

# Antibacterial and antioxidant activities of differential solvent extractions from the green seaweed *Ulva intestinalis*

Watee Srikong<sup>a</sup>, Nuttapon Bovornreungroj<sup>b</sup>, Pimonsri Mittraparparthorn<sup>a</sup>,  
Preeyanuch Bovornreungroj<sup>a,\*</sup>

<sup>a</sup> Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand

<sup>b</sup> Department of Technology and Industry, Faculty of Science and Technology, Prince of Songkla University, Muang, Pattani 94000 Thailand

\*Corresponding author, e-mail: preeyanuch.b@psu.ac.th

Received 12 Jan 2016

Accepted 27 Jun 2017

**ABSTRACT:** The green algae (*Enteromorpha* spp.) is widely distributed in the southern coast of Thailand. Antibacterial, total phenolic, and antioxidant activities of crude extracts from *Ulva intestinalis* (*E. intestinalis*) with differential solvent extractions were investigated. Alga extracts were prepared by maceration with methanol, ethanol, dichloromethane, and hexane. Only the hexane extract had significant antibacterial activities for Gram-positive bacteria but not Gram-negative bacteria. The lowest minimal inhibitory concentrations of the hexane extract against *Bacillus cereus* TISTR 687, *Staphylococcus aureus* ATCC 29213, and methicillin-resistant *Staphylococcus aureus* 001 R (MRSA) were 256 µg/ml. Pore formation was observed on the cell surface of *B. cereus* treated with the hexane extract. Among these extracts, the highest inhibition radical scavenging effects on 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) activities were observed in the dichloromethane extract with IC<sub>50</sub> = 0.92 mg/ml and 1.50 mg/ml, respectively. The highest phenolic content index was observed with the dichloromethane extract as 197 ± 16 mg gallic acid equivalents/g extract and was significantly higher than those of other extracts ( $p < 0.05$ ). The results indicated that this edible seaweed possesses bioactive compounds that could be further explored for future applications in medicine, dietary supplements, cosmetics, and food industries.

**KEYWORDS:** antibacterial activity, antioxidant activity, seaweed extracts

## INTRODUCTION

In recent years, extracts from seaweeds have received worldwide attention because many seaweed extracts are sources of bioactive compounds including polysaccharides, proteins, polyunsaturated fatty acids, pigments, polyphenols, minerals, and plant growth hormones. Some of these compounds have medical applications since they contain antibacterial, antifungal, antioxidant, and anti-inflammatory activities<sup>1–4</sup>. Because green seaweeds have relatively low toxicity and high bioactivities<sup>5</sup>, they can be used as foods, and their extracts as fertilizers and pharmaceuticals<sup>1</sup>.

Reactive oxygen species (ROS), formed as natural byproducts of oxygen metabolism, have important roles in cell signalling and homeostasis. Excessive amounts of ROS, however, initiate biomolecular oxidations which lead to cell injury and death.

They create oxidative stress which results in numerous diseases and disorders such as cancer, stroke, myocardial infections, and haemorrhagic shock, Alzheimer's and Parkinson's diseases<sup>6</sup>. Due to the toxic and carcinogenic effects of synthetic antioxidants, there is a need to search for alternatives from natural sources<sup>6</sup>. In recent years, antioxidant compounds have been isolated from several plant species<sup>7</sup> including seaweeds.

Seaweed bioactive compounds with antimicrobial properties could be used for defence mechanisms against potential pathogens and as antioxidants. In addition, the interests in seaweed as a rich source of pharmaceutical agents have increased during the last few years<sup>8</sup>. There has been some research, on seaweed extracts that can inhibit human pathogenic microorganism such as bacteria, yeast, and fungi<sup>9–13</sup>.

*Ulva intestinalis* (*Enteromorpha intestinalis*), a

green alga in the phylum Chlorophyta, is widely found around the southern coast of Thailand. Their extracts exhibit antioxidant and antibacterial properties<sup>12,14</sup>. This genus of seaweed is widely used for animal feeds, fertilizers, and human foods<sup>15-17</sup>. Furthermore, it can also be used in pharmacy. The aims of the research were to examine the antibacterial and antioxidant activities of several crude extracts from *U. intestinalis*.

