Toxicity Test of Karamunitng Leaf (*Rhodomyrtus tomentosa* (Aiton) Hassk.) Ekstratc with Finder Liquid Variation Using the Brine Shrimp Lethality Test (BSLT) Method

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Abstract. Karamunting leaves (*Rhodomyrtus tomentosa* (Aiton) Hassk.) is a plant that is widely found in areas in Indonesia which has potential as an antioxidant so that it is the basis for the purpose of this study to see the toxicity effect of karamunting leaf extract. Extraction was carried out by maceration method using 3 types of solvents with different levels of polarity, namely ethanol (polar), ethyl acetate (semipolar) and n-hexane (nonpolar) against shrimp larvae (Artemia salina L.) using the BSLT method. Toxicity test using the BSLT method with Artemia salina L. bioindicator. The results obtained were analyzed by analysis to determine the value of Lethal Conceration 50 (LC_{50}). 10; 15; 20; and 25 μg / mL. Based on the results of observations and data analysis, it was found that the ethanol extract of karamunting leaves had a moderate toxic effect with the highest LC_{50} value, namely 70% ethanol extract of 11.55 μg / mL, ethyl acetate extract of 12.44 μg / mL and n-hexane extract. Karamounting to 15.77 μg / mL. Based on the results of this study indicate that the three extracts are included in the strong toxicity category with a range of 0–100 ppm.

Keywords: Rhodomyrtus Tomentosa · Extract · Toxicity · BSLT · Anti cancer

1 Introduction

Cancer is a disease with no known exact cause, but is influenced by many factors such as smoking or exposure to secondhand smoke, alcohol consumption, exposure to ultraviolet (UV) light on the skin, obesity and unhealthy diet, lack of physical activity, and related infections. With cancer. Cancer can be prevented by reducing the risk factors for the occurrence of cancer. In developments in the health sector, anti-cancer drugs have been found and chemotherapy has been carried out, but the high cost factor is an obstacle. This encourages people to take treatment using natural ingredients or traditional medicine (Arter et al. 2013).

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One of the plants that has been used by the community as a traditional medicine is karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk.) leaves *R. tomensa* contain chemical compounds including flavonoids, phenols, terpenoids, tannins, saponins and steroids (Lilingan 2020). A number of medicinal plants containing flavonoid compounds have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anti-cancer activities (Ahmad et al. 2015). The role of antioxidants is very important in reducing the effects of free radicals which are closely related to the occurrence of degenerative diseases based on biochemical processes in the body (Juniarti 2009). Flavonoids act as antioxidants by donating hydrogen atoms or through their ability to chelate metals, in the form of glucosides (containing glucose side chains) or in a free form called aglycone (Redha 2010).

The extraction process can use 3 types of solvents with different levels of polarity, namely n-hexane (nonpolar), ethyl acetate (semipolar) and ethanol/methanol (polar). Differences in solvents in the extraction can affect the total content of bioactive compounds (Santoso et al. 2012). Therefore, a toxicity test will be carried out to see the safety of the extract.

Cytotoxicity test with 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 activity, phototoxicity, pesticides, enzyme inhibition, and ion regulation (Veni & Pushpanathan 2014).

Research conducted by Liligan et al (2020) showed the results of the antioxidant activity test of 70% ethanol extract with an IC value of 15.333 g/mL, ethyl acetate obtained 14.70276 g/mL and n-hexane 24.49371 g/mL. Where the three liquid filters are included in the category of very strong antioxidant activity with a range of <50%. Free radicals in the body are formed naturally during metabolic processes. The formation of free radical compounds in the body that is uncontrolled or exceeds the limit will cause damage to cell oxidation which leads to the risk of developing diseases such as arteriosclerosis, heart failure, cancer and Alzheimer's (Landete 2013). Based on this description, a study of the toxicity test of karamunting leaf extract was carried out with variations of the solvent using the BSLT method on shrimp larvae.

2 Method

2.1 Tools and Materials

The tools used are (*Pyrex*®), beaker (*Pyrex*®), flask (*Pyrex*®), micro pipette (*Dragonlab micropipette*®), analytical balance (*Mittler toleto*®), rod stirrer, drip pipette, aluminum foil, aquarium, desiccator, black flannel, 5 watt lamp, rotary evaporator and vial.

