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Antimycotic Activity of Seaweed Extracts (*Caulerpa lentillifera* and *Eucheuma cottonii*) against Two Genera of Marine Oomycetes, *Lagenidium* spp. and *Haliphthoros* spp.

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Fungal infection mostly caused by marine oomycetes had hindered crustacean production thus searching for natural and safe treatment is currently needed. Thus, this study was conducted to investigate the antimycotic effect of different seaweed extract against marine oomycetes (*Lagenidium* spp. and *Haliphthoros* spp). Two seaweeds species (*Eucheuma cottonii* and *Caulerpa lentillifera*) were extracted using ethanol, methanol and water. Each extracts was tested on four fungi strains of marine oomycetes species for minimum inhibitory concentration (MIC) and fungicidal activities. *C. lentillifera* ethanol extract showed the highest antifungal effect where it can inhibit three from four fungal strains. Meanwhile, *E. cottonii* ethanol extract has lowest MIC (500 ppm) and inhibit *L. thermophilum* IPMB 1401 and *H. sabahensis* IPMB 1402 hyphal growths. Antimycotic effect on zoospores production shows reduction in production after 12 h immersion for three marine oomycetes species. Seaweed extracts toxicity on *Artemia* sp. showed approximately 5% mortality at 12 h immersion. It is suggested that 12 h immersion of seaweed extract is a suitable treatment for marine oomycetes in aquaculture. This study does not only show potential alternative control method for crab larvae health management, it may also contribute to the sustainable development and food security of aquaculture industry.

Key words : Antifungal / Mud crab larvae / *Lagenidiales* / Seaweed Extracts / Oomycetes infection.

INTRODUCTION

Mud crab, *Scylla* spp. are valuable fishery in Asian countries. The mud crab farming industry lack wild juveniles due to declined wild species. Asia, America, China and Europe have high demand for mud crab. The fishing pressure increases the need to develop sustainable aquaculture through hatchery mud crab production (Shelly, 2008; Paterson and Mann, 2011). Despite the effort for producing mud crabs in hatchery, disease hindered crab seed production in hatchery (Jithendran et al., 2009). In Sabah, Malaysia, the problems faced during seed production of mud crab are associated with fungal infection. Fungal infection had hindered crustacean production especially at eggs and zoea stages. The mycelia growth that invades the body causes the tissues to be damaged and caused locomotion difficul-

ties (Jithendran et al., 2010). The fungus caused crab larvae to be susceptible to other secondary infection (Jithendran et al., 2010). The causative agents belong to *Lagenidiales*.

Lagenidiales are reported to cause fungal diseases in mangrove crab, *S. serrata* in Indonesia (Hatai et al., 2000). *Lagenidium thermophilum* is pathogenic to eggs and larvae stages of mud crabs, *S. serrata* and *S. tranquebarica* in Indonesia (Nakamura et al., 1995) and Malaysia (Lee et al., 2016). *L. callinectes* is reported to infect *Callinectes sapidus* eggs and *Portunus pelagicus* eggs and larvae (Nakamura and Hatai, 1995). *Haliphthoros* spp. are also reported to cause fungal diseases on mud crab larvae. Three species under the genus *Haliphthoros* are *H. milfordensis* (Vishniac, 1958); *H. philippinensis* (Hatai et al., 1980) and *H. sabahensis* (Lee et al., 2017). *H. milfordensis* is previously reported to cause fungal infection on *P. pelagicus* zoea (Nakamura and Hatai, 1995a) and *S. serrata*

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eggs (Leaño, 2002). Leaño (2002) also reported other *Haliphthoros* sp., *H. philippinensis*, from *S. serrate*. Recently, a novel *Haliphthoros* sp, *H. sabahensis*, is one of the causative agents to cause mass mortality in *S. tranquebarica* larvae production in Sabah (Lee et al., 2017).