## MATERIALS AND METHODS

### Chemicals and antibacterial agents

All chemical and reagents used in this work were of reagent grade. Folin-Ciocalteu reagent, Na<sub>2</sub>CO<sub>3</sub>, potassium peroxodisulphate, dichloromethane, and *n*-hexane were from Merck Company (Germany). Gallic acid, L-ascorbic acid, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sonic acid)), and DPPH (2,2-diphenyl-2-picrylhydrazyl) were from Sigma Chemical Co. (USA). Others like organic solvents were from BDH-Prolabo (EU).

Antibiotic standard discs, used for the positive control for the antibacterial activity test, were obtained from Oxoid (England). Vancomycin and gentamicin were from Siam Bheasach (Thailand) and Nida Pharma Inc (Thailand), respectively.

### Extract preparation

*U. intestinalis* was collected from Pattani province in the southern coast of Thailand. The alga was air-dried under shade. Dried samples were cut into small pieces and then ground into a powder. Thirty grams of powdered algae was extracted by maceration<sup>18</sup> with 300 ml of 4 different solvents, methanol, ethanol, dichloromethane, and hexane. The extraction was carried out at room temperature and in dark condition for 7 days. The extracts were concentrated under reduced pressure using a rotary evaporator at 40 °C. The concentrated extracts were stored at -20 °C for further use.

### Test microorganisms

Antibacterial studies were carried out on five Gram-positive bacteria (*Bacillus cereus* TISTR 687, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* DMST 4553, *Staphylococcus aureus* ATCC 29213 and methicillin-resistant *Staphylococcus aureus* 001 R (MRSA 001 R)), and eight Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumonia* PSU 1, *Proteus mirabilis* PSU 1, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Typhi PSU 1, *Vibrio alginolyticus* PSU VA 1, *V. harveyi* PSU 4109,

and *V. parahaemolyticus* PSU 5124). All the bacteria were obtained from the Microbiology Department, Prince of Songkla University. The bacteria were grown on Mueller-Hinton agar (MHA) (Bacto, USA), except for *Vibrio* spp., which was grown on MHA supplemented with 1% NaCl (w/v). All bacterial cultures were incubated at 35 °C for 24 h.

### Agar disc diffusion method

The agar disc diffusion method was used to investigate the antibacterial activity of the extracts to the tested microorganisms<sup>19</sup>. Ten microlitres of the algal crude extracts (250 mg/ml) were added to sterile filter paper discs (6 mm) to give a final amount of 2.5 mg/disc. The discs were dried at 30 °C overnight and applied to the surface of MHA plates seeded with a 5 h broth culture of the tested bacteria. The bacterial strains tested were first adjusted to 0.5 McFarland units (1.5 × 10<sup>8</sup> colony forming units per millilitre, CFU/ml). The negative control was prepared using the respective solvents while vancomycin (30 µg/disc) and gentamicin (10 µg/discs) were used as positive controls. The plates were incubated at 35 °C for 18 h. At the end of the incubation period, the zone of inhibition was measured. Each experiment was performed in triplicate.

### Antibacterial activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the Clinical and Laboratory Standards Institute method (CLSI)<sup>20</sup>. The microorganisms that were susceptible to the previous screening using the paper disc diffusion method were taken to determine the MIC value. Vancomycin (0.0625–32 µg/ml) and gentamicin (0.0625–32 µg/ml) were used as reference standards. The alga extracts were prepared as 2-fold dilutions (2–1024 µg/ml) and added to the 96-well microtitre plates. The 96-well microtitre plates were inoculated with 500 µg/ml of the bacterial suspension (10<sup>6</sup> CFU/ml) in each well, homogenized and incubated at 35 °C for 16 h. For the MIC determination, 10 µl of a 0.18% (w/v) resazurin stain were added into each well and the plates were incubated at 35 °C for 2 h. The minimal bactericidal concentrations were performed by streaking the contents of the microtitre wells from which the MIC values were determined onto fresh MHA and incubated at 35 °C for 24 h. The concentration at which there was no visible bacteria growth after 24 h incubation was regarded as the MBC.

