Antimicrobial activity of seaweed extracts from Pattani, Southeast coast of Thailand

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Abstract

Marine algae are known as source of bioactive secondary metabolites. Green marine algae (*Ulva intestinalis*) and red marine algae (*Gracilaria fisheri*) were collected from the coast of Pattani province, Thailand. These marine algae were extracted by four solvents including methanol, ethanol, dichloromethane and hexane. Crude extracts of all seaweed samples were tested for their antimicrobial activities using disk diffusion method and colorimetric broth microdilution method, respectively. Thirteen bacterial strains were used in this study*, Vibrio alginolyticus* PSU VA 1, *V. parahaemolyticus* PSU 5124, *V. harveyi* PSU 4109, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* DMST 4553, *Bacillus cereus* TISTR 687, Methicillin resistant *S.aureus* NPRC 001R (MRSA 001R) and *Enterobacter faecalis* ATCC 29212. All crude extracts showed ability against tested strains (ranging of inhibition zone 6.78–16.45 mm), with the exception of the *U. intestinalis* dichloromethane extract in the disk diffusion assay. Dichloromethane and hexane extracts of *G.fisheri* showed the highest antimicrobial activity against *S.aureus* ATCC 29213 and *B. cereus* TISTR 687 with minimum inhibitory concentration (MIC) values of 256 μg/ml, respectively. From this finding confirm that the extracts of algae are good source of bioactive metabolites.

Keywords: Antimicroial activity, *Ulva intestinalis*, *Gracilaria fisheri*, Seaweed extracts.

1. Introduction

Macroalgae are various group of marine organism that have adapted to the competitive marine environment (Harnedy and FitzGerald, 2011). As a result, these marine organism are recognized as the potential sources of bioactive secondary metabolites and many of these

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substances have demonstrated to possess interesting biological activity (Abedin and Taha, 2008; Abdel-Raouf *et al*., 2008) with antiviral, antibacterial antifungal and anti-inflammatory (Abdel-Raouf *et al*., 2008: Okai and Higashi-Okai, 1997).

Bioactive compounds in marine algae are high and are used widely in pharmaceutical (Al-Saif *et al*., 2013; Salem *et al*., 2011). Bioactive substances isolated from marine algae included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinines, lipids, and glycerol (Cabrita *et al*., 2010). Moreover, it has been demonstrated that extracts of marine algae exhibited high potential antioxidant properties (Yangthong *et al*., 2009).

Recently, consumers are concerned about chemical preservatives in foods. Seaweed extracts could not only offer health benefit but can be used as natural preservative in foods. Two edible seaweed, *Ulva intestinalis* and *Gracilaria fisheri*, are the types of seaweeds widely presented in the Southern coast of Thailand.

Recent study has demonstrated the antibacterial activity of organic solvents extracts of seaweeds (Salem *et al.,* 2011). *Gracilaria* species contain active compounds (Bansemir *et al*., 2006). The antimicrobial activity of the extract from *G. corticata* was highly active against the bacterium *Proteus mirabilis* (Kulik, 1995). Moreover, Kanjana *et al*. (2011) reported that the solvent extracts from *G.fisheri* showed antimicrobial activity against a virulent strain of *Vibrio harveyi* and increased disease resistance in black tiger shrimp (*Penaeus monodon*). The green marine algae are commonly found off the coast. Extracts of green algae such as *Ulva* species are well known as several of bioactive compounds. *U.fasciata* was reported that the extracts showed antimicrobial activity in bacteria better than fungal (Priyadharshini *et al*., 2012). *Enteromorpha intestinalis* (*U.intestinalis*) in Caspian Sea Cost was reported to exhibit significant activity against *Bacillus subtilis* and anti-hemolytic activity (Soltani *et al*., 2012).

Marine algae are potential renewable sources and also known to produce a numerous of secondary metabolites with broad spectrum activity. *G.fisheri* and *U.intestinalis* are cultivated in Pattani province. These species of algae are widely used for as the animal foods in aquaculture and also used as human foods. The objective of this study was to screen for the potential antimicrobial activity from *G.fisheri* and *U.intestinalis* by using disk diffusion method and colorimetric broth microdilution method.