The chemical treatment using trifluralin, formalin and malachite green are common treatment strategies of marine oomycetes (Williams et al., 1986; Lee and Hatai, 2016; Lee et al., 2016). Despite their potency to control fungal infections, their application was hindered due to toxic and mutagenic effect on aquatic environment and aquatic life (Almeida et al., 2009). Furthermore, it becomes a concern in seafood security due to the existing residue in the body or tissues in seafood (Fuangsawat et al., 2011). Therefore, the search of alternative and safe treatment strategies is urgently needed. The recent discoveries of natural antifungal agents have become common (Hatai et al., 2018). Many of them are reported on antifungal agent from terrestrial plants (Hussein et al, 2000; Panchai et al., 2015). There are limited reports on antifungal from aquatic plant. Previously, seaweeds extracts have reported to have antifungal properties. *Ulva lactuca* in methanol, ethanol, methylene chloride, chloroform and hexane extracts showed antifungal activities against plant fungus, *Alternaria solani*, *Sclerotinia sclerotioru* and *Phytophthora infestans*. The methanol and ethanol extracts of *Ulva lactuca* have antifungal activities against *Aspergillus niger*, *Penicillium digitatum* and *Rizoctonia solani*. Water extract has moderate antifungal activity against *P. infestans* and *Fusarium solani* (Selim et al., 2015). Study on the antifungal activities of methanolic extract of *Rhodomela confervoides* and *Padina pavonica* showed positive antifungal effect against *Candida albicans* and *Mucor ramanianus* (Saidani et al., 2012) with minimum inhibitory concentration of 0.1 mg/ml and 0.1 mg/ml, respectively. Brown algae, *Spatoglossum asperum* in chloroform extract shows antifungal activity against *Aspergillus flavus* with 98.93% growth inhibition and methanol extract against dermatophytic fungus, *C. albicans* with 57.14% growth inhibition (Subbiah et al., 2015).

Caulerpa lentilifera and *Eucheuma cottonii* are two local seaweeds that cultured commercially in Sabah. Despite the huge volume of this seaweed produced every year, limited information is available on the application of this seaweed in aquaculture. Previous study only reported antibacterial activities of *C. lentillifera* and *E. cottonii* against *Staphylococcus aureus* and *Streptococcus mutans* (Sabirin et al., 2015). In fact, there are no studies on the antifungal activity of *C. lentilifera* and *E. cottonii* against marine oomycetes. Thus, the aim for this study is to investigate the potential of *C.*

lentirifera and *E. cottonii* extracts for the treatment of pathogenic marine oomycetes (*Lagenidium* sp. and *Haliphthoros* spp.) infections.

MATERIALS AND METHODS

Sample Processing

Two seaweed samples, *C. lentillifera* and *E. cottonii*, were purchased from local market at Tuaran, Sabah. The seaweeds were processed according to Barot (2016) with few modifications. Seaweeds were washed using seawater several times to remove mud, sand and epiphytes. The seaweeds were washed with tap water to remove excess seawater and salt present on surface of seaweed. The seaweeds were placed on drying trays and dried at 50-60°C for three days using drying oven. After drying, the seaweeds were blended into small pieces using an electronic blender (Panasonic MX-AC2105). The powdered seaweeds were kept in an air tight plastic bag and refrigerated at 4°C until further use.

