Taurine Content Of Three Different Macroalgae: *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 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. Research Methods

2.1 Place and time of research

The research was conducted in Mei 2019 until June 2019, in the Laboratory of Botanical, Department of Biology, 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 glass* , *vo r tex* , 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, and aquades.

2.3 Extraction Process of Macroalgae

In this study used *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. obtained from Ketapang Beach, Lampung. *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. are best washed with running water, dried in an oven at 40˚C , and mashed. As much as 100 grams of powder soaked with 1000 ml of ethanol 96 % let stand for 48 hours, maserate is 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 is 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 taurine 1 M measured its absorbance using UV-Vis specrophotometry at a 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 comparative solution is a standard curve so that a linear regression equation is 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 regrese equations and the results are expressed in mg units in gram extract.

3. Results and Discussion

3.1 Determination of maximum wavelength

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

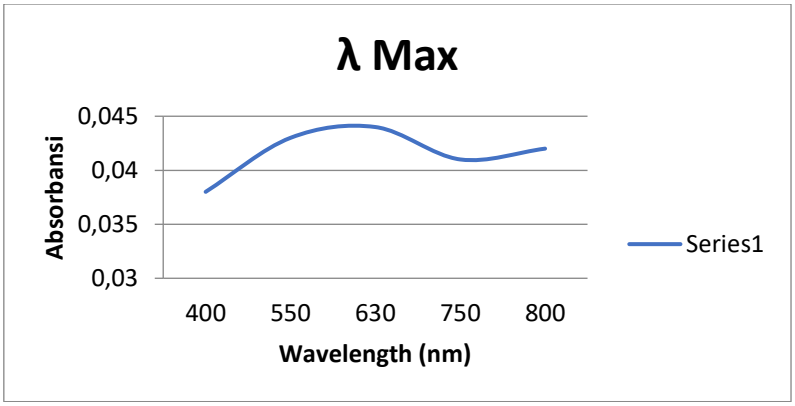


Figure 1. The maximum wavelength of taurine solution

The purpose of determining the maximum wavelength is 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 Making 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. Sta curve ndar taurine can be seen in Figure 2.

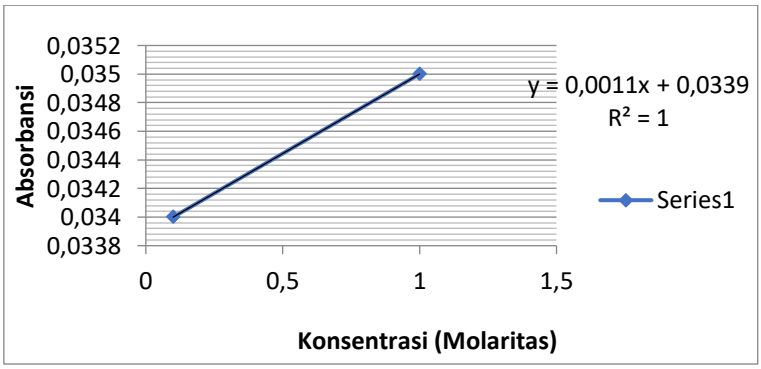


Figure 2. Taurine standard curve

In making the standard curve taurine made a solution with the concentration used is 0.1 M and 1 M. Based on the results of the results obtained in Figure 2 , the standard solution of taurine 0.1 M has an average absorbance of 0.034 while the standard solution of taurine 1 M has an average absorbance of 0.035, this shows that the absorbance value produced increases parallel to the increase in taurine concentration . This is 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 can be used to look for taurine concentrations in extract of *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. by entering the absorption value into the regression equation y = 0.001x + 0.033.

The standard curve is the relationship between the absorbance value of concentration. The standard curve produced can be used for linearity testing. The purpose of the linearity test is to prove the existence of a linear relationship between the concentration of the substance and the response of the tool. Linearity is 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
y = 1

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 levels of taurine macroalgae extract

Table 2. Taurine concentration in ethanol extraction of macroalgae

Sample	Absorbance	Avarage absorbance	Total Levels (mg/100g)	Average Levels (mg/100g)	Total level (mM)
Sample 1	0,798	0,818	7,65	7,85	6,20
	0,833		8		
	0,823		7,9		
Sample 2	0,154	0,154	1,21	1,21	0,96
	0,154		1,21		
	0,154		1,21		
Sample 3	0,493	0,495	4,6	4,61	3,60
	0,500		4,67		
	0,491		4,58		

Information
Sample 1 : Ethanol extract of *Halimeda opuntia* L.
Sample 2 : Ethanol extract of *Sargassum* sp.
Sample 3 : Ethanol extract of *Eucheuma cottonii* L.

3.2 Discussion

Ethanol extract of *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. which have been tested using spectrophotometric methods will produce absorbance values. Taurine levels were calculated using a regression equation obtained from the standard curve y = 0.001x+0.033 by entering the absorbance value of samples of ethanol extract *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L.

The first sample is ethanol extract of *Halimeda opuntia* L.. The average absorbance after 3 measurements is 0.818 and the total taurine level in the sample is 7.85 mg/100g dry mass. The second sample is ethanol extract of *Sargassum* sp. obtained the average absorbance after 3 measurements of 0.154 and the total taurine level in the sample was 1.21 mg/100g dry mass. The third sample is ethanol extract of *Eucheuma cottonii* L. obtained by the average absorbance after 3 measurements of 4.95 and total taurine content in the sample of 4.62 mg/100g dry mass.

