ANTIBACTERIAL POTENTIAL OF MARINE ALGAE COLLECTED FROM SOUTH SULAWESI COAST AGAINST HUMAN PATHOGENS

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ABSTRACT

Twenty-nine algae extracts, isolated from 11 species of marine algae belonging to the classes Phaeophyta (*Dictyopteris acrostichoides, Padina boergeseni, Rosenvingea orientalis* and *Sargassum prismaticum,*), Chlorophyta (*Codium dwarkense* and *Enteromorpha linza*), and Rhodophyta (*Amphiroa tribulus, Eucheuma spinosum, Gracilaria verrucosa, Sarconema filiforme* and *Wrangelia tanegana*) were examined for their *in vitro* antibacterial activity against four human pathogenic strains (*Bacillus subtilis, Staphilococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) by agar diffusion method. Antibacterial activity was analyzed from crude extract of dried samples prepared from three different polarities of solvents (hexane, dichloromethane and ethyl acetate). Of twenty-nine tested extracts, sixteen extracts were against *B. Subtilis*, thirteen against *S. aureus*, three against *E coli* and only one extract was active against *P. aeruginosa*. The highest activity was shown by dichloromethane extract of *Codium dwarkense* against *B. subtilis* (inhibition zone: 19.0 mm), hexane extract of *Rosenvingea orientalis* against *S. aureus* (inhibition zone: 17.5 mm), dichloromethane extract of *Sargassum prismaticum* against *E. coli* (inhibition zone: 12.5 mm) and dichloromethane extract of *Wrangelia tanegana* against *P. aeruginosa* (inhibition zone: 10.0 mm). *Bacillus subtilis and S aureus* were the most susceptible organisms. No algae had broadest activity since all extracts only showed specific activity against each pathogen. In the present study, dichloromethane was found to be the best solvent for extracting antibacterial metabolites from dried samples rather than other solvents. The antibacterial profile of algal extracts suggested that marine algae have compounds that could be utilized for the development of antibiotic drugs.

Keywords: Marine algae, antibacterial, agar diffusion method, human pathogenic bacteria

INTRODUCTION

Over the past several decades, algae and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with different bioactivity such as cytotoxic (Tang *et al.*, 2002), antibacterial (Vallinayagam *et al.*, 2009), antifungal (Aliya and Shamaeel, 1999), antiviral (Serkedjieva, 2004; Garg *et al.*, 1992), antitumour (Kaori, 2002), antioxidant (Yuan and Walsh, 2006) and larvasidal (Thangam *et al.*, 1993). Until now more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations (Faulkner, 2001; Munro and Blunt 1999).

Algae have a big potential as food and medicine since long ago since they rich on vitamin, mineral, crude fibrous, protein and polysaccharide (Ito and Hori, 1989; Lahaye, 1991; Darcy-Vrillon, 1993). Their non-toxic phycocolloid role as low calorie nutrition and stabilization agent in food industry (Van den Hoek *et al.*, 1993; Critchley and Ohno, 1998; Lee, 1999). In Japan traditional food, algae were used as sushi wrapper, seasonings, condiments and salad or vegetables which composed 10-25% of food consume from most Japan people (Skibola, 2004; Teas, 1981).

Global economy crisis, high price of medicinal drugs, difficult access of population on medical treatment and pharmacy, and side effect of synthetic chemical drugs, are the reasonable factor to use herbal medicine for disease treatment. For this, non-synthetic or natural product especially marine natural product could be a promised resource. Several study showed that marine environment which cover almost 70% of earth is a rich sources on bioactive compounds, many of them have unique structure different with those from terrestrial one. Marine secondary metabolites are organic compounds produced by marine organisms like microbes, sponges, and seaweeds. The organism biosynthesizes these compounds to protect themselves and to maintain homeostasis in their environment (Selvin and Lipton, 2004).

