of diseases [3]. Traditionally Gagatan Harimau leaves (*Vitis gracilis* BL.) is a traditional medicinal plant of the Karo people which is efficacious as a medicine for diarrhea, strong medicine, treating wounds, infectious

diseases, diabetes. Gagatan Harimau leaves (*Vitis gracilis* BL.) have been studied to have antibacterial activity on several test bacteria and contain flavonoids [4]. Ethanol extract from gagatan harimau leaves contains several secondary metabolite compounds, namely alkaloids, glycosides, tannins, saponins and flavonoids [5].

Several studies state that species have several properties, including reducing cell inflammation [5], antibacterial pathogens [4] and hepatoprotective [6]. Traditionally, gagatan harimau leaves are also used to treat diarrhea, wounds, diabetes, and as a tonic [3]. The Brine Shrimp Lethality Test (BSLT) is a method to determine the bioactivity of a compound from natural ingredients. Larvae Artemiasalina L. is widely used for environmental studies, toxicity, and screening of bioactive compounds from plant extracts. The BSLT test has a spectrum of pharmacological activity that is easy to perform, simple, fast, and does not require large costs with a 95% confidence level. The toxicity of compounds is expressed by the LC50 value. The LC50 value is an indication of the concentration of compounds that cause shrimp larvae death to 50% of the population. A sample is said to be toxic if it has a value of LC50 <1000 µg/ml. The BSLT test can be used as a sedative, toxicity, insecticide test, and as a preliminary test for cytotoxic or anti-cancer compounds [7].Research using the gagatan harimau (*Vitis gracilis* BL.) aims to initially screen active compounds using the Brine Shrimp Lethality Test method which was carried out by Meyer et al in 1982 to determine compounds that are toxic to Artemia salina Leach. The Brine Shrimp Lethality Test (BSLT) method is often used for pre-screening of active compounds contained in plant extracts because it is cheap, fast, easy and reliable.

II. METHODS

The material used for this research is Gagatan Harimau leaves extract (*Vitis gracilis* BL). A solvent used were distilled water, 96% ethanol, DMSO, artificial sea water, Larvae Artemia salina L.

Simplicia of the Gagatan harimau Leaves

Sample was collected Sukaribu, Telagah Village, Sie Bingei District, Langkat Regency, North Sumatra. The sample used in this study was young and fresh leaves of Gagatan Harimau. The leaves are washed thoroughly to remove soil and amount of impurities, then drained and weighed. The wet weight is obtained. In addition, the leaves are dried in a drying cabinet at a temperature of 40^0 – 50^0C until the leaves are dry (indicated when crushed brittle). The dried simplicia was weighed and then blended into powder.

Formation of the Ethanol Extract of Gagatan Harimau Leaves

500 g of Gagatan Harimau leaf simplicia powder were weighed, added to a glass container with 75 parts (96%) ethanol, covered, and allowed to steep for five days while being kept out of light. The remaining material was then squeezed out. Dregs washed with solvent as much as 25 parts to obtain 100 parts. Then macerate is accommodated in a vessel that is shut, left in a cool and protected 2 days of darkness followed by filtering. A maserate is separated from the solvent using a rotary evaporator with a temperature of ±50°C until the solvent gradually evaporates. Then the concentration activity was carried out use a stove at 50°C heat until a concentrated extract is obtained [8].

Making Artificial Sea Water

Artificial Sea Water is prepared by adding 15 grams of NaCl to 1 liter of distilled water. Then the pH of the seawater was first measured using a pH meter, the pH was 8-9 [9].

Shrimp Larvae Hatching

Hatching of shrimp larvae is carried out in plastic containers. Previously, the plastic container was divided into light and dark parts, then given a barrier in the form of styrofoam with a hole in the bottom edge so that the eggs that hatch could come out of the hole. The container is then filled with sea water until the two holes in the styrofoam are submerged. In a dark room, fill it with 1 tablespoon of egg, then cover it using black duct tape and aluminum foil. In a bright room, it is illuminated using fluorescent lights to stimulate hatching. Then, in a bright room, an aerator is installed to provide oxygen to the eggs which hatch into larvae and move to a bright room. After the eggs hatch into larvae which are 24 hours old, they are then transferred to another container until they are 48 hours old. Larvae that are 48 hours old can be used as test animals in BSLT method experiments [10].

