LARVICIDAL AND ADULTICIDAL ACTIVITIES OF MALAYSIAN SEAWEEDS AGAINST *AEDES AEGYPTI* (L.) AND *AEDES ALBOPICTUS* SKUSE (DIPTERA: CULICIDAE)

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Abstract. Discovering new mosquitocidal agent is the research focus of government authorities, researchers and industrial investors. Seaweeds serve a great potential in providing unique components and compounds with effective bioactivity. In the present study, seaweed species collected from West Malaysia were evaluated for their larvicidal and adulticidal activities according to WHO guidelines. Extracts were prepared by maceration of dried seaweeds in methanol. Of the 15 species tested, the green seaweed *Bryopsis pennata* exhibited the strongest larvicidal activity against *Aedes aegypti* (LC_{50} value: 156.97 μg/ml) and *Ae. albopictus* (LC_{50} value: 177.50 μg/ml). The brown seaweed *Sargassum siliquosum* had the strongest adulticidal effect against *Ae. aegypti* (LC_{50} value: 17.53 mg/cm^2) and *Ae. albopictus* (LC_{50} value: 35.40 mg/cm^2), among the 11 species tested. Larvae and female adults showed signs of intoxication, such as restless movement, tremor, paralysis, and followed by eventual death, after exposure of the seaweed extract. Future investigation on the active component and mode of action of the seaweed extracts should be carried out.

Keywords: *Aedes aegypti*, *Aedes albopictus*, mosquito control, larvicidal, adulticidal

INTRODUCTION

Over 1 million people die from mosquito-borne diseases every year, and hundreds of millions experience pain and suffering from illnesses transmitted by mosquitoes (WHO, 2015). There is an estimated 170,000 yellow fever infection cases and 29,000 to 60,000 deaths in Africa for the year of 2013. Globally, there were 66 countries annually notified the occurrence of yellow fever from 1950 to 2004 (WHO, 2015). The projected number of annual dengue infection cases is 50 to 100 million, with approximately 22,000 deaths mainly among children, in more than 100 countries (WHO, 2013).

These viral infections impose great economic burden on the households, public health care system and society in the endemic regions. In America, dengue infection was estimated to cost USD2.1 billion per year on average (Shepard *et al*, 2011). Likewise, dengue hemorrhagic fever is listed among the ten leading causes of hospitalization in at least eight Asian

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countries (Lee, 2000).

In Malaysia, the numbers of fatal cases and dengue infections increased by 8% and 14% on average per year, respectively (from the year of 2000 to 2010) (Mia *et al*, 2013). In 2015, dengue deaths in Malaysia climbed by 56% as compared to 2014 (MOH, 2015).

In spite of the usage of yellow fever vaccine in the prevention of the disease, to date, dengue vaccine that acts against all four dengue serotypes simultaneously is still under development, while there is no available vaccine for chikungunya (Tolle, 2009; Kumar *et al*, 2010). Control of these diseases still heavily relies on the management of principal epidemic vectors, the disease-carrying mosquitoes.

Dengue fever, yellow fever and chikungunya are harmful viral diseases chiefly vectored by infected *Ae. aegypti* and followed by *Ae. albopictus*. *Aedes* mosquitoes become infected with these viruses through feeding on the infected human/animal and once the mosquitoes are infected, they remain infected for life. In addition, these viruses remain in the mosquito population by transovarial transmission (Tolle, 2009).

Synthetic organic insecticide in vector control is used continuously since its discovery in 1940s. Unfortunately, these synthetic chemicals have been abusively used by the public and consequently, elicited insecticide resistance in the mosquito population. Therefore, there is a growing interest in looking for alternatives sourced from the nature as successful stories have been reported. For example, the discovery of pyrethrins from chrysanthemum flowers (Casida, 1980) and endotoxin originating from bacteria *Bacillus thuringiensis* var. *israelensis* (Schnepf *et al*, 1998) signified great advancements in the insecticide research.

