Sains Malaysiana 44(9)(2015): 1269–1273

Antibacterial Activities of Selected Seaweed and Seagrass from Port Dickson Coastal Water against different Aquaculture Pathogens

(Aktiviti Antibakteria daripada Rumpai Laut dan Rumput Laut Terpilih di Pesisiran Port Dickson terhadap Patogen Akuakultur Berbeza)

F.M.I. NATRAH*, Z. MUTA HARAH, B. JAPAR SIDIK, N.M.S. IZZATUL & A. SYAHIDAH

ABSTRACT

Eight seaweed species in Teluk Kemang and three seagrass species in Teluk Pelanduk, Port Dickson, respectively, were screened for antibacterial activities. The antibacterial activities were screened using disc diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against six aquacultural pathogens strains Aeromonas hydrophila *ATCC35654,* Vibrio harveyi *BB120,* Vibrio harveyi *ATCC14126,* Vibrio alginolyticus *ATCC17749,* Vibrio parahaemolyticus *ATCC17803 and* Vibrio anguillarum *ATCC43313. The results showed that among all the pathogens, seaweed* Padina minor *and seagrass* Thalassia hemprichii *had the strongest antibacterial activity against* Vibrio harveyi *BB120 and* Vibrio harveyi *ATCC14126, respectively. The lowest values for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were obtained from* Padina minor *against* V. harveyi *BB120 and* Thalassia hemprichii *against* V. harveyi *ATCC14126, respectively. The findings suggested that seaweed and seagrass in Port Dickson coastal water have the potential to prevent bacterial diseases particularly in aquaculture.*

Keywords: Antibacteria; aquaculture pathogens; Port Dickson; seagrass; seaweed

ABSTRAK

Lapan rumpai laut dari Teluk Kemang dan tiga rumput laut dari Teluk Pelanduk Port Dickson telah disaring untuk aktiviti antibakteria. Aktiviti antibakteria telah disaring dengan menggunakan ujian resapan disk, pemekatan resapan minimum (MIC) dan pemekatan bakteriasid minimum (MBC) terhadap enam patogen akuakultur Aeromonas hydrophila *ATCC35654,* Vibrio harveyi *BB120,* Vibrio harveyi *ATCC14126,* Vibrio alginolyticus *ATCC17749,* Vibrio parahaemolyticus *ATCC17803 dan* Vibrio anguillarum *ATCC43313. Hasil uji kaji menunjukkan daripada kesemua patogen, rumpai laut* Padina minor *dan rumput laut* Thalassia hemprichii *menunjukkan aktiviti antibakteria yang paling tinggi terhadap* Vibrio harveyi *BB120 dan* Vibrio harveyi *ATCC14126. Nilai paling rendah bagi asai pemekatan resapan minimum (MIC) dan pemekatan bakteriasid minimum (MBC) diperoleh daripada* Padina minor *terhadap* V. harveyi *BB120 dan* Thalassia hemprichii *terhadap* V. harveyi *ATCC14126. Hasil kajian ini mencadangkan rumpai laut dan rumput laut dari pesisiran pantai Port Dickson berpotensi untuk mencegah penyakit bakteria terutamanya dalam akuakultur.*

Kata kunci: Antibakteria; patogen akuakultur; Port Dickson; rumpai laut; rumput laut

INTRODUCTION

As tropical country, Malaysia is blessed with high diversity of marine macrophytes such as seaweed and seagrass. It is estimated that there are about 375 seaweed (Phang 2006) and 14 seagrass (Japar Sidik et al. 2006) species in Malaysian water. Interest on the commercial use of these marine macrophytes has increased, leading to screening of beneficial activities for different bioproducts (Chee et al. 2011). This include antibacterial screening against different bacterial diseases in human and animal.

To date, there are numerous reports concerning the inhibition activities from aquatic macrophytes against human pathogens, fungi and yeast. However, only few data can be found on the effects of the organisms against pathogen in aquaculture (Bansemir et al. 2006). Among the common bacterial pathogens are *Aeromonas* and *Vibrio* species which are responsible for heavy mortality in the wild and cultured aquatic animals (Kayis et al. 2009).