2. Materials and Methods

2.1 Algae samples

Algal samples were collected on the coasts of Pattani province in the Southern of Thailand. Epiphytes were removed from all samples and washed with running water. The final washing step was done using distilled water. The algal were dried under shade. Dried samples were cut into small pieces and then ground in to powder.

2.2 Solvent extraction

The solvent extraction was carried out according to the methods described by Kanjana *et al*., (2011). Thirty grams of powder algal samples were extracted using 500 ml each of methanol, ethanol, dichloromethane and hexane in soxhlet apparatus for 24 h. These extracts were concentrated to pellet in rotary evaporator at the controlled temperature at 40ºC. The extracts were stored at -20ºC for further used.

3. Antimicrobial activity

3.1 Tested bacterial strains

Gram-negative bacterial strains were used this experiment including *V. alginolyticus* PSUVA 1, *V. parahaemolyticus* PSU 5124, V. *harveyi* PSU 4109, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae*, *Salmonella typhi* and *P.mirabilis*. Gram-positive bacterial strains were *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* DMST 4553, *B. cereus* TISTR 687, Methicillin-resistant *S. aureus* NPRC 001R (MRSA 001R) and *Enterobacter faecalis* ATCC 29212. The microorganisms were obtained from the Laboratory of Microbiology, Prince of Songkla University, Hat Yai, Songkhla, Thailand. The bacteria were cultured on Mueller Hinton broth (MHB) 35ºC for 16–18 h followed streaking on Mueller Hinton agar (MHA) at 35ºC for 16-18 h. The bacterial strains were stored on the medium containing 20% glycerol at -20ºC.

3.2 Paper disc diffusion method

Paper disc diffusion method was performed following the method of Ifesan *et al*., (2010). Ten microliters of the algal crude extracts (250 mg/ml) was added to sterile filter paper discs (6 mm), so that each disc was impregnated with 2.5 mg of residue. After that, the discs were dried at 30ºC overnight and applied on the surface of MHA plates seeded with 5 h broth culture of the tested bacteria. The bacterial strains were adjusted to 0.5 McFarland (1.5×10^{8} cfu/ml).

Negative control was prepared using the respective solvents while vancomycin (30 μg/disc) and gentamicin (10 μg/disc) were used as positive controls. The plates were incubated at 35ºC for 18 h. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was performed in triplicate.

3.3 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibition concentration was adapted from the method described by Clinical and Laboratory Standards Institute (CLSI, 2006). The MIC values were studies among the microorganisms that were susceptible in the previous screening by paper disc diffusion method. Vancomycin (0.0625–32 μg/ml) and gentamicin (0.0625–32 μg/ml) were used as reference standards. A serial of 2-fold dilutions (2–1,024 mg/ml) of the extracts were added to the 96-well microtiter plates. Five microliters of bacterial strains (10^6 cfu/ml) were added into each well and the plates were incubated at 35ºC for 16 h. For MIC determination, ten microliters of 0.18% (w/v) resazurin stain were added into each well and the plates were incubated at 35ºC for 2 h. Minimal bactericidal concentrations were performed by streaking the contents from microtiter wells that gave MIC values on fresh MHA and incubated at 35ºC for 24 h. Concentration at which there was no visible bacteria growth after 24 h incubation was regarded as MBC.

