Phytochemical Composition and Antibacterial Activity of Brown Seaweed, *Padina australis* against Human Pathogenic Bacteria

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ABSTRACT

Seaweeds have become targets for chemical screening in search of new compounds that have potential medical value, including antibacterial activity. The aim of this study was to evaluate the chemical composition and antibacterial activity of the extract and fractions of *Padina australis*. The chemical compounds in seaweed samples were extracted with methanol, then sequentially partitioned with hexane, dichloromethane, ethyl acetate (EA) and n-butanol. The EA fraction was further separated using silica gel column chromatography to yield eight subfractions (EA-1 to EA-8). The antibacterial activity was tested against seven pathogenic bacteria through the disc diffusion method and its minimum inhibitory concentration was tested using the standard broth dilution method. The highest activity against gram-positive bacteria was observed for the EA-1 subfraction with inhibition zones of 9.37±0.09 mm, 12.25±0.60 mm and 10.30±0.05 mm for *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATCC25923 and methicillin-resistant *S. aureus* KL046, respectively. None of the extracts showed activity against gram-negative bacteria. Moreover, the antibacterial activity was positively correlated with the total phenolic content in the extracts against *B. subtilis* (r = 0.656), *S. aureus* (r = 0.800) and methicillin-resistant *S. aureus* (r = 0.880). The highest fucoxanthin content was also observed in the EA-1 subfraction. The active compounds of the EA-1 subfraction were identified using gas chromatography coupled with mass spectroscopy, and the major components were fucosterol, (3β, 24Z)-Stigmasta-5, 24(28)-dien-3-ol and phloroglucinol. Based on these results, the bioactive compounds responsible for the antibacterial activity might be the phenolic compounds fucoxanthin, fucosterol and fucosterol derivative.

Keywords: Fucosterol, Fucoxanthin, MRSA, *Padina australis*, Phenolic content

INTRODUCTION

Humanity is currently experiencing problems associated with the increase in bacterial infections and the many side effects of synthetic drugs that are being used to deal with these medical problems. In addition, overuse has made antibiotics less effective, resulting in bacteria that are resistant to one or more antibiotics, which is a growing problem worldwide (Hsieh and Amin, 2016). The high human mortality rates associated with bacterial infections in Thailand caused by drug-resistant bacteria, particularly extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (Thamlikitkul *et al*., 2015) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Jariyasethpong *et al*., 2010; Kitti *et al*., 2011), are a cause for concern. One of the methods to treat antibiotic-resistant bacteria is to use new compounds rather than the existing synthetic antibacterial drugs. Therefore, the search for novel

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Received 8 October 2019 / Accepted 21 July 2020

natural sources, particularly marine organisms, could lead to the isolation of new antibiotics. Among marine organisms, seaweed is an under-exploited plant resource (Gupta and Abu-Ghannam, 2011; Eom *et al*., 2012; Brown *et al*., 2014). Many studies have already found that seaweeds are capable of producing bioactive compounds with antibacterial activity, such as terpenes (Rodrigues *et al*., 2015), sterols (Kavita *et al*., 2014), phlorotannins (Choi *et al*., 2010) and fucoxanthin (Peng *et al*. 2011; Rajauria and Abu-Ghannam, 2013).

Specifically, brown seaweeds have been reported to contain a wide range of bioactive compounds, mainly polyphenols in the group of phlorotannins, with potential antibacterial activity (Nagayama *et al*., 2002; Eom *et al*., 2012; Lee *et al*., 2014). Phlorotannins have high molecular weight chains of phloroglucinol (1, 3, 5-benzenetriol) units and differ from terrestrial plant tannins. The size of phlorotannins ranges from 126 Da to 650 kDa. Dieckol, a phlorotannin derivative purified from *Ecklonia stolonifera*, exerts an antibacterial effect on MRSA with minimum inhibitory concentration (MIC) of 64 $\mu g \cdot mL^{-1}$ as well as a synergistic effect with ampicillin (MIC: 0.5 $\mu g \cdot mL^{-1}$) and penicillin (MIC: 0.5 $\mu g \cdot mL^{-1}$) (Lee *et al*., 2008). Moreover, the ethyl acetate (EA) fraction (containing dieckol, bieckol, and eckostolonol) of *Eisenia bicyclis* possesses anti-MRSA activity with an MIC of 32-64 $\mu g \cdot mL^{-1}$ (Eom *et al*., 2011). Diterpenes are non-volatile compounds with different carbon structures that are abundant in the Family Dictyotaceae (Gupta and Abu-Ghannam, 2011). Several reports have described the antibacterial activity of brown seaweeds; however, only a few studies have examined brown seaweeds from Thailand, such as *Sargassum binderi*, *Turbinaria conoides* (Boonchum *et al*., 2011) and *Sargassum polycystum* (Rattaya *et al*., 2015).

Padina australis is a common brown seaweed belonging to the Family Dictyotaceae that is mostly found in the South China Sea and along the coast of Thailand (Prathep *et al*., 2011; Phang *et al*., 2016). This alga has been increasingly cultivated and studied as a source of alginate and iodine, products that are useful for industries such as food, pharmacy, cosmetics and textiles (Brotosudarmo *et al*., 2018). Moreover, it has been reported to have beneficial biological activities, such as antibacterial (Kantachumpoo and Chirapart, 2010; Chiao-Wei *et al*., 2011; Zailanie, 2016), antioxidant (Gunji *et al*., 2007), antiangiogenic and cytotoxic effects (Canoy and Bitacula, 2018). However, no report has clearly described the antibacterial activity against drug-resistant bacteria. Therefore, the aim of this study was to evaluate the antibacterial activity and phytochemical components responsible for the activity of the extracts and fractions from *P. australis*.

MATERIALS AND METHODS

Seaweed

The seaweed Padina australis was collected at Sattahip district, Chonburi Province, Thailand (12°37′32.6″N 100°57′53.7″E) during March 2017. The seaweed samples were stored in plastic bags and transported to the laboratory. Seaweed was washed with running tap water to remove surface debris, and epiphytes were removed by hand. The cleaned samples were stored frozen at 20 °C and thawed at room temperature before use.

Preparation of crude extracts

The seaweed samples were cut into small pieces (~0.5-1.0 cm) and then extracted for 24 h with 95% methanol (10 g of seaweed:100 mL of methanol). The mixture was incubated on a shaker at room temperature (25±2 °C) in the dark and then filtered through Whatman No. 2 filter paper with vacuum pump to collect the filtrate. The residues were extracted again two additional times under the same conditions. The resulting filtrate was then evaporated to dryness at 40 °C using a rotary evaporator (Buchi R-200, Switzerland) and blown with N_2. The resulting dried extract was weighed to calculate the percent yield, re-dissolved in 95% methanol and stored at -20 °C until further analysis.