Antibiotics and disinfectants are among the several solutions to overcome bacterial disease in aquaculture (Austin & Austin 2007). However, the use of antibiotics and biocides in aquaculture has disadvantages, particularly in relation to the generation of toxicant which may cause risks to the environment and antibiotic resistant bacteria (Jones et al. 2004). Therefore, another solution to prevent bacterial diseases in aquaculture is by using aquatic macrophytes such as seaweed and seagrass (Bansemir et al. 2006). Report by Immanuel et al. (2004) showed that aquatic plants extracts such as *Ulva lactuta* and *Sargassum wightii* added as a diet in the enrichment technique of *Artemia* can help to increase the growth rate and the survival rate of shrimp *Penaeus indicus* juveniles challenged with bacterial pathogen *Vibrio parahaemolyticus*. Therefore, this study aimed to screen selected seaweed and seagrass from two different locations in Port Dickson for antibacterial activity against different aquaculture pathogens.

Materials and Methods

COLLECTION OF SAMPLES

Seaweed were collected from the intertidal zone of Teluk Kemang (2° 27′ 52.63″ N, 101° 50′ 52.12″ E), Port Dickson. Whereas, seagrasses were collected from the intertidal zone of Teluk Pelanduk (2° 25′ 7.85″ N, 101° 52′ 58.19″ E) in Port Dickson, Negeri Sembilan.

BACTERIAL STRAINS

Six pathogenic bacterial strains relevant to aquaculture were used in the antibacterial assay. The strains include one freshwater pathogen, *Aeromonas hydrophila* ATCC35654 and five marine pathogens namely *Vibrio harveyi* BB120, *Vibrio harveyi* ATCC14126, *Vibrio alginolyticus* ATCC17749, *Vibrio parahaemolyticus* ATCC17803 and *Vibrio anguillarum* ATCC43313.

PREPARATION OF SAMPLES EXTRACTS

All collected seaweed and seagrass samples were prepared according to Kannan et al. (2013) and Stirk et al. (2007) with modifications. Upon arrival to the lab, the samples were cleaned properly and were washed using sterile seawater. The samples were then dried using an air circulating oven at 60°C for 24 h. After drying, the samples were cut into small pieces and ground with electrical blender. 5 g of powdered samples were extracted successively with 25 mL of methanol (1:5 w/v) and the extracts were left at room temperature for three days. After three days, the extracts were filtered through Whatmann no.1 filter paper and evaporated using rotary vacuum evaporator at 55°C.

ANTIBACTERIAL ASSAYS

Standard disc diffusion assays were used to detect antibacterial activities of the samples (Kannan et al. 2010). Trypticase Soy Agar (TSA) added with 2% of NaCl were prepared for marine pathogens. Inoculum of bacterial pathogen at 10^8 cfu/mL were spread on top of solidified TSA. Samples extract (1 g/mL) of different seagrass and seaweed were prepared and impregnated using commercial disc. Oxytetracyclin (30 μg/disc) was used as positive control while methanol as negative control. The plates were then incubated for 24 h at 29°C. Antibacterial activity was determined by observing the diameter of inhibition zone around the disc as described by Daud et al. (2005). Inhibition zone was measured and reported in millimetres. All the species were tested in four replicates for concordant result.

Microdilution method was used to determine the minimum inhibitory concentration (MIC) (Devienne & Raddi 2002). In this experiment, only samples with the highest inhibition zone from disc diffusion method was selected for MIC method and tested against the two *Vibrio* strains. The bacterial pathogen (10^8 CFU/mL) was added and mixed with the seagrass and seaweed extracts to obtain 100 mg/mL of starting concentration. Samples were then serially diluted at two-fold. All the bacterial inoculum and extracts were tested in triplicates and the plates incubated at 29°C for 24 h. The response of the bacterial inoculum aginst the extract was checked by visual inspection. The MIC occurs when the formation size of bacterial pellet in the well is different with the bacterial pellet in a negative control well. Minimum inhibitory concentration value was recorded in mg/mL.

Lastly, the samples that showed the highest differences of bacterial pellet formation in MIC assay were selected for the minimum bactericidal concentration (MBC) assay. In this experiment, the samples from MIC assay was pipetted out (10 μL) from their respective test well and spotted on top of Trypticase Soy agar (TSA) added with 2% of sodium chloride (NaCl). Thereafter, the plates were incubated for 24 h at 29°C. The lowest concentration of samples showing less visible growth of bacterial on the TSA was taken as the MBC value and expressed in mg/mL.

