ฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดหยาบจากสาหร่ายขนนก (*Caulerpa racemosa* var. *corynephora*) ต่อเชื้อแบคทีเรียก่อโรคในสัตว์น้ำ

Antibacterial Activity of *Caulerpa racemosa* var. *corynephora* Crude Extracts against Pathogenic Bacteria of Aquatic Animals

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บทคัดย่อ

สาหร่ายขนนก (*Caulerpa racemosa* var. *corynephora*) เป็นสาหร่ายสีเขียวที่มีประโยชน์และพบได้ทั่วไปบริเวณชายฝั่งทะเลอันดามันได้ถูกเก็บรวบรวมมาจากพื้นที่ชายฝั่งทะเลของจังหวัดตรัง และนำมาสกัดด้วยตัวทำละลาย 6 ชนิด ได้แก่ เฮกเซน ไดคลอโรมีเทน เอทิลอะซิเตต เมทานอล เอทานอล และน้ำ หลังจากนั้น สารสกัดหยาบที่ได้ทั้งหมดได้ถูกนำมาทดสอบฤทธิ์ต้านเชื้อแบคทีเรียที่ก่อโรคในสัตว์น้ำ 4 ชนิด ได้แก่ *Streptococcus agalactiae* SAAQ001 ซึ่งเป็นแบคทีเรียแกรมบวก และ *Aeromonas hydrophila* AHAQ001, *Vibrio harveyi* VHAQ001 และ *Vibrio parahaemolyticus* ซึ่งเป็นตัวแทนของแบคทีเรียแกรมลบ ในการศึกษานี้ ได้ทำการทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย (antibacterial activity) ซึ่งแสดงออกมาในรูปของเคลียร์โซน (zone of inhibition) ความเข้มข้นต่ำสุดที่สามารถยับยั้งเชื้อแบคทีเรีย (minimal inhibitory concentration, MIC) และความเข้มข้นต่ำสุดที่สามารถฆ่าเชื้อแบคทีเรีย (minimal bactericidal concentration, MBC) โดยผลที่ได้หลังจากการศึกษาพบว่าสารสกัดหยาบของสาหร่ายชนิดนี้ที่สกัดด้วยสารเอทิลอะซิเตตแสดงประสิทธิภาพดีในการต้านเชื้อแบคทีเรียที่ใช้ทดสอบทั้งหมดด้วยค่า MIC ต่ำสุดอยู่ในช่วง 195.31 μg ml^{-1} ถึง 6,250 μg ml^{-1} และค่า MBC ต่ำสุด อยู่ในช่วง 781.21μg ml^{-1} ถึง 6,250 μg ml^{-1} จากที่ได้ระบุไว้ข้างต้น สาหร่ายขนนกอาจเป็นแหล่งของสารออกฤทธิ์ทางชีวภาพที่มีศักยภาพ อย่างไรก็ตาม ควรมีการศึกษาเพิ่มเติมในส่วนของสารพฤกษเคมีในสาหร่ายชนิดนี้ เพื่อที่จะระบุชนิดของสารออกฤทธิ์ทางชีวภาพที่มีคุณสมบัติในการต้านเชื้อแบคทีเรีย ซึ่งสามารถนำมาใช้ในการจัดการโรคติดเชื้อแบคทีเรียในการเพาะเลี้ยงสัตว์น้ำเพื่อทดแทนการใช้ยาปฏิชีวนะ

