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# Antioxidant Levels, Chlorophyll, Carotenoids, and Antibacterial Activities of *Caulerpa lentillifera* From The North Coast of Java and Bali

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**Abstract:** This study aims to determine the effect of caulerpa habitat location on the content of active compounds and their activities as antibacterial. This research is experimental and data analysis with ANOVA. Samples come from three different locations of CBL, CBB, and CL as well as two different solvents, ethanol and n-hexane. According to research, the highest antioxidant activity produced by n-hexane CBL is 73.48%. The highest value of phenol gives n-hexane CBB at 292,867 mg/mL. The highest total flavonoids are 11.91 mg/ml produced by CBB ethanol. The total chlorophyll is between 0.25–0.87 mg/mL, while the total carotenoid is between 1.40–4.11 µ mol/L. The clear zone is formed in only 2 samples with S-test bacteria. It has 0.5 mm aureus n-hexane CBB and 1.5 mm n-hexane CL. This zone is clearly classified as very small, this can be due to the small content of the extract in the test sample. Active compounds found under the GCMS test are alkanes, alkenes, alcohols, and other fatty acids. These active compounds are a group of potentially antioxidant and antibacterial agents. It can be inferred that habitat location affects active compounds and CBB has greater potential as a source of natural materials than other samples.
**Keywords**: Antioxidant; bioactive compound; Caulerpa lentillifera; antibacterial activity

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## INTRODUCTION

Caulerpa is a marine commodity that comes from the seaweed group. This algae is known for its high ecological value, especially in maintaining the marine ecosystem by providing habitat for various species of fish and other marine organisms. Caulerpa is also commonly referred to as sea grapes. One of the Caulerpa found in Indonesia is *Caulerpa lentillifera*. However, in some regions there are separate names for Caulerpa, known as Lat in the Kei Islands, Lawi-lawi in Sulawesi, Ar-arosep or Latoh in the Philippines, Umi Budo in Japan, while in European countries it is called green caviar (Tapotubun et al., 2020).

Caulerpa is one type of seaweed that has potential as a secondary metabolite material that can be beneficial to the health of the body. Secondary metabolite compounds owned by *C.lentillifera* can be used in various fields, such as industry, research, and medical (Wichachucherd et al., 2019). Seaweed contains certain bioactive compounds, such as polysaccharides, carotenoids, polyphenols, and phlorotannins (Lee & Jeon., 2013). *C. lentillifera* contains caulerpin and caulerpicin. Caulerpin is a secondary metabolite compound that plays an important role in Caulerpa body defense (Januar et al., 2004). Caulerpin is an alkaloid compound contained in Caulerpa as much as 2% and has a role as anti-bacterial, anti-microbial, anti-inflammatory, anti-hypertensive, antiviral, and others (Souza et al., 2009 in Kamal & Sethuraman., 2012). Meanwhile, caulerpicin is an alcohol compound with a long

aliphatic carbon chain that has amine and ether groups (Januar et al., 2004). As an antibacterial, caulerpa extract in antibacterial tests shows a broad spectrum sign against Gram-negative pathogenic bacteria and Gram-positive bacteria tested with an inhibition zone of 12-16 mm (Zainuddin et al., 2019). Based on research conducted by Nurdiansyah et al (2021), it shows that *C. racemosa* from the waters of Lemukutan island obtained the highest activity in the test bacteria *Escherichia coli* and *Staphylococcus aureus* indicated by the diameter of the inhibition zone of 15.96 mm and 16.47 mm, respectively. However, the components of bioactive and antibacterial compounds in *C. lentillifera* seaweed with different aquatic origins will produce different amounts.

*C. lentillifera* can be found naturally marine waters, but nowdays *C.lentillifera* cultivations has developed and has been developed in several regions in Indonesia such as Java. There are various methods of sea grape cultivation that have been developed. They are two types of methods that have been applied, namely the floating method on the coastal waters of the sea and at the bottom of the pond. Cultivated sea grapes are used as a water quality neutralizer in shrimp ponds (Astuti et al., 2021). However, research conducted by Azizah (2006) stated that the floating cultivation method on the sea surface showed a good growth rate compared to the cultivation method attached to the bottom of the pond.

Different water locations can show morphological variations even though they are the same species (Estrada et al., 2020). Green macroalgae groups such as Caulerpa, namely *Caulerpa lentillifera* and *Caulerpa racemose* have a high level of phenotypic plasticity due to the influence of aquatic environmental factors (Belton et al., 2014). Differences in environmental conditions consisting of brightness, salinity, temperature, and nutrients in marine waters and ponds are among parameters that can affect seaweed secondary metabolites (James & Bill., 2001). One example is thallus size, shape, number of branches, nothing exactly the same. Differences in ecological conditions greatly affect the distribution, morphological variation of species (Herbert et al., 2016). In addition to being influenced by the characteristics of phenolic compounds that are owned by the plant itself, the chemical composition of the plant can also be affected by environmental factors. Differences in environmental conditions can affect plant variety (Lisdawati, 2002). For example, phosphate levels in the aquatic environment can affect the phytochemical content of seaweed. At the study site, phosphate levels were recorded to be very low at 0.0037–0.0041. With low phosphate levels, Doda Bahari waters are considered less fertile for seaweed growth (Safia et al., 2020). Thus, it is important to conduct this research with the aim of determine the effect of differences in *Caulerpa lentillifera* habitat and solvent types on chemical content, bioactive compounds, and antibacterial activity.

## METHOD

This research was conducted from May to August 2024. The samples used in this study were samples of Cultivation Caulerpa and Wild Caulerpa. Cultivated caulerpa was obtained from Pokdakan Indo Marine Lamongan Farmer Group and PT Bulung Bali Sejahtera. While wild caulerpa samples were obtained from the waters of the North Sea of Java around the Pokdakan Indo Marine Lamongan Farmer Group. This study used RAL (Completely Randomized Design) experimental design with ANOVA analysis. The treatments given in this study were different types of solvents and caulerpa living habitat. The types of solvents used in this study were ethanol and n-hexane.

*Caulerpa lentillifera* samples were previously washed thoroughly and then dried. The drying process was carried out by aerating at room temperature. After that, the dried samples were sorted and cut into small pieces and mashed. Once smooth, caulerpa samples are ready to be extracted with ethanol and n-hexane solvents. Extraction is done by massage method based on Marraskuranto et al (2021).

**Main Parameters**

***Total phenol testing***

The total phenolic content of Caulerpa *C. lentillifera* was determined using the method of Farasat et al (2014). The test was conducted using Folin Ciocalteu reagent. 200 µL sample extract was mixed with 1000 µL Folin Ciocalteu reagent (1:10). During the mixing process, 800 mL of Na2CO3 (7.5%) was added. After all mixed, the sample was incubated at room temperature for 2 hours in dark conditions. After 2 hours of incubation, absorbance was recorded at 765 nm. Different concentrations of gallic acid (5-100 µg/mL) were used to establish a standard curve. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry extract.

***Flavonoid testing***

Flavonoid content testing was determined based on the method of Cox et al (2010). The test was conducted using alumunium chloride. Aliquots of 250 µL of extract (1 mg/mL) and quercetin (10-100 µg/mL). This solution was used for the calibration curve, then mixed with 1,25 ml of distilled water and 75 µL of 5% NaNO2 solution. After 6 minutes, the addition of 10% AlCl3 solution as much as 150 µL. Then after 5 minutes of incubation, the addition of 1 M NaOH solution as much as 0.5 mL into the mixture. The total volume is made up to 2.5 mL with distilled water. The mixing of the solution was done well then the absorbance of the blank was determined at 510 nm. Flavonoid content was expressed as mg quercetin equivalent (QE)/g dry extract.