Preparation of Seaweed Extracts Stock Solution

Three types of solvents (ethanol, methanol and water) were used in this study. Ethanol and methanol extraction were done using maceration process according to Azmir et al. (2013). Approximately, 10 g of seaweed and 100 ml (1:10 w/v) of particular solvent was mixed and placed in a shaking incubator (Jeio Tech SI 900R) for three days at 25°C. After three days, the extracts were filtered using Whatman glass fiber filter paper GF/CF or water extraction, the method follows Sakagami et al. (2013) with slight modifications. Same ratio (1:10 w/v) was applied for water extraction. The mixtures were heated to 80°C for 30 min, mixing every 10 min. The mixture was filtered through sterile muslin cloth into a bottle. All crude extracts were placed in rotary evaporator (EYELA N-1200A) at 40°C at 70 hPa. The concentrated mixtures were kept in -80°C freezer overnight before freeze dried for one day. The extraction yield for ethanol, methanol and water extracts was calculated from the weight of the extracts. Aseptically, the stock solution with concentration of 5000 ppm was prepared by dissolving in dimethyl sulfoxide (DMSO) (Kim et al., 2014). The extracts were filtered through 0.45 µm and 0.22 µm syringe filters. The extracts were kept at 4°C until further used. Finally, six different extracts were collected and labeled as *E. cottonii* ethanol extract (E.C-E), *E. cottonii* methanol extract (E.C-M), *E. cottonii* water extract (E.C-W), *C. lentilifera* ethanol extract (C.L-E), *C. lentillifera* methanol extract (C.L-M) and *C. lentillifera* water extract (C.L-W).

Preparation of Fungi Strains

Four fungi strains were used in this study, two strains of *Lagenidium thermophilum* IPMB 1401 (LT1401) (Lee et al., 2016) and *L. thermophilum* IPMB 1601 (LT1601) (This study), *Haliphthoros sabahensis* IPMB 1402 (HS1402) (Lee et al., 2017) and *H. milfordensis* IPMB 1603 (HM1603) (Lee et al., 2017) were obtained from Borneo Marine Research Institute culture collection. Peptone Yeast Glucose Seawater (PYGS) agar and broth were used for various tests throughout this study. The media recipe and preparation followed Lee et al. (2016). Fungal strains were maintained on PYGS agar. The fungal strains were incubated at 25°C throughout the study. Seawater temperature around Malaysia is 27°C to 30°C. The optimum growth for marine oomycetes (*Lagenidium* sp. and *Haliphthoros* sp.) is 25°C-30°C (Nakamura et al., 1995; Hatai, 2012; Lee et al., 2017). After 3-4 days with appearance of fungal growth, the mycelia masses with agar blocks were cut and transferred onto new PYGS agar for pure culture (Panchai et al., 2014). Subculture was made every 2-3 weeks.

Antimycotic Screening Assays

Well diffusion method (Magaldi et al., 2004) with modifications was used in the antimycotic screening assay. The preparation of the wells was carried out aseptically. First, 5 x 5 mm area was marked at the center-back of the agar plate for fungal block area. About 10 mm distance away from the fungal block area from both sides (Fig. 1). Two points at the both sides were bored using a sterile five mm cork borer. About 20 µl of PYGS molten agar was filled into the bored hole and allowed to dry to seal off the bottom.

For the inoculation of the fungus blocks on the prepared agar plates, a 5 mm x 5 mm agar block was cut using a sterile blade at the peripheral of fungus colony with fully covered hyphae. The 5 mm x 5 mm agar block was carefully and gently placed (inverted) on the fungal block area. The agar plate with fungus was allowed to grow at least two days until the fungus growth radius reached 5 mm (blue arrow) in all sides (Fig. 1).

When the growth radius reaches 5 mm, 30 µl of extracts (5000 ppm) were pipetted into the wells and allowed to dry. The control test was also prepared using sterile DMSO. The agar plates were incubated at 25°C. The observation was done daily until seven days. The antimycotic effect was determined by observing inhibition of growth of fungus towards the extract and compared with the control test.

Minimum Inhibitory Concentration (MIC)

Seaweed extracts that show positive antifungal

FIG. 1. PYGS agar plate using well diffusion method with fungus growing after 2 days (fungus reach 5 mm mark). (◇ = 5 x 5 mm area; ● = 5 mm; ◀ = 10 mm; ○ = well)

effects were subsequently used for the determination of minimum inhibitory concentration (MIC). The MIC of seaweed extracts against fungal strains were determined using broth microdilution method (NCCLS, 2008). The initial stock was 5000 ppm. The fungal block were inoculated into sterile PYGS broth with supplementation of extracts at different concentration (125, 250, 500, 750, 1000 and 2000 ppm). Controls were prepared by inoculating fungal block into PYGS broth with distilled water and PYGS broth only. All tests were incubated at 25°C for one week and done in triplicates.