Results and Discussion

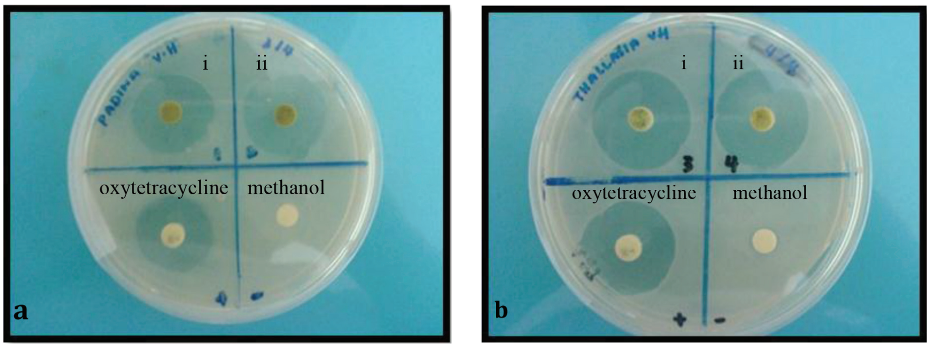
DISC DIFFUSION TEST

The results showed most of the seaweeds and seagrass possessed antibacterial activity against all the Gram-negative pathogen. Among all the pathogens, the different strains of *Vibrio harveyi* (BB120 and ATCC14126) were observed to be the most sensitive (widest zones of inhibition) when tested with the seaweed and seagrass samples.

In seaweed, the highest antibacterial activity was recorded in *Padina minor* (16.25±3.30 mm) against *Vibrio harveyi* BB120 (Figure 1) followed by *Sargassum duplicatum* (12.25±3.40 mm) (Table 1). Seaweed has been proven to be a potential source of antibacterial compounds towards both Gram-negative or Gram-positive pathogenic bacteria (Kolajinathan et al. 2009). Taskin et al. (2007) reported that ethanolic extract of eight seaweed species belonging to Chlorophyta, Phaeophyta and Rhodophyta exhibited broad spectrum activity of both antibacterial and antifungal activities.

In this study, the brown seaweed was found to be more active than the red and green seaweeds. The results were similar with the study by Lavanya and Veerappan (2011) which reported that the brown seaweed extracts showed higher activity than the red seaweed extracts. Nagayama et al. (2002) suggested that the strong antibacterial activities from brown seaweed may be due to the compounds such as phlorotannins, eckol and eckol related-compounds that have strong bactericidal activity.

There can also be variation in antibacterial activity probably due to the method of extraction and location where



a. *Padina minor* against *V. harveyi* BB120; i & ii represents the replicates of *P. minor* extracts; oxytetracycline as the positive control; methanol as the negative control

b. *Thalassia hemprichii* against *V. harveyi* ATCC14126; i & ii represents the replicates of *T. hemprichii* extracts; oxytetracycline as the positive control; methanol as the negative control

FIGURE 1. Antibacterial activity of seaweed and seagrass extracts. Zone inhibition denotes the antibacterial activity of the extracts

TABLE 1. Antibacterial activity of seaweed species extracts against six different aquaculture pathogen strains