Fractionation of the crude methanolic extract

A liquid-liquid solvent extraction procedure was used to separate bioactive substances according to their relative polarity. The crude methanolic

extract was dried with a rotary evaporator and then re-dissolved in 95% methanol. The aqueous suspension was sequentially partitioned with *n*-hexane (Hex), dichloromethane (DCM), ethyl acetate (EA) and *n*-butanol (BuOH) in sequence (Eom *et al*., 2011). Ultimately, Hex, DCM, EA, BuOH and aqueous fractions were obtained. Each fraction was evaporated to dryness, weighed, re-dissolved in 95% methanol and stored at -20 °C until further analysis. The EA fraction was further separated by column chromatography because it displayed the best antibacterial activity. The dried dark green residue of the EA fraction was separated using open column chromatography (70-230 mesh (62-200 µm), 3.5×50 cm, 150 g silica gel) with a gradient of EA-MeOH (50:1, 40:1, 30:1, 20:1, 10:1, 1:1, 0.5:1 and 0:1) to yield eight subfractions (EA-1 to EA-8). The EA-MeOH ratios were modified from Lee *et al*. (2012). Each subfraction was evaporated to dryness, weighed, re-dissolved and stored in a -20 °C freezer until further analysis. The fractionation procedure is shown in Figure 1.

Bacterial strains

All of the bacterial strains used in this study were obtained from the Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University and included *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, methicillin-resistant *S. aureus* (MRSA) KL046, ampicillin-resistant *E. coli* ATCC 35218, extended spectrum β-lactamase-producing *E. coli* (ESBL+*E. coli*) and extended spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL+*K. pneumoniae*). Before use, all strains were maintained on a nutrient agar at 4 °C. The bacteria were activated by subculture at 37 °C for 6 h with fresh Mueller Hinton Agar (MHA, Difco™, France) before any antibacterial tests.

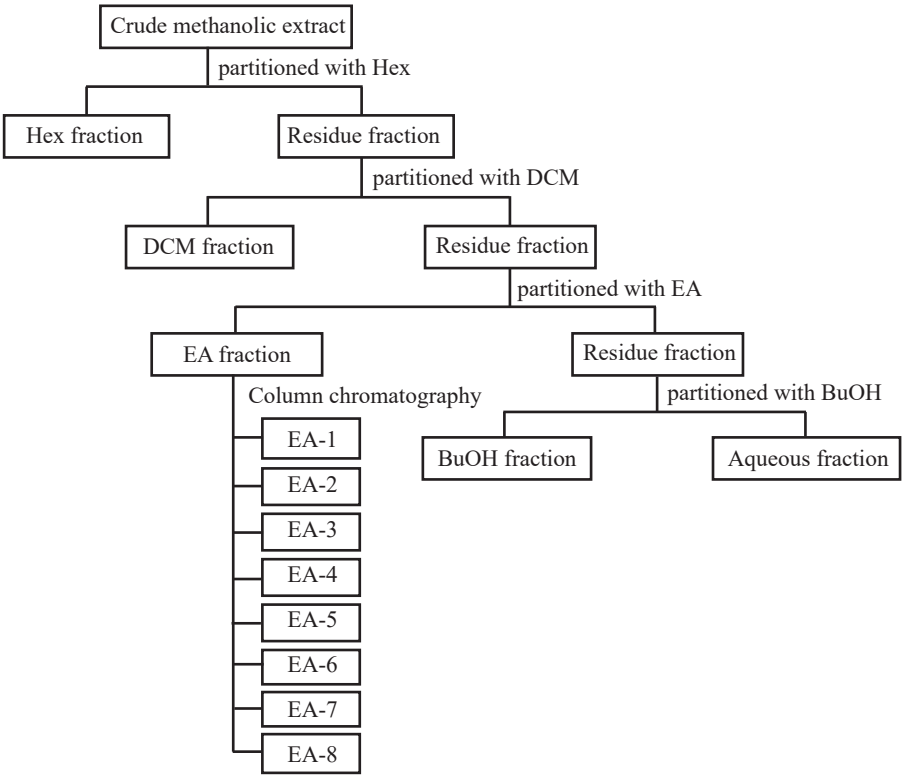

Figure 1. Extraction scheme to obtain various fractions from *Padina australis*. Hex: *n*-hexane, DCM: dichloromethane, EA: ethyl acetate, BuOH: *n*-butanol.

Antibacterial agar disc diffusion assay

The *in vitro* antibacterial activity of each extract or fraction was evaluated using the disc diffusion method (Bauer *et al*., 1966). Bacterial suspensions were prepared with deionized water, the turbidity was adjusted to 0.5 with a McFarland densitometer (Biosan, Den-1B), and then bacteria were spread on MHA plates. Sterile paper discs (6 mm diameter) were placed on the surface of the MHA agar plates. Each paper disc was loaded with 20 µL (50 $mg \cdot mL^{-1}$ in 10% dimethyl sulfoxide, DMSO) of each extract (the final concentration was 1 $mg \cdot disc^{-1}$). Antibiotics, including ampicillin, oxacillin, vancomycin, ceftriaxone, and meropenem, were used as positive controls and 10% DMSO served as the negative control. The zone of inhibition was determined by measuring the diameter of the extract-impregnated disc, then compared with the controls after an incubation at 37 °C for 24 h. Measures of the inhibition zone are presented as the mean values of three replicates and standard deviation.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using the broth microdilution method as recommended by CLSI (Clinical and Laboratory Standard Institute, 2012), with some modifications. Briefly, a serial dilution of each extract/fraction was prepared in concentrations ranging from 2 $\mu g \cdot mL^{-1}$ to 1,024 $\mu g \cdot mL^{-1}$ in sterile 96-well plates containing Mueller-Hinton broth medium. DMSO was used as the solvent for extracts and partially purified compounds and was present in final concentrations at less than 1% (v/v). Then, 50 µL of bacterial suspensions with a density of 10^5 $CFU \cdot mL^{-1}$ were added to each well and incubated at 37 °C for 24 h. MIC was recorded as the lowest concentration that inhibited the visible growth of bacteria.

Analysis of total phenolic content

The total phenolic content (TPC) was evaluated using the method described by Widjaja-Adhi Airanthi *et al*. (2011). The extract was weighed and dissolved in methanol to the appropriate test concentration in a final volume of 100 µL. One hundred microliters of the sample solution was added to 750 µL of 10% Folin-Ciocalteu solution and incubated at 37 °C. After 5 min, 750 µL of 6% Na_2CO_3 was added, and the mixture was incubated at 37 °C for 30 min in the dark. The absorbance was measured at 750 nm using a UV/Vis-spectrophotometer (Shimadzu UV-1700). The TPC of each extract was then calculated based on a phloroglucinol standard curve and reported as milligrams of the phloroglucinol equivalent (PGE) per gram of the extract.

Analysis of fucoxanthin content

All HPLC analyses were performed using an Agilent 1260 Infinity HPLC system (Agilent Technologies Inc., Germany) equipped with a pump (model G1311C 1260 Quant Pump VL), an autosampler (model G1329B 1260 ALS), and a UV detector (model G1365D 1260 MWD VL). The fucoxanthin content of the extract was determined using reverse-phase HPLC (RP-HPLC) with the mixture of methanol and acetonitrile (7:3 v/v) as the mobile phase at a flow rate of 1.0 $mL \cdot min^{-1}$ (Jaswir *et al*., 2011). All RP-HPLC analyses were conducted at 25 °C using Zorbax Eclipse Plus C18 column (5.0 µm particle size, 250 mm x 4.6 mm i.d., Agilent Technologies Inc., Germany). Briefly, an extract was dissolved in the methanol and filtered with a 0.45-µm PTFE membrane filter. The detection wavelength was set to 450 nm for fucoxanthin. The purified fucoxanthin (Sigma-Aldrich, Lot No. BCBQ1635V) was used as standard to calculate the fucoxanthin content in the extract.