Fungicidal Effects of Seaweed Extracts on Hyphal Growth

The results from MIC test were used for the fungicidal effect on fungus hyphae. The method followed Lee and Hatai (2016) with slight modification. Fungus agar blocks were submerged in PYGS broth supplemented with seaweed extract at MIC. The tests periods were at 0, 1, 2, 4, 6, 12 and 24 h. An additional three fungus agar blocks were prepared as control. At 0 h test period, three fungus agar blocks were dipped in solution and removed immediately. Three fungus agar blocks were washed with sterilized seawater for two times and inoculated on PYGS agar. Next, fungus agar blocks were dipped in solution. After 1, 2, 4, 6, 12 and 24 h test period, three fungus agar blocks were removed, washed and transferred onto new PYGS agar. Three fungus agar blocks were cut before washed with sterilized seawater for two times and inoculated on PYGS agar as control. The agar plate was incubated at 25°C for 7 d. The fungicidal effect on hyphae is determined by measuring the hyphae radius growth (cm) on

PYGS agar after seven days using a digital vernier caliper. Furthermore, the fungus growth radius was compared with other test periods and control.

Fungicidal Effect on Zoospores Production

The fungicidal effect of seaweed extracts to zoospores production was carried based on the study by Lee and Hatai (2016) with slight modification. Fungal agar blocks were prepared and submerged in PYGS broth supplemented with seaweed extract at MIC. The tests periods were designated at 0, 2, 4, 6, 12 and 24 h. At 0 h test period, the fungus agar blocks were dipped in solution and removed immediately. They were washed with sterilized seawater two times and incubated in 24 well plate (Nest Biotechnology) with 2 ml of sterilized filtered seawater. The fungi were incubated for one day at 25°C. At 2, 4, 6, 12 and 24 h test periods, another fungus agar blocks were removed from solution and washed with sterilized seawater 2 times and inoculated in 24 well plate. Three fungus agar blocks without treatment act as control. All tests were conducted in triplicate. After 24-h incubation for each test period, a drop of Lugol's solution was added in the 24 well plate and the content was mix thoroughly. The number of zoospore produced in one ml was counted using sedgewick rafter. The fungicidal effect of seaweed extract on zoospores production is determined by calculating the percentage of number of zoospores produced per ml under different test period. The formula to calculate the percentage of zoospores produced was as follows:

$$\text{Percentage of Zoospores produced (\%)} = \frac{\text{Number of zoospores produced after treatment}}{\text{Number of zoospores produced in control}} \times 100$$

Toxicity Test on *Artemia* sp.

Newly hatched *Artemia* sp. from cyst was used. Dried cysts of *Artemia* sp. were hatched in small aquarium

tank filled with filtered sea water at 30°C for 48 h to obtained phototrophic mature instar II nauplii (Zakaria et al., 2011; Sreejamole and Greeshma, 2013). Thirty nauplii were transferred into a Petri dish with sterile seawater supplemented with seaweed extract at MIC. The sterile seawater without extract was used as control. The final volume of each treatment was 25 ml. The tests were done in triplicate. After 30 min, 1, 2, 4, 6, 12 and 24 h, the Petri dishes were examined for cumulative mortality (%) of *Artemia* sp. (Meyer et al., 1982). The toxicity of seaweed extract on *Artemia* sp. was determined by counting the cumulative dead *Artemia* sp. and compared the cumulative mortality of *Artemia* sp. with the control. The cumulative mortality (%) formula is calculated as follows:

$$\text{Cumulative mortality (\%)} = \frac{\text{Cumulative number of dead } Artemia \text{ sp.}}{\text{Initial number of } Artemia \text{ sp.}} \times 100$$

RESULTS

Seaweed Extraction

The extractions yield was recorded in Table 1. *C. lentillifera* methanol extract (C.L-M) has the highest extraction yield at 30.2% while *E. cottonii* ethanol extract (E.C-E) has the lowest extraction yield at 1.5%. *C. lentillifera* extractions has yield between 15.1 to 30.2% while *E. cottonii* extractions has lower yield between 1.5 to 9.8%. For both seaweed extractions, methanol has the highest extraction yield followed by water and ethanol extract.

