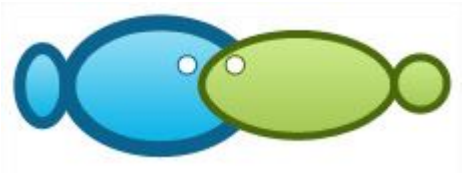

Toxicity of liquid extract of seaweed *Sargassum* sp. on the growth of microalgae *Skeletonema costatum*

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Abstract. *Sargassum* sp. is brown macroalgae that is able to yield secondary metabolites as antibiotics and antimicrobials. One of the methods applied to determine the biological activity, including the toxicity of the compound is the use of microalgae. This study was carried out to know whether *Sargassum* sp. is safe and does not give toxic effect on the growth of microalgae *Skeletonema costatum*. One of the parameters used to measure the response of microalgae to toxic materials, is the growth rate or cell densities. The extract concentration of *Sargassum* sp. used in this study were 0 (control), 1 µg/mL, 10 µg/mL, 100 µg/mL, and 1000 µg/mL. Results showed that during 120 hour-observation, *S. costatum* experienced growth phases of cell density (lag phase, exponential phase, decline phase of growth rate, stationer phase, and mortality phase). Growth of *S. costatum* cell tended to rise and the cell density period has been prolonged with increased concentration of *Sargassum* sp. extract indicating that this extract was not toxic to the growth of *S. costatum*. Duration to reach the growth peak (exponential phase) is different, and it could result from different treatment doses in which the highest cell growth of *S. costatum* occurred in treatment 100 ppm with an average of 77.63 x 10^6 cells/mL at 72 hours and the lowest in control treatment with mean growth peak of 26.38 x 10^6 cells/mL at 36 hours.
Key Words: cell density, biological activity, cell density, response, treatment.

Introduction. Indonesian waters possess abundant and diverse marine biota, one of which is seaweed. It contains secondary metabolites potential as antimicrobial and antibiotic. One of the brown alga species produces several secondary compounds as antibacterials, such as steroid and sterol, phlorotannian (Keusgen et al 1997; Izzati 2007). *Sargassum* sp. is one of the seaweeds abundantly occurring in Indonesia waters, including Central Sulawesi waters. This brown alga is rich in nutrition and bioactive substances.

Since *Sargassum* is rich in active compounds, its toxicity needs to be examined to provide beneficial information for the utilization effort of the natural resources in broader purposes. The toxicity prediction of a substance is performed to detect the toxin of fungi, heavy metal, cyano-bacteria, or pesticide activity. The development of natural substances that can give health benefit for human needs studies on its activity, stability, and safety (Hays et al 2008).

One of the methods used to determine the biological activity, including the toxicity, is the use of microalgae. Major requirements for test animals are: the animal must be sensitive to the treatment material, has high occurrence in water, distributed and present along the year, has high economic value, it is easy to culture, are in good general physical condition. Microalgae can be used as test biota because it has met those criteria and has important ecological role (Hindarti 1997). According to Parish et al (1985), there are several parameters applied to measure the response of the microalgae, one of which is cell density.

Skeletonema costatum is one of the euryhaline diatom (microalgae) with nice golden brown box shape, but the growth peak of *S. costatum* is only one day. Thus, there

is a necessity to have good culture technique to prolong the population peak period. *Skeletonema* growth is closely related with the availability of macro- and micro-nutrients.

Factors affecting the growth of *S. costatum* to obtain good quality production of *S. costatum* are biological, chemical, physical, and culture environment. Biological factor is the availability of good quality seed in sufficient number. Physical factors cover temperature, and light intensity. Chemical factors are salinity, pH, and nutrients in the culture media that must meet the plankton species cultured. It is in line with Andersen (2005) that microalgal cells are affected by the availability of macro- and micro nutrients in the environment. Seaweed is one of the natural aquatic resources containing macro- and micro-minerals. It generally contains essential minerals, such as Fe, I, Al, Mn, Ca, N, P, Cu, Cl, Si, Ta, Co, Si, Rb, Ba, etc. According to Montano & Topas (1990), *Caulerpa racemosa* seaweed extract holds triggering elements of plant growth, i.e. auxin and gibberellin. It is supported by Abetz (1980) and Finnie & Van Staden (1985) that the presence of active compounds and nutrients in seaweed (macroalgae) extract is capable of stimulating the plant growth. Nasmia (2014) reported that *C. racemosa* contained flavonoid compounds. Since seaweed chemical content highly varies with species, the post-harvest utilization can also vary, either as food material, fertilizer, cosmetics, pesticides, etc., so that this study was aimed at knowing the toxic properties of *Sargassum* liquid extract on the growth of *S. costatum*.