Thin-layer chromatography (TLC) assay

TLC was performed using an aluminium silica gel plate (20×20 cm, TLC silica gel 60 F254, Merck). Each fraction of the extract was spotted on the silica gel plate with a solvent system consisting of chloroform/ethanol/acetic acid/water (98:10:2:2 v/v) as the mobile phase (Sathya *et al*., 2017). The spots were observed by spraying the silica gel plates with vanillin-sulfuric acid reagents (3 g of vanillin in 100 mL of ethanol and 0.5 mL sulfuric acid). A blue spot was observed when the fraction contained terpenoids and red spots were observed when the

fraction contained a phenolic compound (Matysik *et al*., 2016). The spots were observed by spraying silica gel plates with Dragendorff's reagent (Alghazeer *et al*., 2013). Orange spots indicated alkaloids.

Gas Chromatography-Mass Spectrometry (GC-MS)

The components of the EA-1 extract of *Padina australis* were analysed using GC-MS (GC Agilent Model 7890 B, MS Agilent Model 5977 B). One microliter of the sample was injected into a HP-5MS UI column (60 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas with a flow rate of 1 $mL \cdot min^{-1}$. The injector was operated at 250 °C and the oven temperature was initially programmed at 60 °C, increased to 120 °C within 2 min at a rate of 10 $°C \cdot min^{-1}$, increased to 230 °C (rate of 4 $°C \cdot min^{-1}$), and increased to 280 °C within 14.5 min at a rate of 10 $°C \cdot min^{-1}$ (total run time 55 min) as modified from Maheswari *et al*. (2017). The detector used Mass Selective Detection (MSD) and scanned a mass range of 30-600 mass units. Compounds were further identified and authenticated by comparing their mass spectra to W10N14.L (Wiley 10^{th} and NIST 2014 libraries). Peaks with higher than 95 % similarity compared to the database were noted and the compounds with peak area below 1 % were not reported (as in Table 5).

Statistical analysis

The values of the chemical composition and antibacterial activity of Padina australis were statistically analysed using a one-way analysis of variance (ANOVA) at a probability level $p<0.05$ and reported as mean±standard deviation. Pairwise comparisons after the ANOVA were performed using Duncan's multiple range test. The correlation between TPC and inhibition zone of the disc diffusion method was performed by using the program R version 3.5.0.

RESULTS AND DISCUSSION

Extraction yields

The extraction yields of the methanolic extract, fractions and subfractions of *Padina australis* are presented in Table 1. The yield of the methanolic extract was 24.27±1.19 % dry weight (DW). Among the fractions, the aqueous fraction had the highest yield (44.76±0.50 % of crude methanolic extract or

Table 1. Yield of methanolic extract (mean±SD, n = 3) and its fractions from *Padina australis*.

Fractions	Yield of crude extract (%)	Yield of EA extract (%)	Yield of g dry weight (%)
Crude	-	-	24.27±1.19
Hex	7.46±1.13	-	1.81±0.27
DCM	8.00±1.86	-	1.94±0.45
EA	16.57±0.34	-	4.03±0.08
EA-1	-	47.36±0.05	1.91±0.00
EA-2	-	3.91±1.12	0.16±0.04
EA-3	-	1.15±0.47	0.05±0.02
EA-4	-	1.69±0.25	0.07±0.01
EA-5	-	20.61±0.61	0.83±0.00
EA-6	-	8.95±2.86	0.36±0.11
EA-7	-	3.01±1.66	0.12±0.06
EA-8	-	3.66±0.25	0.15±0.10
BuOH	21.26±0.21	-	5.17±0.05
Aqueous	44.76±0.50	-	10.87±0.13

Note: Hex, DCM, EA, BuOH and Aqueous are expressed in percent yield of crude methanolic extract; EA-1 to EA-8 subfractions are expressed in percent yield of EA fraction

10.87±0.13 % DW), followed by the BuOH and EA fractions. The EA fraction was further partitioned using column chromatography into eight subfractions because it displayed the highest antibacterial activity, as shown in Table 2. The highest yield was obtained from the EA-1 subfraction followed by the EA-5 subfraction.

The extraction yield of the seaweed appeared to be influenced by the polarity of the solvent, and by the degree of polarity of the various components of the extract, such as phenolic compounds. In this study, the crude methanolic extract was partitioned with different solvents using the liquid-liquid extraction method to yield five fractions (Hex, DCM, EA, BuOH and aqueous). Compounds with a low polarity were extracted with Hex and DCM, while medium-high polarity compounds were extracted with EA and BuOH (Khanum *et al*., 2015). Regarding the extraction yield (Table 1), the crude methanolic extract of *P. australis* was mostly composed of medium to high polarity compounds due to the higher yields of the EA, BuOH and aqueous fractions. Our results were consistent with many reports regarding the extraction yields from numerous species of brown seaweed (e.g. *Eisenia bicyclis*, *E. stolonifera* and *E. cava*) which showed highest yields for EA, BuOH and aqueous fractions (Lee *et al*., 2008; Li *et al*., 2009; Eom *et al*., 2011). However, the extraction yield also depended on the seaweed structure and solvent used for extraction (Cox *et al*., 2010; López et al., 2011).

Antibacterial activity

The antibacterial activity of each extract toward pathogenic bacteria was quantitatively

Table 2. Antibacterial activity (mean±SD, n = 3) of the crude extract, fractions, and subfractions of *Padina australis* determined by agar disc diffusion method.

Fractions	Diameter of inhibition zone (mm)						
	Gram-positive bacteria			Gram-negative bacteria			
	***Bacillus subtilis* 6633**	***Staphylococcus aureus* 25923**	**MRSA KL046***	***Escherichia coli* 25922**	***Escherichia Klebsiella* 35218***	**ESBL+ *Escherichia coli* ***	**ESBL+ *Klebsiella pneumoniae****
Crude	7.76±0.11^{cd}	9.81±0.08^{de}	8.15±0.39^{c}	-	-	-	-
Hex	7.46±0.42^{de}	7.20±0.16^{h}	-	-	-	-	-
DCM	7.24±0.31^{de}	-	-	-	-	-	-
EA	8.58±0.16^{bc}	10.70±0.21^{cd}	9.34±0.13^{b}	-	-	-	-
BuOH	7.19±0.13^{ef}	10.05±0.12^{de}	8.47±0.13^{c}	-	-	-	-
Aqueous	6.55±0.10^{e}	-	6.82±0.21^{e}	-	-	-	-
EA-1	9.37±0.09^{b}	12.25±0.60^{b}	10.30±0.05^{b}	-	-	-	-
EA-2	6.44±0.10^{e}	8.73±0.27^{fg}	-	-	-	-	-
EA-3	-	8.18±0.29^{g}	-	-	-	-	-
EA-4	6.52±0.16^{e}	9.52±0.19^{e}	7.63±0.22^{cd}	-	-	-	-
EA-5	6.59±0.20^{e}	11.05±0.08^{c}	9.47±0.17^{b}	-	-	-	-
EA-6	-	9.49±0.22^{ef}	7.62±0.12^{cd}	-	-	-	-
EA-7	-	10.25±0.10^{de}	7.75±0.17^{c}	-	-	-	-
EA-8	-	-	-	-	-	-	-
Positive control							
Ampicillin (10 µg)	31.84±0.39^{a}	ND	ND	15.65±0.25	ND	ND	ND
Oxacillin (1 µg)	ND	21.48±0.42^{a}	ND	ND	ND	ND	ND
Vancomycin (30 µg)	ND	ND	18.47±0.44^{a}	ND	ND	ND	ND
Ceftriaxone (30 µg)	ND	ND	ND	ND	30.96±0.13	ND	ND
Meropenem (10 µg)	ND	ND	ND	ND	ND	29.03±0.06	29.90±0.26