***Antioxidant testing***

Radical scavenging activity was determined using the method of Brand Williams et al (1995) using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Dry *Caulerpa lentillifera* as much as 0.5gr was extracted using 10 ml ethanol solution, then the mixture was centrifuged at 5,000 rpm for 10 minutes. The 1.5 ml sample solution was then mixed with 0.5 ml of 0.5 mM DPPH. Then the sample was kept in a dark place at room temperature for 30 minutes. Spectrophotometric absorbance was then measured at 517 nm. DPPH free radical inhibition activity was calculated using the following equation:

$$\text{Radical scavenging (\%)} = [(A0 - A1)/A0] \times 100$$

Note: A0 is the control absorbance and A1 is the sample absorbance.

***Chlorophyll testing***

Total chlorophyll in this study was conducted based on the method conducted by Hendriyani & Setiari (2009). A 5-gram sample was extracted using distilled water using a blender. The extract obtained was then transferred into a cuvette and the absorbance was measured with a spectrophotometer. After the results were obtained, the chlorophyll content was measured using the formula below.

$$\text{Chlorophyll a} = 1.07\ (OD663) - 0.094\ (OD644) \quad (1)$$

$$\text{Chlorophyll b} = 1.77\ (OD644) - 0.28\ (OD663) \quad (2)$$

***Carotenoid testing***

Carotenoid testing on *Caulerpa lentillifera* samples was carried out based on the method of Thirumaran & Anantharaman (2009). Samples of 500mg were pulverized and put into 10 ml of 80% acetone until homogeneous. After that, the sample was centrifuged at 3000 rpm for 15 minutes. The supernatant obtained was then re-extracted by washing using 5 ml of 80% acetone until colorless. After that, the extract obtained was used to determine photosynthetic pigments with the appropriate absorbance, namely 645 nm and 663 nm. The carotenoid extract was measured with an absorbance of 480 nm. Then the absorbance results were entered into the following formula:

$$\text{Carotenoids (mg/g)} = \Delta A\ 480 + (0.114 \times \Delta A\ 663) - (0.638 \times \Delta A645)$$

***Bioactive compound testing***

Testing of Caulerpa bioactive compounds was carried out using GC-MS which refers to the method of Nagaraj & Osborne (2014) with minor modifications. *Caulerpa lentillifera* extract was analyzed for the presence of bioactive compounds by using a Perkin Elmer GC model (30 m x 0.25 mm x 0.25 µm) Clarus 680 (Clarus 600 EI mass spectrometer). The carrier gas used in this test was pure helium with a constant flow rate of 1 ml/min. After that, one microliter of sample was injected and the oven temperature was programmed from 60°C to 300°C for 2 minutes at a rate of 10°C/minute and then held isothermally for 6 minutes until the end of the analysis.

***Antibacterial activity testing***

The method of making liquid media refers to the method by Ortez (2005). Making liquid media is done by adding 0.5g peptone, 0.3g meat extract, 0.3g sodium chloride, and 100ml distilled water and stirring well. After that, it was homogenized using a *magnetic stirrer*. After homogeneous, the liquid media was then autoclaved at 121° Cfor 15 minutes and pH measurements were taken with pH paper. The finished liquid media was then taken with a pipette as much as 1mL of media and put into a test tube and covered with alumunium foil. The liquid media is ready to be used for the bacterial culture process.

The bacterial culture method used in this study refers to Silap et al (2020). Liquid media that has been made before is added with cultured bacteria. The bacteria used in this study were *E. coli* and *S. aureus*. Bacterial cultures were added to the liquid media as much as 100µL each with different test tubes. After that, test tubes containing liquid media and bacterial cultures were covered using alumunium foil, then put into an incubator for 1x24 hours at 37$^{o}$C. The next step is preparation positive and negative control.

The manufacture of positive and negative controls in the study refers to Silap et al (2020). Based on the method used, the positive control uses chloramphenicol *paper disc*. As for the negative control using methanol solvent. The preparation of methanol solution was carried out by taking 200 µL of methanol and then bottled on paper discs. The preparation of the test solution refers to the method of Ortez (2005). The step of making the test solution by taking 2mg of *Caulerpa lentillifera* extract and dissolving it with 400µL methanol. So that the total concentration of the test solution was 250 µg / 50µL. The same method for the other fractions. After this, agar media creation process.

Preparation of agar media was carried out based on the method of Silap et al (2020) by mixing 0.5g peptone, 0.3g *meat* extract, 0.3g sodium chloride, 1.5g agar, and 100 mL of distilled water. Then the mixture was homogenized using a *magnetic stirrer* and autoclaved at 121$^{o}$C for 15 minutes. After that, pH testing with pH paper. The agar medium is cooled and ready to be used for antibacterial activity testing.

Antibacterial activity was carried out using the agar diffusion method (Kirby and Bauer disk diffusion). Antibacterial activity testing was carried out using a 6 mm paper disc with an absorbency of 50 µL. Test solution samples were then bottled on each disc with a micropipette. Agar media that has been autoclaved is cooled to a temperature of 40$^{o}$C. After that, agar media is put into a petri dish and take bacteria that have been cultured as much as 100 µL and inoculated on agar media. Previously, the agar media must harden first. After that, each petri dish was labeled. Then the disc paper that has been bottled with the *C. lentillifera* test sample is placed into the petri dish and incubated for 1x24 hours then clear zone observation.

Observation and measurement of clear zones were carried out based on the method of Susanto et al (2012). The observation process is carried out after the incubation period is complete. If clear zones is formed around the disk, it can be concluded that there is a bacterial reaction to the antibiotic or antibacterial material used as a tets sample. After obtaining clear zone measurements were made of the diameter of the clear zone formed using scaled ruler. The measurement is done by measuring the horizontal clear zone diameter plus the length of the vertical clear zone diameter and divided by two. If the diameter results is ≤ 5 mm, it is declared to have weak inhibition. If the diameter is between 6-10 mm it is stated to have moderate inhibition. Strong inhibition, if the diameter of the result is 11-20 mm, and if the diameter ≥ 21 mm then it has a very strong inhibition.

## Supporting Parameters

### *Temperature*

Temperature testing was carried out using a mercury thermometer. The thermometer is inserted into the water surface for approximately 2 minutes until the scale on the thermometer stabilizes.

### *pH*

The pH test was conducted based on the method of Eviati (2005). The pH test was conducted using a pH meter. Before use the pH meter is calibrated first and dried. After that the pH meter is inserted into the water sample and waited until the value stabilizes.

### *Dissolved oxygen (DO)*

The DO testing method refers to the method conducted by Andria & Rahmaningsih (2018). Dissolved oxygen measurements were carried out using a DO meter. Before use, the DO meter must first be calibrated and dried. The measured DO and temperature values can be seen based on the scale that appears on the tool.

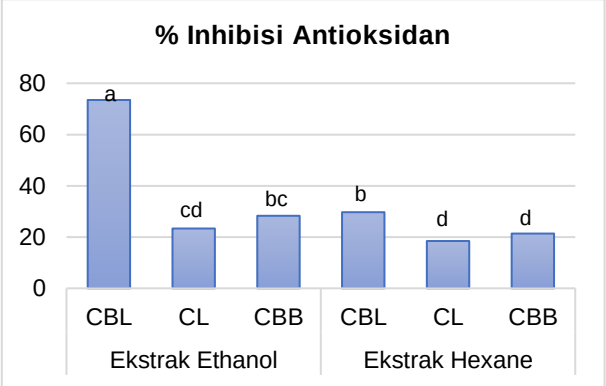
### *Salinity*

Salinity measurements in waters are carried out referring to the Failu et al (2021) method. Salinity measurements were taken using a hand refractometer. Before use, the tool is calibrated first using distilled water and dried. Then a water sample was taken using a drop pipette, and placed on the objective glass of the hand refractometer. After that, the tool is faced towards a bright area and seen the scale that appears on the tool that indicates salinity.

## RESULT AND DISCUSSION

### Antioxidant Activity

Antioxidants are a type of active compound that can counteract free radicals in the body. Free radicals in the body can damage the structure of body cells, proteins, and DNA. The results of antioxidant activity analysis in this study can be seen in Figure 1.




