Antifouling Activity of Cymodecea rotundata and Halodule pinifolia at Pulau Morotai

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Abstract. Biofouling is the attachment of the organism to the surface of the submerged in water. The presence of a fouling organism causes losses in the form of accelerating damage and adding to the burden of the ship/boat. The activity of biofouling can be avoided by using environmentally friendly antifouling paint. Some marine organisms, such as mangroves and seagrass, are reported to have antifouling activity. The purpose of this study was to determine the antifouling activity of seagrass C. rotundata and H. pinifolia and to identify bioactive compounds contained in C. rotundata and H. pinifolia. Based on the result of the research, the yield of C. rotundata extract was 5.96% and H. pinifolia 19.23%. The activity of antifouling H. pinifolia has a minimum concentration of 1000 ppm, while C. rotundata extract is 250 ppm. The results of the identification of bioactive compounds showed that H. pinifolia contains alkaloids, saponins, and steroids, while C. rotundata contains alkaloids.

1. Introduction

Biofouling is commonly found in the marine live structure and has become one of the significant concerns of the environment. Intensive efforts have been taken to address this issue by applying the antifouling synthesis paint, which contains metal compound and TBT (tributyltin). This option, however, has caused environmental pollution as it damaged the living organisms that are economically essential to humans. The current use of TBT as the antifouling has grown into faint [1]. Therefore, the search continued to derive environmentally friendly antifouling paint from natural ingredients.

Several marine plants have been suggested to have an antifouling activity such as mangrove and seagrass. Explorations have been carried out, one of which is a bioactive exploration that contains in the seagrass plant. The leaves of the Seagrass Thalassia hemprichii and Enhalus acoroides inhibit the occurrence of biofilms with weak to moderate categories [2]. The seagrass Cymodecea rotundata can inhibit the growth of Aegypti larvae [3]. It is also reported that the bioactive compounds of seagrass have a significant antibacterial [4].

Seagrass is found in the waters of Morotai Island. There are eight types of seagrass in the waters of Morotai Island, namely Cymodecea rotundata, Cymodecea serrulata, Syringodium isoetifolium, Halodule uninervis, Halodule pinifolia, Halodule ovalis, Enhalus acoroides, and Thalassia hemprichii [5]. Other studies have also reported that there are six types of seagrass in the waters of Dodola Island,
Morotai Island District, namely *Enhalus acoroides, Thalasia hemprichii, Cymodecea rotundata, Halodule uninervis, Halodule pinifolia*, and *Halodule ovalis* [6]. The number of seagrass on Morotai Island is not in line with its utilization, and there is not even the utilization of seagrass economically by the local people. Therefore, this research determined to utilize the local seagrass as an antifouling, which is naturally derived from the Moratai sea.

2. Material and method

2.1. Time and place
The actual research was held from December 2018 until January 2019. It was started with collecting fresh seagrass samples from the locally Morotai sea. In fact, laboratory test was performed in the FPIK Universitas Pasifik Morotai while proceed to the antifouling test, it was conducted in the MIPA Laboratory Universitas Papua.

2.2. Seagrass collection
At first seagrass samples were taken from the surrounding sea of Morotai island. Essential features including leaf, rizom and root were to be clean by using fresh water and proceed to the drying step under the sun light while they were covered evenly with black color fabric until the constant biomass level of it was thoroughly achieved.

2.3. Extraction
The following extraction process [7] suggested that all samples should achieve 95% of water content lose. After that, those well-dried samples began to be grinding into powder for which it eases the extraction procedure. As a result, 20 grams of it was transferred into 100 ml containing 96% methanol during 24 hours duration. Surprisingly, the paste extraction was dried again under 40°C temperature heat in the rotary evaporator. From this step, seagrass suspension was filtered by using the osmosis paper.

2.4. Bioactive compounds identification
This main step was to find out biomass substance which contained the seagrass. In addition to this, each tests were to examine the alkaloids, flavonoids, saponins, and steroids.

2.5. Antifouling test
Undertaking the seagrass antifouling extraction of *Cymodecea rotundata* dan *Halodule pinifolia*, it is then known that biofilm bacteria were involved in it. Initially, jelly diffusion has been adopted from Nur and Nugroho [8]. This method was used to test the antifouling activity by injecting nutrient agar (NA). The media was transferred into a 20 ml sterilized petri dish. Besides, 75 ml of cultured bacteria inserted into the fluid that had been inducted for a day using the spread method. Paper disks were accordingly placed and given a 25 ml drop of seagrass extract and, thus, to be incubated inside the room temperature for 2x24 hours. All in all, observation is set to monitor the starting of a clear zone, and it can be measured up by using calipers.

