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Abstract. Biofouling is a serious problem for marine industries, it can damage many constructions which are submerged in the water and the bacteria are the inisiator of that problem. Commercial antifouling agents which have been using widely is not that effective regarding to the after-effect in the ecosystem. Thus, investigating on natural products which can against fouling bacteria is really essential. This research reported the potentials of seaweeds *Turbinaria ornata* and *Chaetomorpha antennina* against fouling bacteria which have been tested on the inhibition zone and phytochemical contents. The seaweed samples were extracted using various solvents such as hexane, ethyl acetate and methanol. The results showed that *C. antennina* had more potentials against the fouling bacteria than *T. ornata* regarding to the maximum inhibition zone. *C. antennina* extract had 6 mm at 10 µl/disk when *T. ornata* only had 2 mm at 10 µl/disk. In addition, *C. anteninna* extracts also had more phytochemical contents (phenol, flavonoid, steroid, saponin, alkaloid and triterpenoid) than *T. ornata* extracts (phenol, steroid, saponin, and alkaloid). Having larger maximum inhibition zone and more phytochemical contents indicated that *C. anteninna* can be the natural source candidate for antibacterial agent especially fouling bacteria.

1. Introduction

Biofouling, a result from fouling organisms settlement process on a submerged surfaces in the marine environment, is a serious problem for marine industries. It has been damaging every marine structure such as ship, quay wall and also accelerate structure damaging of woods because of wood borers [20]. Moreover, biofouling also caused the decreasing of ship velocity, increasing 40% of fuel consumption, and adding to the weight of the ship, alteration and structure damage on submerged surfaces [3,24]. Bacteria are inisiator of biofouling by forming complex biofilm which was then followed by other organisms such as microalgae and molluscs, therefore the solution of this problem is by eliminating the fouling bacteria [16].

Commercial antifoulant is widely used in marine industries, but it contains heavy metal like tributyltin [1] . One of the most promising alternative techniques to tributyltin is the development of antifouling compounds from natural products [14]. Antifouling compounds derived from natural products have less environmentally harm than heavy metal like tributyltin which also act against non-target species [5]. Marine organism like seaweeds showed rarely epiphytised, which indicates the presence of antifouling mechanisms [14]. Many sessile seaweeds increase their defense mechanism from biofouling by producing secondary metabolites which influence the settlement, growth and survival [10].

Vijayan *et al.* [24] reported that seaweed produces various secondary metabolites, *Turbinaria conoides* extract showed antibacterial activity against *Salmonella* sp., *Escherichia coli*, *Serratia liquefaciens*, and *Aeromonas hydrophila.* Santi *et al.* [17] also reported that brown seaweed *Sargassum duplicatum* showed good response against fouling bacteria *Achromobacter* and

Flavobacterium cytophaga. Sargassum wightii also showed antibacterial activity against *Pseudomonas* sp., and *Bacillus* sp. [2]. Thus, this study was aimed to investigate the potential antibacterial activity of seaweeds *Turbinaria ornata* and *Chaetomorpha antennina* against fouling bacteria based on the inhibition potency and phytochemical contents of *T. ornata* and *C. antennina* which extracted using three different solvents (hexane, ethyl acetate and methanol).

2. Materials And Methods

Collection and Extraction Samples

Seaweeds were collected by hand during low tide at Karapyak Beach, Pangandaran and transported using ice box to the laboratory. At the laboratory, seaweeds were thoroughly rinsed with seawater then freshwater to remove sediments and associated organisms. The samples identified and dried at room temperature. Dried seaweeds were cut into small pieces, then grinded into fine powder to increase effectiveness in extraction process. Then, 50 g of each seaweed sample was soaked in each of 200 ml (1:4 w/v) of three different solvents (methanol, ethyl acetate, hexane) for 24 hours (Lavanya and Veerapan, 2011).

Preparation and Isolation of Fouling Bacteria

Bacteria were isolated using a piece of fiberglass which was submerged for two weeks at marine environment (Bragadeeswaran *et al*. 2011; 17]. After two weeks, fiberglass panel was transported to the laboratory in an ice box. It was rinsed with sterile seawater and the surface was scrapped into bottle sample for the isolation process. It was diluted to 10^{-5}. Dilution of 10^{-1} to 10^{-5} were cultured in Zobel 2216E and incubated at 30^{o} C for 48 hours. Bacteria colonies were purified using streak method [17].