Note: * = drug-resistant pathogenic bacteria, - = no activity, ND = not determined;
Means in the same column with different superscript letters are significantly different ($p<0.05$)

assessed by measuring the inhibition zones, if present. As shown in Table 2, none of the fractions of *Padina australis* extracts displayed activity against gram-negative pathogenic bacteria. The results of this study indicated that gram-positive bacteria were more sensitive to the extracts than gram-negative bacteria. A potential explanation is the presence of the hydrophobic lipopolysaccharide in the outer membrane of gram-negative bacteria that protects against different agents, possibly by inhibiting the cell entry of the active substance (Berber *et al*., 2015). Richter *et al*. (2017) also reported that gram-negative bacteria have an outer membrane consisting of porin proteins that make them more complex than gram-positive bacteria. The results from our study were consistent with the report of Mashjoor *et al*. (2016), which showed that the methanolic and EA extracts of *Padina antillarum* and *P. boergeseni* exhibited antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, while no activity against *Klebsiella pneumoniae* or *Pseudomonas aeruginasa*, representative species of gram-negative bacteria. Similar results were also found by El-Shafay *et al*. (2016), who reported that the methanolic extracts of *Padina pavonica* showed antibacterial activity against MRSA while no activity against *Escherichia coli*. In another study, it was reported that the ethanolic extracts of *P. pavonica* did not show activity against *E. coli* or *P. aeruginosa* (Madkour *et al*., 2019). However, our results differed from the report of Chiao-Wei *et al*. (2011), which showed that *P. australis* extracts possess antibacterial activity against *E. coli* ATCC 25922 and *E. coli* ATCC 35218, with MIC ranging between 0.83 mg·mL^{-1} and 2.08 mg·mL^{-1}, and showed better activity against *S. aureus* with MIC value of 0.414 mg·mL^{-1} (Table 3). This difference in activity might be due to the use of different extraction methods and solvents, as well as active compounds in seaweeds collected from different locations or seasons (Kaufman *et al*., 1999; Praiboon *et al*., 2018).

In the present study, the EA fraction that displayed the best antibacterial activity was further purified using column chromatography to yield eight subfractions (EA-1 to EA-8). The EA-1 subfraction exhibited the highest antibacterial activity against *Bacillus subtilis* (9.37±0.09 mm), *Staphylococcus aureus* (12.25±0.06 mm) and MRSA KL046 (10.30± 0.05 mm), with MICs of 1,024 (*S. aureus* and MRSA KL046) or >1,024 µg·mL^{-1} (*B. subtilis*), followed by the EA fraction (Table 2 and Table 3). Similar results were found by Eom *et al*. (2011), who showed that the EA fraction of *Eisenia bicyclis* exhibited the highest antibacterial activity against *S. aureus* (15 mm) and MRSA (16–17 mm), with MICs ranging from 32 µg·mL^{-1} to 64 µg·mL^{-1}.

Moreover, the antibacterial activity measured by the disc diffusion assay also positively correlated with the TPC of the extract. This result was consistent with the study of Eom *et al*. (2013a), which reported that the antibacterial activity of *Eisenia bicyclis* was positively correlated with the TPC of the extract. Phenolic compounds possess antibacterial activity, particularly phlorotannin,

Table 3. MIC values (µg·mL^{-1}) of *Padina australis* extract including crude methanolic extract and EA fraction.

Fractions	***Bacillus subtilis* 6633**	***Staphylococcus aureus* 25923**	**MRSA KL046***
Crude	>1,024	1,024	1,024
EA	>1,024	1,024	1,024
EA-1	>1,024	1,024	1,024
EA-2	>1,024	>1,024	>1,024
EA-3	>1,024	1,024	>1,024
EA-4	>1,024	1,024	>1,024
EA-5	>1,024	1,024	1,024
EA-6	>1,024	>1,024	>1,024
EA-7	>1,024	1,024	>1,024
EA-8	>1,024	>1,024	>1,024

Note: * denotes drug-resistant pathogenic bacteria

a polyphenol formed through the polymerization process of phloroglucinol (1, 3, 5-trihydroxybenzene) units that is mostly found in brown seaweed (Nagayama *et al*., 2002; Li *et al*., 2011; Eom *et al*., 2012).

Phytochemical composition of Padina australis

The TPC of each extract was determined using the Folin-Ciocaltue method. Among the extractions by solvent type, the highest TPC was observed in the BuOH fraction (168.86±1.76 mg PGE·g^{-1} extract), followed by the EA fraction (136.94±0.54 mg PGE·g^{-1} extract) (Figure 2a). The EA fraction (Figure 2b) was selected for further fractionation due to its high antibacterial activity. The highest TPC was observed in the EA-1 subfraction (246.88±4.35 mg PGE·g^{-1} extract), followed by the EA-5 subfraction (163.93±5.35 mg PGE·g^{-1} extract). Moreover, the antibacterial activity against *Bacillus subtilis* (r = 0.656), *Staphylococcus aureus* (r = 0.800) and MRSA (r = 0.880), as assessed using the disc diffusion method, exhibited a significant correlation with the TPC of the extracts (data not shown).