### Effect of the algal extracts on bacterial cells

The effect of the hexane extract of *U. intestinalis* on the morphological and ultrastructural changes of *B. cereus* TISTR 687 cells were observed by scanning electron microscopy (SEM). The bacterial strains were grown in Mueller-Hinton broth at 35 °C for 18 h. The bacterial cells were centrifuged at 5000 rpm for 10 min. The bacterial cells were treated with the hexane extract at a concentration 1024 µg/ml (4× the MIC). The bacterial cells were treated with 2% ethanol as the control. The samples were incubated at 35 °C for 16 h. The cells were collected by centrifugation, and washed 3 times with sterilized 0.1 M phosphate-buffered saline (PBS) pH 7.0. The pellet of cells was spread on a glass slide and fixed with 2% glutaraldehyde in PBS for 2 h, the pelleted cells were washed 3 times with PBS. The samples were dehydrated with a series of ethanol concentrations (50, 70, 80, 90%, and absolute ethanol), respectively, for 30 min each, then coated with a 42 nm thickness of gold and examined in a Philips Quanta 400 (FEI Company, Czech Republic) at 20.00 kV. Observed by SEM.

### DPPH radical scavenging activity

The free-radical scavenging capacity of the algal extract was analysed using the DPPH test according to the method of Shao et al<sup>5</sup>, with some modifications. Ascorbic acid was used as a reference standard. The alga samples (0.2 ml each) of various concentrations (100–2000 µg/ml) dissolved in methanol was mixed in a 10 ml test tube. Then 0.004% of DPPH reagent was added to achieve a final volume of 2.0 ml. The mixture was incubated in the dark at room temperature for 30 min, the absorbance at 517 nm was measured. The DPPH scavenging rate was calculated as  $1 - (A_s - A_b)/A_c$ , where  $A_s$  is the absorption of the sample,  $A_b$  is the absorption of the sample blank (without DPPH solution), and  $A_c$  is the absorption of the DPPH solution without sample. Each test was run in triplicate and the average values are given in the text. The half-maximal inhibitory concentration values ( $IC_{50}$ ) of the alga extracts were calculated by linear regression analysis and expressed as a mean of the three determinations.

### ABTS radical scavenging activity

The antioxidant activity of the extracts was determined by the ABTS assay<sup>21</sup>. The ABTS radical cation solution was prepared by 16 h of reaction of ABTS (7 mmol) with potassium persate

(2.45 mmol) at room temperature in the dark. The solution was diluted with ethanol to obtain an absorbance of  $0.700 \pm 0.005$  at 734 nm. The decolourization assay was started by mixing the diluted ABTS solution (1.9 ml) with different concentration of the extracts (10–2000 µg/ml). The mixture was allowed to react at 25 °C for 3 min and the absorbance was measured at 734 nm with a spectrophotometer. The ABTS scavenging rate was calculated as  $1 - (A_s - A_b)/A_c$ , where  $A_s$  is the absorption of the sample,  $A_b$  is the absorption of the sample blank (without ABTS solution), and  $A_c$  is the absorption of the ABTS solution without sample. Each test were run in triplicate and the average values are presented in the text. The concentration of the extract that provided the  $IC_{50}$  was calculated from the plotted graph of the percentage inhibition against the extract concentration.

### Total phenolic content

The total phenolic compounds (TPC) of the alga extracts were determined by the Folin-Ciocalteu method as modified by Pourmorad et al<sup>6</sup>. In brief, 0.2 ml of the extract was mixed with 1 ml Folin-Ciocalteu reagent (0.2 N) followed by the addition of 0.8 ml  $Na_2CO_3$  (8%). The mixture was allowed to stand at room temperature for 1 h in the dark, the TPC were measured by colorimetry at 765 nm. Gallic acid was used as the standard reference. TPC values are expressed in terms of mg gallic acid equivalents per gram (mg GAE/g) of dried extract.

### Statistics

All experiments were performed in triplicate and the results were expressed as a mean value  $\pm$  standard deviation of determination. Statistical analysis was performed by one-way ANOVA using the SPSS 11.5 for Windows. The differences in the mean values were considered to be significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Determination of the antimicrobial activity

The organic extracts of *U. intestinalis* were screened for their antibacterial activities against pathogens and food spoilage microorganisms using the disc diffusion method. The antimicrobial activity of these extracts is shown in Table 1. The algal extracts demonstrated antimicrobial activity against Gram-positive bacteria, with inhibition zones that ranged from  $6.85 \pm 0.17$  to  $16.4 \pm 2.4$  mm. None of the extracts, however, showed activity against Gram-negative bacteria. The hexane extract had

**Table 1** Antimicrobial activity (inhibition zone) of various crude solvent extracts of *U. intestinalis* at a 250 mg/ml concentration.

