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The Effect of Various Macroalgaes Extract in Lombok to Mortality of *Artemia salina* Larvae

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Abstract

This research aimed to determine the effect of various macroalgae extract in Lombok marine water to mortality of *Artemia salina* larva. About 9 species of macroalgaes including *Gracilaria sp, Sargassum crassifolium, S.policistum* sp1, *S.policistum* sp2, *S.cristaefolium, Achantophora muscoides, A.spesifera, Padina sp, and Gelidium ratifolium*, were extracted through maseration method using methanol and dichloromethane. Each extract with 3 level of concentration (5ppm, 10ppm, 100 ppm for DCM phase and 100ppm, 200ppm, 300ppm for methanol phase) was added to *Artemia salina* cultures (2 days in age) containing 10 individuals respectively. The results suggest that addition of *Sargassum crassifolium, S.policistum* sp1, *Achantophora muscoides, A.spesifera and Gelidium ratifolium* extract brings the high number of mortality to *Artemia salina* larva (>60% in average). The average number of larva mortality that was caused by addition of *S.policistum* sp1, *Achantophora muscoides, and Gelidium ratifolium* methanol extract are 7, 10, 10 respectively. Additon of *Sargassum crassifolium, S.policistum* sp1, *Achantophora spesifera and Gelidium ratifolium* DCM extracts give the average number of mortality about 6, 6.33, 7.33, and 10 respectively. Furthermore, the median lethal concentration (LC50) ef each extracts should be measured and performed in other research.

Keywords : macroalgae, extract, mortality, *Artemia salina*.

1. Introduction

Cancer is defined as an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases, to metastasize (spread). Cancer is considered as a major cause of death and main public health problem in Indonesia. Major therapy for cancer so far is conventional therapy by medication and chemotherapy, at which they are commonly has unpleasant and bad side-effect to patient (Kamima, 2009). Therefore, there is an urgent attempt to obtain natural chemotherapy medication with less side-effects. One potential source of anticancer agent is macro algae (sea weed) from Lombok's marine water.

Lombok is one of main island from West Nusatenggara Province. It bordered by Wallace's and Weber's lines as well as a major and two minor through-flow currents, and thus retain diverse and unique marine flora and fauna, including macro algae diversity. There are at least 88 species of seaweed were found in WNT marine water (Sunarpi *et al*., 2005, 2006) including 24 wild and cultivated species of red macro algae (*Rhodophyta)* (Eem et al., 2014, Sunarpi et al., 2014), and 10 species of brown algae (*Phaeophyta)* (Sunarpi et al., 2005). This high diversity provides great potential that seaweed from WNT could be a great source of anti-carcer agent.

There are a few methods to determined wether a substance has anticancer activity. One of those method is Brine Srimp lethal test (BSLT) using *Artemia salina larvae*. This test is considered as a useful tool in preliminary assessment of biological activities of plant extracts. The technique is economic and utilizes small amount of test material (Pisutthanan et al., 2004). Since its introduction, this in vivo test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents (Ahmed et al., 2010; Ramachandran et al., 2011).

The potency of macroalga as a source for anticancer agent could be determined with preliminary test using *Artemia salina* larvae. Therefore this research was carried out to know the effect of addition various macroalga extracts to mortality of *Artemia salina* larvae. This research is aimed to find sample/species of macroalga that given the high mortality of *Artemia salina* larvae and has potency to become source of anticancer agent. The result of this research provides information about species of macro alga that can be used as the source of anticancer compound in further research.

2. Materials and Methods

This research were carried out from December 2016 to February 2017 in Laboratory of Immunobiology, University of Mataram.

2.1 Sample preparation and extraction

Nine species of macroalgaes (*Gracilaria* sp, *Sargassum crassifolium*, *S.policistum* sp1, S.*policistu*m sp2, *S.cristaefoliu*m, *Achantophora muscoides*, *A.spesifera, Padina sp, Gelidium ratifolium)* were cleaned then dried in room temperature for 2-4 weeks. 50 mg of each dried macroalgaes were grinded into pieces. 250 mL (1;5, w/v) of Dichloromethane solution was added into each samples then leave in room temperature for 48 hours. Suspension was filtered with filter paper. Filtrate were laid into vacum evaporator for 48 hours. Pellet were extracted using 250 mL methanol (1:5, w/v) then maserated for 48 hours. Filtrate from methanol extraction were filtered then laid into vacum evaporator for 48 hours. Each extracts (methanol and DCM) from 9 samples of macroalga were stored at refrigerator to maintain the evaporation procces.