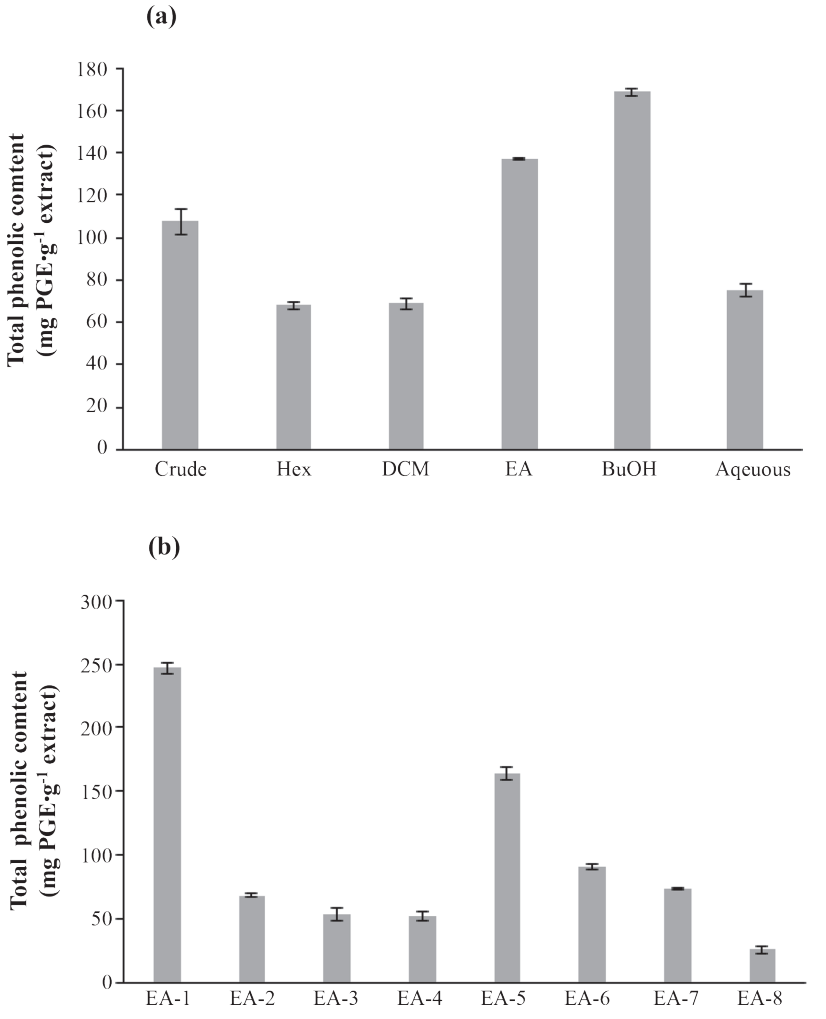

Figure 2. Total phenolic content of *Padina australis* extracts from solvents (a) and the subfractions from EA (b); Error bars indicate SD.

Fucoxanthin is one of the most abundant carotenoids in brown seaweeds as a natural compound, and it possesses high antioxidant activity and exerts many biological effects on human health. Moreover, the antibacterial activity of fucoxanthin has been reported against *Listeria monocytogenes*, with an inhibition zone of 10.89 mm (Peng *et al*., 2011; Rajauria and Abu-Ghannam, 2013). In our study, the highest fucoxanthin content was detected in the EA-1 subfraction (50.40±0.22 mg·g^{-1} extract) (Figure 3), which revealed that fucoxanthin might play an important role in the antibacterial activity.

The chemical compounds responsible for the antimicrobial activity of seaweed are sterols, terpenes, and halogenated, heterocyclic, phenolic and lipophilic compounds. Thin-layer chromatography profiling of all extracts of *Padina australis* extracts revealed the presence of various phytochemicals, such as alkaloids, terpenoids, and phenolic compounds (Table 4). Alkaloid compounds were only detected in the crude methanolic extract, the EA fraction and the EA-8 subfraction, while terpenoids were present in all fractions, except the EA-2 to EA-8 subfractions and the aqueous fraction. Moreover, phenolic

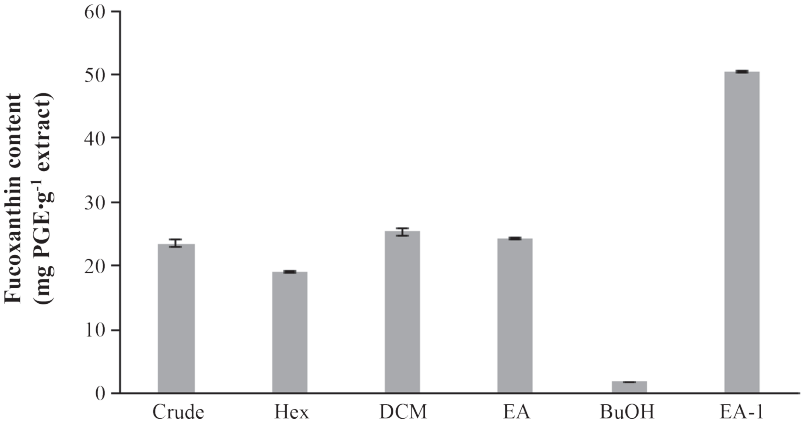

Figure 3. Fucoxanthin content of *Padina australis* extracted from the solvents and a subfraction from EA (EA-1); Error bars indicate SD.

Table 4. Phytochemical compounds in *Padina australis* extracts and fractions detected by TLC.

Extract	Alkaloids	Terpenoids	Phenolic compounds
Crude	+	+	+
Hex	-	+	+
DCM	-	+	+
EA	+	+	+
EA-1	-	+	+
EA-2	-	-	+
EA-3	-	-	+
EA-4	-	-	+
EA-5	-	-	+
EA-6	-	-	+
EA-7	-	-	+
EA-8	+	-	+
BuOH	-	+	+
Aqueous	-	-	+

Note: + = the compound was present in the extract; - = the compound was absent in the extract.

compounds were detected in all extracts. Our study suggests that the chemical compounds that respond to antibacterial activity might belong to the groups of terpenoid and phenolic compounds (Smith, 2004; Kim *et al*., 2008).

Some of the components of the EA-1 subfraction were identified using GC-MS. Based on the spectral match of each obtained compound with the NIST library and relative percentage of the compound, the three most prevalent compounds were fucosterol (23.43 %), which eluted at 53.32 min, (3β, 24Z)-stigmasta-5, 24(28)-dien-3-ol (17.33 %), which eluted at 42.99 min, and 1, 3, 5-benzenetriol or phloroglucinol (16.39 %), which eluted at 24.89 min (Figure 4 and Table 5). These results were consistent with several studies showing that fucosterol was the main sterol found in many members of the genus *Padina*, such as *Padina sanctae-crucis* from the Caribbean coast (Caamal-Fuentes *et al*., 2014), *P. pavonia* from the Adriatic Sea (Kamenarska *et al*., 2002), *P. gymospora* from the Qatar coast (Al Easa *et al*., 1995) and *P. vickersiae* from Senegal waters (Aknin *et al*., 1992). Seaweed sterols have been reported to possess antibacterial activity against *Mycobacterium tuberculosis* (Kim and Ta, 2011). In another study, Kumar *et al*. (2010) reported that the fucosterol isolated from *Turbinaria conoides* showed moderate antibacterial activity against the tested bacteria. Phloroglucinol, the smallest unit of phlorotannin, has been reported as a major EA-soluble compound (Cho *et al*., 2012; Eom *et al*., 2013b; Lee *et al*., 2014). Abdelhamid *et al*. (2018) reported that the EA fraction of *P. pavonica* was a phlorotannin-rich fraction. In another study, Lee *et al*. (2008) reported the highest antibacterial activity for dieckol, a phlorotannin derivative isolated from EA fraction of *E. stolonifera*, against *Staphylococcus aureus* and MRSA. Similar results were found by Eom *et al*. (2013b), who reported that the highest anti-MRSA activity was observed for the EA fraction of *E. cava* fermented by *Candida utilis*.