Malaysia is a tropical country with long coastline that harbors a high diversity of marine organisms. Seaweeds in Malaysia are proven to have bioactivity such as antioxidant (Chew *et al*, 2008; Sheikh *et al*, 2009) and antibacterial properties (Vairappan *et al*, 2010; Chong *et al*, 2013; Natrah *et al*, 2015). These natural heritage advantages the study to screen through potential seaweeds and further test on their mosquitocidal effect, especially in Malaysia where dengue infection is a serious community health problem. Furthermore, earlier reports have envisaged the efficiency of seaweed extracts and compounds in mosquito larvicidal activity (Thangam and Kathiresan, 1991b; Beula *et al*, 2011; Yu *et al*, 2014).

The present study was conducted to evaluate the larvicidal and adulticidal efficacy of Malaysia common Malaysian selected seaweeds towards *Ae. aegypti* and *Ae. albopictus* mosquitoes. In this study, the potential of seaweeds as mosquitocidal agent was determined.

MATERIALS AND METHODS

Preparation of seaweed samples

Fresh seaweeds were collected from 2010 to 2012 from various locations of West Malaysia (Table 1). The seaweeds were brought back to the lab with ice pack and cleaned thoroughly to remove epiphytes and sand, followed by air-drying at 23±1°C for 2 days. Then, the dried seaweeds were ground, sieved and macerated with methanol (60 g/l) for 72 hours. The sample was extracted three times. Then, the solvent was filtered and concentrated by using rotary evaporator (BÜCHI, Flawil, Switzerland) at 50°C. The damp extract was transferred to vial and left to air-dry in the fume hood and later

Table 1
List of seaweeds used in the preliminary screening of larvicidal assay.

Order	Family	Species	Voucher code (Site[a] - No)
Green seaweeds			
Bryopsidales	Caulerpaceae	*Caulerpa lentillifera*	CRM - C2
		Caulerpa racemosa	CRM - C3
Ulvales	Ulvaceae	*Ulva lactuca*	PMJ - C4
		Ulva reticulata	PMJ - C5
	Bryopsidaceae	*Bryopsis pennata*	CRM - C1
Brown seaweeds			
Fucales	Dictyotaceae	*Dictyota dichotoma*	TKNS - P6
		Padina australis	TKNS - P5
	Sargassaceae	*Turbinaria conoides*	CRM - P4
		Sargassum binderi	CRM - P1
		Sargassum polycystum	TKNS - P3
		Sargassum siliquosum	CRM - P2
Red seaweeds			
Gracilariales	Gracilariaceae	*Gracilaria changii*	KKS - R1
		Gracilaria salicornia	KKS - R2
Rhodymeniales	Lomentariaceae	*Ceratodicyton spongiosum*	PCM - R5
Gigartinales	Solieraceae	*Solieria robusta*	PMJ - R4

[a]CRM: Cape Rachado, Melaka (2º 24.95′ N 101º 51.21′ E); PMJ: Pulau Merambung, Johor (1° 18′58.4″ N 103° 36′36.2″ E); TKNS: Teluk Kemang, Negeri Sembilan (2º 26.29′ N 101º 51.42′ E); KKS: Kampung Kelanang, Selangor (2° 46′50.8″ N 101° 25′16.9″ E); PCM: Pantai Cermin, Melaka (2° 24′49.9″ N 101° 51′33.2″ E).

kept at 4°C before use (Sheikh *et al*, 2009).

All stock solutions of seaweed extracts were prepared a day prior to the experiment at concentration of 10,000 µg/ml. The extract was weighed, dissolved in methanol and diluted in distilled water. The solution was mixed well and sonicated to increase the solublity. After that, the stock solution was mixed with distilled water to prepare working solutions at concentrations of 100, 200, 300, 400, and 500 µg/ml.

Preparation of *Ae. aegypti* and *Ae. albopictus*

The procedures for colonization, feeding and use of mosquitoes followed the guidelines from Entomology Unit, Institute for Medical Research (IMR). These procedures were in accordance with the Section 14, Destruction of Disease-Bearing Insects Act 1975 (amended 2000) and approved by the Ministry of Health, Malaysia in 2006. Laboratory strains of *Ae. aegypti* and *Ae. albopictus* were used in the assay.