2.6. Data analysis
Data extraction is served in the form of picture and tabel by using Microsoft Excel 2010. Throughout this, all the data was processed through descriptive manner.

3. Results and discussion

3.1. Extraction seagrass bioactive particles
This process involved a maceration approach that came along with methanol. Heat is not necessary to be used by which the chances to damage the thermolabile can be avoided. Initially, the methanol acted as a solvent, and therefore, it certainly filters significant bioactive compounds [9].
### Table 1. Result of bioactive extraction particles

| Seagrass species | Sample (gr) | Extract (gr) | Yield (%) | Details |
|------------------|-------------|--------------|-----------|---------|
| C. rotundata     | 41.5        | 2.48         | 5.96      | End-texture including paste, sticky, and light green color. |
| H. pinifolia     | 50          | 9.66         | 19.32     | Display texture was paste but sticky. There found clear granule like crystal, and light green color. |

The yield extracts show distinctive outcomes including paste, sticky, dry, and light to dark green color (Table 1). Firstly, *Cymodecea rotundata* methanol extract (5.96%) presented similar characteristics with the absence of dark green color; meanwhile, *Halodule pinifolia* methanol extract (19.32%) showed paste but the sticky texture, there also found some tiny pieces which formed shiny green crystal. Another study reported that there were two forms of extracts, i.e., pasta and paste preferably liquid. Pasta-shaped extracts come from *Enhalus acoroides*, *Halophila ovalis*, and *Cymodecea rotundata* [10]. Methanol solvents produce more yield than those of n-hexane solvents [11].

#### 3.2. Antifouling test activity

The test series are carefully treated with the jelly diffused method, which uses the disk paper. Those treatments were applied three times all around. Besides, each extract is examined explicitly by adjusting the concentration to 1000, 500, and 250 ppm.

### Table 2. Activity test on antifouling *C. rotundata* and *H. pinifolia*

| Seagrass species | Extract concentration (ppm) | Diameter clear zone (mm) |
|------------------|-----------------------------|--------------------------|
| C. rotundata     | 1000 ppm                    | 5.2                      |
|                  | 500 ppm                     | 0                        |
|                  | 250 ppm                     | 0                        |
| H. pinifolia     | 1000 ppm                    | 9.4                      |
|                  | 500 ppm                     | 6.5                      |
|                  | 250 ppm                     | 5.3                      |

According to the table above, *C. rotundata* has the antifouling activity at the minimum concentration of 1000 ppm. On the other side, *H. pinifolia* started at 250 ppm. Methanol extract on *Cymodecea* revealed the antifouling activity towards biofilm bacteria (1—25 μg/ml) [12][13]. Similar to this, clarified that *Cymodecea* dan *H. pinifolia* can be used as a natural antifoulant [14].

#### 3.3. Seagrass bioactive compounds identification

At this stage, the procedure covered up compounds like saponin, alkaloid, flavonoid, and steroid. In this case, the identification attached to the qualitative approach by using the chemical reagents.

### Table 3. Identification outcome on seagrass bioactive particles in Morotai Island

| No  | Species  | Type of bioactive particles | Alkaloid | Flavonoid | Saponin | Steroid |
|-----|----------|------------------------------|----------|-----------|---------|---------|
| 1   | H. pinifolia | +                           | -        | +         | +       |         |
| 2   | C. rotundata | +                           | -        | +         | -       |         |

Ket: (+) contains bioactive particles (-) no contains bioactive particles.

Table 3 shows that seagrass *H. pinifolia* contains bioactive compounds, including alkaloids, saponins, and steroids, whereas *C. rotundata* has saponins and alkaloids. Other researchers have successfully isolated some bioactive compounds such as alkaloids, steroids, and tannins on seagrass leaves and roots [15]. *Cymodecea rotundata* contains phenols, flavonoids, and tannins.
[16]. *Cymodecea rotundata* and *Halodule pinifolia* consist of alkaloids, saponins, and steroids [10][17].

4. Conclusion

The yield of *Cymodecea rotundata* extracts as much as 5.96% and *Halodule pinifolia* as much as 19.23%. Antifouling activity on *H. pinifolia* has a minimum concentration of 1000 ppm, while *C. rotundata* extract is 250 ppm. The identification of bioactive compounds shows that *H. pinifolia* contains alkaloids, saponins, and steroids. As for comparison, *C. rotundata* only consists of alkaloids and saponins.

5. References

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