In addition to fucosterol and phlorotannin, fatty acid and its derivatives have been identified as antimicrobial substances in algae (Thirunavukkarasu *et al*., 2014). Suresh *et al*. (2016) reported the presence of (E)-9-Octadecadienoic acid and Di-(2-ethylhexyl) phthalate in *P. tetrastomatica*, with potent antibacterial and antifouling activity. Moreover, tocopherol derivatives have been reported

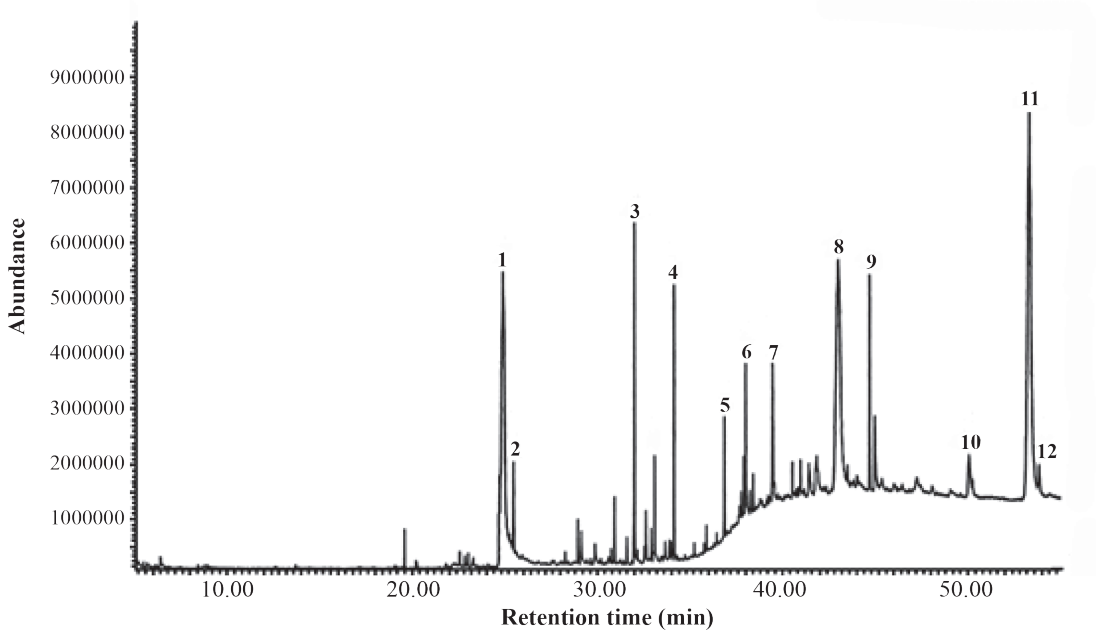

Figure 4. GC-MS Chromatogram of EA-1 subfraction of *Padina australis.*

Table 5. Chemical constituents of the EA-1 subfraction of *Padina australis* detected by GC-MS.

Peak no.	Name[a]	Retention time (min)	Peak Area (%)	Molecular formula	Molecular weight (Da)
1	1, 3, 5-Benzenetriol	24.89	16.39	$C_6H_6O_3$	126.11
2	Hexadecane	25.46	1.09	$C_{16}H_{34}$	226.45
3	Neophytadiene	31.99	3.80	$C_{20}H_{38}$	278.52
4	Methyl hexadecanoate	34.13	3.27	$C_{17}H_{34}O_2$	270.45
5	Arachidonic acid	36.85	1.32	$C_{20}H_{32}O_2$	304.47
6	(E)-9-Octadecadienoic acid	37.99	1.81	$C_{18}H_{34}O_2$	282.47
7	4, 4'-(1-Methylethyldiene) bis-phenol	39.46	1.74	$C_{15}H_{16}O_2$	228.29
8	(3β, 24Z)-Stigmasta-5, 24(28)-dien-3-ol	42.99	17.33	$C_{29}H_{48}O$	412.70
9	Di-(2-ethylhexyl) phthalate	44.69	2.46	$C_{24}H_{38}O_4$	390.56
10	dl-α-Tocopherol	50.06	1.48	$C_{29}H_{50}O_2$	430.72
11	Fucosterol	53.32	23.43	$C_{29}H_{48}O$	412.70
12	Δ-Tocopherol	53.84	1.17	$C_{27}H_{46}O_2$	402.65

Note: [a] Positive identification by mass spectrometry with than higher 95 % similarity to the database and greater than 1.00 % peak area

to exert potent antibacterial effects on MRSA by inducing interleukin-24 production, a cytokine that is involved in host defenses against bacteria (Pierpaoli *et al*., 2011; Fonseca-Camarillo *et al*., 2014). However, tocopherol and its derivatives were present at low percentages in our study (Table 5).

CONCLUSION

This study is a novel report on the antibacterial activity of *Padina australis* extracts and fractions against drug-resistant pathogenic bacteria, especially MRSA. The EA subfraction (EA-1) of *P. australis* exhibited the highest antibacterial activity against *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATCC25923 and methicillin-resistant *S. aureus* (MRSA) KL046. The antibacterial activity from disc diffusion method showed positive correlation with the TPC of the extract. The phytochemical compounds responsible for the antibacterial activity were proposed as phlorotannin, fucoxanthin, fucosterol and fucosterol derivatives. Since only the EA-1 subfraction of *P. australis* was determined in this study, further studies are needed to purify and isolate the active compounds from the extract to optimize their antibacterial activity. Although the extracts of *P. autralis* in this study displayed relatively low antimicrobial activity, the extracts from this species may have other potentially beneficial activities, such as anti-cancer, anti-viral, or anti-inflammatory. Therefore, other biological activities should be studied further.

ACKNOWLEDGEMENTS

This research was supported by the Graduate Scholarship of The Graduate School, Kasetsart University.

LITERATURE CITED

Abdelhamid, A., M. Jouini, H.B.H. Amor, Z. Mzoughi, M. Dridi, R.B. Said and A. Bouraoui. 2018. Phytochemical analysis and evaluation of the antioxidant, anti-inflammatory, and antinociceptive potential of phlorotannin-rich fractions from three Mediterranean brown seaweeds. **Marine Biotechnology** 20(1): 60-74.

Aknin, M., K. Dogbevi, A. Samb, J.M. Kornprobst, E.M. Gaydou and J. Miralles. 1992. Fatty acid and sterol composition of eight algae from the Senegalese coast. **Comparative Biochemistry and Physiology Part B: Comparative Biochemistry** 102: 841-843.

Al Easa, H.S., J. Kornprobst and A.M. Rizk. 1995. Major sterol composition of some algae from Qatar. **Phytochemistry** 39: 373-374.

Alghazeer, R., F. Whida, E. Abduelrhman, F. Gammoudi and M. Naili. 2013. *In vitro* antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Libya. **African Journal of Biotechnology** 12(51): 7086-7091.

Bauer, A.W., M.M. Kirby, J.C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. **American Journal of Clinical Pathology** 45: 493-496.

Berber, I., C. Avsar and H. Koyuncu. 2015. Antimicrobial and antioxidant activities of *Cystoseira crinite* Duby and *Ulva intestinalis* Linnaeus from the coastal region of Sinop, Turkey. **Journal of Coastal Life Medicine** 3(6): 441-445.

Boonchum, W., Y. Peerapornpisal, D. Kanjapothi, J. Pekkoh, D. Amornlerdpison, C. Pumas, P. Sangpaiboon and P. Vacharapiyasophon. 2011. Antimicrobial and anti-inflammatory properties of various seaweeds from the Gulf of Thailand. **International Journal of Agriculture and Biology** 13(1): 100-104.

Brotosudarmo, T.H.P., Heriyanto, Y. Shioi, Indriatmoko, M.A.S Adhiwibawa, R. Indrawati and L. Limantara. 2018. Composition of the main dominant pigments from potential two edible seaweeds. **The Philippine Journal of Science** 147(1): 47-55.

Brown, E.M., P.J. Allsopp, P.J. Magee, C.I.R. Gill, S. Nitecki, C.R. Strain and E.M. McSorley. 2014. Seaweed and human health. **Nutrition Reviews** 72(3): 205-216.