The problem which always appears in marine drug discovery is raw material supply for industrial scale. Marine algae or seaweeds are the renewable resources that easy to cultivate in Indonesia. With the coast line of ±81.000 km, Indonesia has a big challenge or a high potential on marine algae cultivation. Until now there are 555 species of algae that have been found in Indonesia water (Bengen, 2001) and one of the region with a big potential for algae culture is South Sulawesi. The aim of this study was to assess the antimicrobial effects of organic crude extracts of marine algae collected from South Sulawesi coast, towards the human pathogens as a potential source of marine bioprospecting for antimicrobial drugs candidate.

MATERIALS AND METHODS

Algae Collecting

Algae were collected during the low tide along the coasts of Takalar, Bantaeng, Bulukumba (Bira) and Pangkep Regencies in South Sulawesi, Indonesia. The materials were washed with cleaned sea water and put into plastic bags before kept in an ice box to prevent photolysis and thermal degradation during transportation.

Sample Preparation

In the laboratory of Marine Science and Fisheries Faculty of Hasanuddin University, algae species were washed with filtered seawater to remove the epizoones, epiphytes, animal castings, sand, calcareous and other adhering detritus matters. Small samples of the species were separated for identification and the rest were washed with freshwater to remove the salt. After draining off the water, the cleaned plant materials were wiped with a blotting sheet and weighed for wet weight (ww). The samples were then sun-dried carefully under shade for 24-48 hour. Dried materials were weighed and cut into small pieces before finely grounded in a mechanical grinder. The powdered algae were kept airtight in plastic bags and put in the room temperature for further experiment.

Extraction of Algae

Extraction of algal materials was conducted as described previously (Zainuddin, 2006). Fifty grams of finely powdered algal material were extracted with 500 mL n-hexan in a 1-L capacity round bottom flask (1:10, w/v). The extraction was run on a stirrer plate for 24 h under room temperature. The extracts were filtered through a Whatman no. 1 filter paper then evaporated under reduced pressure in a rotary evaporator until 5-10 mL volume. The concentrated extracts were kept on small vials and let dry under room temperature to yield thick oily crude extract and stored airtight at -20ºC for further analysis. The algae residue from n-hexane extraction were dried at room temperature for 24 h and re-extracted successively with higher polarity solvents (dichloromethane and ethyl acetate) using the method as described above.

Agar Diffusion Test

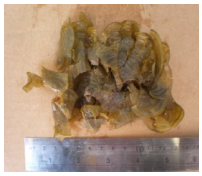
The assay was carried out in 90 mm Petri dishes containing 20 mL of nutrient agar (NA) seeded with inoculums of test organisms (*B. Subtilis, S. Aureus, E. Coli, P.aeruginosa* and *Candida maltosa*). For inoculation, an aliquot of test organisms from a stock culture was suspended in 3 ml of sterile 0.9% NaCl and mixed thoroughly. Two hundred micro-liter of this suspension was diluted with 20 mL sterile warm agar medium and poured

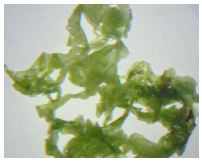
immediately into the Petri dish. A sterile filter disc with a diameter of 6 mm was impregnated with 50 µL test solution. Two milligrams of algal extracts dissolved in the extraction solvent were applied on the paper disc. Solvents were evaporated at room temperature before the discs were applied on the inoculated agar plates. The discs were applied aseptically onto the solidified surface of the agar and the Petri dishes were kept at 4°C over 4 h for pre-diffusion. After this, the plates were incubated for 24 h at 37°C for bacteria and at 28°C for *Candida maltosa* in an inverted position. At the end of the incubation period, the inhibition zones were measured and expressed as the diameter of the clear zone including the diameter of the paper disc (∅ 6 mm). Negative control experiments were performed by using paper discs loaded only with an equivalent volume of the solvent and positive control experiments were performed by using ampicillin against bacteria and amphotericin against yeast.

