Potential Taurine Content from Three Different Macroalgaes: *Halimeda opuntia* L., *Sargassum* sp. And *Eucheuma cottonii* L.

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Abstract. As one of the marine resources, macroalgae presumably contains of free amino acid such as taurine. Taurine is known to be one of the most important amino acid related to hyperosmotic stress for most of living organisms. The study was conducted to explore the taurine contain from the most abundance macroalgae found in Indonesia seawater, especially in Lampung Province, namely *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. Maceration followed by ethanol extraction was applied to those three different macroalgae and the filtrate was identified for its taurine content by using UV-Vis spectrophotometry. Standard of pure taurine of 0,1 and 1 M (NOW) was used and the maximum wavelength of under the UV-Vis spectrophotometry was 630 nm. Simple correlation from the standard taurine was y = 0.001x+0.033 and used to determine the taurine content of those three macroalgae. The result indicated that potential taurine content of *Halimeda opuntia* L was 7.85 mg/100g dry mass, *Sargassum* sp was 1.21 mg/100g dry mass and *Eucheuma cottonii* L was 4.61 mg/100g dry mass.

1. Introduction

Indonesia is known as one of the world's *megacenter* biodiversity including the biodiversity of its marine biota. One of the potential marine biota in Indonesia is macroalgae. In terms of macroalgae productivity is more beneficial because there are no seasonal variations, easier to extract, and abundant raw materials [1]. According to research conducted by Kawasaki *et al* [2] macroalgae have taurine content. Among the 29 types of macroalgae studied, red macroalgae have relatively high taurine content while green macroalgae and brown macroalgae do not contain taurine. Taurine is one of the stimulant substances that can trigger stamina, so it is widely used in energy supplements. Taurine also plays an important role in maintaining the smoothness of various bodily processes [3] Until now there is very little research on macroalgae potential containing taurine, for this reason the researchers tested the taurine content of ethanol extract *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L.

2. Methods

2.1 Place and time of research

The research was conducted in Mei 2019 until June 2019, in the Botanical Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences, University of Lampung.

2.2 Tools and Materials

The tools used in this research are UV-Vis spectrophotometry, oven, *rotary evaporator, Erlenmeyer & beaker glasses, vortex*, measuring cup, test tube, test tube rack, *buchner* funnel, pipette volume, dropper pipette, filter paper, carbon paper, *blender*, and a stirrer. The materials used are *Halimeda opuntia* L., *Sargassum* sp., *Eucheuma cottonii* L., ethanol 96%, taurine powder (NOW), and aquades.

2.3 Extraction Process of Macroalgae

Halimeda opuntia L., *Sargassum* sp. and *Eucheuma cottonii* L. used in this study were obtained from Ketapang Beach, Lampung - Indonesia. *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. were best washed with running water, dried in an oven at 40˚C , and crushed in to powder. As much as 100 grams of powder of each macroalgae was soaked with 1000 ml of ethanol 96 % and let stand for 48 hours, maserate then was filtered with a *Buchner* funnel and the obtained filtrate is concentrated using a *rotatory evaporator* [4].

2.4 Setting Level of Taurine Total

2.4.1 Determination Wavelength Maximum

The maximum wavelength was determined by the detection of the absorbance value of one standard taurine solution in the wavelength range of 400-800 nm using UV-Vis spectrophotometry. The standard solution of 1 M taurine was determined for its absorbance using UV-Vis spectrophotometry and it was at wavelength of 400-800 nm with distilled water absorption.

2.4.2 Determination Standard Curve Taurine

A total of 12.5 grams of taurine powder were added with 10 ml of distilled water, then homogenized using *vortex* until it dissolved completely. After that 20 dilutions were carried out so that the concentration of standard solution of taurine 0.1 M and 1 M was obtained. Each standard solution was measured for absorbance at the maximum wavelength, then a calibration curve was made for the relationship between taurine (M) concentration and absorbance value.

2.4.3 Determination of Total Taurine Levels

1 gram of ethanol extract of *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. were dissolved in 10 ml of distilled water. Absorbance of each solution of macroalgae ethanol extract was measured by UV-Vis spectrophotometry at maximum wavelength. Repeated 3 times.

2.5 Data Analysis

The data obtained were analyzed quantitatively and presented in the form of data tabulation. The primary data obtained from the absorbance value of the taurine as comparative solution used as a standard curve so that a linear regression equation was obtained $y = ax+b$. Total levels of taurine in *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. samples were calculated by entering each absorbance value into linear regression equations and the results were expressed in mg units in gram extract of macroalgae.

3. Results and Discussion

3.1 Determination of maximum wavelength

The results of determining the maximum wavelength of a standard solution of 1M taurine in the wavelength range of 400-800 nm (Figure 1).