คำสำคัญ: สาหร่ายขนนก, ฤทธิ์ต้านเชื้อแบคทีเรีย, เชื้อแบคทีเรียก่อโรคในสัตว์น้ำ

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ABSTRACT

Caulerpa racemosa var. *corynephora*, the beneficial green alga generally found along the seashore of the Andaman Sea, was collected form the coastal area of Trang province, Thailand. This marine seaweed was extracted with six different solvents, namely hexane, dichloromethane, ethyl acetate, methanol, ethanol and water. Afterward, all crude extracts were tested for their antibacterial activities against four pathogenic bacteria of aquatic animals, namely *Streptococcus agalactiae* SAAQ001 which is gram-positive bacteria; *Aeromonas hydrophila* AHAQ001, *Vibrio harveyi* VHAQ001 and *Vibrio parahaemolyticus* which are representative of gram-negative bacteria. In this study, the antibacterial activityinterpreted as zone of inhibition, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) was tested. After having been investigated, the obtained results revealed that ethyl acetate crude extract of this alga exhibited the satisfactory effects on antibacterial activities against all tested pathogenic bacteria with the lowest MIC values ranging from 195.31µg ml^{-1} to 6,250 µg ml^{-1},and the lowest MBC values ranging from 781.21µg ml^{-1} to 6,250 µg ml^{-1}. As aforementioned, *C. racemosa* var. *corynephora* might be the potential source of bioactive metabolites; however, its phytochemicals should be further analyzed in order to identify the bioactive compounds responsible for antibacterial activity which could be used in management of bacterial disease in aquaculture as alternative to antibiotics.

Key words: *Caulerpa racemosa* var. *corynephora*, antibacterial activity, pathogenic bacteria of aquatic animals

INTRODUCTION

Nowadays, the aquaculture practice has highly proliferated and partly replaced capture fishery. Moreover, the aquaculture model has been also transformed from an extensive culture to an intensive culture because the demand of aqua-product consumption has increased steadily. Nevertheless, this brings about several problems, especially in aquatic animal health. This is because the intensive culture usually induces stress condition, leading to deterioration of homeostasis and defense mechanism of cultured aquatic animals, causing increment of susceptibility to infections. According to this incident, antibiotics and disinfectants are applied for solving infectious disease problems in aquaculture, bacterial diseases in particular (Austin and Austin, 2007). However, the disadvantage of antibiotics utilization has subsequently presented as the appearance of antibiotic-resistant strains of microorganisms (Waldvogel, 2004). Furthermore, the use of drugs and chemicals has more disadvantages relating to the generation of toxicant causing risks to environment, animals and consumers (Jones *et al*., 2004). To solve these problems, the natural way for aquaculture should be conducted through the use of probiotics, immunostimulants, and bioactive metabolites from terrestrial medicinal plants and aquatic macrophytes (Bansemir *et al*., 2006). Particularly in marine algae or seaweeds, they are recognized as the potential renewable sources of bioactive secondary metabolites possessing broad spectrum biological activities such as antimicrobial activity, antifungal activity, antiviral activity, antioxidant property, anti-inflammatory property, anticoagulant, and apoptotic effect (Abdel-Raouf *et al*., 2008; Abedin and Taha, 2008; Arunkumar *et al*., 2010; Folmer *et al*., 2010; Mayer *et al*., 2011; Srikong *et al*., 2015). For these reasons, the bioactive compounds derived from seaweeds have been used widely in pharmaceutical industry. The bioactive compounds are usually found in algae arealkaloids, polyketides, cyclic

peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinines, lipids, and glycerol (Cabrita *et al*., 2010; Salem *et al*., 2011; Al-Saif *et al*., 2014; Chingizova *et al*., 2017). However, a few bioactive substances from algae are applied in aquaculture, especially in inhibition of pathogenic bacteria causing infectious disease in aquatic animals (Bansemir *et al*., 2006); therefore, the finding of marine algae containing potential source of bioactive substances which can be used in aquaculture as natural antibiotic should be performed. In this case, *Caulerpa racemosa* var. *corynephora*, one of algae in Chlorophyceae, generally distributing along the seashore of the Andaman Sea, is interesting to study. Previously, it has been used as fertilizer and bactericide in horticulture, and it can be consumed as food and folk medicine for reducing blood pressure and treating rheumatism (Chew *et al*., 2008; Syamsuddin *et al*., 2016). This is because it contains several types of interesting metabolites possessing a diverse range of bioactivities such as antimicrobial activity, antifungal activity, insecticidal activity, antifouling and anti-inflammatory (Kandhasamy and Arunachalam, 2008; Yang *et al*., 2015). Moreover, it also contains nutrients and hormones which act as growth regulators (Syamsuddin *et al*., 2016). With aforementioned advantages, this algal species had been tested to prove its antibacterial properties against the representative of pathogenic bacteria causing infectious diseases in aquaculture in order to find alternative practice for prophylaxis instead of antibiotic application.