Bacterial strains	Diameter of inhibition zone (mm) <sup>a</sup>				
	Methanol	Ethanol	Dichloromethane	Hexane	Positive control <sup>b</sup>
<i>E. coli</i> ATCC 25922	– <sup>c</sup>	–	–	–	20.39 ± 0.26
<i>K. pneumonia</i> PSU 1	–	–	–	–	19.00 ± 0.51
<i>P. mirabilis</i> PSU 1	–	–	–	–	21.74 ± 0.23
<i>P. aeruginosa</i> ATCC 27853	–	–	–	–	20.68 ± 0.38
<i>S. Typhi</i> PSU 1	–	–	–	–	10.61 ± 0.27
<i>V. alginolyticus</i> PSU VA 1	–	–	–	–	14.6 ± 2.0
<i>V. harveyi</i> PSU 4109	–	–	–	–	14.48 ± 0.74
<i>V. parahaemolyticus</i> PSU 5124	–	–	–	–	13.89 ± 0.93
<i>B. cereus</i> TISTR 687	6.85 ± 0.17	–	–	7.28 ± 0.02	20.56 ± 0.37
<i>E. faecalis</i> ATCC 29212	–	–	9.89 ± 0.24	–	21.20 ± 0.52
<i>L. monocytogenes</i> DMST 4553	–	7.96 ± 0.38	–	10.55 ± 0.29	19.20 ± 0.80
MRSA 001 R	12.71 ± 0.98	–	–	16.4 ± 2.4	18.85 ± 0.19
<i>S. aureus</i> ATCC 29213	8.41 ± 0.56	–	–	12.13 ± 0.16	16.67 ± 0.78

<sup>a</sup> Each value represents mean ± SD ( $n = 3$ ).

<sup>b</sup> Positive control: vancomycin (30 µg) for Gram-positive bacteria and gentamicin (10 µg) for Gram-negative bacteria.

<sup>c</sup> – indicates no activity.

the highest activity against all Gram-positive bacteria except for *E. faecalis* ATCC 29212, with a maximum inhibition diameter against MRSA 001 R of 16.4 ± 2.4 mm. Similarly, the methanol extract showed moderate activity against MRSA 001 R (12.71 ± 0.98 mm) followed by *S. aureus* ATCC 29213 and *B. cereus* TISTR 687. In addition, the dichloromethane and ethanol extracts showed little activity. These results agree with that of Dussault et al<sup>13</sup>, who reported that *Ulva* sp. extracted with dichloromethane followed by dichloromethane/methanol (50:50) showed antimicrobial activity against Gram positive food-borne pathogen (*L. monocytogenes*, *B. cereus*, and *Staphylococcus aureus*). Their algal extracts had no antibacterial activity against Gram-negative bacteria (*Escherichia coli* and *Salmonella enterica* serovar Typhimurium). Hellio et al<sup>22</sup> reported that the ethanolic extract of *E. intestinalis* exhibited antimicrobial activity with the highest inhibition zone in the case of *B. megaterium* (10–12 mm), a moderate inhibition zone against *B. subtilis* (8–10 mm), and the lowest inhibition zones against *B. cereus*, *Streptococcus* sp., and *S. aureus* (4–8 mm). Their extract did not display activity against Gram-negative bacteria including *E. coli* K12, *K. pneumonia*, *Serratia marcescens*, *P. vulgaris*, and *P. aeruginosa*. In contrast, Lima-Filho et al<sup>23</sup> reported that, when the green alga *U. fasciata* (*Ulva* sp.) was extracted with different solvents such as hexane, chloroform, and ethanol and their activity was evaluated for

its antimicrobial activity by the single disk method, no antimicrobial activity was found against any organism (*B. subtilis*, *S. epidermidis*, *S. aureus*, *Citrobacter freundii*, *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae*, *Morganella morganii*, *P. aeruginosa*, *S. Typhimurium*, and *S. Enteritidis*). It is possible to assume that these differences could be due to the different production of secondary metabolites with different antimicrobial activities; perhaps because of intraspecific variability related to seasonal variations<sup>23,24</sup>. Secondly, there may also be differences in the capability of the extraction protocols to recover any inhibitory phytochemicals, and the different assay methods could also result in the different susceptibilities of the target strains<sup>25–27</sup>. Finally, there may be differences in the stage of active growth or sexual maturity<sup>28,29</sup>.

The results of this study showed that the alga extracts were ineffective against Gram-negative bacteria but had a significant activity against most of the Gram-positive bacteria tested. These may be due to differences in the permeability barriers. In Gram-negative species, the outer membrane is a barrier that does not allow the tested compounds to pass<sup>30</sup>.