Material and Method

Study site. Seaweed *Sargassum* sp. samples were collected from the waters of Morowali regency and Tojo Una-Una regency. Extraction and phytotoxicity test were carried out in the laboratory of Fisheries and Biotechnology, Tadulako University, Palu.

Seaweed Sargassum sp. extraction. Extraction is an isolation process of solid or liquid organic compounds from the complex form by taking advantage of the solubility property difference of each component using certain solvents. Seaweed extraction used maceration method while stirring for 24 hours. Maceration is a filtering process in which the powder is submerged in the solvent up to absorb and soften the cell structure, so that the soluble substances will be dissolved. Extraction was done for 24 hours and repeated three times. Maceration could take several hours or several days for optimum extraction. It was done at 15-20°C for 3 days until the substances were dissolved. Extraction of *Sargassum* sp. fine powder (Figure 1) used maceration method in water solvent. It was done for 24 hours and repeated three times. The extract solution was filtered through Whatman no.1 filter paper. After extraction, the organic solvent was vacuum-evaporated using a rotavapor to obtain the extract (Zainuddin 2010). The extract was then inserted into a previously weighed vial. When the solvent was dried up, the extract was weighed and stored in the freezer up to be used.

Figure 1. *Sargassum* sp. (source: Nasmia et al 2016).

Sterilization of equipment and materials. Room sterilization was carried out by cleaning the room and spraying 70% alcohol. Equipment sterilization was conducted through dry sterilization, in which the equipment was wrapped and put into an oven at 180^0C for about 1 hour. Other materials (seawater and distilled water) were sterilized using an autoclave at 121^0C for 15 minutes.

Fitotoxicity test. Phytotoxicity test was done in Fisheries Laboratory of Tadulako University. This examination was intended to know the toxic property of *Sargassum* sp. liquid extract. It used *S. costatum* as test organisms (Figure 2).

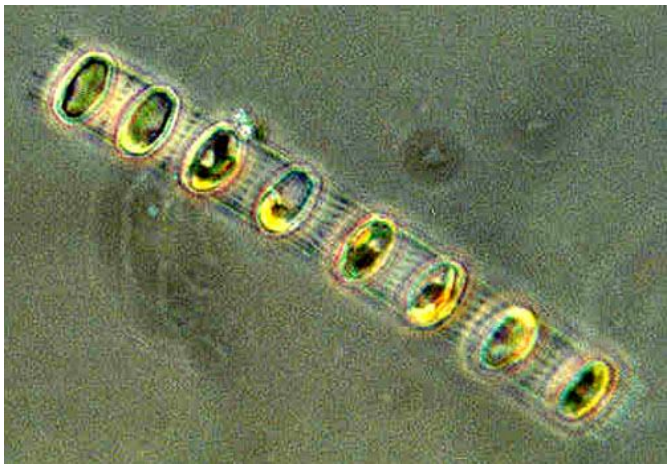

Figure 2. *Skletonema costatum* (source: Armanda 2013).

S. costatum cultivation was conducted in seawater media enriched with nutrient of Conway fertilizer in sterile room. During the study, the media were exposed to 600 lux illumination, continuous aeration, and controlled temperature of 25^oC-28^oC for optimal growth of *S. costatum*. The phytotoxicity test was conducted in sterile air-conditioned room using pure seed of *S. costatum* in the jar containing 500 mL of sterile seawater, then added with active liquid extract of *Sargassum* sp. at the concentration of 0, 1, 10, 100, and 1000 µg/mL used in this study. Water quality was daily measured, and all equipment and materials for culture were previously sterilized.

Measurements of the microalgae cell density were carried out by dropping the microalgal solution on a haemocytometer and counted under the microscope. Growth curve of the microalgae was determined through daily cell counts, and then the growth curve was made. To obtain culture densities (cells/mL), the following formula was applied:

$$\text{cells/mL} = N \times ¼ \times 10^6$$

Where: N = number of cells observed.

Compound isolation using thin-layered chromatography (TLC). Active antibacterial compounds were determined using thin-layered chromatography (TLC) with F_{254} silica gel plate. As much as 0.5 mg of liquid extract of *Sargassum* sp. was taken and dissolved in the water solvent, spotted on the plate of silica gel thin-layered chromatography as stationary phase, then the plate was eluted using appropriate solvent system as mobile phase. Chromatogram readings were done at 254 nm and 366 nm UV light and sprayed with the reagent to make the spot or tape look clear.