Ingredients

The materials used include: aquadest, sea water, karamunting leaf extract (Rhodomyrtus tomentosa), 10% dimethyl sulfoxide (DMSO), Yeast, *Artemia salina Leach* larvae.

***Artemia salina Leach* Larvae Toxicity Test**

a. **Hatching**

The selection and maintenance of the test animals used were *Artemia salina Leach* shrimp larvae aged 48 h reared in a container containing seawater with a conditioned pH of 7–8, preparing 50 mg of shrimp larvae, soaked in a container containing 200 ml of seawater under light. The lamp is equipped with an aerator at a temperature of 25 c for 48 h.

b. **Implementing the Toxicity Test**

Ten larvae were put into each test vial that had been given seawater, then each concentration of the sample stock solution was added and 1 drop of yeast suspension was added as a food source, then the volume of the solution in the vial was filled with seawater as much as up to 10 ml. For control, 10 ml of sea water was added, 10 *Artemia salina Leach* larvae were added and 1 drop of yeast was added. The test vials are then stored in a place where there is sufficient light. After 24 h, the number of dead larvae was observed. For each sample and control, the test was repeated 3 times.

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

C. Results

3 Discussion

This study aims to see and find out whether the difference in the level of toxicity of the karamunting leaf extract is based on the difference in the solvent and to determine which extract has the highest toxicity by using the LC_{50} while the sample used in this study is the karamunting leaf extract obtained from previous studies (Lilingan 2020). The filters used when extracting were n Hexane, ethyl acetate and 70% ethanol. The selection of the most suitable solvent for the extraction of secondary metabolites in simplicia is based on the level of polarity. In this case, n-hexane is non-polar, ethyl acetate is semi-polar and 70% ethanol is polar. In the study (Lilingan 2020) a thick extract was obtained to calculate the % yield. The results of the calculation of % yield can be seen in Table 1.

After obtaining the extract, then performed a toxicity test on *Artemia salina Leach* larvae, the larvae used in this study were 48 h old because at that age the larvae were the

Table 1. Results of Karamunting Leaf Extract Yield Sample

Weight	Simplified (g)	Extra weight (kg)	Yield (%)
Ethanol Extract	200	31,27	15,635
Extract Ethyl Acetate		42,095	21,047
Extract n-Hexan		7,409	3,704

most sensitive due to at the age of 48 h the organs in the larvae are shaped and sensitive, the use of *Artemia salina Leach.*

The use of *Artemia salina Leach* as a test animal is due to its very fast growth and also because of its good immune system (Pangabean, 1984). Administration of one drop of yeast suspension is to optimize the results obtained or the response to death is not caused by a lack of food. The use of aerators in this study is to get oxygen assistance and help circulate eggs in seawater, and placed under a light that is sufficient to stimulate egg hatching. If the pH of seawater is less than 8, the hatching efficiency decreases. In this study using lat water was intended to see whether the response to the death of the test animals really came from the sample and was not caused by the solvent, namely sea water needed for the development of *Artemia salina Leach*, therefore aeration must be given continuously until hatching occurs, as well as irradiation that can stimulate and reactivate the development of the embryo of *Artemia salina L.* Stimulation aims to stimulate the hatching process, and the temperaturegood for hatching is 25–30 °C.

The results of the research carried out on each karamunting leaf extract with concentrations of 5, 10, 15, 20 and 25 g/mL and sea water as a negative control were tested on *Artemia salina Leach* larvae with larval mortality parameters after 24 h of treatment as a toxicity response. . The number of deaths of shrimp larvae at each concentration and calculation of LC_{50} can be seen in the following Table 2, 3, and 4.

Data on mortality of shrimp larvae extract n-hexane of karamunting leaves for 24 h can be seen in the table above, it can be seen that the larvae that died at a concentration of 5 g/mL, for 1–3 replications, the total number of dead larvae was 4 with a mortality percentage of 13.3%, at a concentration of 10 g/mL. Larvae that died were 10 with a mortality percent of 33.3%, at a concentration of 15 g/mL the number of larvae that died was 14 with a mortality percent of 46.4%, at a concentration of 20 g/mL larvae that died was 17 with a mortality percentage of 56.6%, at a concentration of 25 g/mL the number of larvae that died was 21 with a mortality percent of 70%.