MATERIALS AND METHODS

1. Algal materials preparation

Caulerpa racemosa var. *corynephora* was collected from Palian district, Trang province, Thailand. The whole thallus of this green alga was firstly cleaned with seawater to remove all epiphytes and contaminants, and then it was thoroughly washed with tap water and followed by distilled water. Next, the clean seaweed was air-dried under shade for 7 days and then oven-dried at 50 ^{o}C for 24 hours.The dried alga was chopped to small pieces, and finely ground with the electric blender. The algal powder was stored in the cool-dried area for further crude extraction.

2. Extraction procedure

The procedure for extraction in this study had been separated as 2 parts according to the use of organic solvents, following the method of Khongsai *et al*. (2017). Part 1, the 500 g of algal powder was macerated in 1 liter of different solvents, namely hexane, dichloromethane, ethyl acetate, ethanol, and methanol for 5 days, and then the solutions were filtered through Whatman No.1 filter paper. The filtrates of algal extracts were then concentrated using a rotary evaporator under low pressure at 40 ^{o}C. Part 2, the remaining 500 g of algal powder was boiled in distilled water for an hour. The obtained filtrate of algal extract was then concentrated using a rotary evaporator under low pressure at 50 ^{o}C and dried using freeze dryer. Each crude extract from both part of extraction was re-dissolved in the dimethyl sulfoxide (DMSO) to produce the extract concentration of 100 mg ml^{-1}, and then stored at -20 ^{o}C until it had been tested.

3. Antibacterial activity investigation

3.1 Inoculums preparation

The extract samples were evaluated for investigating the activity against four pathogenic bacteria of aquatic animals, namely *Streptococcus agalactiae* SAAQ001, *Aeromonas hydrophila* AHAQ001 and *Vibrio harveyi* VHAQ001 derived from Kasetsart University, and *Vibrio parahaemolyticus* derived from Songkhla Aquatic Animal Health Center, Thailand. All tested bacterial inoculums were prepared following the modified method of Sritunyalucksana *et al*. (2005), and adjusted with No. 0.5 McFarland standard in order to approximately produce the bacterial concentration of 1.5×10^{8} CFU ml^{-1} for testing antibacteial activity through hole-plate diffusion method. These inoculums were diluted once again to produce the bacterial concentration of 1×10^{6} CFU ml^{-1}

for broth microdilution susceptibility test and also microbicidal activity test.

3.2 Determination of antibacterial activity

The antibacterial characteristic existing in all *Caulerpa racemosa* var. *corynephora* crude extracts was interpreted as zone of inhibition determined in quadruplicate through the hole-plate diffusion method (Brantner *et al*., 1994). Firstly, the tested inoculums containing bacterial concentration of 1.5×10^8 CFU ml^{-1} were gently swabbed onto the Mueller-Hinton agar (MHA) to produce the uniform distribution of microorganisms. The agar plates were dried in the laminar flow for 5 min and then the wells with 6 mm in diameter were made by using the sterile puncture. Then, 50 µl of 100 $mgml^{-1}$crude extracts dissolved in DMSO was added into four created wells. Likewise, the 30 µg $ml^{-1}$of oxytetracycline, antibiotic commonly used in aquaculture, and DMSO were introduced into the other wells as positive control and negative control, respectively. Finally, the agar plates already filled with algal crude extracts were incubated at 35 °C for 24 and 48 hours. After incubation, the antibacterial activity presenting as the inhibition zone (clear zone) surrounding the well was measured at time checked and interpreted as millimeterin diameter.