The broth microdilution method (Table 2) was further employed to determine the MIC and MBC values. Satisfactory MIC and MBC values were detected almost entirely from the hexane extract in the range of 256–1024 µg/ml. All other values were > 1024 µg/ml. The MIC values of the algal hexane extract against *B. cereus* TISTR 687 (MBC =

**Table 2** Minimal inhibition concentrations (MIC) and minimal bactericidal concentrations (MBC) of extracts from *U. intestinalis*.

Bacterial strains	MIC/MBC ( $\mu\text{g/ml}$ )				
	Methanol	Ethanol	Dichloromethane	Hexane	Positive control <sup>b</sup>
<i>B. cereus</i> TISTR 687	1024/1024	NT	NT	256/256	2/2
<i>E. faecalis</i> ATCC 29212	NT <sup>a</sup>	NT	1024/>	NT	0.5/8
<i>L. monocytogenes</i> DMST4553	NT	>/>	NT	1024/1024	0.25/8
MRSA 001 R	>/> <sup>c</sup>	NT	NT	256/512	0.5/0.5
<i>S. aureus</i> ATCC 29213	>/>	NT	NT	256/512	0.5/0.5

<sup>a</sup> NT: not tested.

<sup>b</sup> Positive control: vancomycin for Gram-positive bacteria and gentamicin for Gram-negative bacteria.

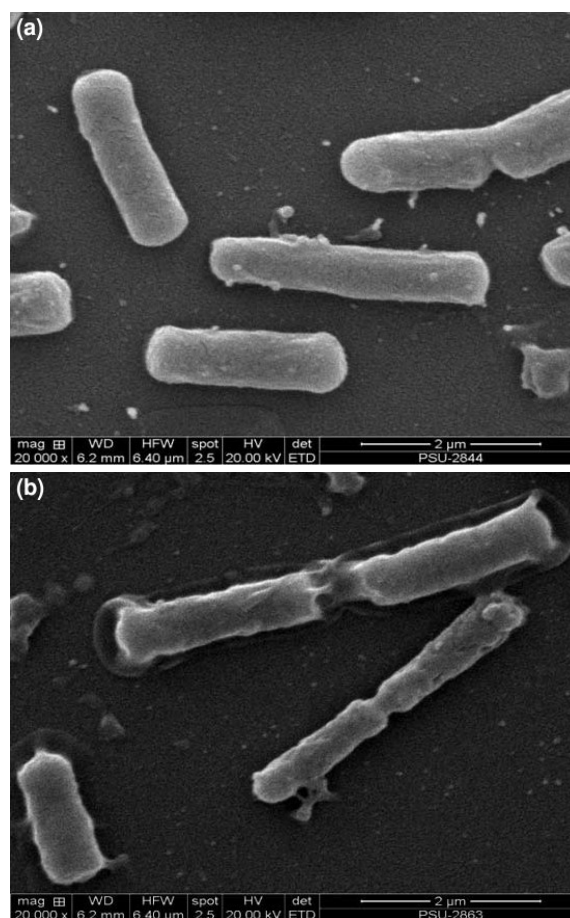
<sup>c</sup> > indicates a concentration higher than 1024  $\mu\text{g/ml}$ .

256  $\mu\text{g/ml}$ ), *Staphylococcus aureus* ATCC 29213 (MBC = 512  $\mu\text{g/ml}$ ) and MRSA 001 R (MBC = 512  $\mu\text{g/ml}$ ) were 256  $\mu\text{g/ml}$ . Similar results were obtained by Hellio et al<sup>22</sup>, and their extracts from *E. intestinalis* showed strong activity against Gram-positive bacteria such as *B. cereus* and *S. aureus* with an MIC value of 96  $\mu\text{g/ml}$ . In addition, Rosaline et al<sup>31</sup> reported that the green alga *Chaetomorpha linum* extracted by maceration with acetone showed the highest activity against MRSA with an MIC value of 2.5 mg/ml, while the ethanol and hexane extracts of *C. linum* exhibited MIC values of 5.0 mg/ml.

Phenolic compounds from marine algae also have an effect on bacterial cells. These compounds attack the cell walls and cell membranes of the bacteria. In addition, bio-oil from a plant extract can inhibit bacteria<sup>8</sup>. The bio-oil can be extracted by a low or non-polar solvent, and the potent biological activities of bio-oil were from free fatty acids (such as myristic acid, palmitic acid, and *cis*-8-octadecanoic acid) with an ability to kill or inhibit the growth of bacteria<sup>32</sup>.