Data analysis. Data collected were cell density, and then descriptively analyzed in the form of growth curve *S. costatum*. Following the growth pattern of the macroalgae, lag phase, exponential phase, growth decline phase, stationary phase, and mortality phase (Utomo et al 2005).

Results and Discussion. This study found that the application of *Sargassum sp*. extract gave positive effect on the density of *S. costatum*. It could be seen from significant increment of cell numbers compared with the growth of the control treatment (Table 1). Increased cell density is part of the growth, and it is in agreement with Salisbury & Ross (1992) that growth means size increment in the form of weight, volume, and number of cells.

Table 1

Mean cell density of *Skletonema costatum* cultured with and without *Saragassum* sp. extract

Extract concentration (ppm)	*Observation hours (cell density x10^6 cells/mL)*										
	0	*12*	*24*	*36*	*48*	*60*	*72*	*84*	*96*	*108*	*120*
0 (Control) A	1.00	1.65	24.00	26.38	15.75	14.90	13.75	6.95	3.00	0.00	0.00
B (1)	1.00	1.55	24.88	23.75	49.25	25.25	13.75	9.25	7.75	3.50	0.00
C (10)	1.00	1.65	22.38	25.50	22.75	16.75	12.38	9.63	7.63	4.35	1.00
D (100)	1.00	1.25	16.75	38.88	39.13	46.80	77.63	50.50	46.75	26.25	12.75
E (1000)	1.00	1.85	20.00	30.38	30.00	29.60	34.00	59.63	49.25	43.13	17.38

Phytotoxicity test of each 12 hours shows increasing trend with increased liquid extract of *Sargassum* sp. (Table 1).

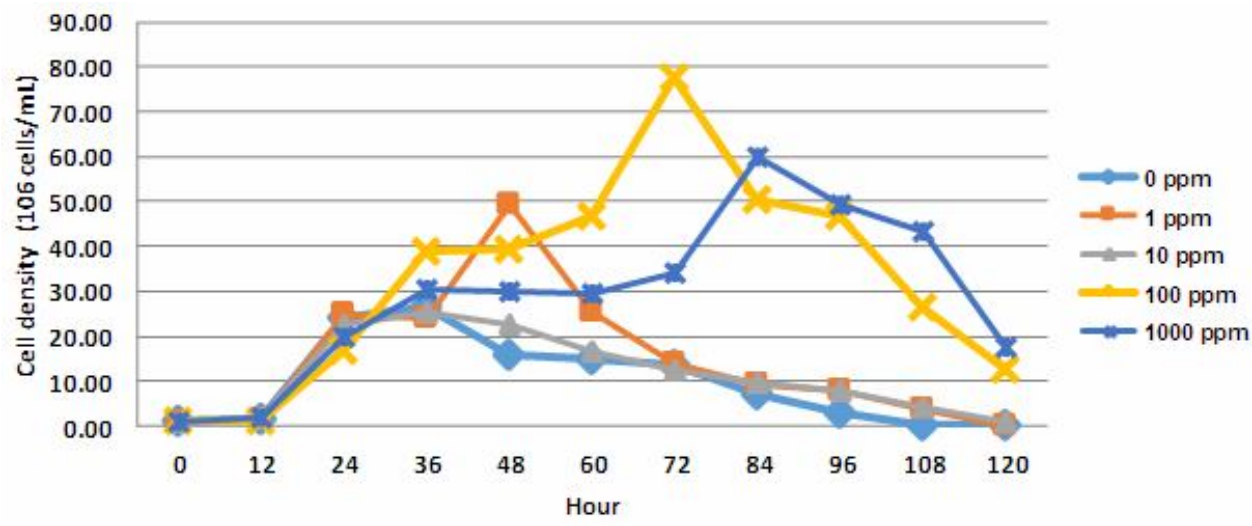

Figure 3. Growth (cell density) of *Skletonema costatum.*

Phytotoxicity test (Table 1 and Figure 3) revealed that cell density tended to rise with increased extract concentration, with the highest cell density at the concentration of 100 ppm, 77.63 x 10^6 cells/mL (at 72 hours) and the lowest occurred in control concentration. This result revealed that at the control concentration, mortality phase occurred faster than that with *Sargassum* extract. It means that the compound of *Sargassum* sp. extract is not toxic to *S. costatum* and safe to use in order to increase growth and cell density due to good nutrient content.