Antibacterial Activity Assay

Antibacterial activity assay was conducted using the disk diffusion technique. Sterile filter paper disk (d = 6 mm) were loaded with different seaweed extracts and air-dried. Disk containing solvents were used as controls [8]. The concentration of seaweeds extracts was 50 µg/disk (10 µl) [17,23]. The crude extract which has antibacterial activity will form halo zone around the disks.

Phytochemical Analysis

Alkaloids

Alkaloid was obtained using 0.1 g extract which mixed with 5 ml chloroform and 5 ml ammonia. It was then heated and filtered. Subsequently, 5 drops of sulfuric acid 2 N were added to each filtrate, which was then shaken. The supernatan of each filtrate was treated and measured with Meyer, Wagner and Dragendorf reagent. A brown, red and white deposition indicated the presence of alkaloids [4].

Saponins

Saponin was tested using 2 ml distilled water which was shaken with seaweed extract for 15 minutes. A stable foam layer was formed for the presence of saponins [19].

Flavonoids

Flavonoid was tested using 0.1 g extract which was dilluted with 20 ml water, heated for 5 minutes and filtered. To 5 ml of the filtrate 0.05 g Mg and 3 drops of HCl were added. A red, yellow or pink color indicated the presence of flavonoids [4].

Steroid/Triterpenoid

0.1 g extract was soaked in 20 ml ether for 2 hours and then filtered. Subsequently, 5 ml of filtrate was steamed until dry and then 2 drops of sulfuric acid was added and followed by 2 drops of acetate anhydride acid. A purple to blue or green color indicated the presence of steroids, whereas red color indicated the presence of triterpenoid [4]. [4].

Phenol

Phenol was determined using 1 ml extract which was added by 2 ml distilled water and 10 drops of FeCl3 1%. The appearance of green, blue or dark color indicated the presence of phenols (Subrathaa and Poonguzhali, 2013).

Quinon

Quinon was observed by adding 10 ml ethanol to 0.1 g seaweed extract. It was then heated for 5 minutes and filtered. Subsequently, 5 drops NaOH was added to 5 ml of filtrate. The appearance of red color indicated the presence of quinon [4].

3. Results and Discussion

The antibacterial activities of seaweeds extracted using three different solvents against 14 fouling bacteria F1- F14 were presented in Table 2 and Fig. 1-2. Total fouling bacteria that thoroughly inhibited by seaweed extracts were 4 isolates. No activity was recorded against bacteria F5, F6, F7, F8, F10, F11, F12, F13 and F14. Phytochemical analysis was presented in Table 3.

Extraction

The yield of crude extract varied significantly among the different seaweeds and different solvents. Thus, the highest crude extract was obtained from methanol extract of *C. antennina* and the lowest was ethyl acetate extract of *C. antennina* (Table 1).

Turbinaria ornata

The maximum inhibition zone (Fig. 1) was found in methanol extract against bacteria F3 (2 mm). Hexane and ethyl acetate extracts also showed weak activity against bacteria F3 (1 mm). Furthermore, hexane and ethyl acetate extract were active against F3 only.

Chaetomorpha antennina

The maximum inhibition zone (Fig. 2) was shown by ethyl acetate extract against bacteria F9 (6 mm) followed by hexane and methanol extracts which showed activity only against bacteria F1, F2 and F3. Ethyl acetate extract and hexane were only active against F1 and F9, on the other hand methanol extract had more activity for four bacteria.