*CBL (Caulerpa Cultivation Lamongan); CL (Wild Caulerpa); CBB (Caulerpa Cultivation Bali)

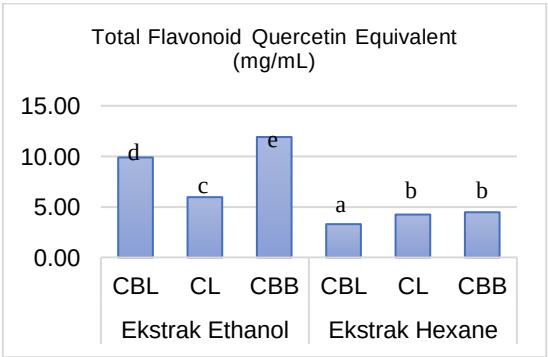
**Figure 1**. Percent antioxidant inhibition of ethanol and n-hexane extracts of *C.lentillifera*

Based on Figure 1, it can be seen the ethanol and n-hexane extracts of CBL (Caulerpa Cultivation Lamongan) have the highest IC50 value compared to the extracts. The % antioxidant inhibition of CBL ethanol extracts was 73,48% while that of n-hexane extracts was 29,7%. In second place, the ethanol extract samples of CBB (Caulerpa Cultivation Bali) has an IC50 value of 28,3% and the n-hexane extract of CBB is 21,37%. The IC50 value of the CL (Wild Caulerpa) ethanol sample was 23.34% and the n-hexane extract produced a value of 18.46%. Based on ANOVA analysis, the results of antioxidant testing of ethanol and n-hexane extracts of *C. lentillifera* showed significantly different results ($P < 0.05$). It can be concluded that the treatment given and differences in the living habitat of *C. lentillifera* can affect the amount of antioxidant content.

All samples of ethanol extract of *C. lentillifera* have higher antioxidant value compared to n-hexane extract. These results are similar to research conducted by Gazali et al (2021), namely the antioxidant activity of crude ethanol extract has a value of 46.45±4.03 µmol trolox/g and followed by crude ethyl acetate extract (23.74±0.035 µmol trolox/g) and crude n-hexane extract (21.56±0.028µmol trolox/g). If the percentage of DPPH method results is more than 90% then this indicates very high antioxidant activity (>90%), high antioxidant activity between 50%-90%, moderate antioxidant between 20-50%, low antioxidant activity less than 20%, and if antioxidant activity is 0% it is concluded that there is no antioxidant activity (Firda et al., 2022).

## Total Flavonoids

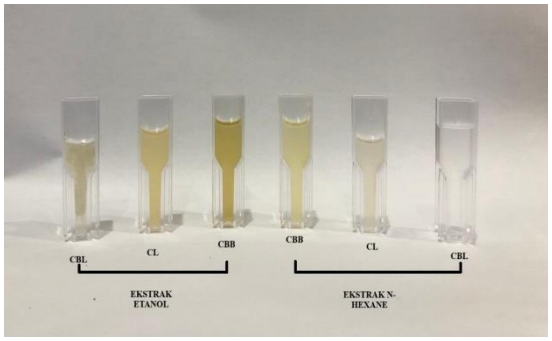
Flavonoids in *Caulerpa lentillifera* extract showed differences in each treatment given. The results of flavonoid testing can be seen in Figure 2.




*CBL (Caulerpa Cultivation Lamongan); CL (Wild Caulerpa); CBB (Caulerpa Cultivation Bali)

**Figure 2**. Total flavonoid quercetin equivalent (mg/mL)

Based on Figure 2, it shows that the highest flavonoid content was produced by the ethanol extract of sample CBB (Caulerpa Cultivation Bali) at 11.91 mg/ml. Also, the lowest flavonoid value was produced by n-hexane extract of CBL (Caulerpa Cultivation Lamongan) sample at 3.29 mg/ml. Flavonoid test on sea grapes (Caulerpa) shows that this compound is present in Caulerpa when the extract changes color to red, yellow, or orange. Color changes in Caulerpa samples can be seen in Figure 3.



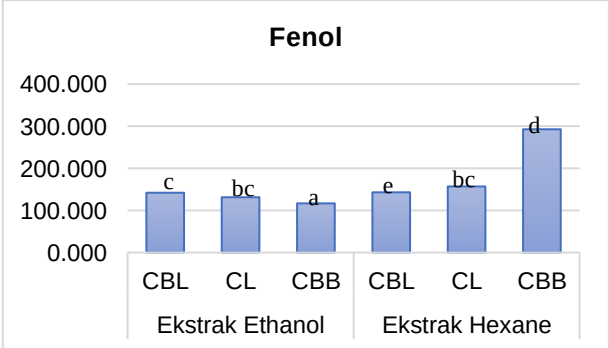

*CBL (Caulerpa Cultivation Lamongan); CL (Wild Caulerpa); CBB (Caulerpa Cultivation Bali)

**Figure 3**. Color of caulerpa sample after flavonoid testing

Based on the Figure 3, extracts with lighter and more concentrated colors contain high flavonoids. The flavonoid content produced by the test samples in ethanol extracts ranged from 5.96-11.91 mg/ml. The sample with n-hexane extract showed flavonoid content ranging from 3.29-4.47 mg/ml. The ethanol extract of *Caulerpa lentillifera* has a higher flavonoid content than the n-hexane extract, this is because flavonoids can dissolve well in polar solvents, such as ethanol, methanol, acetone dimethylformamide, butanol, and others (Arifin & Ibrahim, 2018). According to Koodkaew et al (2024), the flavonoid content of sea grapes from Trang ranged from 22.21±0.95 to 23.05±0.85 QE/g. Extracts of sea grapes from Petchaburi ranged from 11.56±2.36 to 19.15±2.78 mg QE/g. Based on this, the flavonoid results in this study are low. Also, the results in this study are similar to the results obtained by Srinorasing et al (2021), which states that the flavonoid content is less, namely 5.40±0.76 mg QE/g.

## Total Phenol

Phenol is one of the active compounds included in the antioxidant class. The content of phenol compounds in seaweed is positively correlated with its antioxidant capacity. The higher the phenol content, the higher the antioxidant activity (Cahyaningrum et al., 2016; Sedjati et al., 2018). Based on the analysis that has been done on the sample shows that the sample with n-hexane extract has higher phenol than the ethanol extract sample (Figure 4).




*CBL (Caulerpa Cultivation Lamongan); CL (Wild Caulerpa); CBB (Caulerpa Cultivation Bali)

**Figure 4**. Phenol value of ethanol and n-hexane extracts of caulerpa

CBB (Caulerpa Cultivation Bali) n-hexane extract has the highest phenol content of 292.867 mg/mL. The lowest phenol content was produced by the ethanol extract sample of CBB. These result are higher than the result of research conducted by Nurjanah et al (2019), which produced total phenol in *C.lentillifera* is 42,58mg GAE/g. The difference in total phenol results obtained with existing literature can be caused by various factors. One of them can be influenced by the type of solvent used. Another factor that can affect the amount of phenols and flavonoids in *Caulerpa* sp. is environmental factors. Environmental factors such as differences in *Caulerpa* sp. habitat (Nurjanah et al., 2019). Rajauria et al (2016) stated that polyphenol levels in seaweed are influenced by various factors, such as species, season, harvest age, harvest time, and geographic location.

**Total Chlorophyll and Carotenoids**

Chlorophyll and carotenoids are antioxidant compounds that are found in many plants. The content of chlorophyll and carotenoids in a plant is influenced by the age of the plant.