The growth pattern of *S. costatum* followed a logarithmic model, lag phase, exponential phase, growth decline phase, stationary phase, and mortality phase. The growth curve for 120 hour-observations demonstrated that *S. costatum* cells nearly did not have an adaptation phase, in which mean cell density at the twelve observation has risen (Table 1). The adaptation phase was not clearly seen in all treatments. It could be concluded that the inoculated cells of *S. costatum* quickly adapt to new culture media so that they can grow and quickly divide.

The cell growth of *S. costatum* in the culture media was indicated by cell size increment or cell numbers. It reflects that *S. costatum* has sufficiently good cell density at each treatment, even though number of cell densities at different treatments the presence of *Sargassum* sp. extract could prolong the growth period of *S. costatum* cells compared with that of the control concentrations at 48 hours that started getting cell number declining phase. It is supported by Azizah et al (2015) that application of *Eucheuma sp*. extract positively influenced the density of *Chlorella sp*. indicated with significant increment of cell numbers (6,923 log cells/mL) compared with treatment without *Eucheuma sp*. extract (5,904 log cells/mL).

S. costatum needs organic and inorganic materials obtained from the environment that mainly function as energy source and cell grower. Becker *in* Armanda (2013) reported that macro-nutrients needed by *S. costatum* are N, P, K, S, Na, Si, and Ca, in which N, P, and S are important components for protein formation. P is important for energy transfer and nucleic acid biosynthesis. One of the materials containing mineral nutrition is seaweed extract. Handayani et al (2004) stated that nutrient composition in *Sargassum* sp. consisted of 5.19% protein, 36.93% ash (mineral), vitamin A of 489.55 µg RE/100 g, vitamin C of 49.01 mg/100 g, 1.63% fat, and 37.91% alginate.

Nutrient analysis on *Sargassum* sp. extract found N of 35.59 mg/L, P of 3.81 mg/L, K of 3.22 mg/L, Ca of 2.02 mg/L, Fe of 2.17 mg/L, S of 6.67 mg/L, Cu of 0.19 mg/L, and Zn of 19.62 mg/L, respectively. It reflects that the liquid extract of *Sargassum* sp. contains sufficient nutrients to be used by *S. costatum*. Phytoplankton growth and development require various nutrients from the environment, meaning that the availability of macro- and micro-nutrients in the culture media is highly needed. Fogg (1975) claimed that N in nitrate and P were basic components that should be present in the phytoplankton culture media. The nutrients needed in high numbers are nitrogen, phosphorous, iron, sulphur, magnesium, kalium, and calcium.

The use of *Sargassum* sp. liquid extract showed normal growth curve at all treatment concentration. Therefore, the extract compound is proved to be not toxic to *S. costatum*. This result is similar to Asri (2011) who studied cytotoxic test on methanol extract and H_2O extract of green seaweed *Ulva reticulata* that did not show cytotoxic activity. The thin-layered chromatographic (TCL) test on the liquid extract of *Sargassum* sp. found flavonoid compound (data in preparation for publication). Vickery & Vickery (1981) also added that flavonoid in plants can increase fine cells, and in the medical world, flavonoids can function as antibiotics, antivirus, and antifungi. Flavonoids are compounds that are soluble in the water and natural phenols that can systematically act as immunostimulator (Harborne 1987). According to Patier et al (1993), the organic compounds in the seaweed extract (macroalgae) are capable of stimulating growth due to the presence of protein synthesis, cell division, and nutrient mobilization.

Water quality conditions were found to be in good range for *S. costatum* growth with salinity of 25 ppt, pH of 7-8, temperature of 28-29^oC. It is in agreement with Haryati (1980) who stated that *S. costatum* is an eurythermal diatom that is capable of growing at temperature range of 3-30^oC with optimal temperature of 25-27^oC. It is categorized as euryhaline diatom that is capable of growing at wide salinity range, 15-34 ppt with the highest growth rate at 20-30 ppt.

As aquatic organism, water salinity is one of the limiting factors for phytoplankton growth and development. Thus, salinity fluctuation could directly cause change in the osmotic pressure in the cell. Extremely high or low salinity could make the osmotic pressure in the cell be lower or higher and disturb the cell activity.

Conclusions. Phytotoxicity test found that application of *Sargassum* sp. liquid extract at the concentration of 1 ppm, 10 ppm, 100 ppm, and 1000 ppm did not cause cell mortality of *S. costatum*, so that this result revealed that the extract was not toxic to the growth of *S. costatum*. Active compound content of *Sargassum* sp. liquid extract belongs to flavonoid group.

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