The Effect Extract Bioactive Compounds Seaweed *Codium* Sp On Total *Hemocyte C*ount (THC) OF Tiger shrimp (*Penaeus monodon*)

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Abstract -The research was conducted to analyze the content of bioactive compounds in the ethanol extract of seaweed *Codium* sp. and to determine the effect of the bioactive compound content of *Codium* sp on Total Hemocyte Count (THC) in tiger shrimp. *Codium* sp seaweed is obtained from the waters of Takalar, South Sulawesi. Codium sp seaweed powder was extracted using polar ethanol solvent (maceration method) with a ratio of 1: 3 (w / v). The results of phytochemical test analysis showed that *Codium* sp seaweed contained alkaloid bioactive compounds, tannins and saponins. The results of analysis of variance (ANOVA) of Codium sp seaweed extract with different doses showed no significant difference (P> 0.05). *Codium* sp seaweed extract with a dose of 0.5 g / kg of feed showed the highest THC value, namely 2.9 x 106 cells / mm3.

Keywords: Phytochemicals, ethanol extract, *Codium* sp, THC

I. INTRODUCTION

Seaweed or sea algae has a high level of species diversity. Seaweed contains various sources of natural bioactive compounds so that it has the potential to be used as an agent in the pharmaceutical world (Rangaiah *et al*., 2010). Some research results report that seaweed can increase antibacterial activity (Al-Haj et al., 2010). In addition, according to Lantah *et al*., (2017), several types of algae have the potential as prophylactic and immunostimulating materials because several types of algae contain chemical compounds and biological activity or have bioactive activity. Seaweed is considered a source of bioactive compounds because it can produce a wide variety of secondary metabolites characterized by a wide spectrum of biological activity (Cox *et al*., 2010). Most of the bioactive substances isolated from these marine algae are chemically classified as brominated, aromatic, nitrogen-heterocyclic, nitrosulfuric-heterocyclic, sterols, dibutanoid, protein, peptide and sulfate polysaccharides (Kolanjinathan *et al*., 2009). Alkaloids, flavonoids, terpenoids, and tannins, seta saponins are a secondary metabolite found in seaweed.

The content of secondary metabolites found in seaweed has the potential as an ingredient that can be used as an anti-bacterial and immunostimulant. Immunostimulants are chemical compounds or other substances that can trigger or increase the immune response of fish (Anderson, 1992). Alifuddin (1999) added that giving immunostimulants can increase both cellular and humoral immune response mechanisms in fish. Immunostimulants are an alternative to chemical antibiotics

that are effective in controlling disease for aquatic organisms, besides being safe for consumers and environmentally friendly, phagocytic activity (Felix *et al*., 2004; Yeh *et al*., 2006). One of the seaweeds that have the potential to have bioactive compounds and increase the total hemocytes in shrimp is the type of *Codium* sp.

Codium sp is one type of seaweed from the green algae class, this type is used by coastal communities as a vegetable, not much has been explored regarding the content of secondary metabolite compounds, secondary metabolite compounds contained in *Codium* sp seaweed can be determined by phytochemical test methods.

II. METHOD

This research was conducted from July to August 2020. *Codium* sp. Seaweed was obtained in Takalar waters, Takalar Regency, South Sulawesi, Indonesia. Phytochemical tests were carried out at the Laboratory of the Central Health Laboratory (BBLK) Makassar. Measurement of Total Hemocyte Count was carried out at the Laboratory of Fish Pests and Diseases, Faculty of Marine and Fisheries Sciences, Hasanuddin University, The test animal used was tiger shrimp (*Penaeus monodon*) with a size of ± 8 g / shrimp. The test animals were kept in a tank with a density of 10 animals / aquarium. This experiment was designed using a completely randomized design (CRD) with 4 treatments and 3 replications each. *Codium* sp seaweed extract was mixed into commercial shrimp feed with doses of 0, 0.5, 1, and 1.5 g / kg of *Codium* sp extract feed and maintained for 14 days.

Seaweed Extraction *Codium* sp

Seaweed is washed using seawater and fresh-water to remove salt, microorganisms, and other unwanted substances on the material, and dried. Then, the seaweed is finely ground and sieved using a fine sieve (60 mesh size). The extraction method was carried out by macerated seaweed powder for 24 hours using room temperature with ethanol solvent with a ratio (1: 3, w / v) where 100 g of *Codium* sp seaweed powder and 300 ml of ethanol solution. After 24 hours the extraction solution is filtered using filter paper, then evaporated using a rotary evaporator at a temperature of 50-60 ^{0}C to obtain a pure extract in the form of a paste. After that, phytochemical testing is carried out.