All samples of ethanol extract macroalgae proved to contain natural taurine. Based on the results obtained by *Halimeda opuntia* L. has the highest taurine content with a total taurine content of 7.85 mg/100g dry mass.

Why does *Halimeda opuntia* L. have higher levels of taurine than *Eucheuma cottonii* L. and *Sargassum* sp. are not yet known. However, *Halimeda opuntia* L. is thought to have higher taurine levels than other types of macroalgae due to the influence of several factors both ecologically, physiologically, and histologically .

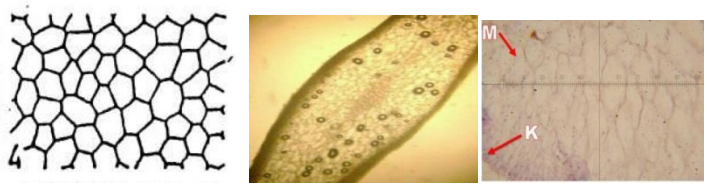
Halimeda opuntia L. grows in shallow waters called the interdal zone with sea water that fluctuates following tides. In one day sea water can experience two tides and twice low tide with almost the same height that occurs regularly. The average tidal period is 12 hours 24 minutes.

When the recedes *Halimeda opuntia* L. will dry and will be easily exposed to ultraviolet radiation, on the contrary when pairs *Halimeda opuntia* L. will be submerged in sea water with varying salinity levels. This extreme environmental condition allows *Halimeda opuntia* L. to adapt by producing metabolites in the form of taurine. Taurine plays an important role as an osmolytic compound in macroalgae.

According to Strange and Jackson [8] taurine is an organic osmolite compound derived from amino acid derivatives containing sulfurhidril groups which serves to protect cells from a changing environment. Organic osmolites 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]. If extracellular osmolarity increases, it will be balanced by an increase in intracellular organic osmolite in response to compensating for changes in environmental conditions. The bulging of cells will result in the entry of organic osmolites from the outside. This income is largely due to an active increase in osmolite transport [10]. Cells that experience osmotic stress generally will accumulate more taurine as a compatible osmolite compound [11].

Histologically, differences in the shape of *Halimeda opuntia* L., *Sargassum* sp. and *Eucheuma cottonii* L. cells can be seen in Figure 3.



(a) (b) (c)

Figure 3. An overview of macroalga network histology

Information
(a) : *Halimeda opuntia* L. [12].
(b) : *Sargassum* sp. [13].
(c) : *Eucheuma cottonii* L. [14].

In Figure 3 it can be seen that cells in *Sargssum* sp. and *Eucheuma cottonii* L. have almost similar cell shapes, namely oval round [15] with small diameter sizes and coinciding. Whereas in *Halimeda opuntia* L. cells are polygonal [16] with large cell diameter sizes .

On the surface of *Halimeda opuntia* L. cells there is a high enough calcium content in the form of calcium carbonate. Calcium carbonate ($CaCo_3$) comes from metabolic results deposited in *thallus* cell *tissue* , in the form of aragonite and calcite [16]. Aragonite is a mineral from calcium carbonate that is formed at low temperatures [17].

The cell number in macroalgae is influenced by salinity [18]. Water quality parameters that greatly influence the development and growth of macroalgae are salinity, this is directly related to osmoregulation that occurs in cells [19]. Taurine is a good osmoregulator biology [20].

The cell number in macroalgae is influenced by salinity [21]. Water quality parameters that greatly influence the development and growth of macroalgae are salinity, this is directly related to osmoregulation that occurs in cells [22]. Taurine is a fairly good biological osmoregulator [23].

Adaptation success will determine the sustainability of organisms in shallow waters [24]. Therefore *Halimeda opuntia* that live in the interdal zone can accumulate more taurine as an osmolite compound to overcome the changing variations in the environment.

The results of research by Kevin [25] state that taurine can be correlated with chlorophyll a. Chlorophyta is the largest group of algal vegetation containing chlorophyll a [26]. In the chloroplast assimilation occurs wherein photosynthetic reducing agents together with ATP play a role in absorbing sulfur compounds [27]. This is 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).

According to research by Tevatia *et al* [28] taurine synthesis occurs via the serine/sulfate pathway. The pathway for taurine synthesis can be seen in the following Figure 4.

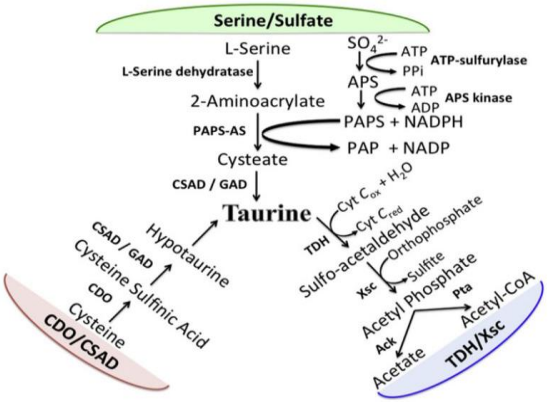


Figure 4. Pathway for taurine synthesis

Taurine in algae is synthesized via the serine/sulfate pathway, this pathway uses organic sulfate in the L-serine carbon backbone. L-serine is converted to 2-aminoacrylate by the enzyme L-serine dehydratase, then converted to cysteate by PAPS-AS (adenosine-3'-phosphate-5'-phosphosulfate). Cysteate will be decarboxylated with the help of CSAD/GAD enzyme to taurine [29].

4. Conclusion

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.

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