Toxity Test Of Primary And Secondary Metabolite Extracts On *Caulerpasp.* Which Is Cultivated By The Community Of The District Takalar

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Abstract.

Lawi-lawi (Caulerpa sp) has economic value for some coastal communities who cultivate it. Apart from having the opportunity to be exported abroad as a complement to processed seafood, it turns out that lawi-lawi can also be used as an antidote to cancer or malignant diseases. This research is about the level of toxicity of the seaweed extract fraction (Caulerpa sp) on Artemia salina Leach larvae, method while for screening anticancer activity used the Brine Shrimp Lethality Test (BSLT) method. In methanol extract Caulerpa sp based on the results of toxicity test measurements using the BSLT method, it is included in the toxic category. Primary extract In Caulerpa sp based on the results of toxicity test measurements using the BSLT method, it is included in the very toxic category.

Keywords: *Lawi-lawi, Caulerpa sp, , Metanol extract, Primary extract and toxicity.*

I. INTRODUCTION

Macroalgae, which consists of various groups and types, is one of the marine biota that functions as the main producer in marine waters. Apart from functioning ecologically in aquatic ecosystems, macroalgae with all their chemical contents have economic benefits in the food sector and health (Kalimantan, Sari dan Sofiana, 2023). The seaweed used in the research is *Caulerpa sp*. *Caulerpa sp*. consequences of the boiling process have not been reported. The boiling process can inactivate enzymes and reduce the number of microbes, but there are concerns that it could affect the fiber components contained in seaweed. This research aims to determine changes in dietary fiber (soluble and insoluble food fiber), crude fiber, and fiber components (cellulose, hemicellulose, lignin) due to the boiling process. Caulerpa has great potential as a source of fiber from food and non food aspects for making cosmetics. Apart from fiber, seaweed is also known to be very useful as a food and nonfood source, including as a source of salt (Nurjanah *et al.*, 2018).*Caulerpa sp* is one of various species of seaweed that grows naturally in Indonesian waters. Caulerpa is found growing on coral substrates or on sand and coral fragments. Caulerpa racemosa produces primary and secondary metabolites, one type of secondary metabolite which contains folic acid, thiamine, ascorbic acid and phenol (Mokoginta, Yudistira dan Mpila, 2021).

The use of non-commercial antibiotics to inhibit the growth of bacteria or other types of disease in the pharmaceutical sector is still limited, however. The potential for Indonesian seaweed (macroalgae) is very large to be developed as a raw material for medicine. In traditional medicine, sea algae has long been used for the treatment of various types of diseases. So now a lot of research has been carried out to explore the benefits of seaweed as a basic ingredient for medicines. Several antibacterial research results have been carried out using marine organisms, such as several algae originating from Indonesian waters were found to have active compounds that act as antimicrobials against pathogenic bacteria, one of which is from the genus. The nature of secondary metabolites as a means of self-defense for marine organisms turns out to have enormous potential as a source of medicinal ingredients to treat various diseases (Ikbal dan Zainuddin, 2015).Algal protein has biological activity so that it becomes a potential source of new natural medicinal ingredients as antibacterial, antifungal, and anticancer . The use of protein as a medicinal ingredient has the advantage that it is well accepted by the body and has few side effects .

Previous studies have shown that proteins from red algae (*Rhodophyceae*) have potential as anticancer drug agents, however, this study was still at the first stage as screening for anticancer activity used the Bhrine Shrimp Lethality Test (BSLT) method. In this study, the anticancer activity of bioactive proteins from red algae (*Rhodophyceae*) will be further tested in vivo against *HeLa* cancer cells(Sugrani, 2021).The toxicity test carried out using the BSLT (Brine Shrimp Lethality Test) method is aimed at determining the potential of a compound to be toxic by knowing its level of toxicity. BSLT is a method that is widely used for toxicity testing using *Artemia Salina* Leach larvae as test animals. Toxicity tests are carried out to determine the presence of toxic effects in the use of a compound. Test Toxicity test is a test to determine the ability of a poison to cause damage when it enters the body and organs that are susceptible to it. Toxicity testing is carried out using the BSLT method which is indicated to determine the potential for a poison to be toxic by determining its level of toxicity by determining toxicity value using Lethal Concentration (LC_{50}) (Sepvina, Ridwanto dan Rani, 2022).The research objectives are to determine the toxicity of primary and secondary metabolite extracts contained in the lawi-lawi plant (*Caulerpa sp*), and it is hoped that with this research we will obtain new potential anticancer drug agents that can be developed in the future.

II. METHODS

Tools and Materials

The tools used in this research were aerators, stirring rods, porcelain cups, chemical glasses, measuring cups, incandescent lamps, micropipettes and tips, cool boxes, rotary evaporators, horn spoons, a set of BSLT test tools, a set of cold centrifuge tools, 5 vials ml and 10 ml, maceration containers, aquariums, freezers. The ingredients used in this research are methanol extract *Caulerpa sp.* , protein crude extract, egg *A. Salina Leach*, yeast suspension, sea water

Research Procedure

1. Sampling

Lawi-lawi sampling (*Caulerpa sp*) was carried out in Laikang Village, Mangarabombang District, Takalar Regency, South Sulawesi Province. Samples are taken directly by seaweed farmers, then cleaned with clean sea water to remove dirt, sand, animals and attached organisms. Next, the sample is placed into polyethylene plastic and put in a cool box for identification. Then the samples are stored in the freezer.