Table 1. The Yield of Seaweeds Extracts

Solvent	Dry weight (g)	Yield Extract (%)
Turbinaria ornata		
Hexane	1.3	2.6
Ethyl acetate	1.7	3.4
Methanol	4.7	9.4
Chaetomorpha antennina		
Hexane	1.8	3.6
Ethyl acetate	1	2
Methanol	5.9	11.8

Table 2. Diameter of Inhibition Zone from Seaweeds Extracts

Bacteria Isolates	*Turbinaria ornata*			*Chaetomorpha antennina*		
	Methanol	Ethyl Acetate	Hexane	Methanol	Ethyl Acetate	Hexane
F1	+	-	-	+	+	-
F2	+	-	-	+	-	-
F3	+	+	+	+	-	+
F4	-	-	-	-	-	-
F5	-	-	-	-	-	-
F6	-	-	-	-	-	-
F7	-	-	-	-	-	-
F8	-	-	-	-	-	-
F9	-	-	-	++	+++	+++
F10	-	-	-	-	-	-
F11	-	-	-	-	-	-
F12	-	-	-	-	-	-
F13	-	-	-	-	-	-
F14	-	-	-	-	-	-

- Negative
+ 1-2 mm/weak
++ 3-4 mm/moderate
+++ >5 mm/strong

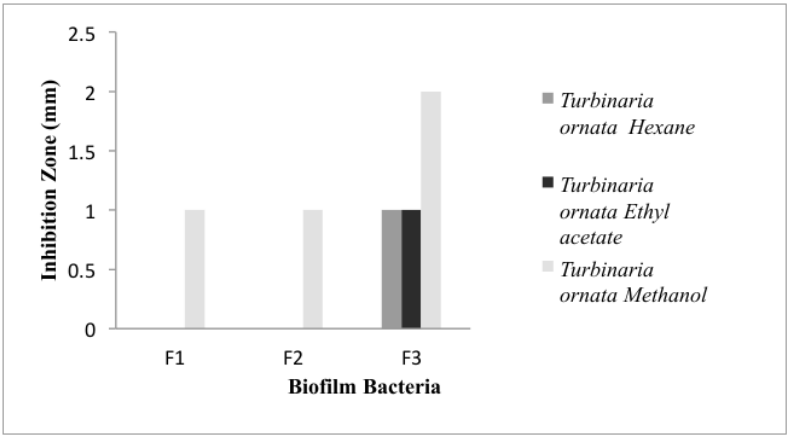

Figure 1. Antibacterial Activity of *Turbinaria ornata*

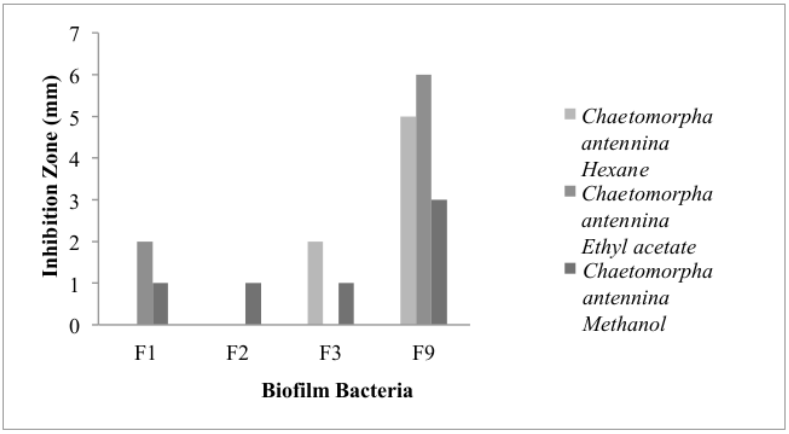

Figure 2. Antibacterial Activity of *Chaetomorpha antennina*

Table 3. Phytochemical Compounds of Seaweeds Extracts

Phytochemical Compounds	*Turbinaria ornata*			*Chaetomorpha antennina*		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
Phenols	-	-	+	-	-	+
Flavonoids	-	-	-	+	-	-
Quinons	-	-	-	-	-	-
Steroids	+	+	+	+	+	-
Saponins	-	-	+	+	+	+
Alkaloids	-	+	-	-	+	+
Triterpenoids	-	-	-	-	-	+

(-) Absent
(+) Present

Marine environment has diverse biologically habitats. Many marine organisms live in complex habitats exposed to extreme conditions and, in adapting to new environmental surroundings, they produce a wide variety of secondary (biologically active) metabolites which cannot be found in other organisms [13]. Seaweeds had been reported to have advantage for antibacterial [6].