**Table 1**. Chlorophyll and carotenoid assay results of ethanol and n-hexane extracts caulerpa

| Sample | Total Chlorophyll (mg/L) | Carotenoid (µmol/L) |
|---|---|---|
| CBL | 0,25 | 1,40 |
| CBB | 0,87 | 4,11 |
| CL | 0,50 | 2,21 |

*CBL: Caulerpa Cultivation Lamongan; CBB: Caulerpa Cultivation Bali; CL: Wild Caulerpa

Based on Table 1 above, the highest value of total chlorophyll was 0.87 mg/L produced by the CBB sample. The lowest total chlorophyll was produced by CBL. While the highest amount of carotenoids was produced by the CBB sample with 4.11 µmol/L. The lowest amount of carotenoids was produced by the CBL sample at 1.40 µmol/L. Chlorophyll and carotenoids can be detected by the appearance of color changes. The colors produced by chlorophyll and carotenoid pigments are green, yellow, and red. In this study, it is evident that CBB has the highest value in accordance with the color change. The color produced by the CBB sample looks more intense yellow than the others. Color changes in CBL, CBB, and CL samples can be seen in Figure 5.

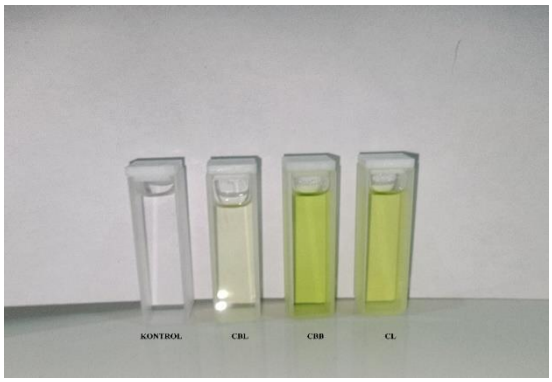



**Figure 5**. Chlorophyll and carotenoid testing results of caulerpa

The result of chlorophyll analysis in this study are lower than the result of the research conducted by Samad et al (2021) which states that *Caulerpa* sp. seaweed has a higher color pigment content compared to *Ulva lactuca* seaweed which is 12,96

mg/L, while the chlorophyll content of *Ulva lactuca* is 6,79 mg/L. Based on the result of this analysis in genotypes that have high chlorophyll content, the carotenoid is also high (Reswari et al., 2019; Minsas et al., 2023). The level of carotenoids will be comparable to chlorophyll because of its role in supporting chlorophyll in the process of light absorption (Maliya et al., 2019). This statements is in accordance with the result of the research obtained, the results of chlorophyll and carotenoids are directly proportional. Chlorophyll and carotenoids in *C.lentillifera* are influenced by several factors, one of which is the living habitat. The living habitat of the samples used in this study comes from different habitats. CBL and CBB samples are samples cultivated with different methods, while CL samples are caulerpa that live in wild waters. These habitat differences can result in different amounts of chlorophyll and carotenoids. One of the factors that affect the amount of chlorophyll in a plant is the cultivation method (Firdaus et al., 2022). Light intensity conditions, extraction process, and harvest season can affect pigment levels (Mahfudh et al., 2021). Plants adjust the amount of pigment according to the surrounding light conditions, which indicates the ability of the species to adapt physiologically to a particular environment (Riechert & Dawes, 1986).

**Antibacterial Activity**

Antibacterials are compounds that can inhibit or stop the development of pathogenic bacteria that cause infection. Antibacterial activity testing was carried out using *S.aureus* and *E.coli* bacteria as test bacteria. Based on tests that have been carried out on *E.coli* test bacteria, it shows that there is no inhibition zone formed in the entire test sample. The test results of antibacterial activity against *E.coli* can be seen in Figure 6. The inhibition zone can be caused by the possibility that the extract contained in the test sample is too small so that it is unable to carry out its activity as an antibacterial substance.

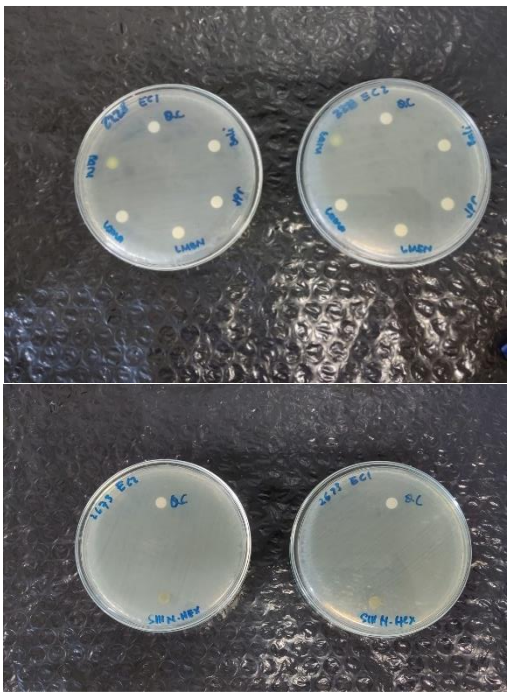


**Figure 6**. Results of antibacterial activity testing on ethanol and n-hexane extracts of caulerpa against *E.coli*

The result of this analysis are similar to the result of research conducted by Ritan et al (2021), which stated that the fraction extract from *Caulerpa racemose* algae showed no effect on gram negative bacteria *Eschericia coli.* This is due to the difference in cell structure between the two bacteria *E.coli* and *S.aureus. E.coli* bacteria have cell walls that are rich in fat and arranged in layers. The inhibition zone that is not formed or the small inhibition zone formed can be caused by various factors, such as the type of bacteria used in the study, the content of active compounds in the test extract, and the concentration of the test extract given. (Jawetz et al., 2012). In addition, the non-formation of inhibition zones can also be influenced by the length of storage. The longer the extract is stored, the greater the potential for a decrease in the activity of the extract used (Andriyana et al., 2021).

In addition to *E.coli* as the test bacteria, this test uses other test bacteria, namely *Staphylococcus aureus* as the second test bacteria. Testing ethanol and n-hexane caulerpa extracts against *S.aureus* showed that inhibition zone formation only occurred in two test samples. The inhibition zone was formed in the n-hexane extract of Caulerpa Cultivated Bali (CBB) with an inhibition zone of 0.5 mm and the n-hexane extract of Caulerpa Wild (CL) of 1.5 mm. It can be concluded that in this study the inhibition zone was only formed in the n-hexane extract of CBB and CL with the test bacteria *S.areus*. the inhibition zone formed from these two samples is very small. The results of antibacterial activity testing on *Staphylococcus aureus* test bacteria can be seen in Figure 7.

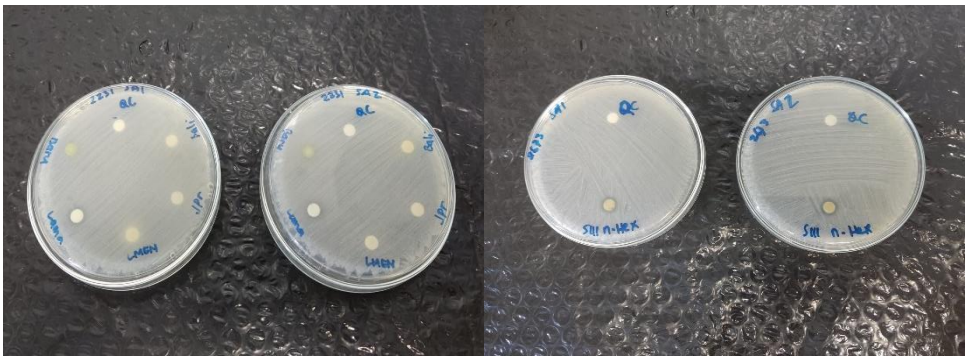


**Figure 7**. Results of antibacterial activity testing on ethanol and n-hexane extracts of caulerpa against *S.aureus*

The clear zone formed in the CBB and CL samples is relatively small compared to the results of the research by Ritan et al (2021), the results obtained in testing caulerpa extracts against *S.aureus* showed that the formation of inhibition zones in the methanol fraction was around 7.32 mm, in the n-hexane solvent of 7.53 mm, and in the extract with ethanol solvent of 6.72 mm. The results of research conducted by Montolalu et al (2019) stated that *S.aureus* was more sensitive to *C.sertulariodes* extracts with methanol, n- hexane, and ethyl acetate. On the other hand, *E.coli* is more sensitive to water solvents. This is due to differences in bacterial sensitivity to a bioactive compound caused by differences in bacterial cell wall structure. The absence of inhibition zone in antibacterial activity testing can be influenced by many factors. The difference in antibacterial activity of a sample against test bacteria is caused by habitat factors, sampling time, seaweed growth stage, extraction method, extraction solvent, and others (Adaikalaraj et al., 2012).