4. Results and Discussion

The paper disc susceptibility test of all solvent extracts from *U. intestinalis* and *G. fisheri* are shown in Table 1. Seaweed extracts demonstrated the good antimicrobial activity against all gram-positive and gram-negative pathogenic bacteria excepted *V. parahaemolyticus* PSU 4109, *P. aeruginosa* ATCC 27853 and *P. mirabilis*. Among the extracts from four solvents, the hexane extract of *U. intestinalis* (UH) gave the highest inhibition zone (16.45±0.10 mm) against MRSA 001R (Figure 1) followed by *E. faecalis* ATCC 29212 (13.88±0.45 mm) and *S. aureus* ATCC 29213 (13.72±0.21 mm). However the UH gave only little activity against *V. harveyi* PSU 4109 and *V. alginolyticus* PSUVA 1 with the inhibition zone around 6.78±0.17 mm and 6.82±0.20 mm, respectively. Moreover, the hexane extract of *G. fisheri* (GH) gave less inhibition zone against MRSA 001R (13.47±0.05 mm) and *S. aureus* ATCC 29213 (13.27±0.31 mm) when compared with UH. These results demonstrated that hexane extracts of both marine algae showed higher bacterial inhibitory activity when compared to extracts from other organic solvents.

Table 1 Antimicrobial activity of various crude solvent extracts of marine algae.

	Zone of inhibition (mm)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
U. intestinalis													
Methanol (UM)	-	-	-	-	-	-	-	-	-	8.57±0.05	8.16±0.23	-	-
Ethanol (UE)	-	-	-	-	-	-	-	-	-	-	6.75±0.28	-	-
Dichloromethane (UD)	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexane (UH)	6.82±0.20	-	6.78±0.17	8.26±0.65	-	7.15±0.40	9.61±0.39	-	13.72±0.21	-	7.97±0.38	16.45±0.10	13.88±0.45
G. fisheri													
Methanol (GM)	-	-	-	-	-	-	-	-	-	-	7.18±0.36	-	-
Ethanol (GE)	-	-	-	-	-	-	-	-	7.52±0.14	-	-	-	-
Dichloromethane (GD)	-	-	-	-	-	-	-	-	8.65±0.17	-	-	8.00±0.00	-
Hexane (GH)	-	-	-	-	-	-	-	-	13.27±0.31	-	7.26±0.02	13.47±0.05	-
Positive control													
Gentamicin (10 µg)	13.45±0.21	14.15±0.57	14.20±0.48	20.38±0.25	20.67±0.37	19.00±0.5	10.61±0.27	21.73±0.22					
Vancomycin (30 µg)									16.67±0.78	19.01±0.62	20.68±0.31	18.85±0.19	20.96±0.52

Note: Each value representing mean±SD of 3 replicates; Gram-negative bacteria: 1.*V. alginolyticus* PSUVA 1, 2.*V. parahaemolyticus* PSU 4109, 3.*V. harveyi* PSU 4109, 4.*E. coli* ATCC 25922, 5.*P. aeruginosa* ATCC 27853, 6.*K. pneumonia*, 7.*S. typhi*, 8.*P. mirabilis*; Gram-positive bacteria, 9.*S. aureus* ATCC 29213, 10.*L. monocytogenes* DMST 4553, 11.*B. cereus* TISTR 687, 12. MRSA OO1R, 13.*E. faecalis* ATCC 29212, "-" indicating no activity.

Table 2 showed the MIC and MBC values of the extracts from algae. These MIC and MBC values were demonstrated to range from 2–1,024 µg/ml. Lowest MIC and MBC value was recorded for the dichloromethane extract of *G.fisheri* (GD) against *S.aureus* ATCC 29213 (256/1,024 µg/ml) and GH against *B.cereus* TISTR 687 (256/1,024 µg/ml).

Table 2 Minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of extracts from seaweeds.