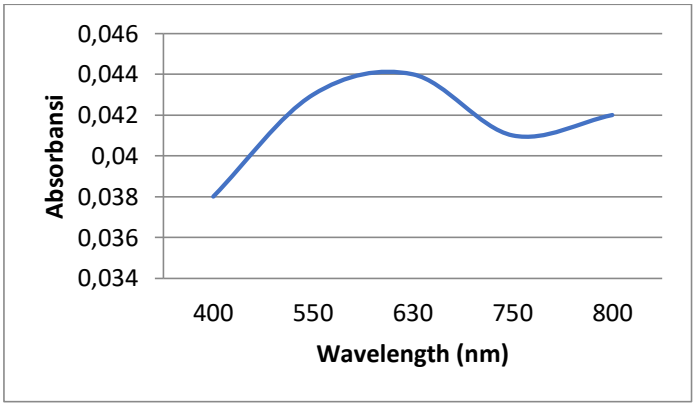

Figure 1. The maximum wavelength of taurine solution

The purpose of determining the maximum wavelength was to determine the taurine wavelength that is able to provide maximum absorption so that it can be absorbed by UV-Vis spectrophotometry. Obtained wavelength measurement results with 3 times the control at maximum absorption 0.044 with a wavelength of 630 nm. In accordance with the literature which states that Taurine has a maximum wavelength of 630 nm [5].

3.2 Taurine standard curves and linearity test

The concentration used in the standard curve of curricula is 0.1 M and 1 M with a maximum wavelength of 630 nm. Standard curve taurine can be seen in Figure 2.

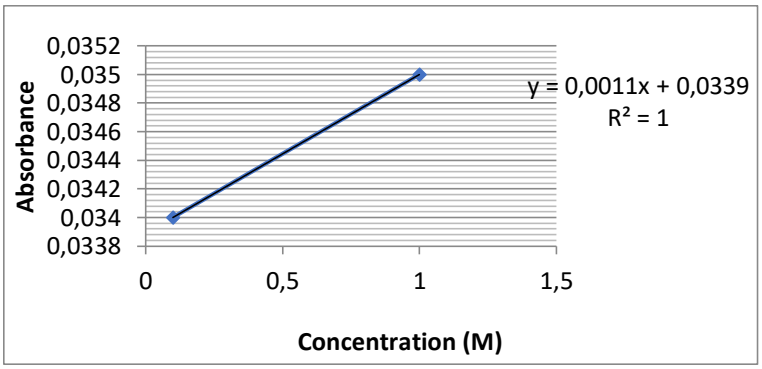

Figure 2. Taurine standard curve

Taurine standard curve was applied by making taurine solution with the concentration of 0.1 M and 1 M. Based on the result obtained in Figure 2, the standard solution of taurine 0.1 M had an average absorbance of 0.034 while the standard solution of taurine 1 M had an average absorbance of 0.035, this shows that the absorbance value produced increases parallel to the increase in taurine concentration. This was also in accordance with the Lambert-Beer law where A= abc, ie the absorbance value (A) is directly proportional to the concentration value (c) [6].

From the taurine standard curve obtained a linear regression equation between concentration and absorbance, namely y = 0.001x + 0.033 with the value of correlation coefficient that is r = 1. Based on the results obtained the correlation coefficient value obtained has met the requirements of AOAC [7] which is close to or equal to 1.

The resulting linear regression equation then was used to determine taurine concentrations in ethanolic extraction of *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. by entering the absorption value into the regression equation of y = 0.001x + 0.033.

The standard curve was the relationship between the absorbance value of concentration. The standard curve produced could be used for linearity testing. The purpose of the linearity test was to prove the existence of a linear relationship between the concentration of the substance and the response of the tool. Linearity was usually expressed in correlation coefficient (r).

3.3 Linearity test

Table 1. The linearity test results of taurine standard solutions

Concentration (M)	Replication	Absorbance	Average
0.1	1	0.034	0.034
	2	0.034	
	3	0.034	
1	1	0.034	0.035
	2	0.035	
	3	0.035	
Slope (b)		**0.033**	
Intercept axis (a)		**0.001**	
Correlation coefficient (r)		**1**	

Then the regression equation obtained y = a+bx
y = 0.001x+0.033

Correlation coefficient (r) equal to 1 from the standard curve shows a correlation between concentration and absorbance. If the number of correlation coefficient (r) is equal to 1, then the two variables have a perfectly positive linear relationship.