## Bioactive Compound

Bioactive compound are active compounds that can have biological effects on the body. In this study, various types of bioactive compound derivatives were found

from caulerpa extract. Bioactive compounds found in this study come from the group of alkanes, alkenes, alcohol, stearic acid, palmitic acid, and other fatty acids derivatives. The results of the analysis of this research sample can be seen in the table below.

**Table 2**. Analysis results of bioactive compounds in ethanol extract of CBB (Caulerpa Cultivation Bali)

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 1. | Nonadecane | $C_{19}H_{40}$ | 0,68 |
| 2. | Heneicosane | $C_{21}H_{44}$ | 16,78 |
| 3. | Iron, tricarbonyl [N-phenyl-2-pyridinylmethylene) benzenamine-N,N'] | $C_{21}H_{14}FeN_2O_3$ | 1,72 |
| 4. | 9 Octadecenoic acid (Z)-(CAS) | $C_{18}H_{34}O_2$ | 1,56 |
| 5. | Nonadecane | $C_{19}H_{40}$ | 6,58 |
| 6. | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 0,80 |
| 7. | n-Hexadecanoic acid (asam palmitat) | $C_{16}H_{32}O_2$ | 2,61 |
| 8. | 9-Octadecenoic acid | $C_{18}H_{34}O_2$ | 1,27 |
| 9. | n-Hexadecanoic acid (asam palmitat) | $C_{16}H_{32}O_2$ | 2,40 |
| 10. | 1-Nonadecane | $C_{19}H_{40}$ | 0,44 |
| 11. | Nonadecane | $C_{19}H_{40}$ | 6,58 |
| 12. | 1-Decanol 2-Hexyl | $C_{16}H_{34}O$ | 30,00 |
| 13. | Octadecanoic acid | $C_{18}H_{36}O_2$ | 2,98 |
| 14. | Heneicosane | $C_{21}H_{44}$ | 16,78 |
| 15. | Eicosane (CAS) | $C_{20}H_{42}$ | 0,87 |
| 16. | Heneicosane | $C_{21}H_{44}$ | 15,67 |
| 17. | Hexadecane (CAS) | $C_{16}H_{34}$ | 1,38 |
| 18. | Nonadecane | $C_{19}H_{40}$ | 12,01 |
| 19. | Eicosane (CAS) | $C_{20}H_{42}$ | 0,83 |
| 20. | 1,2 Benzenedicarboxylic acid, mono (2-ethyl hexyl) ester | $C_{16}H_{22}O_4$ | 0,46 |

**Table 3**. Analysis results of bioactive compounds in ethanol extract of CL (Wild Caulerpa)

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 1. | n-Hexadecanoic acid | $C_{17}H_{32}O_2$ | 29,28 |
| 2. | Heptadecane-8-carbonic acid – (1) | $C_{17}H_{32}O$ | 45,51 |
| 3. | Tetra pentacontane, 1,54-dibromo | $C_{54}H_{108}Br_2$ | 9,30 |
| 4. | Tetra pentacontane, 1,54-dibromo | $C_{54}H_{108}Br_2$ | 8,99 |
| 5. | 14-Beta-H-Pregna | $C_{21}H_{36}$ | 6,92 |

**Table 4**. Analysis results of bioactive compounds in ethanol extract of CBL (Caulerpa Cultivation Lamongan)

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 1. | Acetic acid | $CH_3COOH$ | 1,84 |
| 2. | Cyclotetrasiloxane, octamethyl | $C_8H_{24}O_4Si_4$ | 0,36 |
| 3. | 1-Octanol, 3,7-dimethyl (CAS) | $C_{10}H_{22}O$ | 0,40 |

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 4. | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 2,21 |
| 5. | 9-Hexadecenoic acid (CAS) | $C_{16}H_{30}O_2$ | 4,28 |
| 6. | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 48,56 |
| 7. | Oleic Acid | $C_{18}H_{34}O_2$ | 28,72 |
| 8. | Octadecanoic acid | $C_{18}H_{36}O_2$ | 4,63 |
| 9. | 1-Hentetracontanol (CAS) | $C_{41}H_{84}O$ | 6,99 |
| 10. | Heneicosane | $C_{21}H_{44}$ | 2,00 |

**Table 5**. Analysis results of bioactive compounds in n-hexane extract of CBB (Caulerpa Cultivation Bali)

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 1. | Cyclopropane, nonyl | $C_{12}H_{24}$ | 0,50 |
| 2. | Naphthalene (CAS) | $C_{10}H_8$ | 1,30 |
| 3. | 1-Tetradecene | $C_{14}H_{28}$ | 1,61 |
| 4. | Heptadecane | $C_{17}H_{36}$ | 1,27 |
| 5. | Heptadecane (CAS) | $C_{17}H_{36}$ | 1,13 |
| 6. | Phenol, 2, 4-bis (1,1-dimethylethyl) (CAS) | $C_{14}H_{22}O$ | 3,09 |
| 7. | 9-Octadecene (E) | $C_{18}H_{36}$ | 2,98 |
| 8. | Pentadecane (CAS) | $C_{15}H_{32}$ | 2,38 |
| 9. | Heptadec-8-ene | $C_{17}H_{34}$ | 1,70 |
| 10. | 1-Heptadecene (CAS) | $C_{17}H_{34}$ | 0,63 |
| 11. | Heptadecane | $C_{17}H_{36}$ | 4,06 |
| 12. | 1-Hexadecanol (CAS) | $C_{16}H_{34}O$ | 3,64 |
| 13. | Pentadecane (CAS) | $C_{15}H_{32}$ | 2,36 |
| 14. | Neophytadiene | $C_{20}H_{38}$ | 3,43 |
| 15. | 2-Pentadecanone, 6, 10, 14 trimethyl (CAS) | $C_{18}H_{36}O$ | 7,25 |
| 16. | 3, 7, 11, 15-Tetramethyl-2-hexadecene-1-01 | $C_{20}H_{40}O$ | 1,23 |
| 17. | 9-Eicosene (E) | $C_{20}H_{40}$ | 1,38 |
| 18. | 3, 7, 11, 15- Tetra methyl-2-Hexadecen-1-01 | $C_{20}H_{40}O$ | 3,77 |
| 19. | 1-Nonadecene | $C_{19}H_{38}$ | 0,84 |
| 20. | Iron, tricarbonyl [N-(phenyl-2-pyrydinyl methylene) benzenamine-N, N] | $C_{21}H_{14}FeN_{20}$ | 1,52 |
| 21. | 2-Pentadecanone, 6, 10, 14 trimethyl (CAS) | $C_{18}H_{36}O$ | 0,95 |
| 22. | Hexadecanoic acid, methyl ester (CAS) | $C_{17}H_{34}O_2$ | 2,70 |
| 23. | Isophytol | $C_{20}H_{40}O$ | 0,48 |
| 24. | 1-Hexadecanol (CAS) | $C_{16}H_{34}O$ | 4,01 |
| 25. | Octadecane | $C_{18}H_{38}$ | 2,52 |
| 26. | 1-Heptadecene (CAS) | $C_{17}H_{34}$ | 0,67 |
| 27. | Dodecane, 1-iodo | $C_{12}H_{25}I$ | 1,86 |
| 28. | 11-Octadecenoic acid, methyl ester | $C_{19}H_{36}O_2$ | 2,41 |
| 29. | Piperitol isomer 1 (cis) | $C_{12}H_{20}O_2$ | 0,48 |
| 30. | Phytol | $C_{20}H_{40}O$ | 10,15 |
| 31. | Palmitaldehyde, diallyl acetat (CAS) | $C_{19}H_{32}O_3$ | 1,34 |
| 32. | 1-Nonadecene | $C_{19}H_{38}$ | 4,52 |
| 33. | Hexadecane (CAS) | $C_{16}H_{34}$ | 2,50 |
| 34. | Neophytadiene | $C_{20}H_{38}$ | 4,06 |