Larvicidal assay

The larvicidal assay was conducted according to the guidelines of WHO (2005). All experiments were conducted at temperature of 26±2°C and relative humidity of 80±2%. The larvae were divided into a few batches with each batch constituted of 25 larvae and put into different 200 ml-paper cups filled with

seaweed extract solution (100, 200, 300, 400 and 500 μg/ml), in order to determine the concentration value that kills 50% of the larvae (LC_{50} value). The homogeneity of larvae was ensured by collecting the third instar larvae from the same batch and calibrated their size to 8.0±0.2 mm. Temephos (stock solution of 6.25 mg/l) (WHO, Geneva, Switzerland) was used as positive control and 0.20% (v/v) of methanol was used as negative control. The larval mortality (%) was recorded at the end of 24 hour-monitoring. The larvae were considered dead if they did not move when the water was disturbed. The test with negative control larvae that pupated more than 10% or with mortality rate more than 20% was discarded and the test was repeated. The mortality of treated groups was corrected by Abbott's formula, when the mortality of larvae in negative control was 5% to 20% (Abbots, 1925).

$$\text{Corrected mortality (\%)} = \frac{X-Y}{X} \times 100$$

X: percentage survival in negative control, Y: percentage survival in the group treated with seaweed.

The experiment was repeated five times with triplicates. The LC_{50} value was calculated by using the Probit analysis (Finney, 1971) of SPSS 20® (IBM, Armonk, NY). The effect of different seaweed extract treatments were compared through one way analysis of variance (ANOVA) followed by Tukey test, by using IBM SPSS 20. A $p<0.05$ was considered statistically significant.

Behavioral study of treated larvae

The swimming behavior and movement of treated larvae were studied. These larvae were observed every 2 hours for 24 hours starting from the exposure of treatment, and their behavior was recorded. The larvae treated with extract solution were compared to the larvae in negative control.

Adulticidal assay

Adulticidal assay was carried out according to WHO (1998). All experiments were conducted at temperature of 26±2°C and relative humidity of 80±2%. Stock solutions of seaweed extract (10,000 μg/ml) were prepared by dissolving methanol extract in ethanol and diluted with distilled water. Impregnated papers were prepared freshly prior to testing. Each filter paper (14.0x11.5 cm) was impregnated in 4 ml of solution making final concentrations of 0.248, 0.496, 0.993 and 1.987 mg/cm^2. Permethrin-impregnated papers of different concentrations (0.004-0.038 mg/cm^2) were prepared as a positive control by using permethrin solution (WHO, Geneva, Switzerland). Negative control was the filter paper impregnated with 5% (v/v) ethanol solution. Then, the impregnated papers were left to air-dry at the temperature of 25 ± 1°C.

Five-day-old female adults of *Ae. aegypti* and *Ae. albopictus* were obtained from the insectary of IMR. Different batches formed by 15 adult females each were introduced to the exposure tubes (WHO, Geneva, Switzerland) containing an impregnated paper (rolled into cylinder shape) for 3 hours. At the end of the 3-hour exposure, the mosquitoes were transferred to the holding tubes (without the impregnated paper) and given 10% sugar solution enriched with vitamin B complex as food. The mortality (%) of the adults was recorded at the end of 24 hours monitoring. The experiment was repeated three times with triplicates. The LC_{50} value was calculated by using the Probit analysis (Finney, 1971) of SPSS 20 (IBM, Armonk, NY). The effect of different

Table 2
Larvicidal activity of 15 methanol seaweed extracts towards *Aedes aegypti* and *Ae. albopictus*.