2.2 Mortality assay using *Artemia salina* larvae

Mortality assay of *Artemia salina* larvae were conducted with 3 level of concentration of each macroalgae extract (5, 10, and 100 ppm for DCM extract and 100, 200, 300 ppm for methanol extract). Each extracts of macroalgae were poured into ELISA well that contained 10 larva of *Artemia salina (*2 days old) with 3 replication for each treatments respectively. After 48 hours, the mortality of Artemia salina larva in each treatments were calculated. Data were analyzed with ANOVA test using minitab 14 computer programme .

3. Results and Discussion

ANOVA analysis using minitab 14 showed that variety of macroalgae species and level of concentration of each extracts gave the significant effect to mortality of *Artemia salina* larva (table 1). 5 Samples of macroalga (*Sargassum crassifolium, S.policistum* sp1, *Achantophora muscoides, A.spesifera and Gelidium ratifolium)* brings the high number of mortality to *Artemia salina* larva (Table 2 and table 3).

Table 1. Two-way ANOVA test of Larva Mortality using minitab 14. Computer Programme.

R denotes an observation with a large standardized residual.

Two-way ANOVA: larva mortality versus concentration, sample

Source	DF	SS	MS	F	P
concentration	2	11.611	5.8056	8.96	0.002
sample	8	200.736	25.0920	38.73	0.000
Error	16	10.367	0.6479		
Total	26	222.713			

S = 0.8049 R-Sq = 95.35% R-Sq(adj) = 92.44%

According to this result, the concentration of each macoalgae extracts gives the significant effect to mortality of *Artemia salina* larva. Therefore the analysis of LC50 of each extracts of macroalgae should be measured and performed on other research in order to know the optimal concentration that gives the highest number of mortality.

The number of mortality of *Artemia salina* larva in methanol extracts of macroalga showed that there are 3 macroalgae extracts (*Sargassum policistum* sp 1, *Acanthophora muscoides* and *Gellidium ratifolium)* brings the highest number of mortality. The number of death larva that caused by addition to these extracts in highest concentration were 7, 10 and 10 larva respectively (table 2). Additon of *Sargassum crassifolium, S.policistum* sp1, *Achantophora spesifera and Gelidium ratifolium* DCM extracts give the average number of mortality in their highest concentration about 6, 6.33, 7.33, and 10 respectively (table 3)..

Table 2. Mortality of *Artemia salina* larvae in Each Methanol Extract of Macroalgae

Sample	Control	Concentration (ppm)		
		100	200	300
K	2.67			
1		3.00	5.00	3.33
2		3.00	4.33	4.33
3		3.33	7.33	7.00
4		3.33	3.00	3.67
5		3.33	4.33	3.33
6		8.67	10.00	10.00
7		1.67	2.00	2.33
8		1.00	3.67	2.67
9		9.67	10.00	10.00

Table 3. Mortality of *Artemia salina* larva in Each DCM Extract of Macroalgae

Sample	Control	Concentration (ppm)		
		5	10	100
K	4.44			
1		4.33	5.00	3.67
2		3.67	4.00	6.00
3		5.00	6.00	6.33
4		4.00	3.33	5.33
5		4.67	2.67	4.33
6		3.00	3.33	3.00
7		7.00	8.67	7.33
8		8.00	7.33	5.67
9		7.67	8.00	10.00

According to the result, we can conclude that there are at least 5 macroalgae species that has potency a source of anticancer agents. Sanchez *et al (*1993) suggests that bioassay againts *Artemia salina* is a simple and inexpensive method to test cytotoxicity, to biodirect fractionation of natural products and as a predictor of antitumor and pesticidal activity. It also indicates antiviral, antiplasmodial, antifilarial, antimalarial activities (Sleet *et al,* 1983). Therefore further research should be carried out directly in human cancer cell using these 5 extracts to measured the effect of these extracts in cancer cell.

The results provide new promising source for novel biological activity for anticancer. Extracts of microalgae are mainly characterised by their complex chemical mixture. In a preliminary assay, the results can be diffuse and not well bio-directed. Thus, this research should be continued with pytochemical assay to identify the active compounds that produced by macroalgae.

4. Conclusion

The results of this research suggest that addition of *Sargassum crassifolium, S.policistum* sp1, *Achantophora muscoides, A.spesifera and Gelidium ratifolium* extract brings the high number of mortality to *Artemia salina* larva (>60% in average). This research should be continued to determine the lethal concentration (LC50) of its extracts, pytochemical assay to identify the active compounds that produced by macroalga and bioassay using human cell.

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