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 35. | Heneicosane | $C_{21}H_{44}$ | 2,44 |
| 36. | 3,7-Dimethyl-1-octyl methylphosphono fluoridene | $C_{11}H_{24}FO_2P$ | 1,36 |
| 37. | Heneicosane | $C_{21}H_{44}$ | 4,84 |
| 38. | Heneicosane | $C_{21}H_{44}$ | 1,83 |
| 39. | 1,2-Benzene dicarboxylic acid, 3-nitro- | $C_8H_5NO_6$ | 0,73 |
| 40. | Tetra tetra contane (CAS) | $C_{44}H_{90}$ | 4,07 |

**Table 6**. Analysis results of bioactive compounds in n-hexane extract of C (Wild Caulerpa)

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 1. | Neophytadiene | $C_{20}H_{38}$ | 0,98 |
| 2. | Hexadecenoic acid methyl ester (CAS) | $C_{17}H_{34}O_2$ | 21,39 |
| 3. | 1,2-Benzenedicarboxylic acid, bis (2-methoxyethyl) ester (CAS) | $C_{14}H_{18}O_6$ | 0,88 |
| 4. | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 17,86 |
| 5. | 9,12-Octadecadienoic acid, methyl ester (CAS) | $C_{19}H_{34}O_2$ | 4,56 |
| 6. | 9 Octadecenoic acid (2)-methyl ester (CAS) | $C_{19}H_{36}O_2$ | 28,45 |
| 7. | 2 Hexadecen-1-01,3,7,11,15-tetra methyl-[R-[R*,R*-E] (CAS) | $C_{20}H_{40}$ | 1,54 |
| 8. | Octadecenoic acid, methyl ester (CAS) | $C_{19}H_{30}O_2$ | 3,40 |
| 9. | Oleic acid | $C_{18}H_{34}O_2$ | 10,73 |
| 10. | 9,12 Octadecadienoic acid (2,2) methyl ester (CAS) | $C_{19}H_{34}O_2$ | 1,64 |
| 11. | Hexadecenoic acid, 2 hydroxyl-1-(hydroxymethyl) ethyl ester (CAS) | $C_{19}H_{38}O_4$ | 1,48 |
| 12. | Hexadecenoic acid, 2-hydroxyl-1,3-propanedyl ester (CAS) | $C_{37}H_{72}O_6$ | 1,90 |
| 13. | 9 Tetradecenal (Z) | $C_{14}H_{26}O$ | 1,71 |
| 14. | 9 Octadecenal (Z)-(CAS) | $C_{18}H_{34}O$ | 1,94 |
| 15. | Hexadecenoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester (CAS) | $C_{19}H_{38}O_4$ | 1,53 |

**Table 7.** Analysis results of bioactive compounds in n-hexane extract of CBL (Caulerpa Cultivation Lamongan)

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 1. | 1,3,5,7-Cyclooctatetraene (CAS) | $C_8H_8$ | 81,87 |
| 2. | Silane, trichloro docosyl | $C_{22}H_{45}Cl_3Si$ | 0,87 |
| 3. | Heptane, 3 ethyl 2 methyl | $C_{10}H_{22}$ | 0,85 |
| 4. | Dodecane (CAS) | $C_{12}H_{26}$ | 0,32 |
| 5. | Benzaldehyde | $C_7H_6O$ | 2,91 |
| 6. | Cycloheptane, methyl | $C_8H_{16}$ | 0,66 |
| 7. | Decane | $C_{10}H_{22}$ | 2,86 |

| Peak | Compound | Chemical Formula | Area (%) |
|---|---|---|---|
| 8. | Nonane, 2, 6-Dimethyl | $C_{11}H_{24}$ | 0,34 |
| 9. | 1-Tetracosanol (CAS) | $C_{24}H_{50}O$ | 0,55 |
| 10. | Benzyl isobutyl ketone | $C_{12}H_{16}O$ | 0,39 |
| 11. | Heptane, 3, 3, 5-trimethyl | $C_{10}H_{22}$ | 0,32 |
| 12. | Decane, 2 methyl | $C_{11}H_{24}$ | 0,87 |
| 13. | Decane, 3 methyl | $C_{11}H_{24}$ | 0,80 |
| 14. | Benzene, methyl (1-methylethyl) (CAS) | $C_9H_{12}$ | 0,53 |
| 15. | Dodecane (CAS) | $C_{12}H_{26}$ | 2,19 |
| 16. | Dodecane (CAS) | $C_{12}H_{26}$ | 0,32 |
| 17. | Butyl hydroxy toluene | $C_{15}H_{24}O$ | 0,56 |
| 18. | Pentadecane (CAS) | $C_{15}H_{32}$ | 0,23 |
| 19. | 2-Pentadecanone, 6, 10, 14 trimethyl (CAS) | $C_{18}H_{36}O$ | 0,44 |
| 20. | Iron-tricarbonyl [N-(phenyl-2-pyridinylmethylene) benzenamine-N, N'] | $C_{21}H_{14}FeN_2O_3$ | 0,24 |
| 21. | 1-Nonadecene | $C_{19}H_{38}$ | 0,29 |
| 22. | Hexadecane (CAS) | $C_{16}H_{34}$ | 0,25 |
| 23. | 1-Nonadecene | $C_{19}H_{38}$ | 0,26 |
| 24. | 1,2-Benzenedicarboxylic acid, mono (2 ethylhexyl) ester | $C_{16}H_{22}O_4$ | 0,42 |
| 25. | 1-Tricosene | $C_{23}H_{46}$ | 0,35 |

Based on the tables above, it can be seen that various types of bioactive compounds were found in each sample. However, there are several types of compounds found in all ethanol and n-hexan extracts. These compounds include dodecane, decane, octadecanoic acid, hexadecane, heptadecane, and heneicosane. Heneicosane is a secondary metabolite compound that has antimicrobial effects against pathogenic microorganisms (Wijayanti & Dewi, 2022). Heneicosane is included in the high alkane group which has biopesticide properties (Rhetso et al., 2020). The alkane group is the dominant group in this test sample. Next are the stearic acid and palmitic acid groups. Stearic acid and palmitic acid are saturated fatty acids (Riski et al., 2020). The ability of fatty acid compounds, palmitic acid and stearic acid on the crystal surface shows antibacterial activity against gram-negative rod-shaped cells *Pseudomonas aeruginosa* and gram-positive round-shaped cells *Staphylococcus aureus* (Ivanova et al., 2017).

In addition to the alkane group, bioactive compounds derived from the alkaloid group were also found. Alkaloids are secondary metabolite compounds that have many nitrogen atoms and are found in plant tissues (Maisarah et al., 2023). Alkaloid compounds can be used as active substances that can fight bacteria, viruses, fungi, and cancer cells (Olivia et al., 2004). Alkaloids have antimicrobial activity by inhibiting esterase, DNA, RNA polymerase, and cell respiration. Alkaloids bind to ergosterol to form holes that can cause cell membrane leakage and lead to permanent damage so that it can cause death in fungal cells (Setiabudy et al., 2007; Maisarah et al., 2023). Diisobutyl phthalate, dibutyl phthalate, and mono-(2-etylhexyl) phthalate are the main compounds in the phthalate group that have strong bioactive activities as antifungal, antidiabetic, anticancer, and others (Parwanayoni & Sudirga, 2020).