3.1.4 Determination of possible taurine concentration on macroalgae extract

Table 2. Taurine concentration in ethanol extraction of macroalgae

Sample	Absorbance	Average absorbance	Total Levels (mg/100g)	Average Levels (mg/100g)	Total level (mM)
H. opuntia L	0.798	0.818	7.65	7.85	6.20
	0.833		8.00		
	0.823		7.90		
Sargassum sp	0.154	0.154	1.21	1.21	0.96
	0.154		1.21		
	0.154		1.21		
E. cottonii L	0.493	0.495	4.60	4.61	3.60
	0.500		4.67		
	0.491		4.58		

3.2 Discussion

Ethanol extract of *Halimeda opuntia* L. had average absorbance of 0.818 with potential taurine concentration of the sample was 7.85 mg/100g dry mass. While ethanol extract of *Sargassum* sp. obtained the average absorbance of 0.154 with potential total taurine concentration was 1.21 mg/100g dry mass. While the ethanol extract of *Eucheuma cottonii* L. obtained the average absorbance of 4.950 with potential total taurine content in the sample of 4.62 mg/100g dry mass.

In this study, all of ethanol extract of macroalgae indicated possibly to contain natural taurine. Based on the results, *Halimeda opuntia* L. had the highest taurine content compared to other two macroalgae, namely *Sargassum* sp and *Eucheuma cottonii* L. This high content of taurine in *Halimeda* presumably due to several factors, ecologically, physiologically and histology of *Halimeda*.

Halimeda opuntia L. grows in shallow waters, specifically in the interdal zone with sea water fluctuates following tides. It can experience two tides and twice low tide with almost the same height that occurs regularly. The average tidal period is 12 hours 24 minutes. During low tide, *Halimeda opuntia* L. suffered from dry and easily exposed to ultraviolet radiation, on the contrary, during high tide *Halimeda opuntia* L. submerged in sea water with varying salinity levels. This extreme environmental condition allows *Halimeda opuntia* L. to adapt by producing secondary metabolites, one of which in the form of taurine. Taurine plays an important role as one of organic osmolytes compound in macroalgae.

According to Strange and Jackson [8] taurine is an organic osmolyte compound from amino acid derivatives containing sulfurhydril groups which serves to protect cells from a changing environment. Organic osmolytes are found in high concentrations of around 10mM to 100mM in the cytosol of all organisms from low to high levels, from bacteria to humans.

Taurine also acts as an osmoprotective in osmoregulation so that the distribution of energy replaced by taurine can be used for growth [9]. The increasing in extracellular osmolarity will be balanced by an increase in intracellular organic osmolyte in response to compensate for changes in environmental conditions. The bulging of cells will result in the entry of organic osmolytes from the outside which largely due to an active increase in osmolyte transport [9]. Cells that experience osmotic stress generally will accumulate more taurine as a compatible osmolyte compound [9].

Based on the other study related to histology of those three macroalgae indicated that they had different structures [10,11,12,13]. *Sargassum* sp. and *Eucheuma cottonii* L. cells had almost similar cell shapes, namely oval round with small diameter sizes and coinciding. Whereas in *Halimeda opuntia* L. cells were polygonal [11] with large cell diameter sizes.

On the surface of *Halimeda opuntia* L. cells there was high enough calcium content in the form of calcium carbonate. Calcium carbonate ($CaCo_3$) came from metabolic results, deposited in *thallus* cell *tissues*, in the form of aragonite and calcite [11]. Aragonite was a mineral from calcium carbonate that was formed at low temperatures [11].

The cell number in macroalgae is influenced by salinity [14]. Water quality parameters that greatly influence the development and growth of macroalgae was salinity, this was directly related to osmoregulation that occurs in cells [15,16,17]. Presumably taurine was also accumulated in this action.

Adaptation success will determine the sustainability of organisms in shallow waters [18]. Therefore, *Halimeda opuntia* that survived in the intertidal zone could accumulate more taurine as osmolyte compound to overcome the changing variations in the environment.

Meanwhile, some study indicated that taurine could be correlated with chlorophyll a [19]. Chlorophyta is the largest group of algal vegetation containing chlorophyll a [20]. In the chloroplast assimilation occurs wherein photosynthetic reducing agents together with ATP played a role in absorbing sulfur compounds [21]. This was related to the synthesis of taurine which requires sulfur, the sulfur is absorbed in the form of sulfate ions for the formation of PAPS-AS (adenosine-3'-phosphate-5'-phosphosulfate). The study also indicated that taurine synthesis in algae occur via the serine/sulfate pathway. This pathway used organic sulfate in the L-serine carbon backbone. L-serine was converted to 2-aminoacrylate by the enzyme L-serine dehydratase, then converted to cysteate by PAPS-AS (adenosine-3'-phosphate-5'-phosphosulfate). Cysteate then would be decarboxylated with the help of CSAD/GAD enzyme to taurine [20].

4. Conclusion

The result indicated that potential taurine content of *Halimeda opuntia* L was 7.85 mg/100g dry mass, which was much higher compared to *Sargassum* sp (1.21 mg/100g dry mass) and *Eucheuma cottonii* L. (4.61 mg/100g dry mass).

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