Caamal-Fuentes, E., R. Moo-Puc, Y. Freile-PelegrÍn and D. Robledo. 2014. Cytotoxic and antiproliferative constituents from *Dictyota ciliolata*, *Padina sanctae-crucis* and *Turbinaria tricostata*. **Pharmaceutical Biology** 52(10): 1244-1248.

Canoy, J.L. and J.G. Bitacura. 2018. Cytotoxicity and antiangiogenic activity of *Turbinaria ornata* Agardh and *Padina australis* Hauck ethanolic extracts. **Analytical Cellular Pathologly** 2018: 3709491. DOI: 10.1155/2018/3709491.

Chiao-Wei, C., H. Siew-Ling and W. Ching-Lee. 2011. Antibacterial activity of *Sargassum polycystum* C Agardh and *Padina australis* Hauck (Phaeophyceae). **African Journal of Biotechnology** 10: 14125-14131.

Cho, S., H. Yang, Y.J. Jeon, C.J. Lee, Y.H. Jin, N.I. Baek, D. Kim, S.M. Kang, M. Yoon, H. Yong, M. Shimizu and D. Han. 2012. Phlorotannins of the edible brown seaweed *Ecklonia cava* Kjellman induce sleep via positive allosteric modulation of gamma-aminobutyric acid type A-benzodiazepine receptor: A novel neurological activity of seaweed polyphenols. **Food Chemistry** 132: 1133-1142.

Choi, J.G., O.H. Kang, O.O. Brice, Y.S. Lee, H.S. Chae, Y.C. Oh, D.H. Sohn, H. Park, H.G. Choi, S.G. Kim, D.W. Shin and D.Y. Kwon. 2010. Antibacterial activity of *Ecklonia cava* against methicillin-resistant *Staphylococcus aureus* and *Salmonella* spp. **Foodborne Pathogen Disease** 7(4): 435-441.

Clinical and Laboratory Standard Institute. 2012. **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard,** 9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne. 87 pp.

Cox, S., N. Abu-Ghannam and S. Gupta. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. **International Food Research Journal** 17: 205-220.

El-Shafay, S., S.S. Ali and M.M. El-Sheekh. 2016. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. **Egyptian Journal of Aquatic Research** 42: 65-72.

Eom, S.H., J.H. Park, D.U. Yu, J.I. Choi, J.D. Choi, M.S. Lee and Y.M. Kim. 2011. Antimicrobial activity of brown alga *Eisenia bicyclis* against methicillin-resistant *Staphylococcus aureus*. **Fisheries and Aquatic Science** 14: 251-256.

Eom, S.H., D.S. Lee, Y.M. Kang, K.T. Son, Y.J. Jeon and Y.M. Kim. 2013a. Application of yeast *Candida utilis* to ferment *Eisenia bicyclis* for enhanced antibacterial effect. **Applied Biochemistry and Biotechnology** 171(3): 569-582.

Eom, S.H., K.H. Lim and Y.M. Kim. 2013b. Potential of *Candida utilis* to ferment *Ecklonia cava* by-product for enhanced anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity. **Journal of Applied Phycology** 25: 1949-1956.

Eom, S.H., Y.M. Kim and S.K. Kim. 2012. Antimicrobial effect of phlorotannins from marine brown algae. **Food Chemistry Toxicology** 50: 3251-3255.

Fonseca-Camarillo, G., J. Furuzawa-Carballeda, J. Granados and J.K. Yamamoto-Furusho. 2014. Expression of interleukin (IL)-19 and IL-24 in inflammatory bowel disease patients: a cross-sectional study. **Clinical and Experimental Immunology** 177: 64-75.

Gunji, S., J. Santoso, Y. Yoshie-Stark and T. Suzuki. 2007. Effects of extracts from tropical seaweeds on DPPH radicals and Caco-2, cells treated with hydrogen peroxide. **Food Science and Technology Research** 13(3): 275-279.

Gupta, S. and N. Abu-Ghannam. 2011. Bioactive potential and possible health effects of edible brown seaweeds. **Food Science and Technology** 22: 315-326.

Hsieh, L. and A. Amin. 2016. **Antimicrobial stewardship: Hospital strategies to curb antibiotic resistant.** In: Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches (eds. K. Kon and M. Rai), pp. 1-18. Elsevier, London.

Jaswir, I., D. Noveindri, H.M. Salleh, M. Taher and K. Miyashita. 2011. Isolation of fucoxanthin and fatty acids analysis of *Padina australis* and cytotoxic effect of fucoxanthin on human lung cancer (H1299) cell lines. **African Journal of Biotechnology** 10(81): 18855-18862.

Jariyasethpong, T., C. Tribuddharat, S. Dejsirilert, A. Kerdsin, P. Tishyadhigama, S. Rahule, P. Sawanpanyalert, P. Yosapol and N. Aswapokee. 2010. MRSA carriage in a tertiary governmental hospital in Thailand: emphasis on the prevalence and molecular epidemiology. **European Journal of Clinical Microbiology and Infectious Diseases** 29: 977-985.

Kamenarska, Z., M.J. Gasic, M. Zlatovic, A. Rasovic, D. Sladic, Z. Kljajic, K. Stefanov, K. Seizova, H. Najdenski, A. Kuumgiev, I. Tsvetkova and S. Popov. 2002. Chemical composition of the brown alga *Padina pavonica* (L.) Gaill from the Adriatic Sea. **Botanica Marina** 45: 339-345.

Kantachumpoo, A. and A. Chirapart. 2010. Components and antimicrobial activity of polysaccharides extracted from Thai brown seaweeds. **Kasetsart Journal Natural Science** 44: 220-233.

Kaufman, P.B., L.J. Cseke, S. Warber, J.A. Duke and H.L. Brielmann. 1999. **Natural Products From Plants.** CRC Press, Boca Raton, Florida. 343 pp.

Kavita, K., V.K. Singh and B. Jha. 2014. 24-Branched Δ5 sterols from *Laurencia papillosa* red seaweed with antibacterial activity against human pathogenic bacteria. **Microbiological Research** 169(4): 301-306.

Khanum, R., F. Mazhar and M. Jahangir. 2015. Antioxidant evaluations of polar and non-polar fractions of *Cajanus cajan* seeds. **Journal of Medicinal Plants Research** 9(6): 193-198.

Kim, S.K. and Q.V. Ta. 2011. **Potential beneficial effects of marine algal sterols on human health.** In: Advances in Food and Nutrition Research (ed. S.K. Kim), pp. 191-198. Elsevier, San Diego, California.

Kim, Y.B., Y.G. Moon and M.S. Heo. 2008. Antioxidant and antimicrobial activities of seaweed, *Ecklonia cava*. **Journal of Biotechnology** 136: 589-601.

Kitti, T., K. Boonyonying and S. Sitthisak. 2011. Prevalence of methicillin-resistant *Staphylococcus aureus* among university students in Thailand. **The Southeast Asian Journal of Tropical Medicine and Public Health** 42(6): 1499-1504.

Kumar, S.S., Y. Kumar, M.S.Y. Khan and V. Gupta. 2010. New antifungal steroids from *Turbinaria conoides* (J. Agardh) Kutzing. **Natural Product Research** 24(15): 1481-1487.