Preparation of Test Sample Solutions

The main solution is prepared by weighing 2 g of extract, dissolving it in 2 ml of DMSO and adding distilled water to make up to 100 ml to obtain main solution concentration of 2000 ppm [11]. After obtaining a stock solution of 2000 ppm, dilution is carried out to obtain a test solution with a concentration of 180 ppm, 200 ppm, 220 ppm, 240 ppm, 260 ppm, 280 ppm, 300 ppm.

Toxicity Test using the BSLT Method

Test solutions with concentrations of 180 ppm, 200 ppm, 220 ppm, 240 ppm, 260 ppm, 280 ppm, 300 ppm. 10 ml of each pipette was put into a test tube and 10 Artemia salina Leach shrimp larvae that were 48 hours old were added. Each concentration was repeated 3 times and compared with the negative control. The number of larvae that died after 24 hours was counted in each test tube. Calculations are carried out using a loupe, digital colony counter or under lamp light. Dead larvae were identified by the absence of movement during observation [12].

III. RESULT AND DISCUSSION

Toxicity Test using the BSLT Method

The results of observations of the death of artemia salina leach larvae after 24 hours in the ethanol extract of gagatan harimau can be seen in the table below.

Treatment	**Mortality Rate of Artemia salina Leach Shrimp Larvae from 10 Larvae**							**Negative Control**
	Concentration(ppm)							
	180 ppm	200 ppm	220 ppm	240 ppm	260 ppm	280 ppm	300 ppm	**0%**
I	6	7	8	8	9	10	10	0
II	5	6	7	8	9	9	10	0
III	5	6	8	9	8	8	10	0
Total	16	18	23	25	26	28	30	0
Average Mortality	0,533	0,6	0,766	0,833	0,866	0,933	1	0
Death Percentage %	53,33	60	76,66	83,33	86,66	93,33	100	

Table 1. Effect of Various Concentrations of Ethanol Extract of Gagatan Harimau (*Vitis gracilis* BL.) Leaves on Artemia salina Leach Larvae.

Table 1. shows the percentage of death of artemia salina larvae with 3 repetitions. At concentrations of 180, 200, 220, 240, 260, 280, 300 and 0 ppm as negative controls, total larval deaths were 16, 20, 23, 25, 26, 29, 30, 0 and average deaths were 0.53, 0.66, 0.76, 0.83, 0.86, 0.96, 1, 0 with a death percentage of 53.33, 66.66, 76.66, 83.33, 86.66, 96.66, 100, 0.The level of influence of 96% ethanol extract concentration of gagatan harimau leaf (*Vitis gracilis* BL.) ethanol extract on the death of artemia salina Leach larvae can be seen in fig. 1. The highest percentage level of larval death was found at a concentration of 300 ppm. Meanwhile, the lowest percentage level was found at a concentration of 180 ppm. This shows that the higher the extract concentration level, the higher the total larval mortality.

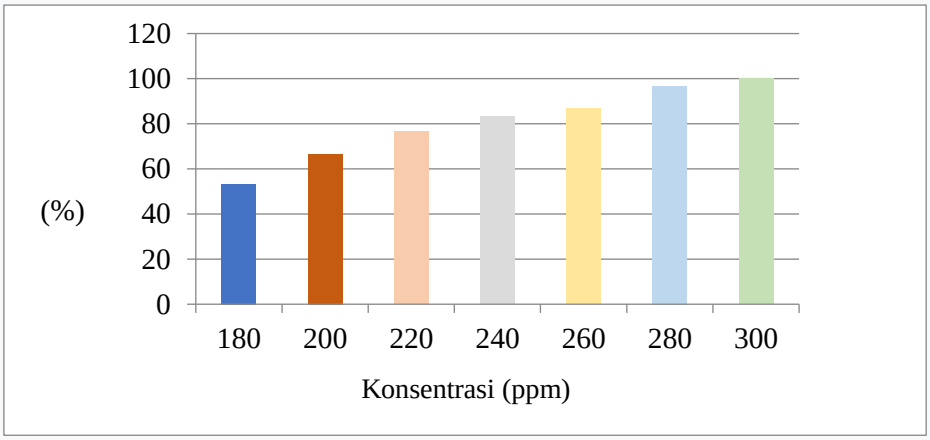

Fig 1. Graph of the Effect of Ethanol Extract Concentration of Gagatan Harimau (*Vitis gracilis* BL.) Leaves on Artemia salina Leach Larvae

To determine the truth value, the LC50 value is calculated using Microsoft Office Excel by creating a straight line percentage Y = ax+b in Table 2.