	MIC and MBC values (µg/ml)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
U. intestinalis										
Methanol	ND	ND	ND	ND	ND	ND	1,024/1,024	>/>	ND	ND
Ethanol	ND	ND	ND	ND	ND	ND	ND	1,024/1,024	ND	ND
Dichloromethane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hexane	1,024/1,024	1,024/>	>/>	>/>	>/>	>/>	ND	1,024/>	>/>	512/>
G. fisheri										
Methanol	ND	ND	ND	ND	ND	ND	ND	>/>	ND	ND
Ethanol	ND	ND	ND	ND	ND	>/>	ND	ND	ND	ND
Dichloromethane	ND	ND	ND	ND	ND	256/1,024	ND	ND	512/>	ND
Hexane	ND	ND	ND	ND	ND	512/>	ND	256/1,024	512/>	ND
Positive control										
Gentamicin	2/2	2/4	0.5/2	0.5/0.5	0.5/1					
Vancomycin						0.5/0.5	0.25/8	2/2	0.5/0.5	0.5/8

Note: ND: not done; Gram-negative bacteria: 1.*V. alginolyticus* PSUAV 1, 2.*V. harveyi* PSU 4109 , 3.*E. coli* ATCC 25922, 4.*K. pneumonia*, 5.*S. typhi*; Gram-positive bacteria: 6.*S. aureus* ATCC29213, 7.*L. monocytogenes* DMST 4553, 8.*B. cereus* TISTR 687, 9.MRSA 001R, 10.*E. faecalis*. ">" indicating the concentration more than 1,024 µg/ml

Generally plant extracts are potential more inhibitory against gram-positive than against gram-negative bacteria (Marino *et al*., 2001). Antibacterial activity of extracts from 26 species of red marine algae (8 Ceramiales, 7 Gelidiales, 9 Gigartinales, 1 Bonnemaisoniales and 1 Rhodymeniales) against three gram-positive and two gram-negative bacteria was demonstrated in previous study. Ninety six percent of the extracts were active against only one strain of *S.aureus* (Rhimou *et al*., 2010). Recently, eight seaweeds collected from red sea were extracted with methanol and ethyl acetate and were screened for their antibacterial activities against gram-positive and gram-negative bacteria. The green marine algae *Caulerpa racemosa* showed strongly inhibition of bacteria in both solvents and *S.aureus* was the most susceptible to the extract using disc diffusion test. The lowest MIC value was recorded for the ethyl acetate extract from *C.racemosa* against *B. cereus* (5 mg/ml) and methanol extract from *C.racemosa* aginst *S.aureus* (5 mg/ml) (Salem and Nasr El-deen, 2011).

Rhodomaceae, especially *Gracilaria* species, are known as a potential source of bioactive compounds such as bromophenols (Oh *et al*., 2008). The red algae *G.corticata* exhibited broad spectrum of antimicrobial activity especially gram-positive bacteria, gram-negative bacteria and yeast. Among two extracts, acetone extract showed higher activity when compared with methanol extract. The acetone extract had good activities against *Candida albicans*, however methanol extract could inhibit *S.aureus* and *P.mirabilis* (Govindasamy *et al*., 2012). In previous study, *G.fisheri* was extracted with difference organic solvents (ethanol, methanol, chloroform and hexane) and were evaluated for prevention and inhibition of *V. harveyi* infections in black tiger shrimp (*P.monodon*). Among the four extracts, ethanol and chloroform extracts showed higher activity against *V. harveyi* in disc diffusion with the MIC value at 90.0±5.5 and 90.0±9.7 μg/ml, respectively (Kanjana *et al*., 2011).

The green marine algae are commonly found off the coast. Extracts of green algae such as *Ulva* species are well known as several of bioactive compounds. *U.fasciata* was reported that the four solvent extracts were showed antibacterial activity against gram positive bacteria (*B.cereus*) and gram negative bacteria (*P.aeruginosa, S.typhi* and *S.marcescens*). The extracts from *U. fasciata* were presented of bioactive compounds including steroids, alkaloids, phenolic compounds, fravonoids, saponins, tannins and triterpenoids (Anantham *et al*., 2012) *E. intestinalis* (*U.intestinalis*) was extracted with 70% ethanol. The extract showed activity against *S.typhimurium*, *S.aureus*, *B.subtilis* and *P.mirabilis*, except *P.aeruginosa* (Soltani *et al*., 2012).