Pathogen strains	Zone of inhibition (mm)					
	Aeromonas hydrophila ATCC35654	*Vibrio harveyi* BB120	*Vibrio harveyi* ATCC14126	*Vibrio parahaemolyticus* ATCC17803	*Vibrio alginolyticus* ATCC17749	*Vibrio anguillarum* ATCC43313
Positive control						
Oxytetracycline (30 µg/disc)	20.50±0.58^{a}	25.25±0.50^{a}	20.75±0.96^{a}	22.00±2.00^{a}	20.50±0.58^{a}	20.00±0.82^{a}
Seaweed extracts						
Dictyota dichotoma	7.00±0.82^{e}	12.00±3.16^{c}	8.50±1.29^{c}	9.00±0.82^{c}	10.50±3.11^{b}	7.75±0.50^{b}
Padina minor	8.00±1.41^{c,d}	16.25±3.30^{b}	11.00±9.75^{b}	11.50±2.38^{b}	8.75±1.26^{b,c}	8.00±0.82^{b}
Halimeda macroloba	8.50±0.58^{b,c}	10.50±2.65^{c}	7.75±0.96^{c}	9.50±1.29^{b,c}	9.00±0.82^{b,c}	7.50±0.58^{b}
Caulerpa racemosa	8.00±0.82^{c,d}	9.50±1.73^{c}	9.25±0.96^{c}	9.25±2.23^{c}	7.75±0.96^{c}	8.00±0.82^{b}
Caulerpa macrophysa	9.75±0.96^{b}	9.25±0.96^{c}	9.25±0.50^{c}	8.25±0.96^{c}	8.00±0.82^{c}	7.50±0.58^{b}
Ulva intestinalis	7.33±0.58^{c,d}	10.00±1.83^{c}	8.25±1.71^{c}	8.50±0.58^{c}	8.25±0.50^{c}	8.25±0.96^{b}
Amphiroa fragilissima	8.00±0.82^{c,d}	9.75±2.06^{c}	9.00±0.82^{c}	9.00±0.82^{c}	8.00±0.82^{c}	7.75±0.50^{b}
Sargassum duplicatum	8.00±0.82^{c,d}	12.25±3.40^{c}	9.75±0.96^{b}	8.50±0.58^{c}	7.25±0.50^{c}	8.50±0.58^{b}

Mean ± SD of four replicates in column with different superscript (a>b>c>d>e) was significantly different (p<0.05) using Duncan multiple range test

samples were collected (Kandhasamy & Arunachalam 2008). In this experiment, all methanol extracts showed positive inhibition of antibacterial activity. According to Febles et al. (1995) methanol extraction yielded higher antimicrobial activities for seaweed. In a different study by Bansemir et al. (2006), dichloromethane extract of *Dictyota dichotoma* did not show any inhibition against *Aeromonas hydrophila* which was in contrast with the present study where methanol extracts of *Dictyota dichotoma* effectively inhibited antibacterial activity.

On the other hand, among the seagrass samples, *Thalassia hemprichii* showed the highest antibacterial activity (Figure 1) against both strain of *V. harveyi* with the same inhibition zones of 11.50±1.29 mm followed by *Enhalus acoroides* against *Vibrio harveyi* ATCC14126 with zone inhibition of 10.25±0.96 mm (Table 2). The results were in accordance with the report by Premanathan et al. (2000) where *Thalassia hemprichii* showed antibacterial activity against four pathogens (*Bacillus subtilis*, *Staphylococus aureus*, *Agrobacterium tumefaciens* and *Escherichia coli*) were tested. Meanwhile, methanolic extract of *Enhalus acoroides* has been shown by Alam et al. (1994) to be the most effective against different bacterial pathogens (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) compared to ethanolic extract.

Interestingly, Bushman and Ailstock (2006) stated that the estuarine submerged aquatic plants inhibited growth of Gram-negative bacterial species like *Vibrio*, *Listonella* and *Pasteurella*. The results were similar with this study, where three seagrass species *Thalassia hemprichii, Enhalus*

TABLE 2. Antibacterial activity of seagrass species extracts against six different aquaculture pathogen strains

Pathogen Strain	Zone of inhibition (mm)					
	Aeromonas hydrophila (ATCC35654)	*Vibrio harveyi* (BB120)	*Vibrio harveyi* (ATCC14126)	*Vibrio parahaemolyticus* (ATCC17803)	*Vibrio alginolyticus* (ATCC17749)	*Vibrio anguillarum* (ATTC43313)
Positive control						
Oxytetracycline (30 μg/disc)	21.25±0.50[a]	25.75±1.50[a]	20.50±0.58[a]	20.50±1.29[a]	21.00±0.82[a]	21.00±0.82[a]
Seagrass extract						
Thalassia hemprichii	7.25±0.96[b]	11.50±1.29[b]	11.50±1.29[b]	9.00±0.82[b]	9.25±1.71[b]	7.25±0.50[b]
Cymodocea rotundata	7.50±0.58[b]	8.50±0.58[c]	9.75±0.96[b]	7.75±0.50[b]	8.25±1.26[b]	7.50±0.58[b]
Enhalus acoroides	8.00±0.82[b]	8.75±1.50[c]	10.25±0.96[b]	9.00±0.82[b]	8.00±0.82[b]	8.00±0.82[b]

Mean ± SD of four replicate in column with different superscript (a>b>c) was significantly different (p<0.05) using Duncan multiple range test

TABLE 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the seaweed and seagrass towards different *V. harveyi* strains

Species	*Vibrio harveyi* (BB120)		*Vibrio harveyi* (ATCC14126)	
	MIC	MBC	MIC	MBC
Padina minor	0.78	1.56	1.56	3.13
Thalassia hemprichii	1.56	3.13	1.56	3.13

MIC and MBC values were expressed in mg/mL

acoroides and *Cymodocea rotundata* inhibited successfully the different Gram-negative bacterial pathogens.

MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Table 3 shows the minimum inhibitory concentration (MIC) values of *Padina minor* (seaweed) and *Thalassia hemprichii* (seagrass). Both samples were selected based on the highest inhibitions zones using disc diffusion method. The lowest MIC values was obtained from *Padina minor* extracts against *V. harveyi* BB120 and *V. harveyi* ATCC14126 with 0.78 and 1.56 mg/mL, respectively. On the other hand, MICs for *Thalassia hemprichii* extracts showed similar values against the two *V. harveyi* strains at 1.56 mg/mL.

The miminum bactericidal concentration (MBC) values for *Padina minor* and *Thalassia hemprichii* towards *V. harveyi* BB120 and *V. harveyi* ATTC14126 were also shown in Table 3. Methanolic extracts of *Padina minor* exhibited the lowest MBC against *V. harveyi* BB120 at 1.56 mg/mL, while *Thalassia hemprichii* exhibited the lowest MBC against *V. harveyi* ATCC 14126 at 3.13 mg/mL.

Minimum inhibitory concentration and MBC were conducted to assess the bacteriostatic and bactericidal concentration of the seaweed and seagrass extracts against the respective pathogens. According to Chiao-Wei et al. (2011), the lower the MIC and MBC values, the higher the antibacterial potential of the plants extracts.

According to Kannan et al. (2012), *Thalassia hemprichii* inhibited the growth of different bacteria (*Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia*, *Serratia* sp.) with MIC value of 0.025 mg/mL whereas in the present study *Thalassia hemprichii* against *V. harveyi* ATCC14126 showed 1.56 mg/mL growth inhibitory activities. The variation of antibacterial activity of the extracts might be due to the different response of antibacterial substances from seagrass towards different bacteria (Zubia et al. 2008).

CONCLUSION

In conclusion, 11 different species of marine macrophytes which consist of eight seaweed and three seagrass species collected from different location in Port Dickson coastal water exhibited antibacterial activities against the Gram-negative pathogenic bacteria relevant to aquaculture. The results showed that *Padina minor* and *Thalassia hemprichii* are promising species as antibacterial agents. Further investigations on the suitability of the seaweed and seagrass with antibacterial activity on aquaculture organisms need to be further elucidated.

ACKNOWLEDGEMENTS

We would like to thank the Vice-Chancellor, Universiti Putra Malaysia, for providing the facilities and logistics for this study. This study was funded by the Ministry of Education (KPM), Malaysia with collaboration of the Japan Society for the Promotion of Science (JSPS) Asian CORE program and the Establishment of Research Education Network on Coastal Marine Science in Southeast Asia (COMSEA).

REFERENCES

Alam, K., Agua, T., Maven, H., Taie, R., Rao, K.S., Burrows, I., Huber, M.E. & Rali, T. 1994. Preliminary screening of seaweeds, seagrass and lemongrass oil from Papua New Guinea for antimicrobial and antifungal activity. *Pharmaceutical Biology* 32: 396-399.

Austin, B.B. & Austin, D.A. 1999. *Bacterial Fish Pathogens: Disease of farmed and Wild Fish*. Chichester, United Kingdom: Praxis Publishing Ltd.

Bansemir, A., Blume, M., Schröder, S. & Lindequist, U. 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture* 252: 79-84.

Bushmann, P.J. & Ailstock, M.S. 2006. Antibacterial compounds in estuarine submersed aquatic plants. *Journal of Experimental Marine Biology and Ecology* 331: 41-50.

Chee, S-Y., Wong, P-K. & Wong, C-L. 2011. Extraction and characterisation of alginate from brown seaweeds (Fucales, Phaeophyceae) collected from Port Dickson, Peninsular Malaysia. *Journal of Applied Phycology* 23: 191-196.