Antimycotic Screening Assay

There were 24 tests in the antimycotic screening assays. There were nine positive results against marine oomycetes (Table 1). *C. lentillifera* ethanol extract (C.L-E) shows antimycotic effect against three marine

TABLE 1. Extraction yield and antimycotic effects of the seaweeds extracts against for oomycetes species

Extraction	Symbol	Extraction yield (g)	Yield Percentage (%)	Antimycotic Activities*			
				LT1401	HS1402	LT1601	HM1603
<i>C. lentillifera</i> - water	C.L-W	1.93	19.3	+	-	-	-
<i>C. lentillifera</i> - ethanol	C.L-E	1.51	15.1	+	-	+	+
<i>C. lentillifera</i> - methanol	C.L-M	3.02	30.2	-	+	+	-
<i>E. cottonii</i> - water extract	E.C-W	0.45	4.5	-	-	-	+
<i>E. cottonii</i> - ethanol	E.C-E	0.15	1.5	+	+	-	-
<i>E. cottonii</i> - methanol	E.C-M	0.98	9.8	-	-	-	-

* '+' = Positive effect; '-' = Negative effect; LT1401 = *L. thermophilum* IPMB 1401; HS1402 = *H. sabahensis* IPMB 1402; LT1601 = *L. thermophilum* IPMB 1601; HM1603 = *H. milfordensis* IPMB 1603

oomycetes; LT1401, LT1601 and HM1603. Meanwhile, C.L-M and E.C-E shows positive antimycotic effect against 2 marine oomycetes (HS1402; LT1601 and LT1401; HS1402, respectively), while *C. lentrifera* water extract (C.L-W) and *E. cottonii* ethanol extract (E.C-W) shows positive antifungal effect against one marine oomycetes, LT1401 and HM1603, respectively. *E. cottonii* methanol extract (E.C-M) has no antimycotic effect against 4 marine oomycetes strains. LT1401 was the most susceptible to seaweed extracts where its growth was inhibited by three different seaweed extracts, which are C.L-W, C.L-E and E.C-E. Fig. 2 showed the example of antimycotic effect of seaweed extract to oomycetes.

Minimum Inhibitory Concentration

Seaweed extracts showing positive results in screening tests were carried forward to the minimum inhibitory concentration (MIC) test. Although seaweed extracts have antimycotic effect against marine oomycetes, each fungal strains has different susceptibility towards seaweed extracts (Table 2). E.C-E shows the lowest MIC at 500 ppm against LT1401 and HS1402. C.L-E and C.L-M have MIC of 2000 ppm and 750 ppm, respectively, against LT1601. Both C.L-E and E.C-W have MIC of 3000 ppm against HM1603. Seaweed extracts with the lowest MIC against each fungal strain

were used for subsequent tests.

Antimycotic Effect on Hyphae Production

Based on previous sections, LT1401 and HS1402 were tested with E.C-E at 500 ppm. LT1601 was tested with C.L-M at 750 ppm, and HM1603 was tested with C.L-E at 3000 ppm. The results showed that the growth of hyphae of LT1401 was inhibited after 4 h immersion in E.C-E 500 ppm. The growth of hyphae of HS1402 and HS1601 was inhibited by E.C-E at 500 ppm and C.L-M at 750 ppm, respectively after 12 hours immersion. No inhibition of the growth of hyphae of HM1603 was observed by C.L-E at 3000 ppm after 24 h immersion but shows reduced growth radius.