According to the previous reports, marine algae are rich sources of fiber, minerals, proteins, antioxidant and bioactive compounds. The bioactive compounds in marine algae are polyphenols (present hydroxyl group in structure), terpenoids, carotenoids and tocopherols. A number of bioactive compounds which have been isolate from marine algae include sulphate polysaccharides (laminarin and fucoidans), polyphenol (such as phorotannins), carotenoid pigments (such as fucoxantin and astaxanthin), sterols and mycosporine-like amino acids (MAAs) (Gupta and Abu-Ghannam, 2011; Zou *et al*., 2008; Airanthi *et al*., 2011). Polyphenols such as catechin, epicatechin, epigallocatechin gallate and gallic acid were presented in the green marine algae *Halimada* (Yoshie *et al*., 2002). Bromophenol has been reported as biofuntion of antimicrobial compounds was founded in the red marine algae (Oh *et al*., 2008; Xu *et al*., 2003). In addition to those mentioned above, the mechanism of phenolic compounds influences the cell wall and cell membranes of microorganism. Moreover, it can interfere with the membrane function such as destroy the electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity. These active phenolic compounds might be several invasive targets which could lead to inhibition of bacteria (Gupta and Abu-Ghannam, 2011). In this study, the active compound presented in crude extracts might be interacting synergistically for bacterial inhibition.

5. Conclusion

In conclusion, the result of the present study confirm that marine algae *U. intestinalis* and *G. fisheri* are potential source of bioactive compounds against various human pathogens, which can be used as natural non-toxic preservative and may be more acceptable to consumers. Lowest MIC and MBC value was observed in *S. aureus* ATCC 29213 and *B. cereus* TISTR 687 treated with GD and GH, respectively. Further work is needed to identify the active compounds and role of antibacterial activity of these marine algae.

Acknowledgements

The authors are grateful this work was supported by a fund from Prince of Songkla University Budget and also the Department of Microbiology, Faculty of Science, Prince of Songkla University for supporting this research.

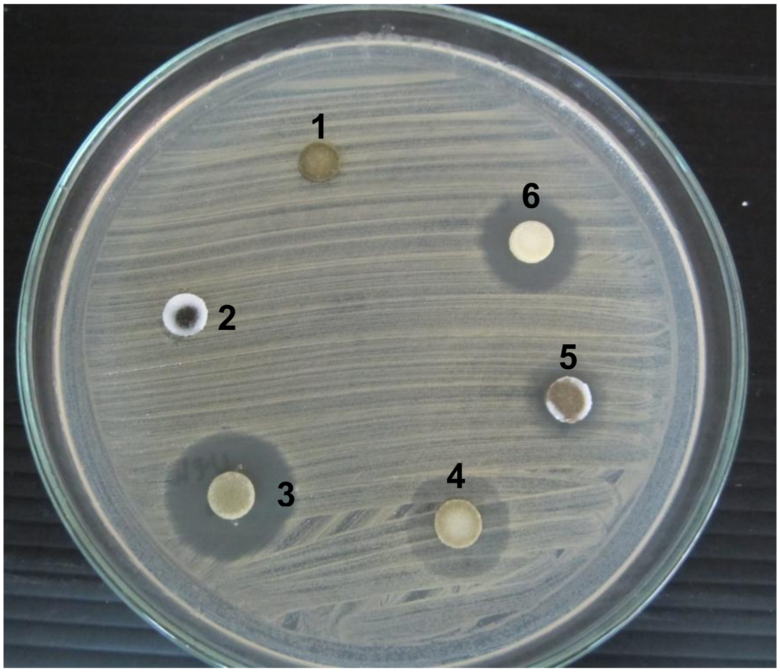

Figure 1 Inhibition zone of different solvent extracts from *U. intestinalis*

and *G. fisheri* against MRSA 001R. 1, ethanol extract of *U. intestinalis*; 2, dichloromethane extract of *U. intestinalis*; 3, hexane extract of *U. intestinalis*; 4, ethanol extract of *G. fisheri*; 5, dichloromethane extract of *G. fisheri*; 6, hexane extract of *G. fisheri.*

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