RESULTS

The algae species are collected along the coasts of South Sulawesi Province, Indonesia (Takalar, Bantaeng, Bulukumba (Bira) and Pangkep Regencies). They are distributed in the intertidal zone of sandy beaches and belonging to the Classes Phaeophyceae, Chlorophyceae and Rhodophyceae (Table 1). Eleven species consist of four Phaeophyceae (*Dictyopteris acrostichoides, Padina boergesenii, Rosenvingea orientalis* dan *Sargassum prismaticum*), two Chlorophyceae (*Codium dwarkense* and *Enteromorpha linza*) and five Rhodophyceae (*Amphiroa tribulus, Gracilaria verrucosa, Eucheuma spinosum, Sarconema filiforme* and *Wrangelia tanegana*) were used for antimicrobial test.

Table 1. Figure, classification and location of marine algae collected from South Sulawesi coast

No.	Algae species	Class, Order and Family (Location)	Algae Figure
1.	*Dictyopteris acrostichoides*	Phaeophyceae, Dictyotales, Dictyotaceae (Takalar)	
2.	*Padina boergesenii*	Phaeophyceae, Dictyotales, Dictyotaceae (Takalar)	

No.	Algae species	Class, Order and Family (Location)	Algae Figure
3.	*Rosenvingea orientalis*	Phaeophyceae, Scytosiphonales, Chnoosporaceae (Takalar)	
4.	*Sargassum prismaticum*	Phaeophyceae, Fucales, Sargassaceae (Takalar)	
5.	*Codium dwarkense*	Chlorophyceae, Bryopsidales, Codiaceae (Takalar)	
6.	*Enteromorpha linza*	Chlorophyceae, Ulvales, Ulvaceae (Takalar)	
7.	*Amphiroa tribulus*	Rhodophyceae, Corallinales, Corralinaceae (Pangkep)	
8.	*Eucheuma spinosum/Eucheuma denticulatum*	Rhodophyceae , Gigartinales, Areschougiaceae (Takalar)	
9.	*Gracilaria verrucosa*	Rhodophyceae, Glacilariales, Gracilariaceae (Bantaeng)	
10.	*Sarconema filiforme*	Rhodophyceae, Gigartinales, Solieriaceae (Pangkep)	
11.	*Wrangelia tanegana*	Rhodophyceae, Ceramiales, Wrangeliaceae (Bira)	

Percentages of dry/wet weight ranged from 6.57% to 25.39% (Table 2). The highest percentage was shown by red alga *Amphiroa tribulus*, followed by brown alga *Rosenvingea orientalis* and red alga *Sarconema filiforme* (both had

percentage of dry/wet weight of 19.62%). The lowest percentage was shown by green algae *Codium dwarkense* with 6.57%.

Table 2. Wet- and dry-weight of marine algal biomass.

No.	Algae Species	Class	Wet Weight (ww) (g)	Dry Weight (dw) (g)	Percent of dw/ww
1.	*Dictyopteris acrostichoides*	Phaeophyta	1008	110,9	11
2.	*Padina boergesenii*	Phaeophyta	540	58,22	10.78
3.	*Rosenvingea orientalis*	Phaeophyta	265,04	52	19.62
4.	*Sargassum prisnaticum*	Phaeophyta	597,3	74	12.39
5.	*Codium dwarkense*	Chlorophyta	1129	74,14	6,57
6.	*Enteromorpha linza*	Chlorophyta	433	37,47	8.65
7.	*Amphiroa tribulus*	Rhodophyta	370,24	94	25.39
8.	*Eucheuma spinosum*	Rhodophyta	126,93	15	11.82
9.	*Gracilaria verrucosa*	Rhodophyta	608.54	86	14.13
10.	*Sarconema filiforme*	Rhodophyta	158	31	19.62
11.	*Wrangelia tanegana*	Rhodophyta	514	78,67	15.31

A total of 29 organic extracts were obtained by extraction of seven marine algae species with three different polarities of solvent (hexane, dichloromethane and ethyl acetate). The dry weight of crude extracts ranging from 40.6 mg to 1186.5 mg (Table 3). Among the organic extracts, the highest yield was supplied by ethyl acetate extract of red alga *Amphiroa tribulus* with 0.08%.