Analysis of Alkaloid Compounds

The alkaloid test was carried out based on the method used by Sangi *et al*., (2008) as much as 4 g of extract samples were then added to taste chloroform, then added 10 mL of ammonia and 10 mL of chloroform. Then the solution is filtered and put into a tube In the next reaction, the results of the filtrate were added 10 drops of H2SO4 2N, then shaken and left for a few minutes until two layers were formed. The layer above was transferred into a test tube as much as 1 ml. Then the tube is added a few drops of Meyer reagent. If a white precipitate is formed, this indicates that the sample contains alkaloids.

Analysis of Flavonoid Compounds

The flavonoid test was carried out according to the method of Sangi et al., (2008). The seaweed extract sample was taken as much as 200 mg then extracted with 5 ml of ethanol and heated for 5 minutes in a test tube. Then add a few drops of concentrated HCl. Then 0.2 g of Mg powder was added. A positive result is indicated by the appearance of a dark red color for 3 minutes.

Tannin Compound Analysis

The tannin test was carried out according to Sangi et al., (2008) where 20 mg of extracted seaweed samples were taken then added with ethanol solution until all samples were submerged. Next, add 2 to 3 drops of 1% FeCl3 solution. A positive result is indicated by the formation of a bluish-black or green color.

Saponin Compound Analysis

The saponin test was carried out according to Sangi et al., (2008) that the sample from the seaweed extract was taken as much as 2 g then put in a test tube, then added distilled water until the entire sample was submerged, then the

sample was boiled for 2 to 3 minutes, then the sample was cooled, then shaken. Positive results are indicated by the formation of stable foam.

Total Hemocyte Count (THC) Analysis

Take 0.1 ml of shrimp hemolime from the base of the first swim leg with a 1 ml tuberculin syringe (26G x ½ needle) which already contains 0.3 ml of anticoagulant, and put it into the tube. Take one drop of hemolime solution from the tube then drop it into a hemocytometer (improved Neubaur type, Merck, Lutterworth) then count the number of hemocyte cells counted per ml under a light microscope at 400 times magnification

THC = average sel x 25 x 10^4 sel/mL

The data obtained were then analyzed statistically with the ANOVA test method (analysis of variance) at a 95% confidence interval (a = 0.05).

III. RESULTS

The qualitative phytochemical test results show that the seaweed extract of *Codium* sp contains bioactive compounds, namely alkaloids, flavonoids, saponins and tannins, as shown in Table 1 below:

Table 1. Qualitative test of bioactive compounds in extracts of *Codium* sp

Bioactive compounds	Result	Indicator
Alkaloids	+	There is a white precipitate
Flavonoids	-	There are no red deposits
Saponins	+	There is foam
Tanin	+	There is a bluish-black color

Information (+) = positive there is an active compound, (-) negative there is no active compound

Alkaloids Flavonoids Tanin Saponins

Figure 1. Phytochemical Test Results Seaweed *Codium* sp

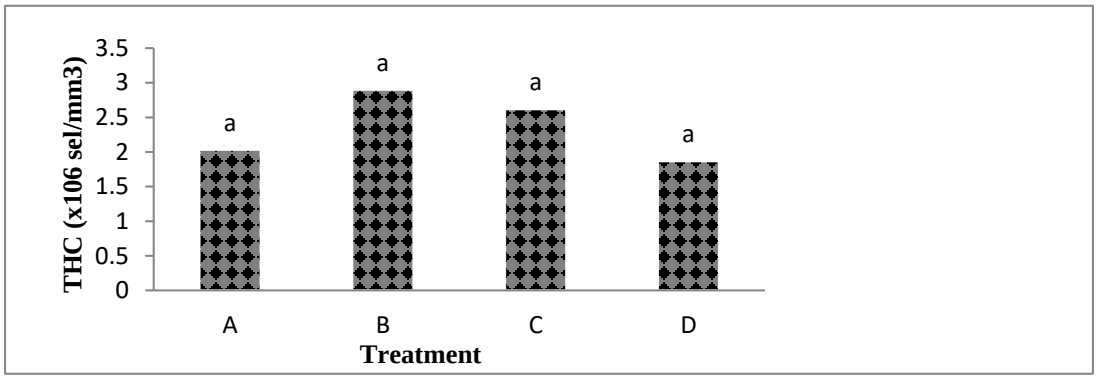

Figure 2. Total Haemocyte Count (THC) Tiger shrimp (*Penaeus monodon*) Description: A: control; B: a dose of 0.5 g kg^{-1} of feed; C: dose 1.0 g kg^{-1} feed; D: dose 1.5 g kg^{-1} of feed;

Total Hemocyte value of tiger shrimp fed with *Codium* sp seaweed extract with the highest different doses was seen in treatment B (2.9 x 106 cells / mm^3), C (2.6 x 106 cells / mm^3), A (2.0 106 cells / mm^3), the lowest was in treatment D (1.8 106 cells / mm^3). The results of the analysis of variance showed that the treatment with the addition of *Codium* sp seaweed extract was not significantly different ($p > 0.05$) between treatments