Based on the analysis result of Table 3, in the ethanol extract of CL (Wild Caulerpa), the number of bioactive compounds found was the least. The active

compounds shown by CL ethanol extract were Heptadecene-8-carbonic acid-(1), n-Hexadecanoic acid, tetra pentacontane, 1,54-dibromo, dan 14-Beta-H-Pregna. The highest peak in this sample was produced by the compound Heptadecene-8-carbonic acid-(1) with a peak of up to 45,51%. This compound is a fatty acid that can interact with other compounds to produced antioxidant, antibacterial, and antiproliferative (Saputri, 2020; Zahra et al., 2021).

Tetrapentacontane compounds in this study appeared twice, namely at minutes 38.201 and 38.265. This can be caused by the similarity of the chemical structure between the two compounds although not identical so that they can be detected at different retention times. This possibility arises because in the GCMS analysis of sample data obtained with existing data in the literature so that its nature is still in conjecture and requires further research. Beta-H-Pregna is a secondary metabolite compound that belongs to steroids (Junairiah et al., 2019). In addition, several reports confirm the biological activity of Beta-H-Pregna showing antibacterial, antifungal and antioxidant effects (Gharari et al., 2019; Ghanbari et al., 2023).

Table 4 shows the results of bioactive compound analysis of Caulerpa Cultivation Lamongan (CBL) ethanol extract. Bioactive compounds were found that were different from other extracts, namely oleic acid. For many years it has been known that long-chain unsaturated fatty acids have antibacterial activity, such as linoleic and oleic acids which are bactericidal against pathogenic microorganisms, including *Staphylococcus* aureus, *Helicobacter pylori*, *Candida albicans*, *Candida albicans* resistant to methicillin. (Choi et al., 2013; Hendy et al., 2020).

Table 5 shows the presence of 40 bioactive compounds in the n-hexane extract of Caulerpa Cultivation Bali (CBB). The compounds found belong to various groups, namely alkane, ketone, alcohol, fatty acid, stearic acid, alkaloid, alkene, nitrophthalic acid, and aromatic compounds. Aromatic compounds found in the form of naphthalene (cas) compounds. This compound is volatile so that it is easy to evaporate (Arimbawa et al., 2019), although it is volatile but this compound has the ability as an antibacterial. The antibacterial ability of naphthalene rings on bacterial and fungal strains has been supported by many studies (Abdelfatah et al., 2023). Many naphthalene derivatives were synthesized and showed important and satisfactory antimicrobial activities (Makar et al., 2019; Kalariya et al., 2022).

The new compound found in the CBB n-hexane extract is a class of nitro phtalic acid or commonly called 1,2 benzenedicarboxylic acid 3 nitro. This compound has the ability as antifouling and antibacterial. Two of the four compounds that have antibacterial properties are Phythalic acid and 1,2-Benzenedicarboxylic acid because both are phenol compounds (Ridhwan et al., 2024). The active compound 1,2 benzendicarboxylic acid belongs to a class of fatty acids whose mechanism works by stopping or inhibiting the growth of pathogenic bacteria (Arista et al., 2020).

Based on Table 6, it can be seen that n-hexane extract of CL (Wild Caulerpa) produces 15 biactive compounds. These compounds can be used as anticoagulants, antibacterials, and antidiabetics. The bioactive compounds found in this extract have similarities with compounds found in other extracts. The highest peak was produced by the compound 9-octadecenoic acid (Z)- methyl ester while the chemical compound with the lowest area was produced by the compound neophytadiene. Neophytadiene is a diterpenoid compound found in many plants and marine algae (Dos Santos et al., 2020). This compound is one of the compounds that has potential as an antimicrobial and anti-inflammatory (Pratama et al., 2019). In addition, terpenoids have properties as antifungals and antioxidants (Venkata et al., 2012). According to research conducted Selmy et al. (2023), stated that neophytadiene has the ability as an

anticancer. Neophytadiene shows high affinity to Human LRH-1 and Human A2a Receptor.

The results shown in Table 7 indicate that the n-hexane extract of Caulerpa Cultivation Lamongan (CBL) contains 25 types of bioactive compounds. The compounds found came from the aldehyde, alcohol, and other groups. Based on the results obtained, there are compounds that have the highest peak, namely cyclooctatetraene and the lowest peak produced by pentadecane. Cyclooctatetraene is an organic compound that has an eight-carbon ring structure with four conjugated double bonds. This molecule does not belong to the class of aromatic compounds. The cyclooctatetraene molecule has a cis or Z double bond in a typical nonplanar tube-like shape (Wu & Schleyer, 2013; Li et al., 2017). While pentadecane is a volatile compound that is widely found in various types of plants (Szmigielski et al., 2012).

The volatile compounds found in CBL n-hexane extract are almost the same as the results of research conducted by Goksen (2023) on the components of bioactive compoundanns found in *L. papillosa*. Goksen (2023) stated that in *L. papillosa*, hydrocarbon groups including pentadecane, 1-pentadecene and heptadecane were found. The compound derived from the alcohol group is 1-Octen-3-ol, and the aldehyde group is benzaldehyde, and the compound derived from the ester group is 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro4,4,7a-trimethyl. However, it can be said that quantitative and qualitative differences in the components of volatile compounds are due to different species, growing regions, harvesting seasons, environments, extraction methods, and others (Kamernaska et al., 2006; Goksen, 2023).

**Temperature**

Temperature is one of the supporting parameters that support aquatic ecosystems. Water temperature can affect the physical, chemical, and biological properties of water and the organisms in it. Water temperature plays a role in supporting the good or bad quality of a body of water. This is because water quality is very important for the successful growth of *Caulerpa racemose* (Safitri & Rachmadiarti, 2023). Water quality includes physical and chemical parameters of waters that have an important role, this is because it can affect the development of seaweed (Cahyani & Ummah, 2020). If the water quality is poor or does not meet the standards for growth, then this can inhibit the development of seaweed and can result in a decrease in the quality of seaweed (Alamsyah, 2016).

**Table 8**. Results of temperature observations on the living habitat of *C.lentillifera*

| No. | Sample | Temperature ($^oC$) |
|---|---|---|
| 1. | Caulerpa Cultivation Bali (CBB) | 28,5 |
| 2. | Caulerpa Cultivation Lamongan (CBL) | 27,9 |
| 3. | Wild Caulerpa (CL) | 30,0 |

Based on the table above, it can be seen that the water temperature in each location or caulerpa living habitat is different. The water temperature ranges from 27,9$^o$C to 30$^o$C. The highest temperature is produced in the CL sample, where the CL sample has a living habitat in sea water. While the CBB and CBL samples are cultivated samples. However, the two samples have different cultivation methods. The CBB sample is cultivated using a round tarpaulin pond without other organisms while the CBL sample is cultivated in a fish pond. Location differences, handling differences, and natural conditions can affect water temperature.

Temperature values at the time of cultivation ranged from 27.4 - 27.5$^{o}$C. This temperature value is optimal and suitable for seaweed growth. This water temperature is good for Caulerpa growth (Apriliyanti et al., 2021). According to Tomascik et al., (1997) the optimal water temperature around seaweed plants (*Caulerpa* sp) ranges from 26-30$^{o}$C.

**pH**

pH or often called the degree of acidity is a standard for the acidity or basicity of a solution. pH can also affect aquatic ecosystems. Generally, waters have a pH value in the range of 6 to 8. This is because if the pH value is outside the range, it can result in disruption of the lives of aquatic organisms. The pH value produced in *Caulerpa lentillifera* waters used as research samples can be seen in Table 9.