Table 3. Percentage of crude extracts obtained from 25-50 g biomass dry weight in 250-500 mL organic solvent (1:10 w/v).

No.	Algae species	Class	Extracts	Crude Extracts (mg)	Crude Extracts (%)
1.	*Dictyopteris acrostichoides*	Phaeophyta	Hex	519.6	1.04
2.	*Dictyopteris acrostichoides*	Phaeophyta	DCM	662	1.32
3.	*Dictyopteris acrostichoides*	Phaeophyta	EtOAc	328.4	0.66
4.	*Padina boergesenii*	Phaeophyta	Hex	226.9	0.45
5.	*Padina boergesenii*	Phaeophyta	DCM	144.4	0.29
6.	*Padina boergesenii*	Phaeophyta	EtOAc	282.6	0.57
7.	*Rosenvingea orientalis*	Phaeophyta	Hex	218.1	0.44
8.	*Rosenvingea orientalis*	Phaeophyta	DCM	213	0.43
9.	*Sargassum prismaticum*	Phaeophyta	Hex	215.7	0.43

No.	Algae species	Class	Extracts	Crude Extracts (mg)	Crude Extracts (%)
10.	*Sargassum prismaticum*	Phaeophyta	DCM	238.6	0.47
11.	*Sargassum prisnaticum*	Phaeophyta	EtOAc	171.4	0.34
12.	*Codium dwarkense*	Chlorophyta	Hex	147.5	0.3
13.	*Codium dwarkense*	Chlorophyta	DCM	222.4	0.45
14.	*Enteromorpha linza*	Chlorophyta	Hexan	59.4	0.24
15.	*Enteromorpha linza*	Chlorophyta	DCM	66.6	0.27
16.	*Amphiroa tribulus*	Rhodophyta	Hexan	170.8	0.34
17.	*Amphiroa tribulus*	Rhodophyta	DCM	196.6	0.40
18.	*Amphiroa tribulus*	Rhodophyta	EtOAc	40.6	0.08
19.	*Euchema spinosum*	Rhodophyta	DCM	1186.5	2.37
20.	*Euchema spinosum*	Rhodophyta	EtOAc	82.3	0.17
21.	*Gracilaria verrucosa*	Rhodophyta	Hexan	171.4	0.34
22.	*Gracilaria verrucosa*	Rhodophyta	DCM	467.1	0.93
23.	*Gracilaria verrucosa*	Rhodophyta	EtOAc	74.8	0.15
24.	*Sarconema filiforme*	Rhodophyta	Hexan	56.8	0.11
25.	*Sarconema filiforme*	Rhodophyta	DCM	142.4	0.29
26.	*Sarconema filiforme*	Rhodophyta	EtOAc	204.3	0.41
27.	*Wrangelia tanegana*	Rhodophyta	Hex	110.5	0.22
28.	*Wrangelia tanegana*	Rhodophyta	DCM	361.3	0.72
29.	*Wrangelia tanegana*	Rhodophyta	EtOAc	201.6	0.40

Altogether twenty-nine extracts (hexane, dichloromethane and ethyl acetate) were screened against two gram-positive bacteria *B. subtilis* and *S. aureus*, two gram-negative bacteri *E. coli* and *Pseudomonas aeruginosa.* Of all tested extracts only three extracts exhibited no activity against all indicator organisms. Others showed low until high activities against at least one pathogenic bacterium. Generally, hexane and dichloromethane extracts exhibited the highest activity while the ethyl acetate only showed a low activity. No extract showed considerable effects, since all extracts only showed spesific and narrow activity against all test organisms.

Sixteen of twenty-one extracts showed antibacterial activity against gram-positive bacterium *B. subtilis* and the highest activity was shown by dichloromethane extract of *Codium dwarkense* with inhibition zones of 19.0 mm (Fig. 1). Hexane extract of *Codium dwarkense*, eth yl acetate extract of *Padina boergesenii,* dichloromethane extract of *Sarconema filiforme* and ethyl

acetate extract of *Gracilaria verrucosa* showed also high activity against *B. subtilis* with inhibition zones between 16.0 and 18.0 mm.