Concentration (ppm)	ppm	Log (ppm)	Probit	% death	Mortality	Total
180	1800000	6,255	5,08	53%	16	30
200	2000000	6,301	5,25	60%	18	30
220	2200000	6,342	5,74	77%	23	30
240	2400000	6,380	5,95	83%	25	30
260	2600000	6,415	6,13	87%	26	30
280	2800000	6,447	6,48	93%	28	30
300	3000000	6,477	8,09	100%	30	30

Table 2. Calculation of LC50 Value of Gagatan Harimau Extract (*Vitis gracilis* BL.) Using Probit Analysis

Graph in Figure. 2. shows of the relationship between the percentage of death of Artemia salina Leach larvae. with log concentration of ethanol extract of tiger gagatan leaves (Vitis gracilis BL). The linear regression equation from the graph above is Y = 11.674x - 68.227 so the X value is 6.275, the LC50 value is antiloged and gets a value of 187.068 ppm. This means that the death of test animals reached 50% when the concentration of the compound extract reached 187,068 ppm.

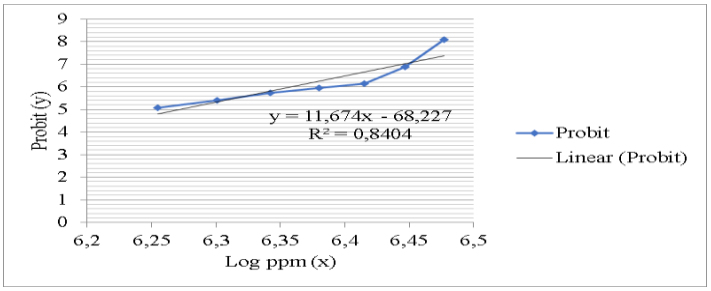

Fig 2. Linear Regression Graph of Gagatan Extract Concentration Gagatan harimau (*Vitis gracilis* BL.)

In this study, it was found that the ethanol extract of gagatan harimau leaves (*Vitis gracilis* BL.) has high effectiveness and is therefore toxic. This is related to the compounds contained in gagatan harimau leaves, namely saponins and flavonoids, which at certain levels have toxic potential and can cause the death of Artemia salina Leach shrimp larvae. The mechanism of larval death is thought to be related to the function of saponin and flavonoid compounds in gagatan harimau which can inhibit the larvae's ability to eat (antifeedant).The phase used in this research is the nauplius phase because in this phase Artemia salina Leach is in a very active phase dividing mitotically which is identical to cancer cells which also divide mitotically.

This causes the BSLT test to be often carried out as a preliminary test of anticancer activity [12]. The toxicity test using the BSLT method using A. salina is a preliminary test to determine the bioactivity of a sample. This test is useful for determining various biological activities in plants such as cytotoxic, phototoxic, pesticide, enzyme inhibition and ion regulation activities [13].Based on the toxicity test of the ethanol extract of gagatan harimau leaves (*Vitis gracilis* BL.) using the BSLT (Brine Shrimp Lethality Test) method in this study, it is toxic because the LC50 is <1000 ppm so it has the potential to be anticancer [10]. Meyer (1982) and Anderson (1991), reported that an extract showed toxicity activity in BSLT if the extract could cause the death of 50% of test animals at a concentration of less than 1000 ppm. Based on the statement above, the ethanol extract of gagatan harimau leaves (*Vitis gracilis* BL.) is toxic. This is shown by the data obtained for the ethanol extract reaching LC50 at a concentration of 187,068 ppm.

IV. CONCLUSION

Extracts from the gagatan harimau (*Vitis gracilis* BL.) have potential toxicity against Artemia Salina Leach using the Brine Shrimp Lethality Test (BSLT) method. LC50 value of ethanol extract of gagatan harimau (*Vitis gracilis* BL.) determined by probit analysis was 187.086 ppm. With a concentration of 300 ppm % mortality is 100%.

V. ACKNOWLEDGMENTS

The authors are grateful to the talent program of the Institute of Community Service/Services to the Universitas Sumatera Utara for providing research grants for lecturers of basic research programs so that the authors can carry out research well.

REFERENCES

[1] A. R. E. Hendrawati, Acute Toxicity Test of Ethanol Extract of Basil Leaves (Ocimum sanctum Linn.) Against Artemia salina Leach Larvae Using the Brine Shrimp Lethality Test (BST) Method, Medical faculty, 2009.

[2] Harahap, Arman ,2018, Macrozoobenthos diversity as bioindicator of water quality in the Bilah river, Rantauprapat, Medan. ***J. Phys.:*** Conf. Ser. 1116 052026.