4. Determination of minimal inhibitory concentration (MIC)

The MIC value of algal extract samples was determined by broth microdilution susceptibility test according to the modified method of Eloff (1998) and Clinical and Laboratory Standards Institute (CLSI) (2006). Firstly, the stock solution of algal extracts dissolved in DMSO with the initial concentration of 100 mg ml^{-1} was serially two-fold diluted with Mueller Hinton broth (MHB) in sterilized 96-well microtiter plate to produce 50 µl of final investigated concentration in each well. This was done in quadruplicate. Next, 50 µl of tested inoculums containing bacterial concentration of 1×10^6 CFU $ml^{-1}$was filled into each well and then gently re-mixed with multichannel auto-pipette to produce final bacterial concentration of 5×10^5 CFU ml^{-1} in each well. Then, the 96-well microtiter plates were covered with sterile lid, sealed with parafilm, and incubated at 35 ºC for 24 hours. After incubation, the turbidity of solutions was checked, and *p*-Iodonitrotetrazolium chloride (INT) was filled in each well to confirm the bacterial growth from discoloration of the mixture. The well with pink mixture showed the existing of live microorganisms while the well without precipitate and discoloration of the mixture indicated that the diluted concentration of the extracts could inhibit bacterial growth in the well. In this case, the MIC value was defined as the lowest concentration of the extracts showing complete inhibition of bacterial growth. In addition, 30 µg ml^{-1} of oxytetracycline and DMSO (organic solvent) were also tested in quadruplicate following the aforementioned method as positive and negative controls, respectively.

5. Determination of minimal bactericidal concentration (MBC)

In case of the MBC value, it was subsequently determined through broth microdilution test. Briefly, one loopful of solutions presenting inhibition of bacterial growth was streaked onto the Mueller Hinton agar (MHA) plates. Afterwards, the agar plates were incubated at 35 ºC for 24 hours. The bactericidal concentration of tested extracts was estimated from the appearance of bacterial colonies on the agar plates. The lowest concentration of diluted extracts presenting no bacterial colonies was the MBC value (CLSI, 2006).

6. Statistical analysis

The data of antibacterial activity obtained from hole-plate diffusion method were statistically analyzed using one-way analysis of variance (ANOVA) in statistical program (IBM SPSS Statistics 21), and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means. The comparisons were done at a significant level of 0.05 (Duncan, 1995).

RESULTS

To check the primary properties against all tested microorganisms of *C. racemose* var. *Corynephora* crude extracts derived from extraction with different solvents, hole-plate diffusion method was done, and the results of this testing were specified in Table 1-2. In this part, the derived results from 24 and 48 hours checked revealed that the ethyl acetate crude extract of this alga exhibited the results on growth inhibition of tested bacterial strains slightly better thanthe algal crude extracts derived from extraction with other solvents. The susceptibility of gram-positive *S. agalactiae* to all crude extracts was more than those of gram-negative tested bacteria comprising of *A. hydrophila*, *V. harveyi* and *V. parahaemolyticus*. Moreover, the aqueous crude extract presented the lowest efficacy against all tested bacteria, and the results of inhibition zone from 24 hours checked were slightly higher than the results from 48 hours checked.

As the results specified in Table 3, the ethyl acetate crude extract of *C. racemose* var. *corynephora* showed the satisfactory results on bacteriostatic activity with the lowest MIC values ranging from 195.31 µg ml^{-1} to 6,250 µg ml^{-1}. Nevertheless, the detected values were not different when compared to the hexane crude extract tested with *A. hydrophila* and *V. parahaemolyticus*. In case of MBC values shown in Table 4, the hexane crude extract of *C. racemosa*var. *corynephora* exhibited the lowest value of MBC against *A. hydrophila* equal to 195.31 µg ml^{-1}, and the dichloromethanecrude extract showed the lowest value of MBC against *S. agalactiae* equal to 781.21 µg ml^{-1}. In term of MBC values against *V. harveyi* and *V. parahaemolyticus*, the satisfactory results belonged to the ethyl acetate crude extract. The effective concentration of this crude extract against *V. harveyi* and *V. parahaemolyticus* were 3,125 µg ml^{-1} and 6,250 µg ml^{-1}, respectively. In addition, this investigation found that *A. hydrophila*and *S. agalactiae* were more susceptible to all extract of *C. racemosa*var. *Corynephora* than *V. harveyi* and *V. parahaemolyti*

Table 1 Inhibition zone of *C. racemosa* var. *corynephora* crude extracts derived from extraction with different solvents against four pathogenic bacteria causing infectious disease in aquaculture at 24 hours