Seaweed species	Mosquito species	LC_{50} (95% CL) (μg/ml)	Slope (±SE)	χ^2
Brown seaweed				
Dictyota dichotoma	*Ae. aegypti*	>600	--	--
	Ae. albopictus	351.05 (313.93-396.41)	2.88 (0.27)	0.16
Padina australis	*Ae. aegypti*	500.46 (478.99-530.52)	8.18 (0.70)	1.00
	Ae. albopictus	458.44 (400.82-535.20)	2.49 (0.24)	2.34
Sargassum binderi	*Ae. aegypti*	417.04 (376.45-467.85)	3.37 (0.33)	0.01
	Ae. albopictus	334.47 (298.69-377.17)	2.84 (0.27)	3.76
Sargassum polycystum	*Ae. aegypti*	>600	--	--
	Ae. albopictus	>600	--	--
Sargassum siliquosum	*Ae. aegypti*	>600	--	--
	Ae. albopictus	>1,000	--	--
Turbinaria conoides	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	616.65 (535.98–740.16)	4.56 (0.37)	3.17
Green seaweed				
Bryopsis pennata[a]	*Ae. aegypti*	156.97 (133.54–179.46)	2.57 (0.25)	1.47
	Ae. albopictus	177.50 (156.41–198.68)	3.05 (0.27)	4.02
Caulerpa lentilifera	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Caulerpa racemosa	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Ulva lactuca	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Ulva reticulata	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Red seaweed				
Ceratodicyton spongiosum	*Ae. aegypti*	>600	--	--
	Ae. albopictus	>600	--	--
Gracilaria changii	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Gracilaria salicornia	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Solieria robusta	*Ae. aegypti*	>1,000	--	--
	Ae. albopictus	>1,000	--	--
Temephos	*Ae. aegypti*	0.006 (0.004-0.008)	2.63 (0.23)	1.35
	Ae. albopictus	0.013 (0.009-0.015)	7.63 (0.34)	2.24

[a]Methanol extract with the strongest larvicidal effect.
LC_{50}, lethal concentration that kills 50% of the exposed larvae; 95% CL, 95% confidence limits; χ^2, chi-square value.

Table 3
Adulticidal activity of 11 methanols seaweed extracts towards female adults of *Ae. aegypti* and *Ae. Albopictus*.

Seaweed species	Mosquito species	LC_{50} (95 % CL) mg/cm^2	Slope (±SE)	χ^2
Brown seaweed				
Padina australis	*Ae. aegypti*	30.80 (22.29-42.55)	1.338 (0.21)	0.95
	Ae. albopictus	36.21 (30.02-43.68)	1.580 (0.15)	0.98
Sargassum binderi	*Ae. aegypti*	34.32 (26.78-43.98)	1.517 (0.03)	0.97
	Ae. albopictus	109.30 (96.43-123.80)	1.134 (0.60)	0.99
Sargassum polycystum	*Ae. aegypti*	44.65 (41.48-48.06)	1.272 (0.04)	0.99
	Ae. albopictus	108.60 (92.08-128.10)	1.242 (0.09)	0.99
Sargassum siliquosum[a]	*Ae. aegypti*	17.53 (16.31-18.84)	1.383 (0.04)	0.99
	Ae. albopictus	35.40 (30.75-42.80)	1.116 (0.11)	0.98
Green seaweed				
Bryopsis pennata	*Ae. aegypti*	86.48 (76.76-97.42)	1.142 (0.05)	0.99
	Ae. albopictus	156.30 (150.1-162.80)	1.416 (0.02)	0.99
Caulerpa rasemosa	*Ae. aegypti*	29.33 (24.65-34.90)	1.555 (0.15)	0.98
	Ae. albopictus	51.48 (43.23-61.30)	1.775 (0.18)	0.98
Ulva lactuca	*Ae. aegypti*	144.40 (140.0-149.0)	1.174 (0.01)	0.99
	Ae. albopictus	204.80 (185.80-225.80)	1.169 (0.03)	0.99
Ulva reticulata	*Ae. aegypti*	72.90 (68.25-77.87)	1.351 (0.04)	0.99
	Ae. albopictus	170.90 (163.40-178.80)	1.219 (0.02)	0.99
Red seaweed				
Gracilaria changii	*Ae. aegypti*	69.73 (62.99-77.18)	1.272 (0.05)	0.99
	Ae. albopictus	74.06 (68.95-79.54)	1.302 (0.01)	0.99
Gracilaria salicornia	*Ae. aegypti*	134.24 (126.83-143.70)	1.591 (0.22)	0.97
	Ae. albopictus	276.00 (243.40-313.10)	1.061 (0.03)	0.99
Solieria robusta	*Ae. aegypti*	129.72 (122.47-139.31)	1.778 (0.31)	0.96
	Ae. albopictus	256.70 (243.6-270.60)	1.181 (0.01)	0.99
Permethrin	*Ae. aegypti*	0.022 (0.015-0.031)	1.640 (0.50)	0.99
	Ae. albopictus	0.036 (0.029-0.045)	2.581 (0.75)	0.97