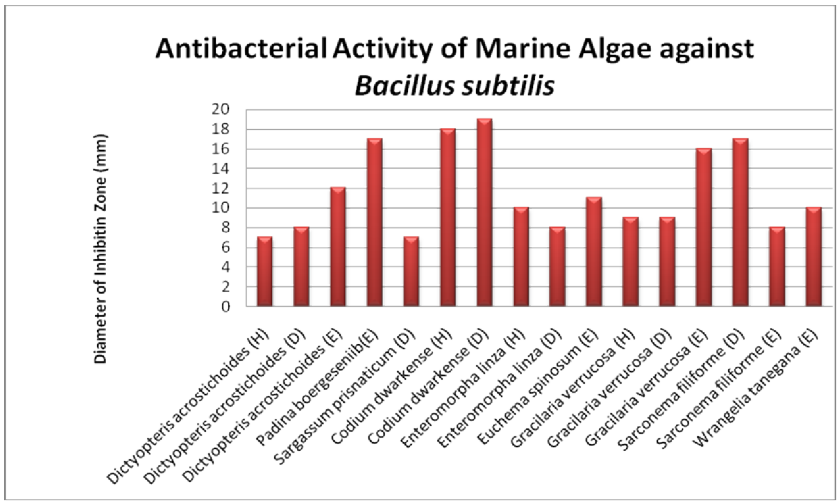


Fig. 1. Antibacterial activity of algal extracts against *B. subtilis* [Agar diffusion assay; n= 2; 2 mg/disc; Ø of inhibition zone (mm) includes Ø disc (6 mm]

From thirteen extracts that were active against gram-positive bacterium *S. aureus* the hexane extract of *Rosenvingea orientalis* showed the highest activity with inhibition zone of 17.5 mm and following by the dichloromethane extracts of *Amphiroa tribulus* with inhibition zone of 16.0 mm (Fig. 2). A moderate activity against this bacterium was shown by ethyl acetate extract of *Amphiroa tribulus* (inhibition zone: 13.0 mm).

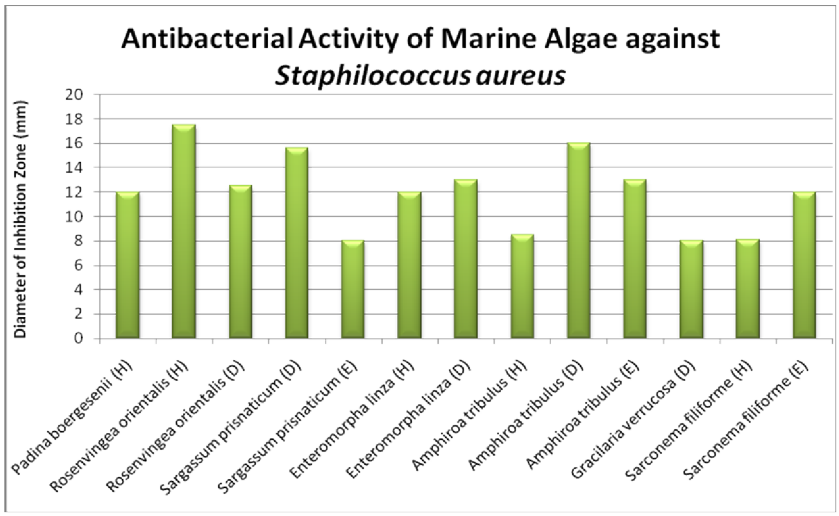


Fig. 2. Antibacterial activity of algal extracts against *S. aureus* [Agar diffusion assay; n= 2; 2 mg/disc; Ø of inhibition zone (mm) includes Ø disc (6 mm)]

Out of 29 tested extracts, ten extracts were found to be active against gram-negative bacterium *E. coli* with moderate (inhibition zones of 10.0 - 12.5 mm) and low activities (inhibition zones of 8.0-9.0 mm (Fig. 3). The

dichloromethane extract of *Sargassum prisnaticum* had the highest activity against this pathogen (diameter of zone inhibition: 12.5 mm).