	Samples	Inhibition zone (mm in diameter)			
		A. hydrophila	***S. agalactiae***	***V. harveyi***	***V. parahaemolyticus***
Extracts	Hexane	14.13 ± 1.75^{ab}	21.06 ± 4.17^{a}	15.56 ± 1.33^{ab}	11.44 ± 1.26^{b}
	Dichloromethane	12.88 ± 3.27^{ab}	12.44 ± 4.39^{c}	15.00 ± 1.04^{b}	10.25 ± 0.79^{bc}
	Ethyl acetate	15.00 ± 2.34^{a}	18.38 ± 2.15^{ab}	16.63 ± 1.20^{a}	15.75 ± 0.84^{a}
	Ethanol	11.56 ± 2.85^{ab}	13.56 ± 2.86^{bc}	11.00 ± 0.84^{d}	9.06 ± 0.24^{cd}
	Methanol	10.63 ± 4.75^{cd}	13.69 ± 5.52^{bc}	12.63 ± 0.85^{c}	8.50 ± 0.65^{d}
	Water	7.06 ± 1.66^{d}	9.25 ± 2.50^{c}	10.44 ± 1.09^{d}	8.06 ± 1.56^{d}
Oxytetracycline		34.88±0.14	37.50±2.27	41.88±0.60	33.50±1.02
DMSO		1.50±1.29	2.00±0.91	1.94±0.66	1.88±1.31

Note: Mean values within the same column sharing the different superscript were significantly different at $p<0.05$.

Table 2 Inhibition zone of *C. racemosa* var. *corynephora* crude extracts derived from extraction with different solvents against four pathogenic bacteria causing infectious disease in aquaculture at 48 hours

	Samples	Inhibition zone (mm in diameter)			
		A. hydrophila	***S. agalactiae***	***V. harveyi***	***V. parahaemolyticus***
Extracts	Hexane	13.50±2.12^{ab}	20.06±2.38^{a}	14.56±0.97^{ab}	10.06±0.66^{b}
	Dichloromethane	12.50±2.71^{ab}	13.50±5.26^{bc}	14.31±0.69^{b}	11.00±0.82^{b}
	Ethyl acetate	14.69±2.31^{a}	17.50±0.54^{ab}	16.00±0.89^{a}	14.63±0.78^{a}
	Ethanol	11.38±2.86^{abc}	11.75±4.26^{bc}	9.63±1.55^{c}	7.81±0.24^{c}
	Methanol	9.88±5.92^{bc}	13.56±5.70^{bc}	9.19±1.40^{c}	8.50±1.70^{c}
	Water	7.50±1.78^{c}	10.19±3.35^{c}	8.44±0.66^{c}	7.75±1.17^{c}
Oxytetracycline		33.06±0.13	35.75±1.55	41.00±1.14	32.00±0.58
DMSO		1.44±0.88	1.88±1.31	1.63±0.48	1.81±1.25

Note: Mean values within the same column sharing the different superscript were significantly different at $p<0.05$.

Table 3 Minimal inhibitory concentration (MIC) of *C. racemosa* var. *corynephora* crude extracts derived from extraction with different solvents against four pathogenic bacteria causing infectious disease in aquaculture

	Samples	Minimal inhibitory concentration (μg ml^{-1})			
		A. hydrophila	***S. agalactiae***	***V. harveyi***	***V. parahaemolyticus***
Extracts	Hexane	195.31	781.21	6,250.00	6,250.00
	Dichloromethane	781.21	391.00	6,250.00	12,500.00
	Ethyl acetate	195.31	195.31	3,125.00	6,250.00
	Ethanol	12,500.00	1,563.00	12,500.00	12,500.00
	Methanol	12,500.00	3,125.00	12,500.00	12,500.00
	Water	12,500.00	12,500.00	12,500.00	12,500.00
Oxytetracycline		0.19	0.05	0.19	0.01
DMSO		ND	ND	ND	ND

Note: ND was no detection.

Table 4 Minimal bactericidal concentration (MBC) of *C. racemosa* var. *corynephora* crude extracts derived from extraction with different solvents against four pathogenic bacteria causing infectious disease in aquaculture

	Samples	Minimal bactericidal concentration (μg ml^{-1})			
		A. hydrophila	***S. agalactiae***	***V. harveyi***	***V. parahaemolyticus***
Extracts	Hexane	195.31	1,563.00	6,250.00	12,500.00
	Dichloromethane	781.21	781.00	12,500.00	12,500.00
	Ethyl acetate	781.21	1,563.00	3,125.00	6,250.00
	Ethanol	25,000.00	12,500.00	12,500.00	25,000.00
	Methanol	25,000.00	12,500.00	12,500.00	12,500.00
	Water	25,000.00	25,000.00	12,500.00	12,500.00
Oxytetracycline		0.19	0.05	0.38	0.01
DMSO		ND	ND	ND	ND

Note: ND was no detection.