[a]Extract with the strongest lethal effect.
LC_{50}, lethal concentration that kills 50% of the exposed adults; 95% CL, 95% confidence limits; χ^2, chi-square value.

seaweed extract treatments were compared through one-way analysis of variance (ANOVA) followed by Tukey test, IBM SPSS 20. A p <0.05 was considered statistically significant.

Behavioral study of treated female adults

The behavior of treated adults in the test tubes for adulticidal assay was observed hourly for 24 hours starting from the exposure of treatment, until the death of mosquitoes. The mosquito was considered dead if it showed no sign of movement.

RESULTS

Larvicidal assay

Larvicidal effects of 15 seaweeds on larvae of *Ae. aegypti* and *Ae. albopictus*

mosquitoes were assessed. The results are shown in Table 2. Values of LC_{50}, 95% of confidence limits, slope and chi-square were also determined. The larvicidal activity of all tested samples was proportional to the concentration used. The larvae in negative control showed no mortality.

Among the methanol extracts of 15 seaweeds tested, the green seaweed *Bryopsis pennata* was the only sample exhibited larvicidal activity towards larvae of *Ae. aegypti* and *Ae. albopictus* at LC_{50} values less than 200 μg/ml (LC_{50} values of 156.97 and 177.50 μg/ml, respectively). The brown seaweeds, namely *Padina australis* and *S. binderi*, exhibited larvicidal activity at LC_{50} values ranging from 200 to 500 μg/ml; The remaining seaweed extracts had LC_{50} values more than 500 μg/ml.

Effects of methanol extract at the concentration of LC_{50} value on swimming behavior of *Ae. aegypti* and *Ae. albopictus* larvae were recorded. Overall, the larvae under different treatments of seaweed extracts exhibited similar abnormal behavior as a sign of intoxication. However, these changes of behavior varied at the time and duration of manifestation depending on the seaweed species and extract concentration. The onset of behavioral changes was observed to be correlated to the toxicity of the seaweed extract. Methanol extract with a stronger larvicidal ability caused earlier onset of intoxication symptoms, as compared to the methanol extract with a weaker larvicidal ability.

Most of the mosquito larvae were observed to undergo three distinct phases after extract treatment. In phase I, the larvae showed signs of unnatural restlessness, wriggling movement and frequent sinking followed by floating; the larvae became sluggish with random tremor at the bottom of the container in phase II; this is followed by phase III which the larvae paralyzed, sank to the bottom of the container and died.

Adulticidal assay

Adulticidal effects of methanol extracts of 11 seaweeds against *Ae. aegypti* and *Ae. albopictus* are presented in Table 3. The results showed that the mortality rate of female mosquitoes was directly proportional to the concentration of seaweed extracts applied.

The brown seaweed *S. siliquosum* exhibited the strongest lethal effect against female adults of *Ae. aegypti* and *Ae. albopictus* at LC_{50} values of 17.53 and 35.40 mg/cm^2, respectively. This is followed by *P. australis* exhibiting lethal effect at LC_{50} values of 30.80 and 36.21 mg/cm^2, against female adults of *Ae. aegypti* and *Ae. albopictus*, respectively. In the present study, all seaweeds exhibited significant higher LC_{50} values towards *Ae. albopictus* as compared to *Ae. aegypti*.