Table 2. Mortality of shrimp larvae from Karamunting Leaf N-Hexane Extract for 24 h

Observation	Replication	Control	Karamunting leaf n-hexane extract				
			5 g/mL	10 g/mL	15 g/mL	20 g/mL	25 g/mL
larvae of early	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	8	7	8
	II	0	2	5	2	5	5
	III	0	1	2	4	5	8
	Total	0	4	10	14	17	21
% Mortality		**0%**	**13.3%**	**33.3%**	**46.6%**	**56.6%**	**70%**

Table 3. Death Result Data of Shrimp Larvae Ethanol Extract 70% Karamunting Leaves for 24 h

Observation	Replication	Control	Ethanol Extract 70%				
			5 g/mL	10 g/mL	15 g/mL	20 g/mL	25 g/mL
Number of initial 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	_
Total mortality	I	0	4	5	6	8	9
	II	0	2	5	5	6	6
	III	0	2	3	7	6	8
	Total	0	8	13	18	20	23
% Mortality		0%	**26.6%**	**43.3%**	**60%**	**66.6%**	**76.6%**

Table 4. Data on Mortality of Shrimp Larvae Ethyl Acetate Extract of Karamunting Leaves for 24 h

Observation	Replication	Control	Ethyl Acetate Extract				
			5 g/mL	10 g/mL	15 g/mL	20 g/mL	25 g/mL
Number of larvae initial	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	2	6	8	9
	II	0	0	2	8	7	8
	III	0	2	6	6	8	8
	Total	**0**	**3**	**10**	**20**	**23**	**24**
% Mortility		**0%**	**10%**	**33.3%**	**66.6%**	**76.6%**	**80%**

Data on mortality of shrimp larvae with 70% ethanol extract of karamunting leaves for 24 h can be seen in the table above, larvae can be seen that died at a concentration of 5 g/mL, for replication 1-3 the total number of dead larvae was 8 with a mortality percentage of 26.6%, at a concentration of 10 g/mL the dead larvae were 13 with a mortality percent of 43.3%, at a concentration of 15 g/mL the number of dead larvae was 18 with 60% mortality percent, at a concentration of 20 g/mL the dead larvae were 20 with 66.6% mortality percent, at a concentration of 25 g/mL the number of dead larvae was 23 with percent mortality 76.6%.

Data on mortality of shrimp larvae from ethyl acetate extract of karamunting leaves for 24 h can be seen in the table above, it can be seen that the larvae that died at a concentration of 5 g/mL, for 1–3 replications, the total number of dead larvae was 3 with a mortality percentage of 10%, at a concentration of 10 g. /mL larvae that died were 10 with a percent mortality of 33.3%, at a concentration of 15 g/mL the number of larvae that died was 20 with a percent mortality of 66.6%, at a concentration of 20 g/mL the dead larvae were 23 with a percent mortality of 76,6%, at a concentration of 25 g/mL the number of larvae that died was 24 with a mortality percent of 80%.

Judging from the mortality of *Artemia Salina* extract with the highest % mortality among the three extracts tested was ethyl acetate extract with a concentration of 25 g/mL.

Data for calculating LC_{50} with probit analysis can be seen in appendices 5, 6 and 7. Based on the results of probit analysis, the LC_{50} values for n-hksan extract, ethyl acetate and 70% ethanol, respectively, are 15.77 g/mL, 12,44 g/mL and 11.55 g/mL. Based on the results obtained, it can be seen that the lower the LC_{50}, the greater the level of toxicity so that 70% ethanol extract is the extract with the lowest LC_{50} and has the strongest toxic effect. The mechanism of death of *Artemia salina L* shrimp larvae is related to the function of phenolic compounds, flavonoids and tannins contained in extracts that can inhibit the feeding power of larvae, while the way these compounds work is to act as stomach povoning or stomach poison. Therefore, these compounds enter the body of the larvae and the digestive tract of the larvae will be disturbed. In addition, this compound is able to inhibit taste receptors in the mouth of the larvae, this causes the larvae to fail to receive a taste stimulus so they are unable to recognize their food and than the larvae die.

4 Conclusion

Based on the research results obtained from each extract, the LC_{50} value of n-hexane extract was 15.77 g/mL, the ethyl extract 12.44 g/mL and the ethanol extract 11.55 g/mL had a very strong toxicant.

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