IV. RESULT

Based on Table 1, it is known that the results obtained in this study are that the results of extraction of *Codium* sp seaweed using ethanol solvent can produce extracts with phytochemicals in the form of alkaloids, saponins and tannins show that ethanol solvent has a high polarity, a solvent that has high polar power tends to be more effective at attracting phytochemical compounds. Alkaloids contain nitrogen atoms which have the function of making coordinate covalent bonds with metal ions form. Alkaloid compounds are compounds that have basic substances, and contain heterocyclic nitrogen and are synthesized in plants from amino acids and their derivatives. Alkaloids function as antibiotics and anti-inflammatory which can reduce pain (Sudarsono, 2002). According to Mulyadi *et al* (2020) Alkaloids are compounds that are easily soluble in water and have functions as antimicrobials, antivirals, and immunostimulants. Alkaloids can be toxic and can be used widely in the field of medicine (Harborne, 1997). The mechanism of action of alkaloids as antibacterials through alkaloid components is known as a DNA intercalator which can inhibit the action of the bacterial cell topoisomerase enzyme. Inhibition of DNA replication can result in bacteria being unable to divide, thus inhibiting bacterial growth. Meanwhile, the alkaloids contained in the extract can interfere with the formation of cross-bridges of peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not formed completely and causes certain cell death (Karou, 2005).

Saponins are triterpene and sterol glycosides that have been identified in plants. Glycosides are glycons or reducing sugars. Saponins contained in plants can be detected by the formation of foam when concentrating the extract (Harborne, 1987). Wardana and Tukiran (2016) added that the saponin test has a principle where the hydrolysis reaction of saponin compounds into aglycons and glycons can be characterized by the formation of stable foam. Then according to Simaremare (2014) that saponins have hydrophilic and hydrophobic groups. At the time of the saponin test, the sample after being shaken will form a foam, this is thought to be due to the presence of a hydrophilic group that binds to water then the hydrophobes will bind to the air so that it will form foam. Saponins function as antimicrobials in cultivated organisms. According to Harborne (1997), saponins have substances that can hemolyze blood. Saponins can be used as anti-microbial materials by forming complex polysaccharides on the cell walls, saponins will interact with the cell walls and will cause damage to cell walls and membranes resulting in bacteriolysis. The working principle of saponins as antibacterial agents is their ability to leak proteins and enzymes from within cells (Madduluri et al, 2013). Furthermore, saponins can become anti-bacterial is caused by the active substance found on the surface that resembles a detergent, which causes the stress on the saponins to drop. Damaged cell membranes will disrupt the survival of bacteria (Harborne, 1997). Saponins will diffuse

through the outer membrane and vulnerable cell walls and then bind to the cytoplasmic membrane so that it disturbs and reduces the stability of the cell membrane. This causes the cytoplasm to leak out of the cell resulting in cell death (Cavalieri et al, 2005).

Tannins can inhibit bacterial growth, by denaturing protein and damaging bacterial cell membranes and dissolving fat in the cell walls (Mulyadi et al, 2020). Tannins consist of two groups, namely the hydrolyzed tannins group, this tannin group will produce a blackish-blue color when added FeCl3 and the condensation tannin group will produce a blackish green color when added FeCl3. When the addition of FeCl3 is thought to react with one of the hydroxyl groups present in the tannin compound so that the final result will cause a color. The reagent, FeCl3, is widely used to identify phenolic compounds including tannins. Therefore it is possible that a positive result can also be given by other phenolic compounds in the sample (Sangi et al., 2008). Flavonoids are one of the largest groups of phenolic compounds detected in plants and are composed of 15 carbon atoms as the basic nucleus. The same, so that at the time of observation no red deposits were formed.

The results of the study with the addition of *Codium* sp seaweed extract to the feed at different doses for 14 days can increase the total hemocytes of tiger shrimp. This increase in total hemocytes indicates that the bioactive compound content of seaweed Codium sp can act as an immunostimulant which helps stimulate the formation of hemocyte cells to increase the defense of the immune system. This is in line with Braak's (2002) statement, that an increase in the total number of hemocytes is assumed to be a form of cellular immunity response in shrimp because hemocytes are the body's defense mechanism of shrimp. Subagyio's research (2009) also reported that the alkaloids, flavonoids and other phenolic compounds in Halimeda sp. Seaweed was able to increase the total number of hemocytes and phagocytosis activity of Vannamei shrimp.

IV. CONCLUSION

The ethanol extract of *Codium* sp. contains alkaloid bioactive compounds, tannins and saponins. *Codium* sp extract can increase Total Hemocyte Count (THC) in tiger shrimp and the best dose is 0.5 g / kg of feed.

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