Antifungal Effect on Zoospores Production

Zoospore production was reduced after each submersion time. The production of zoospore has lowered even after quick submersion (at 0 h) when compared to control in every treatment. Based on the results at Table 3, LT1401 and LT1601 have the lowest zoospore production after 24 hours submersion at 0.7% and 2.5%, respectively.

Toxicity Test against *Artemia* sp.

From previous sections, E.C-E, C.L-M and C.L-E at 500 ppm, 750 ppm and 3000 ppm were selected for

FIG. 2. Representative of positive and negative antimycotic effect of seaweed extract against oomycetes. (A) The fungal growth is inhibited towards the well; (B) The fungal growth is not inhibited

TABLE 2. Minimum inhibitory concentration of seaweed extracts

Seaweed extract	Minimum inhibitory concentration (ppm)			
	LT1401	HS1402	LT1601	HM1603
C.L-W	1000	NT	NT	NT
C.L-E	3000	NT	2000	3000*
C.L-M	NT	3000	750*	NT
E.C-W	NT	NT	NT	3000
E.C-E	500*	500*	NT	NT
E.C-M	NT	NT	NT	NT

*Seaweed extract used for subsequent tests; NT: Not tested due to negative result from screening test

TABLE 3. Percentage of zoospore production at different dipping time

Fungal Strains + Seaweed extract	MIC tested	Control	Dipping time (hour)					
			0	2	4	6	12	24
LT1401+E.C-E	500 ppm	100%	2.9%	2.2%	4.0%	1.8%	1.1%	0.7%
HS1402+E.C-E	500 ppm	100%	35.0%	86.7%	31.7%	37.5%	5.0%	13.3%
LT1601+C.L-M	750 ppm	100%	36.2%	38.4%	42.7%	8.5%	1.5%	2.5%
HM1603+C.L-E	3000 ppm	100%	39.8%	36.4%	21.0%	15.9%	38.6%	36.9%

TABLE 4. Cumulative mortality percentage (%) of *Artemia* sp. submerged in selected seaweed extract at fungal MIC.

Seaweed extract	Dipping time (hour)						
	1/2	1	2	4	6	12	24
Control	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.2%
E.C-E	0.0%	0.0%	0.0%	0.0%	1.1%	2.2%	21.1%
C.L-M	0.0%	0.0%	1.1%	1.1%	1.1%	1.1%	5.6%
C.L-E	0.0%	0.0%	0.0%	0.0%	0.0%	2.2%	2.2%

toxicity test on *Artemia* nauplii. The results showed that the mortality of *Artemia* sp. reached up to 21.1, 5.6 and 2.2% after 24 h treatment with E.C-E, C.L-M and C.L-E, respectively. *Artemia* sp. are tolerant at 12 h treatment for all 3 seaweed extract with minimal mortality that comparable to control (Table 4).

DISCUSSION

The aim of this study is to assess the potential of local seaweed as antimycotic compound for the treatment of oomycetes infection. The findings showed that *C. lentillifera* have higher extraction yield compared to *E. cottonii* and methanol extract for both seaweeds were the highest effect compared to ethanol and water extracts. The results are comparable to previous reports. Ethanol extraction of 100 g *U. lactuca* has 5.1% yield (Hassan and Ghareib, 2009) while 3 g *U. lactuca* methanol extract at 1:10 w/v ratio has 53.5% yield (Barot et al., 2016). The present study showed both seaweeds have antimycotic properties. However, even though ethanol extracts have the lowest yield but it shows better antifungal effects. Despite the low extraction yield, there might be a possibility that most of the bioactive secondary metabolites are dissolved and collected in ethanol better than other solvents in *E. cottonii*. However, at current study, this is difficult to ascertain. Thus, further studies are required on the type of chemical constituent in *E. cottonii* and *C. lentillifera* which have antimycotic properties against marine oomycetes species.