DISCUSSION

Previously, the antibacterial properties of several marine algae had been tested with the pathogenic bacteria causing infectious diseases in human or the bacteria concerning food spoilage (Srikong *et al*., 2015; Khongsai *et al*., 2017). There were a few reports implying the effectiveness of them on bacteriostasis and bactericidal activity against pathogenic bacteria causing disease outbreak in aquaculture. For example, the report of Kanjana *et al*. (2011) indicated that ethanol, methanol, chloroform and hexane extracts of *Gracilaria fisheri* had the potential effect on antimicrobial activity against *Vibrio harveyi*and the effect on enhancement of disease resistance in black tiger shrimp (*Penaeus monodon*). The report by Natrah *et al*. (2015) specified that the metanolic extract of brown alga *Padina minor* showed the best values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *V. harveyi* BB120. Moreover, Immanuel *et al*. (2004) reported that *Artemia* enriched with *Ulvalactuta* and *Sargassum wightii* could increase growth rate and survival rate of shrimp *Penaeusindicus* juveniles challenged with *Vibrio parahaemolyticus*.

The greenmarine alga, *C. racemose* var. *corynephora*, had been reviewed from several previous studies that its extract contained noteworthy biological metabolites for antibacterial activity (Del Val *et al*., 2001; Radhika *et al*., 2012), and it could be used for protection of ice-ice disease in *Gracilaria verrucosa*, caused by *Pseudomonas* infection (Syamsuddin *et al*., 2016). In this investigation, the ethyl acetate crude extract of *C. racemose* var. *corynephora* exhibited the satisfactory antibacterial properties against all tested bacteria. This was in accordance with the previous report by Salem *et al*. (2011) which indicated that the activity of ethyl acetate extract of *C. racemosa* was the most powerful inhibition of bacterial growth because this extract presented the best antibacterial properties against human pathogenic bacteria such as *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Next, Chandrasekaran *et al*. (2014) also reported that the maximum antibacterial activity against vancomycinresistant *Enterococcus faecalis* belonged to the ethyl acetate extracts of *C. racemosa*. Moreover, Khongsai *et al*. (2017) reported that the ethyl acetate extracts of this algal species presented the best results on antibacterial activity against bacteria causing human dermatitis.

In case of algal extracts derived from extraction with the other solvents, the results in this test had revealed that the hexane crude extract also showed the minor effectiveness against all tested bacteria, and it exhibited the best inhibition of *S. agalatiae* growth. Formerly, the report of Natrah *et al*. (2015) had specified that the methanolic extract of *C. racemosa* showed the effective antibacterial activities against *A. hydrophila, V. harveyi, V. parahaemolyticus*, *V. alginolyticus* and *V. anguillarum* with the results of inhibition zone ranging from 7.75 mm in diameter to 9.50 mm in diameter. This was similar to the results derived from the metabolic crude extract in this study; however, it still showed less effectiveness on antibacterial activity than the ethyl acetate crude extract. Besides, the aqueous crude extract of *C. racemose* var. *corynephora* in this test exhibited the lowest results of antibacterial activities against all bacterial strains tested. This was similar to the reports of Alghazeer *et al*. (2013) which had tested the effects of *C. racemosa* extracts against representative of gram-positive and gram-negative bacteria. This obtained result could be related to the absence of antibacterial activity in some extracts because of the insolubility of the active substances in these solvents (Shankar *et al*., 2010). As mentioned above, the derived results of antimicrobial activity shown by *C. racemose* var. *corynephora* in this study may be attributed to its bioactive substances comprising caulerpin (Paul, *et al*., 1987), caulerpanyene (Amico *et al*., 1978), flexin and trifarin (Blackman and Wells, 1978). In addition, the crude extracts of this algal species may contain flavonoids (Syamsuddin *et al*., 2016) which are water soluble

compounds, and function as antimicrobial substances for suppression or killing microorganismsthrough destabilization of proteins, cell membrane structures, and cytoplasm composition (Harborne, 1984). In this study, the results of inhibition zone from 24 hours checked were slightly higher than the results from 48 hours checked. This might be due to the active compounds in algal crude extracts deteriorated after extending the tested time for 48 hours.