2. Hatching of Shrimp Larvae

Shrimp eggs were hatched 2 days before testing. Hatching of *Artemia Salina* Leach larvae takes 48 hours. Hatching is done by immersing the eggs in artificial sea water (50 grams of non-iodized salt or fish salt in 800 ml of distilled water with a pH of 7-9 like pure sea water) in a 1 L plastic bottle container which has had the bottom side cut so that it is shaped like a cone as a substitute for an aquarium. Previously, plastic bottle containers had aerators installed, these aerators were useful for maintaining oxygen levels in the container. Plastic bottle containers eggs have been filled and an aerator has been installed, then placed in a room with sufficient light or illuminated with a 5 watt fluorescent lamp to warm the hatching temperature so that the hatching temperature is maintained at 25-30˚C and stimulates the hatching process. This light functions for the growth of *Artemia Salina* larvae. Leach. Within 2x24 hours the eggs will turn into *Artemia Salina* Leach larvae and will be used as test animals for toxicity testing (Zulfiah *et al.*, 2020).

3. BSLT Test

Toxicity test *Caulerpa sp*carried out using the method Brine Shrimp Lethality Test (BSLT). The BSLT method is a method for screening medicinal plants that have anticancer potential because it is cheaper, shorter, easier to develop and there are no ethical rules for using test materials. The mortality value was determined using probit analysis to determine the toxicity value Lethal Concentration (LC_{50}). Toxicity test Sample testing was carried out by inserting the sample into a vial, then the sample was homogenized using a vortex for 1 minute, then 10 48-hour-old *Artemia Salina* shrimp larvae were put into the vial. After 24 hours of treatment, *Artemia Salina* shrimp larvae were observed using a loop. Observation of larval death can be seen from the movement of the larvae for a few seconds and the dead larvae will settle at the bottom of the vial (Abriyani, 2022).

III. RESULT AND DISCUSSION

The Toxicity Test Method used is the Brine Shrimp Lethality Bioassay or BSLB/BSLT which aims to determine the concentration required for tea-tehan extract to kill half the initial population of test animals. The "Brine Shrimp Lethality Test" is a preliminary test of a compound which has the advantage of being which is obtained faster (24 hours), inexpensive, easier to carry out than other tests because it does not require special equipment and training/relatively few samples are used. Toxic effects can be known or measured from the death of larvae due to the influence of the test material (Jelita *et al.*, 2020).

In this study, larvae data were obtained *Artemia Salina* experienced death after administration of crude protein extract and crude methanol extract. This is caused by the high concentration of active compound extracts which diffuse into the larva's body *Artemia Salina*more disturbance to the body *Artemia Salina* Subsequently, they experienced death due to the presence of phenolic compounds, namely flavonoids, in the extract which could cause the death of *Artemia Salina* larvae. There are three mechanisms for the death of *Artemia Salina* larvae related to the function of phenolic compounds, namely first, inhibiting the feeding ability of *Artemia Salina* larvae, second, phenolic compounds act as stomach poisons. Third, phenolic compounds will inhibit the taste receptors in the mouths of *Artemia Salina* larvae, resulting in the larvae failing to receive taste stimuli and the larvae being unable to recognize their food so that the larvae will lack nutritional intake and will subsequently experience death due to starvation.

Test Sample	Incubation Time (Hour)	LC Value 50	Category
Protein Extract	24	1.8478, E-15	Very Toxic
Methanol Extract	24	86.303	Toxic

In methanol extract *Caulerpa sp* based on the results of toxicity test measurements using the BSLT method, it is included in the toxic category. According to Meyer et al (1982) in Jelita (2020), the level of toxicity of plant extracts can be determined by looking at the LC_{50} value. If the LC_{50} value is less than 1000 µg/ml it is said to be toxic, conversely if the LC_{50} value is greater than 1000 µg/ml it is said to be non-toxic. This level of toxicity will give meaning to its potential activity as an anticancer. The lower the LC_{50} value, the more toxic a compound is. Protein extract In *Caulerpa sp* based on the results of toxicity test measurements using the BSLT method, it is included in the very toxic category.Exploration of secondary metabolites as anticancer agents has been widely carried out and alkaloids show promising anticancer and chemopreventive effects (Ballout et al., 2019). Alkaloid compounds are thought to be toxic at certain concentrations and the toxic mechanism of alkaloids is that they act as stomach poisoning.

Alkaloids that enter the larva's body will disrupt the digestive system, interfere with the taste stimulus so that the larva cannot recognize its food and end up starving to death (Puspa et al., 2017). According to Jelita et al. (2020) that a foreign substance or compound in the environment is diffusely absorbed into the body and affects shrimp metabolism. Shrimp larvae will die if a foreign compound is toxic. Toxic extracts can enter through the mouths of A. salina L. larvae and be absorbed into the digestive tract, then distributed into the body, and metabolism becomes disturbed. The difference in concentration gradient between the larval cell membrane and the environment outside the cell causes toxic compounds to spread well into the larva's body, so that within 24 hours it can cause 50% death of A. salina L larvae (Silitonga, Setyati dan Sibero, 2022) The LC_{50} (Lethal Concentration) value is the number of levels that cause the death of 50% of the animal's test at a certain time interval, the classification of the LC_{50} toxicity value is if LC_{50} < 20 g/mL is categorized as very toxic, if the LC_{50} is at 20-100 g/mL is categorized toxic, if the LC_{50} value is at 100-500 g/mL it is categorized as moderate, if the LC_{50} value is at 500-1000 g/mL it is categorized as weak and if the LC_{50} value > 1000 g/mL is categorized as non-toxic (Manal dan El, 2015).

IV. CONCLUSION

In conclusion, in this study, the protein was successfully extracted In methanol extract *Caulerpa sp* based toxic category and protein extract very toxic.

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