**Table 9**. Results of pH observations of *C.lentillifera* habitat

| No. | Sample | pH |
|---|---|---|
| 1. | Caulerpa Cultivation Bali (CBB) | 8,4 |
| 2. | Caulerpa Cultivation Lamongan (CBL) | 8,1 |
| 3. | Wild Caulerpa (CL) | 8,3 |

The pH value of a water body determines the condition of acidic or alkaline waters that can affect biological life in it. Changes in the acidity of a water body can affect the survival of fish and other aquatic organisms (Djoharam et al., 2018). Each aquatic organism has a different pH value, this is influenced by the different types of organisms and the metabolic system of the body of each organism. Based on the results in table 10, it shows that the pH of caulerpa habitat waters ranges from 8.1 to 8.4. The results of the pH value are similar to the results of research conducted by Cahyanurani & Ummah (2020), pH measurements were taken in the morning with results between 8.3 to 8.6 with an average of 8.5.

The pH value of waters is strongly influenced by the amount of hydrogen ions, it can be concluded that if there is an increase in pH, hydrogen ions will decrease. While a decrease in pH occurs when the amount of hydrogen ions in the water increases. According to Kusmawati et al. (2018), the ideal water pH for the growth of sea grapes (*Caulerpa racemose*) ranges from 7-8.5. The research data obtained can be concluded to still belong to the optimum pH for the growth of sea grapes.

Differences in pH values are influenced by several factors. One of them is the factor of different cultivation methods between each sample and weather conditions when measuring pH. In addition, it can also be influenced by water CO2 levels. This is in line with the opinion of Rendiansyah et al (2024), which states that high or low pH in waters is influenced by CO2 levels dissolved in water. A decrease in CO2 levels can interfere with the photosynthesis process in seaweed that can affect its growth.

**Dissolved Oxygen (DO)**

Dissolved oxygen or DO is dissolved oxygen contained in the water. DO indicates the amount of dissolved oxygen needed by aquatic organism. Dissolved oxygen is one of the important chemical parameters in water measured to evaluate the carrying capacity of aquatic ecosystems through primary productivity or to assess the condition of a body water (Hamzah et al., 2022).

**Table 10**. DO observation results of *C. lentillifera* habitat

| No. | Sample | Dissolved Oxygen |
|---|---|---|
| 1. | Caulerpa Cultivation Bali (CBB) | 4,1 |
| 2. | Caulerpa Cultivation Lamongan (CBL) | 4,4 |
| 3. | Wild Caulerpa (CL) | 4,7 |

The DO values obtained ranged from 4.1 - 4.5 mg/L. The highest DO value was produced by the CL (Wild Caulerpa) sample with a DO level of 4.5 mg/L, while the lowest DO value was produced by CBB (Caulerpa Cultivation Bali). The higher the amount of DO (*Dissolved oxygen*), the better the water quality. If the DO level is low, the water quality is not good. Low DO values in waters can be an indicator of the low performance of the oxidation process by microorganisms or bacteria (Febiyanto, 2020). The results of research conducted by Kenedi et al. (2023) are different from the observations that have been made, namely the value of Do ranging from 6.00-8.00 ppm. However, according to Prayogi (2017) the optimal DO value for *Caulerpa racemosa* cultivation ranges from 3-6.7 ppm. While the DO value for seaweed is >5mg/L. It can be concluded that the observation results obtained are still optimal values for the survival of *Caulerpa lentillifera*.

Differences in dissolved oxygen content in each water body are caused by various factors. Dissolved oxygen (DO) content in waters can change because it is influenced by various factors such as temperature, salinity, water turbulence, and atmospheric pressure (Boyd, 1982). The need for oxygen in animals and aquatic plants varies depending on the type of species. Factors that affect the oxygen demand of aquatic biota include temperature, CO2 concentration, pH and body metabolic rate. The higher the water temperature, the greater the oxygen demand. It is also influenced by movement and body size. The larger the body size and movement, the higher the oxygen demand (Mustofa, 2019).

**Salinity**

Various factors can affect aquatic ecosystems, one of which is salinity. Salinity is the total amount of salt dissolved in water with units of parts per thousand (ppt or ‰) or grams per liter (g/L). Salinity is an important parameter in aquatic ecosystems because it affects the physical, chemical and biological characteristics of water. In addition, salinity plays a significant role in ocean circulation and the distribution of aquatic organisms.

**Table 11**. Salinity observation results on *C. lentillifera* habitat

| No. | Sample | Salinity (%) |
|---|---|---|
| 1. | Caulerpa Cultivation Bali (CBB) | 25 |
| 2. | Caulerpa Cultivation Lamongan (CBL) | 24 |
| 3. | Wild Caulerpa (CL) | 28 |

Based on observations of the salinity content of the waters of the living habitat of the research sample is different. The CBB (Caulerpa Cultivation Bali) sample has a salinity value of 25 ppt and the CBL (Caulerpa Cultivation Lamongan) sample is 24 ppt. while the CL (Wild Caulerpa) has a salinity of 28 ppt. the salinity obtained in this study has the same salinity levels as the results of research conducted by Ardiansyah (2020), which states that the result of salinity measurements taken every week show values ranging from 25-30 ppt.

It can be concluded that the results of research on CBB and CL samples belong to the optimum salinity for *Caulerpa lentillifera* survival. However, the results of the salinity of the CBL sample are below the optimum standard and the lowest compared to other samples. According to Guo et al (2014), *Caulerpa lentillifera* seaweed can survive in salinity between 20 to 50 ppt. However, caulerpa growth can only occur at salinities of 20 to 45 ppt.

Differences in salinity of caulerpa living habitat affect the physiological form and biochemical mechanisms. This is because salinity is related to changes in osmosis which is also related to the role of caulerpa cell membrane in the process of nutrient transport. Differences in salinity can affect the physiology of caulerpa, this results in differences in caulerpa body size, caulerpa ramuli size, and nutrient content. Some factors that affect salinity are light intensity. Light intensity can affect the photosynthesis rate of marine biota in the waters. If the light intensity is less, the photosynthesis rate will be low, so it can affect the salt content in the sea. In addition, the disruption of the photosynthesis rate due to too much energy received can affect the salinity of the waters.

According to Fakhrulddin et al (2021), there is a correlation between leaf length, water depth, and water salinity. When the water depth decreased, the leaf length of *Caulerpa lentillifera* increased along with the increase in salinity. It is concluded that physicochemical factors such as light intensity and salinity affect Caulerpa species. Factors that strongly influence salinity as one of the main parameters to describe the characteristics of waters include circulation patterns, tides, freshwater flow from rivers, and light intensity that affects the physical and chemical processes of the water column (Tubalawony et al., 2023). According to Patty et al (2020), states that the value of salinity in the sea is influenced by various factors such as water circulation patterns, evaporation rates, and rainfall. Seasonal changes result in significant variations in salinity values at the 1% confidence level with a considerable influence such as the Fcount result of 14.984 which differs by 0.01 from the 1% F table value (4.44).

## CONCLUSION

Based on the research it can be concluded that differences Caulerpa lentillifera habitat were shown to affect the content of antioxidants, flavonoids, phenols, chlorophyll, carotenoids, bioactive compounds and antibacterial activity. This is evidenced by antioxidant activity between 18.46%-73.48%, total flavonoids ranging from 3.29 mg/mL-11.91 mg/mL, total phenols ranging from 116,860 mg/mL-292,867 mg/mL, total chlorophyll 0.25 mg/L-0.87 mg/L, total carotenoids of 1.40mg/L-4.11 mg/L. Antibacterial activity was only shown by CBB and CL n-hexane extract samples with S. aureus test bacteria. The zone of inhibition formed on CBB n-hexane extract was 0.5 mm and CL n-hexane extract was 1.5 mm. While the E.coli test bacteria did not show any inhibition zone formed. The bioactive compounds produced in this study showed various groups of active compounds, namely alkanes, alkenes, alkaloids, alcohol, palmitic acid, and stearate acid.

## RECOMMENDATION

Further research is needed regarding the exploration of bioactive compounds from *Caulerpa lentillifera* that can be utilized more widely in the fields of Biotechnology and pharmaceuticals.

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