In term of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), the overall derived results in this test revealed that ethyl acetate crude extract showed the best effective results of MIC and MBC, and the minor effective results belonged to hexane crude extract. The lowest concentrations of the ethyl acetate crude extract of *C. racemose* var. *corynephora* which inhibited growth of all tested bacteriarangedfrom 195.31 µg ml^{-1} to 6,250 µg ml^{-1}, and the lowest biocidal concentrations of this extract ranged from 781.21 µg ml^{-1} to 6,250 µg ml^{-1}. For the hexane crude extract, the ranges of MIC and MBC values were similar to the ethyl acetate crude extract except the MBC value tested with *V. parahaemolyticus* which raised to 12,500 µg ml^{-1}. The result of this investigation was quite similar to the report by Salem *et al*. (2011) which specified that the lowest MIC values of ethyl acetate crude extract of *C. racemose* ranged from 5,000 µg ml^{-1} to 50,000 µg ml^{-1}, and *Bacillus cereus* was susceptible to this crude extract with the lowest concentration of 5,000 µg ml^{-1}. Moreover, Khongsai *et al*. (2017) had studied the effects of *C. racemose* extracts derived from several solvents against some bacteria causing dermatitis in human, and reported that ethyl acetate extract of *C. racemosa* provided the effective results with the MIC and MBC values ranging from 780 µg ml^{-1} to 6,250 µg ml^{-1}, and 12,500 µg ml^{-1} to 25,000 µg ml^{-1}, respectively. These results were also similar to the findings of this study.

According to bacterial strains tested, the derived results also revealed that *Vibrio* species, *V. haveyi*and *V. parahaemolyticus*, presented more resistance to all extracts tested than the bacterial strains from fresh water, *A. hydrophila* and *S. agalactiae*. This was consonant with the report by Srikong *et al*. (2015) which specified that the *Vibrio* species, *V. alginolyticus* and *V. harveyi*, resisted to extracts from seaweeds tested without detection of MIC and MBC values. In addition, this study indicated that the gram-positive *S. agalactiae* was susceptible to all extracts of *C. racemose* var. *corynephora*. This was in accordance with the study of Alghazeer *et al*. (2013) who found that the gram-positive *B. subtilis* strain was highly susceptible to the extract of *C. racemosa*. As aforementioned, many authors had similarly observed that the susceptibilities of gram-positive bacteria to the algal extracts was more than those of gram-negative bacteria (Demirel *et al.*, 2009 and Ibtissam *et al.*, 2009; Salem *et al*., 2011). To support this, the additional susceptibility of gram-positive bacteria to the algal extract may be due to their cell wall containing thinner murine layer which prevents the entry of antibacterial substances than gram-negative bacteria (Kandhasamy and Arunachalam, 2008); moreover, the outer membrane of gram-negative bacteria also acts as a barrier to many environmental substances including antibiotics (Tortora *et al*., 2001).

The variation of antibacterial activity in this test might be due to the difference of solvents used, the difference in capability of extraction protocols to recover the active metabolites, and the difference in the assay methods (Salem *et al*., 2011). Furthermore, the location, seasons and temperature of the water were also affected towards susceptibilities of the target bacterial strains (Febles *et al*., 1995).

All in all, the above results confirmation revealed that *C. racemose* var. *Corynephora* may be the potential source of biologically active

marine organisms because its extract derived from extraction with ethyl acetate exhibited the obvious efficacy on inhibition of pathogenic bacteria in aquaculture. This may provide an initial point for investigations aimed at exploiting new natural antibacterial substances for application in aquaculture.

CONCLUSION

C. racemosa var. *corynephora* might be the potential source of bioactive metabolites, and ethyl acetate crude extract of this algal species had exhibited obvious activity against pathogenic bacteria of aquatic animals; however, its phytochemical should be further analyzed in order to identify the bioactive compounds responsible for antibacterial activity which could be used in management of bacterial disease in aquaculture as alternative to antibiotics.

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