### Effect of alga extract on bacterial cells

Morphological observations on the hexane extract-treated *B. cereus* TISTR 687 at  $4 \times \text{MIC}$  using SEM analysis showed that the bacteria became rough and looked porous (Fig. 1b), in comparison to the control cell that was smooth and rod-shaped (Fig. 1a). It was believed that at 16 h, the cells lost their metabolic functions and this directly or indirectly inhibited the cell growth by the hexane extract. Koprivnjak et al<sup>33</sup> suggested that the bacterial cell wall is the main site of action of the algal extract. This result was similar to the report by Lee et al<sup>34</sup>. They used the marine alga *Myagrapsis myagroides* extract that had an effect on the cytoplasmic membrane of *L. monocytogenes*. The treated *L. monocytogenes*



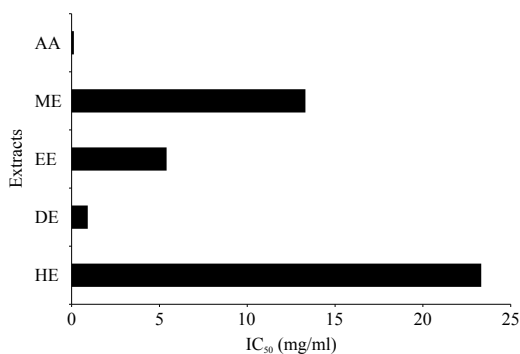
**Fig. 1** Scanning electron microscope images of (a) untreated and (b) treated cell of *B. cereus* TISTR 687 with hexane extract.

cells also showed pore formation and there was extensive damage to the cell envelope, including the membrane and cell wall, that led to cell lysis.

**Table 3** DPPH scavenging activity, ABTS scavenging activity and the amount of total phenolic compounds in the crude extracts from *U. intestinalis* with mean  $\pm$  SD, for  $n = 3$ .

Crude extract	DPPH scavenging effect (%)	ABTS scavenging effect (%)	Total phenolic (mg GAE/g extract)
methanol	22.6 $\pm$ 0.8 <sup>c</sup>	25.0 $\pm$ 0.5 <sup>c</sup>	54.4 $\pm$ 0.3 <sup>d</sup>
ethanol	31.9 $\pm$ 0.4 <sup>b</sup>	35.7 $\pm$ 3.7 <sup>b</sup>	88.6 $\pm$ 1.3 <sup>c</sup>
dichloromethane	87.5 $\pm$ 0.1 <sup>a</sup>	61.9 $\pm$ 1.3 <sup>a</sup>	197 $\pm$ 16 <sup>a</sup>
hexane	22.5 $\pm$ 0.2 <sup>c</sup>	27.9 $\pm$ 0.2 <sup>c</sup>	150 $\pm$ 13 <sup>b</sup>

Different letters in the same column indicate a significant difference ( $p < 0.05$ ).



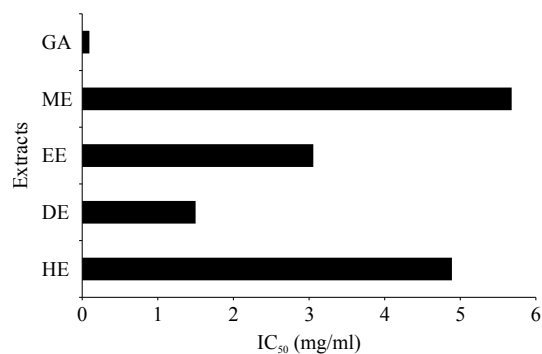
**Fig. 2** IC<sub>50</sub> (mg/ml) values of alga extracts for free-radical scavenging activity by DPPH radical. A lower IC<sub>50</sub> value indicates a higher antioxidant activity. Ascorbic acid (AA) was used as reference standard. Extract: ME = methanol extract, EE = ethanol extract, DE = dichloromethane extract, and HE = hexane extract.