Ethanol extract of *E. cottonii* and methanol extract of *C. lentillifera* showed comparable MIC value to previous studies on other seaweed species. *U. lactuca* methanol extract has antifungal properties against *F. solani* (Rasha et al., 2015). The MIC of *U. lactuca* in methanol extract on *C. albicans* was 600 ppm (Saidani et al., 2012). However, it is very difficult for direct comparison since the reports on seaweed extract against marine oomycetes species were very limited.

Antimycotic effect of seaweed extract against fungal hyphae production determines the time taken for seaweed extract to inhibit fungal hyphae growth.

Fungicidal test showed that *H. sabahensis* IPMB 1402 and *L. thermophilum* IPMB 1601 hyphae growth was inhibited after 12 h submersion. The findings of this study are coherent with Lee et al. (2016) whereby fungal hyphae growth inhibition may occur between the 2 h and 24 h immersion. The most effective inhibition is ethanol extract of *E. cottonii* at 500 ppm against *L. thermophilum* IPMB 1401 whereby the hyphae growth is inhibited after 4 h submersion. Lee et al. (2016) reported the inhibition of the growth of fungal hyphae by formalin at 25 ppm. Furthermore, seaweed extracts showed the reduction of the zoospore production in marine oomycetes. Lee et al. (2016) reported that the zoospore production of *L. thermophilum* IPMB 1401 and *H. sabahensis* IPMB 1402 were inhibited at 24 h immersion at 25 ppm. The results are coherent with the present study whereby the zoospore production was affected. The current study showed similar efficiency in term of fungal inhibition between seaweed extract and formalin treatment. The findings of this study showed potential alternative treatment using seaweed extract than chemical (formalin) treatment on *L. thermophilum* and *H. sabahensis* infection. This study is also reported new findings on the effect of seaweed extracts on two other *Lagendiales* species, *L. thermophilum* IPMB 1601 and *H. milfordensis* IPMB 1603. Furthermore, there are no studies on the zoospore production on these two species. Therefore, as far as our knowledge, this is the first study to report of the quantitative inhibition of zoospore production for *Lagendiales* species.

The purpose of this study is to assess the toxicity of seaweed extract against *Artemia* sp. after determining the efficiency of seaweed extract against marine oomycetes. In the present study, the highest mortality is 21.1% after 24 h in *E. cottonii* ethanol extract at 500 ppm while other extracts shows the mortality not more than 5%. At 12 h tests, the mortality is relatively low, which is below 5%. Experimental infection of *Artemia* sp. nauplii against *H. sabahensis* IPMB 1402 shows that the mortality of nauplii after 24 h and 48 h is 20% and 26%, respectively (Lee et al., 2017). The current results show that zoospore production for IPMB 1402 after treatment is 13.3%. Therefore seaweed extract reduces the probability of *Artemia* sp. being infected. The present finding also shows that seaweed extract has low toxicity towards *Artemia* sp. Thus, seaweed extracts might be suitable against marine oomycetes because it has no cytotoxic effect on living organism.

In summary, *E. cottonii* and *C. lentillifera* extracts may be promising for the prevention of oomycetes infection and the useful treatment due to their significant antimycotic activity against hyphae and zoospores of *L. thermophilum*, *H. sabahensis* and *H. milfordensis*, with low toxicity to *Artemia* sp. The minimum inhibitory concen-

tration test, fungicidal bioassay and toxicity test suggested that the treatment of 12 h immersion with seaweed extract for marine oomycetes was a suitable in aquaculture. The extracts from both seaweed species might be used synergistically for the optimum treatment of infections. However, this may need further clarification on synergistic effects of both extracts to marine oomycetes species. Besides, further study on seaweed extract antimycotic properties and toxicity test should be conducted on mud crab zoea. Nevertheless, this study shows potential on finding the alternative control measurements for crab larvae health management which can contribute to the sustainable development and food security of aquaculture industry.

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