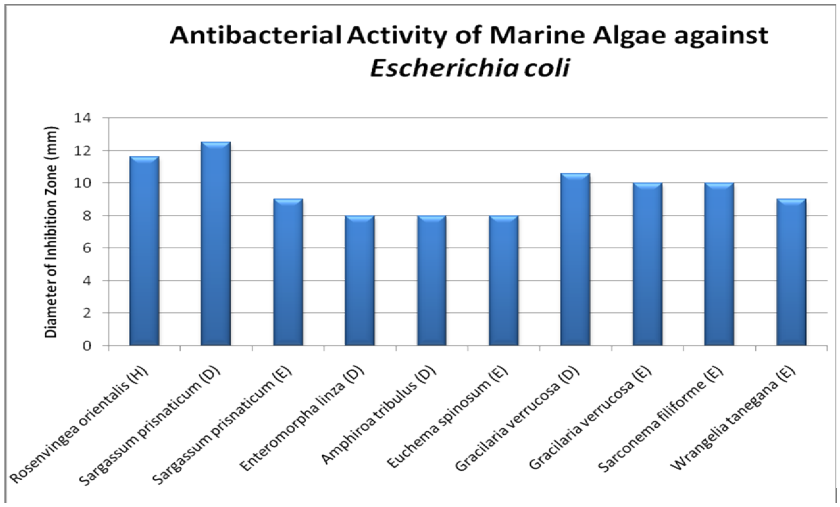


Fig. 3. Antibacterial activity of algal extracts against *E. coli* [Agar diffusion assay; n= 2; 2 mg/disc, Ø of inhibition zone (mm) includes Ø disc (6 mm)]

The human pathogen bacterium *Pseudomonas aeruginosa* was the resistant bacteria since almost all algal extracts had no activity against this organism. Only the dichloromethane extract of *Wrangelia tanegana* had a low activity against this organism (inhibition zone: 10.0 mm) (data not shown). No antifungal activity was detected in all algal extracts.

DISCUSSIONS

Searching and discovering new antibiotics and bioactive compounds from algae cannot be performed satisfactory only by chemical means. The biological screening systems are necessary because the biological activity of specific compounds cannot be predicted by identification and examination of their structure particularly for the new compounds. One of such screening systems commonly used to detect the antimicrobial activity of substances is the agar diffusion assay (Østensvik *et al.*, 1998]. This method proved to be a valuable tool for detecting the antibacterial effects of algal extracts as well as for bioassay guided fractionation (Falch *et al.*, 1993, 1995; Østensvik *et al.*, 1998).

In our screening experiments, a total of twenty-nine lipophilic extracts obtained from biomass of seven strains of marine algae were investigated for antimicrobial activity against two gram-positive bacteria (*B. subtilis* and *S. aureus*), two gram-negative bacteria (*E. coli* and *P. aeruginosa*) and one yeast (*Candida albicans*). Out of twenty-nine extracts, twenty-six extracts showed antibacterial activity (89.66%). All algal extracts did not have antifungal

activity against *Candida albicans*. From twenty-nine extracts with antibacterial activity, eleven extracts (37.93%) showed activities against more than one strain of human pathogenic bacteria.

All screened marine algal strains showed antimicrobial activity but not all extracts of each strain were active. It indicated that most algae have narrow and specific antibacterial activities. Most of the antibacterial activity was found in dichloromethane extracts which eight extracts showed moderate and high activities. These results indicate that the inhibitory substances seem to be semi-polar compounds (in range of n-hexane and ethyl acetate polarities).