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

Daud, A., Gallo, A. & Sánchez Riera, A. 2005. Antimicrobial properties of *Phrygilanthus acutifolius*. *Journal of Ethnopharmacology* 99: 193-197.

Devienne, K.F. & Raddi, M.S.G. 2002. Screening for antimicrobial activity of natural products using a microplate photometer. *Brazilian Journal of Microbiology* 33: 166-168.

Febles, C.I., Arias, A., Gil-Rodruguez, M.C., Hardisson, A. & Sierra Lopez, A. 1995. *In vitro* study of antimicrobial activity in algae Chlorophyta and Rhodophyta collected from the coast of Tenerife in Spanish. *Anuario del Institute de Estudios Canarios* 34: 181-192.

Immanuel, G., Vincybai, V.C., Sivaram, V., Palavesam, A. & Marian, M.P. 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen *Vibrio parahaemolyticus* load on shrimp *Penaeus indicus* juveniles. *Aquaculture* 236: 53-65.

Japar Sidik, B., Muta Harah, Z. & Aziz, A. 2006. Distribution and significance of seagrass ecosystems in Malaysia. *Aquatic Ecosystem Health and Management* 9: 203-214.

Jones, O.A.H., Voulvoulis, N. & Lester, J.N. 2004. Potential ecological and human health risks associated with the presence of pharmaceutically active compounds in the aquatic environment. *Critical Reviews in Toxicology* 34: 335-350.

Kandhasamy, M. & Arunachalam, K.D. 2008. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. *African Journal of Biotechnology* 7: 1958-1961.

Kannan, R.R.P., Arumugam, R., Iyapparaj, P., Thangaradjou, T. & Anantharam, P. 2013. *In vitro* antibacterial, cytotoxicity, haemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India. *Food Chemistry* 136: 1484-1489.

Kannan, R.R.R., Arumugam, R. & Anantharaman, P. 2012. Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. *Food Chemistry* 135: 2470-2473.

Kannan, R.R.R., Arumugam, R. & Anantharaman, P. 2010. Antibacterial potential of three seagrasses against human pathogens. *Asian Pacific Journal of Tropical Medicine* 3: 890-893.

Kayis, S., Ozcelep, T., Capkin, E. & Altinok, I. 2009. Protozoan and metazoan parasites of cultured fish in Turkey and their applied treatments. *The Israeli Journal of Aquaculture* 61: 93-102.

Kolanjinathan, K., Ganesh, P. & Govindarajan, M. 2009. Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. *European Review Medical Pharmacological Sciences* 13: 173-177.

Lavanya, R. & Veerappan, N. 2011. Antibacterial potential of six seaweeds collected from gulf of mannar of southeast coast of India. *Advances in Biological Research* 5: 38-44.

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

Phang, S.M. 2006. Seaweed resources in Malaysia: Current status and future prospects *Aquatic Ecosystem Health and Management* 9: 185-202.

Premanathan, M., Rajendran, S., Ramanathan, T., Kathiresan, K., Nakashima, H. & Yamamoto, N. 2000. A survey of some Indian medicinal plants for anti-human immunodeficiency virus (HIV) activity. *Indian Journal of Medical Research* 112: 73-77.

Stirk, W.A., Reinecke, D.L. & van Staden, J. 2007. Seasonal variation in antifungal, antibacterial and acetylcholinesterase activity in seven South African seaweeds. *Journal of Applied Phycology* 19: 271-276.

Taskin, E., Ozturk, M. & Kurt, O. 2007. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African Journal Biotechnology* 6: 2746-2751.

Zubia, M., Payri, C. & Deslandes, E. 2008. Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *Journal of Applied Phycology* 20: 1033-1043.

F.M.I. Natrah*, Z. Muta Harah, N.M.S Izzatul & A. Syahidah
Department of Aquaculture, Faculty of Agriculture
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

B. Japar Sidik
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
Bintulu Sarawak Campus
97008 Bintulu, Sarawak Bumi Kenyalang
Malaysia

F.M.I. Natrah* & Z. Muta Harah
Laboratory of Marine Biotechnology, Institute Bioscience
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: natrah@upm.edu.my

Received: 23 May 2014
Accepted: 15 May 2015