Treated female adults of *Ae. aegypti* and *Ae. albopictus* in adulticidal assay were observed for 24 hours for their behavioral changes. These female adults showed similar trend of abnormal behavior before death, except for the onset of manifestation depending on the concentration applied. Adults under higher concentration of treatment exhibited an earlier onset of behavioral changes, as compared to those being treated with lower concentration of seaweed extract.

Most of the female adults treated with seaweed extracts were observed to undergo two phases of intoxication. In phase I, the treated females showed restless movement and hardly resting still on the surface of holding tube. This is followed by phase II as the female adults were increasingly found to be wagged or

paralyzed lying at the bottom of holding tube and died. In the present study, about 20% to 50% of the female mosquitoes treated with *S. siliquosum* extract-impregnated paper at the concentration of 0.993 mg/cm^2, exhibited sign of inactive, after 24-hour holding period.

DISCUSSION

In the present report, the larvicidal activity of *B. pennata* methanol extract was considered as a moderate effective larvicide (LC_{50} value between 100 and 200 mg/l) (Table 2) according to the classification of Thangam and Kathiresan (1996). The methanol extracts of *P. australis* and *S. binderi* tested in this report were classified as ineffective larvicide (LC_{50} value higher than 200 mg/l).

However, other species under the same genus were reported to have stronger mosquito larvicidal activity as compared to that of those species in this report. For instance, *S. wightii* has strong larvicidal action against larvae of *Ae. aegypti* (LC_{50} value: 84.82 μg/ml) and *Culex quinquefasciatus* (LC_{50} value: 87.09 μg/ml) (Manilal *et al*, 2011). Similarly, *S. swartzii* demonstrates strong larvicidal effect on larvae of *Anopheles stephensi* (LC_{50} value: 11.76 μg/ml) (Khanavi *et al*, 2011) and *An. sundaicus* (LC_{50} value: 1.56 mg/l) (Kumar *et al*, 2012). On the other hand, *P. tetrastromatica* exhibits larvicidal activity against larvae of *Cx. quinquefasciatus* and *Ae. aegypti*, at LC_{50} values of 97.94 and 97.41 μg/ml, respectively (Manilal *et al*, 2011). Wide difference in bioactivity between the individual species in the same genus can be due to geographical and ecological factors that affect the production of carbon-based bioactive secondary metabolites. Seasonal variation that influences the composition of chemical constituents in seaweeds may also result in various degrees of bioactivity (Stengel *et al*, 2011; Wong and Phang, 2004).

Manilal *et al* (2011) described the abnormal before-death-behavior of larvae *Culex quinquefasciatus* and *Ae. aegypti* treated with the brown seaweed *Lobophora variegata* at the concentration of 200 μg/ml. The treated larvae were observed to be excited and restless after an hour of exposure (primary phase). They became sluggish which began 6 hours after exposure (secondary phase), followed by paralysis and death in 12 to 24 hours (tertiary phase). Similar symptoms were clearly seen on larvae of *Cx. quinquefasciatus* after treatment of rhizome of *Kaempferia galanga* at the concentration of 61.43 ppm, *eg,* restlessness (5-15 minutes after treatment), tremors, followed by paralysis (45-60 minutes) (Insun *et al*, 1999). These results are similar to the observation of present study, but with differences in the concentration of treatment and time of showing the symptoms.

The abnormal behavior of treated larvae in the present study is in agreement with the observation of Watanabe *et al* (1990), which described the behavior of mosquito larvae treated with monoterpenes derived from the red seaweed *Plocamium telfairiae*. In the study, the compounds were proposed to have interacted with picrotoxinin receptor, indicating their mode of action resembled the mechanism of cyclodiene-type insecticide (Watanabe *et al*, 1990). Cyclodiene is a nerve poison and organochloride insecticide that blocks GABA-gated chloride channel, resulting in reduction of neuronal inhibition, hyperexcitation of central nervous system, convulsion and death (Bloomquist, 1993). The behavioral observation of present study indicates the correlation of seaweed extract in affecting the nervous system and motor coordination of treated larvae. Fur-

ther investigations are needed to confirm the neurological effect of seaweed extract that act as a nerve poison.