In our study, the potential antibacterial activity was pronounced against gram-positive bacteria than gram-negative bacteria. Also in the screening of marine algae (Reichelt and Borowitzka, 1984; Rao and Parekh, 1981), no or weak activities were observed against gram-negative bacteria. In the investigation of 44 lipophilic and hydrophilic extracts of cultured terrestrial and freshwater cyanobacteria for antibacterial activity, 54.5% of extracts were found to be active against gram-positive bacteria and no activity was observed against gram-negative bacteria (Mian *et al.*, 2003). Screening for antibacterial activity of algae from different location showed that the inhibitory substances were most active against gram-positive than gram-negative bacteria (Rao and Parekh, 1981; Tuney *et al.*, 2006) and the highest activity were detected in Classes Phaeophyceae (Vlachos *et al.*, 1997) and Rhodophyceae (Salvador *et al.*, 2007). From the investigation of 44 algal species from Canary island 28 species had potential antibacterial activity against gram-positive bacteria (De Val *et al.*, 2001).

The potential antibacterial activity against gram-positive bacteria *B. Subtilis* was detected in the dichloromethane and hexane extracts of *Codium dwarkense*, whereas the hexane extract of *Rosenvingea orientalis* exhibited a good activity against gram-positive bacteria *S. aureus*. Ozdemir *et al.* (2006) reported that the hexane extracts of *D. membranacea* dan *C. barbata* exhibited high activities than their methanolic extracts. In our study, a moderate activity against gram-negative bacteria *E. Coli* was performed by dichloromethane extract of *Sargassum prismaticum* whereas against gram-negative bacteria *P. aeruginosa* was pronounced by dichloromethane extract of *Wrangelia tanegana*. These results indicate that dichloromethane extract was the pronounced extract against all pathogenic bacteria tested. Evaluation of antimicrobial activity of various extracts (hexane, dichloromethane and methanol) of different species of brown algae (Phaeophyta) showed that the dichloromethane extract has more potential activity than the hexane and methanol extracts (Demirel *et al.,* 2009). From investigation of three extracts (dichloromethane, methanol and water) of 26

algal species against five fish pathogenic bacteria, the highest activity was presented by dichloromethane extract (Bansemir *et al.*, 2006).

No algal strain revealed extracts with broad activities against all human pathogens tested. Bloor and England (1989) reported that from investigation of five cyanobacterial strains, *Nostoc muscorum* MAC (LP23) was identified as a producer of broad spectrum antimicrobial compounds against different bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli*), filamentous fungi (*Cladosporium herbarum, Hormoconis resinae*) and yeasts (*Candida albicans* and *C. pseudotropicalis*). No coincidental effect was detected in our screening, since no antifungal activity was detected in the strains with antibacterial activity. In contrast, Gerwick *et al.* (1989) found a synergic activity in the extracts of a tropical cyanobacterium *Hormothamnion enteromorphoides*. This extract inhibited either the growth of *Bacillus subtilis* also the growth of a pathogenic yeast *Candida albicans*.

CONCLUSION

High price of the medicine and difficult access to the pharmacy could be the reason for poor people in the coastal community to provide their drug with marine medicinal plant surround their area. Indonesia has ± 75% of marine area (5,8 million km2) with ± 81.000 km coast line and 17.500 big and small island. These advantages make the marine and fishery industries have good expectations in Indonesia. As we know, culture location of algae or seaweeds is in the sub-tidal zone with 2 m depth. Like terrestrial plant, marine algae require sun light for their metabolisms and Indonesia is one of the tropical countries which obtain sun light through the year. This advantage makes the algae is easy to be cultivated in Indonesia regions (marine bioprospecting). Sulawesi Selatan is one of the east Indonesia region which has a potential area to develop the algae culture. Almost half of total national productions of algae commercial are come from South Sulawesi area. Therefore, the aim of the study is to explore the bioactive compounds of marine algae from South Sulawesi as marine bio-prospecting resources. In conclusion, the results of this study showed that marine algae from South Sulawesi can be used as medicinal marine plant for antibiotic drugs candidate.

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