[3] L. Sari, Utilization of traditional medicines with consideration of their benefits and safety, Magazine. Pharmaceutical Sciences, vol. 3, no. 1, pp. 1–7, 2006.

[4] E. E. Silaban, Y. Afifuddin, and R. Batubara, Exploration of Medicinal Plants in the Mount Sibuatan Area, Brand District, Karo Regency, North Sumatra, ***Peronema For. Sci. J***., vol. 4, no. 2, pp. 78–91.

[5] A. Harahap, P. Hrp, N.K.A.R. Dewi, Macrozoobenthos diversity as anbioindicator of the water quality in the River Kualuh Labuhanbatu Utara, ***International Journal of Scientific & Technology Research,*** 9(4), 2020, pp. 179-183.

[6] T. Juwitaningsih, I. S. Jahro, S. A. Sari, and Y. Rukayadi, "Antibacterial activity of various medicinal plants in North Sumatra against common human pathogens, ***Res. J. Chem. Environ.***, vol. 24, no. 1, pp. 99–105, 2020.

[7] N. Z. Wasnis, S. Ilyas, S. Hutahaean, R. Silaban, and P. C. Situmorang, Antibacterial activity of various medicinal plants in North Sumatra against common human pathogens, ***Res. J. Chem. Environ***., vol. 24, no. 1, pp. 99–105, 2020.

[8] Harahap, et, all, Macrozoobenthos diversity as anbioindicator of the water quality in the Sungai Kualuh Labuhanbatu Utara, AACL Bioflux, 2022, Vol 15, Issue 6.

[9] C. Gao, C. Tian, R. Zhou, R. Zhang, and Y. Lu, Phenolic composition, DNA damage protective activity and hepatoprotective effect of free phenolic extract from Sphallerocarpus gracilis seeds, Int. Immunopharmacol., vol. 20, no. 1, pp. 238–247, 2014.

[10] Harahap,Arman.2020.Species Composition & Ecology Index Of The Family Gobiidae At The Mangrove Belawan Of Sicanang Island ***International Journal of Scientific & Technology Research*** Volume 9, Issue 04, April 2020.

[11] M. Simorangkir, B. Nainggolan, T. Juwitaningsih, and S. Silaban, The Toxicity of n-Hexane, Ethyl Acetate and Ethanol Extracts of SarangBanua (Clerodendrum fragrans Vent Willd) Leaves by Brine Shrimp Lethality Test (BSLT) Method, in ***Journal of Physics: Conference Series***, IOP Publishing, 2021, p. 12053.

[12] P. O. M. Ditjen, Indonesian Pharmacopoeia, Ed. III. Jakarta. Ministry of Health of the Republic of Indonesia, vol. 9, 1979.

[13] Harahap, A., et all (2021), Monitoring Of Macroinvertebrates Along Streams Of Bilah River ***International Journal of Conservation Sciencethis link is disabled***, 12(1), pp. 247–258.

[14] A. Sepadan, Acute toxicity test of 96% ethanol extract of avocado seeds (Persea americana mill.) against Artemia salina leach larvae using the Brine Shrimp Lethality Test (BSLT) method, 2014.

[15] N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. J. Nichols, and J. L. McLaughlin, Brine shrimp: a convenient general bioassay for active plant constituents, Planta Med., vol. 45, no. 05, pp. 31–34, 1982.

[16] S. A. R. Wulandari, Formulation and test of antibacterial activity of Stapylococcus epidermidis microemulsion preparation of cherry leaf extract (Muntingia calabura Linn.) with isopropyl mirystate oil phase, Universitas Islam Negeri Maulana Malik Ibrahim, 2017.

[17] H. Kurniawan, U. P. Nera, and F. Inarah, Acute Toxicity Test of Methanol Extract of Kesum Leaves (Polygonum Minus Huds) Against Artemia salina Leach Larvae Using the Brine Shrimp Lethality Test (BSLT) Method, Thesis, Tanjung Pura University, Pontianak, 2012.

[18] T. Veni and T. Pushpanathan, Comparison of the Artemia salina and Artemia fransiscana bioassays for toxicity of Indian medicinal plants, ***J. Coast. Life Med.***, vol. 2, no. 6, pp. 453–457, 2014.

[19] Mamangkey, J., Suryanto, D., et all (2021). Isolation and enzyme bioprospection of bacteria associated to Bruguiera cylindrica, a mangrove plant of North Sumatra, Indonesia, Biotechnology Reports, 2021, 30, e00617.