The susceptibility of different mosquito species towards insecticidal agents varies (Ali *et al*, 2013). Female adults of *Ae. aegypti* in adulticidal assay of the present study were more vulnerable towards the lethal effect of seaweed extracts, as compared to *Ae. albopictus*. Earlier reports showed varied adulticidal effect of the herb extracts, namely *Eclipta alba* and *Andrographis paniculata*, towards female adult of *Ae. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Govindarajan and Sivakumar, 2011, 2012). Among these three mosquito species tested in the reports, *An. stephensi* was the most vulnerable species (LC_{50} values ranging from 130 to 197 ppm), followed by *Cx. quinquefasciatus* (LC_{50} values ranging from 149 to 238 ppm) and *Ae. aegypti* (LC_{50} values ranging from 172 to 252 ppm) (Govindarajan and Sivakumar, 2011, 2012).

Research on the adulticidal effect of seaweeds against mosquitoes is scarce. However, the repellent activity of the brown seaweed *S. wightii* towards *An. sundaicus* was reported by Kumar *et al* (2012). In the report, the repellency was 89% when 10 mg/l of *S. wightii* methanol extract was applied (Kumar *et al*, 2012). Methanol extracts of Malaysian plants such as *Acorus calamus*, *Litsea elliptica* and *Piper aduncum* exhibit LC_{50} values ranging from 0.04 to 0.20 mg/cm^2 towards females of *Ae. aegypti* females (Hidayatulfathi *et al*, 2004). Essential oil of shrub leave of *Lantana camara* exhibits LC_{50} values ranging from 0.05 to 0.06 mg/cm^2 towards five mosquito species (Dua *et al*, 2010). As compared to these reports, the seaweeds of present study are considered as relatively weak adulticidal agents (LC_{50} values ranging from 17 to 276 mg/cm^2).

In view of the weak adulticidal activity of seaweed extracts, the application of seaweed extract alone may not be an effective adulticidal agent. Binary insecticide mixtures (containing seaweed extract and other insecticides) could be a better option in killing adult mosquitoes, since combination of seaweed extract and commercial insecticides has proven to have effective mosquito larvicidal activity (Thangam and Kathiresan, 1991a; Kumar *et al*, 2012). Further investigation is needed to confirm the efficiency of the binary insecticide mixtures against mosquito adults.

Similar behavioral observation on treated female mosquitoes was described by Dua *et al* (2010) who reported essential oil of the flowering plant *Lantana camara* possessed adulticidal activity. At the exposure concentration of 0.208 mg/cm^2 of *L. camara* essential oil, most of the mosquitoes showed sign of paralysis within 10 to 15 minutes, and all mosquitoes became inactive at the end of 1-hour exposure. In contrast, Choochote *et al* (2004) reported a different trend of intoxication behavior caused by the celery *Apium graveolens*. According to their report, most of the mosquitoes showed sign of paralysis after exposure of 3.5 to 10.6 mg/cm^2 for 5 to 15 minutes. However, when they were transferred from the exposure tube to the holding tube, approximately 30% of the adults recovered within an hour. At the end of a 24-hour holding period, the results revealed that the mortality of adults ranging from 16% to 76%.

Seaweed extracts in the present study showed significant influence on the behavior of female adults. The symptoms observed in adult mosquitoes treated with phytochemicals, such as excitation, convulsion, paralysis and followed by death, were similar to those caused by nerve poison (Choochote *et al*, 2004; Dua

et al, 2010). The seaweed extracts tested in present study might share a common mode of action with nerve poisons, as their signs of intoxication are similar.

In conclusion, seaweeds with strong larvicidal and adulticidal activities demonstrated their potential in future investigation. Future research to identify the active components in the methanol extract should be carried out. The mode of action of the active mosquitocidal agents should be further determined. Information of the effects on non-target organisms of potential mosquitocidal agents is also necessary.

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