Lee, D.S., M.S. Kang, H.J. Hwang, S.H. Eom, J.Y. Yang, M.S. Lee, W.J. Lee, Y.J. Jeon, J.S. Choi and Y. M. Kim. 2008. Synergistic effect between dieckol from *Ecklonia stolonifera* and β-Lactamase against methicillin-resistant *Staphylococcus aureus*. **Biotechnology and Bioprocess Engineering** 13: 758-764.

Lee, J.H., S.H. Eom, E.H. Lee, Y.J. Jung, H.J. Kim, M.R. Jo, K.T. Son, H.J. Lee, J.H. Kim, M.S. Lee and Y.M. Kim. 2014. *In vitro* antibacterial and synergistic effect of phlorotannins isolated from edible brown seaweed *Eisenia bicyclis* against acne-related bacteria. **Algae** 29: 47-55.

Lee, M.S., T. Shin, T. Utsuki, J.S. Choi, D.S. Byun and S.R. Kim. 2012. Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and hepatoprotective properties in tacrine-treated HepG2 cells. **Journal of Agriculture and Food Chemistry** 60: 5340-5349.

Li, Y., Z.J. Qian, B. Ryu, S.H. Lee, M.M. Kim and S.K. Kim. 2009. Chemical components and its antioxidant properties *in vitro*: an edible marine brown alga, *Ecklonia cava*. **Bioorganic and Medicinal Chemistry** 17: 1963-1973.

Li, Y.X., I. Wijerekara, Y. Li and S.K. Kim. 2011. Phlorotannins as bioactive agents from brown algae. **Process Biochemistry** 46: 2219-2224.

López, A., M. Rico, A. Rivero and M.S. Tangil. 2011. The effects of solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. **Food Chemistry** 125: 1104-1109.

Madkour, F.F., G.A. El-Shoubaky and M.A. Ebada. 2019. Antibacterial activity of some seaweeds from the Red Sea coast of Egypt. **Egyptian Journal of Aquatic Biology and Fisheries** 23(2): 265-274.

Mashjoor, S., M.A., Esmaeili and R. Rafiee. 2016. Cytotoxicity and antimicrobial activity of marine macro algae (Dictyotaceae and Ulvaceae) from the Persian Gulf. **Cytotechnology** 68: 1717-1726.

Maheswari, M.U., A. Reena and C. Sivaraj. 2017. GC-MS analysis, antioxidant and andtibacterial activity of the brown algae, *Padina tetrastromatica*. **International Journal of Pharmaceutical Sciences and Research** 8(9): 4014-4020.

Matysik, E., A. Wozniak, R. Paduch, R. Rejdak, B. Polak and H. Donica. 2016. The new TLC method for separation and determination of multicomponent mixtures of plant extracts. **Journal of Analytical Methods in Chemistry** 2016: 1813581. DOI: 10.1155/2016/1813581.

Nagayama, K., Y. Iwamura, T. Shibata, I. Hirayama and T. Nakamura. 2002. Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. **Journal of Antimicrobial Chemotherapy** 50: 889-893.

Peng, J., J.P. Yuan, C.F. Wu and J.H. Wang. 2011. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. **Marine Drugs** 9: 1806-1828.

Phang, S.M., H.Y. Yeong, E.T. Ganzon-Fortes, K. Lewmanomont, A. Prathep, L.N. Hau. G.S. Gerung and K.S. Tan. 2016. Marine algae of the South China Sea bordered by Indonesia, Malaysia, Philippines, Singapore, Thailand and Vietnam. **Raffles Bulletin of Zoology** 34: 13-59.

Pierpaoli, E., O. Cirioni, A. Barucca, F. Orlando, C. Silvestri, A. Giacometti and M. Provinciali. 2011. Vitamin E supplementation in old mice induces antimicrobial activity and improves the efficacy of daptomycin in an animal model of wounds infected with methicillin-resistant *Staphylococcus aureus*. **Journal of Antimicrobial Chemotherapy** 66(9): 2184-2185.

Praiboon, J., S. Palaka, T. Noiraksa and K. Miyashita. 2018. Seasonal variation in nutritional composition and anti-proliferative activity of brown seaweed, *Sargassum oligocystum*. **Journal of Applied Phycology** 30: 101-111.

Prathep, A., S. Pongparadon, A. Darakrai, B. Wichachucherd and S. Sinutok. 2011. Diversity and distribution of seaweed at Khanom-Mu Ko Thale Tai National Park, Nakhon Si Thammarat Province, Thailand. **Songklanakarin Journal of Science and Technology** 33(6): 633-640.

Rajauria, G. and N. Abu-Ghannum. 2013. Isolation and partial characterization of bioactive fucoxanthin from *Himanthalia elongata* brown seaweed: A TLC-based approach. **International Journal of Analytical Chemistry** 2013: 802573. DOI: 10.1155/2013/802573

Rattaya, S., S. Benjakuland T. Prodpran. 2015. Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornata* and *Sargassum polycystum*, grown in Thailand. **International Aquatic Research** 7: 1-16.

Richter, M.F., B.S. Drown, A. Riley, A. Garcia, T. Shirai, R.L. Svec and P.J. Hergenrother. 2017. Predictive rules for compound accumulation yield a broad-spectrum antibiotic. **Nature** 18: 299-304.

Rodrigues, D., C. Alves, A. Horta, S. Pinteus, J. Silva, G. Culioli, O.P. Thomas and R. Pedrosa. 2015. Antitumor and antimicrobial potential of bromoditerpenes isolated from the red alga, *Sphaerococcus coronopifolius*. **Marine Drugs** 13: 713-726.

Sathya, R., N. Kanaga, P. Sankar and S. Jeeva. 2017. Antioxidant properties of phlorotannins from brown seaweed *Cystoseira trinodis* (Forrsskål) C. Agardh. **Arabian Journal of Chemistry** 10: 2608-2614.

Smith, A.J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. **Journal of Applied Phycology** 16: 245-262.

Suresh, M., P. Iyapparaj and P. Anantharaman. 2016. Antifouling activity of lipidic metabolites derived from *Padina tetrastromatica*. **Applied Biochemistry and Biotechnology** 179: 805-818.

Thamlikitkul, V., P. Rattanaumpawan, A. Boonyasiri, V. Pumsuwan, T. Judaeng, S., Tiengrim, W. Paveenkittiporn, S. Rojanathien, S. Jaroenpoj and S. Issaracharnvanich. 2015. Thailand antimicrobial resistance containment and prevention proGram. **Journal of Global Antimicrobial Resistance** 3: 290-294.

Thirunavukkarasu, R., P. Pandiyan, K. Subaramaniyan, D. Balaraman, S. Manikkam, B. Sadaiyappan and G.E.G. Jothi. 2014. Screening of marine seaweeds for bioactive compound against fish pathogenic bacteria and active fraction analysed by gas chromatography-mass spectrometry. **Journal of Coastal Life Medicine** 2(5): 367-375.

Widjaja-Adhi Airanthi, M.K., M. Hosokawa and K. Miyashita. 2011. Comparative antioxidant activity of edible Japanese brown seaweeds. **Journal of Food Science** 76: 104-111.

Zailanie, K. 2016. Study of *Padina australis* using UV-VIS, HPLC and Antibacterial. **Journal of Life Science and Biomedicine** 6(1): 1-5.