### DPPH radical scavenging activity

The antioxidant activity was evaluated for four extracts from *U. intestinalis* and each showed significant differences in DPPH radical scavenging activity ( $p < 0.05$ ) at 2 mg/ml (Table 3). The dichloromethane extracts of *U. intestinalis* possessed the highest antioxidant activity, with 87.54  $\pm$  0.11% of inhibition of the DPPH radical and lower IC<sub>50</sub> values of 0.92 mg/ml (Fig. 2). That was followed by the ethanol, methanol, and hexane extracts with activities of 31.9  $\pm$  0.4, 22.6  $\pm$  0.8, and 22.5  $\pm$  0.2% inhibition of the DPPH radical, respectively. Previous studies have reported that an ethanol extract of *E. prolifer*a showed the highest inhibition for scavenging the DPPH radical by 96%<sup>35</sup>. Farasat et al<sup>15</sup> observed that methanolic extracts of *U. intestinalis* showed the highest DPPH scavenging activity (48% inhibition) and a lower IC<sub>50</sub> value of 2.32 mg/ml.

### ABTS radical scavenging activity

In the present work, a number of free radical assay systems were successfully used to evaluate the antioxidant activity. These systems have been widely



**Fig. 3** IC<sub>50</sub> (mg/ml) values of alga extracts for free-radical scavenging activity by ABTS radical. A lower IC<sub>50</sub> value indicates a higher antioxidant activity. Gallic acid (GA) was used as a reference standard. Extract: ME = methanol extract, EE = ethanol extract, DE = dichloromethane extract, and HE = hexane extract.

used to test the ability of compounds to act as free-radical scavengers or hydrogen donors<sup>36</sup> and the antioxidant activity of plant extracts have been evaluated by different response tests<sup>37</sup>.

The free-radical scavenging activities of the four *U. intestinalis* extracts, assessed by the ABTS assay, showed significant differences (Table 3). The results showed that, among the four extracts, dichloromethane extract had the highest ABTS scavenging activity (61.9  $\pm$  1.3%) and a lower IC<sub>50</sub> value of 1.50 mg/ml (Fig. 3). The result of both ABTS and DPPH radicals showed that there was a potential for inhibition of free radicals. The scavenging effect of the hexane extract was lower than that of the other extracts from both methods, probably because hexane is a non-polar solvent. Accordingly, Tepe et al<sup>38</sup> reported that polar extracts exhibited a stronger antioxidant activity than the non-polar extracts from *Salvia tomentosa*.

### Total phenolic content

The phenolic compound content ranged from 54.4  $\pm$  0.3 to 197  $\pm$  16 mg GAE/g extract (Table 3).

The highest phenolic content was significantly different ( $p < 0.05$ ) in dichloromethane extract followed by hexane, ethanol, and methanol extracts, respectively. All phenolic content values were considerably higher than those reported for the algal extract with 80% methanol of *U. intestinalis* (1.258 mg GAE/g extract) collected from the Northern Coasts of the Persian Gulf<sup>17</sup>. A similar result for two green algae *Halimeda* species (of the same area) has been reported by Yoshie et al<sup>39</sup>. This difference in polyphenolic contents may be due to local variations.

Generally, the higher total phenolic content resulted in a higher antioxidant activity. According to Table 3, determination of antioxidant activities (DPPH and ABTS) of dichloromethane extract were similar to that of TPC. In addition, Naczka and Shahidi<sup>40</sup> suggested that the polarity of the solvent illustrated a significant role in increasing the solubility of phenolic compounds.

## CONCLUSIONS

In conclusion, the results of the present study confirm that the marine alga *U. intestinalis* was a potential source of bioactive compounds but mainly for scavenging free radicals. This study indicates that, among the four extracts, the hexane extract showed the best antimicrobial activity against various human Gram-positive bacterial pathogens, but had no effect on Gram-negative bacteria. The lowest MIC and MBC values were observed for *B. cereus* TISTR 687, MRSA 001 R and *S. aureus* ATCC 29213 treated with the hexane extract. It was of interest that pore formation was found on the cell surface of *B. cereus* TISTR 687 treated with the hexane extract. As the MIC levels were relatively high, it is unlikely that useful antibiotics could be isolated from this green alga. However, for the *U. intestinalis* extracted with different solvents, the most efficient solvent for phenolic extraction and radical scavenging activity was dichloromethane. Further work is needed to isolate and identify the active compounds from marine algae. The results from the present study indicate that this edible alga possesses suitable antioxidant potential and should be considered for future applications in medicine, dietary supplements, cosmetics, and food industries.

**Acknowledgements:** This work was supported by the Faculty of Science Research Fund, Prince of Songkla University (Contract No. 1-2554-02-005).

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