In this research seaweed extracts of *C. antennina* were more potent against the fouling bacteria. Diameter of inhibition zone from *C. antennina* was 1-6 mm. It was larger than *T. ornata* which had zone inhibition 1-2 mm. Mani *et al.* [9] divided characteristic of zone inhibiton, weak <2 mm, moderate 3-4 mm and strong >5 mm. *C. antennina* showed positive respond against fouling bacteria F1, F2, F3 and F9. Hexane and ethyl acetate extract of *C. antennina* had strong zone inhibition (5-6 mm). Zone inhibition of *T. ornata* methanol extracts belonged to weak characteristic as it only inhibited 2 mm on F3 bacteria, it also had no activity against F9 bacteria. Distinct antibacterial activity could be obtained depending on the thickness and composition of cell walls of the target bacteria [15].

Kandhasamy and Arunachalam [7] reported that Chlorophyceae had higher antibacterial activity than other members. Previous research from Lavanya and Veerappan [8] showed that antibacterial from seaweeds Rhodophyceae showed higher antibacterial activity than Phaeophyceae and Chlorophyceae. In the present study, Chlorophyceae also showed higher activity than

Phaeophyceae. According to [6], variation of chemical composition of plants with geographic differences can produce different biological active compounds.

In the present study, seaweeds *T. ornata* and *C. antennina* were extracted and screened for both nonpolar and polar compounds using various solvents based on polarity. Qualitative analysis showed the presence of major bioactive compounds like alkaloids, phenols, flavanoids, saponins, steroids, quinon and triterpenoids. Table 3 showed that *T. ornata* dan *C. antennina* extracts had phenolics, flavonoids, steroids, saponins, alkaloids and terpenoid compounds. *T. ornata* which was extracted using three different solvents showed the presence of phenolics, steroids, saponins and alkaloids and *C. antennina* showed the presence of phenols, flavonoids, steroids, saponins, alkaloids and triterpenoids. Unnikrishnan *et al.* (2014) reported that *T. ornata* ethyl acetate extract showed the presence of alkaloids, phenols, flavonoids, saponins and methanol extract showed the presence of the same phytochemical compounds except saponins.

In Lavanya and Veerappan [8] methanol, chloroform, ethyl acetate and aqueous extracts of tested seaweeds were more active than the acetone, diethyl ether and hexane extracts against the bacterial pathogens. *T. Ornata* acetone extracts showed better activity against pathogen bacteria [6] According to Neelamathi and Kannan [11], *T.ornata* methanol extracts showed thepresence of alkaloids, terpenoid, phenols, saponins, flavonoids and quinon. Tiwari *et al*. [22] also reported that methanol extract is a more potent of bioactive components than other solvents.

C. antennina hexane, chloroform and ethanol extract contained flavonoids, tritriterpenoids, alkaloids, coumarins, quinines and saponins. *C. antennina* hexane extracts showed the presence of saponins, alkaloids and quinons. In the study of Sivakumar and Safhi [18] petroleum extract of *C. Antennina* was active against some Gram positive and Gram negative bacteria. The extracts also showed very little activity against *B. subtilis* and no activity was observed against *K. pneumoniae* and *Bacillus cereus*. Thanigaivel *et al*. [21] mentioned that *C. antennina* ethanol extracts showed good antibacterial actvity.

The environment where seaweeds were exposed by sun light and high oxygen concentration, those factors can trigger free radical and oxydation agents. But, seaweeds were rarely affected by the photodynamics during metabolism. The fact show that seaweeds contained a number of secondary metabolites which have ecological role for their defense mechanism [12]. Neelamathi and Kannan (2016) also mentioned that seaweeds which were collected from rocky beach and affected by sea wave contained alkaloids, phenols, flavonoids, triterpenoids, saponins and quinons.

4. Conclusions

C. antennina had more potential antibacterial compounds than *T. ornata* according to its inhibition zone against tested bacteria and its phytochemical contents. The differences between the result of the present study and the results of other studies may be due to the production of bioactive compounds related to the solvents used for the extraction. Methanol solvent showed the most effective solvents for phytochemicals investigation for both seaweeds *T. ornata* and *C. antennina.* Further studies also should be made to identify and evaluate the actual substances responsible for the antibacterial property.

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