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LARVICIDAL EFFECTS OF KOREAN SEAWEED EXTRACTS ON BRINE SHRIMP *ARTEMIA SALINA*

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ABSTRACT

A total of 38 methanol and water extracts of macroalgal species (18 brown, 14 red, and 6 green) collected from the Korean coast were investigated for their lethality to brine shrimp larvae. Of the solutions tested, methanol extracts of six species (15.79%) showed larvicidal effects against *Artemia salina*. Aqueous extracts of two species (5.26%) were lethal to *A. salina*. The brown algae *Dictyota dichotoma*, *Ecklonia kurome*, *Ishige okamurae*, *Sargassum sagamianum*, and *Pachydictyon coriaceum*, and the green algae *Enteromorpha linza* and *Ulva pertusa*, displayed significant larvicidal activity in 2.5% methanol extracts. None of the methanol extracts of the 14 red algae species tested had larvicidal effects. The brown alga *Colpomenia bullosa* and the red alga *Lomentaria catenata* displayed significant larvicidal activity in 2.5% extracts. No larvicidal effects were observed from extracts of green algae. These results offer insight into the larvicidal potential of Korean seaweed extracts.

Keywords: Seaweed extract, Larvicidal effect, *Artemia salina*, *Enteromorpha linza*, *Gelidium amansii.*

INTRODUCTION

Over the past several decades, seaweeds (marine macroalgae) and their extracts have generated great interest in the pharmaceutical industry as a source of bioactive compounds with immense medicinal potential. Seaweeds produce a great variety of secondary metabolites with a wide range of functions, making them potentially promising sources of pharmaceutical agents (Mayer and Hamann, 2002; Newman *et al*., 2003). Compounds with antiviral, antihelminthic, antifungal, antibacterial, antioxidant, anti-inflammatory, and anti-allergenic properties have been found in green, brown, and red algae (Newman *et al*., 2003; Wijesinghe and Jeon, 2011).

There are about 50 known species of edible seaweed in Korea (Kang 1968; Sohn 1998; Oh *et al*., 1990). Several Korean seaweeds are also used for medicinal purposes (Oh *et al*., 1990). Seaweeds may also contain toxic compounds including bromophenol (Wang *et al*., 2005; Choi *et al*., 2011), iodine (Müssig, 2009), arsenic (Sakurai *et al*., 2006; Almela *et al*., 2006), terpenoid (Ricci *et al*., 1999), lead, and cadmium (Almela *et al*., 2006; Khan *et al*., 2015). These compounds can be used to produce mosquito larvicide (Ali *et al*., 2013; Bianco *et al*., 2013), spermicidal compounds (Prakash *et al*., 2014), and biologics for killing ciliate protists (Ricci *et al*., 1999). However, indigenous Korean seaweeds have not yet been investigated for their larvicidal capabilities.

Brine shrimp lethality bioassays have been used to determine the cytotoxic activities of many seaweed species (Sukoso *et al*., 2012; Devi *et al*., 1998; Ara *et al*., 1999; Ayesha *et al*., 2010). However, the larvicidal activity of indigenous Korean seaweeds has not been researched until now.

Therefore, we conducted a brine shrimp lethality assay to determine the larvicidal activity of methanol and water extracts of 38 indigenous Korean seaweed species. Our results provide insight into the larvicidal potential of Korean seaweed extracts.

MATERIALS AND METHODS

Seaweed extracts: A total of 38 species of seaweed were collected from various locations in South Korea between November 2013 and April 2014. Seaweed tissues were washed with tap water to remove salt, epiphytes, and sand, then dried for 1 day at room temperature using an electric fan. A mill (Sinil Co., Seoul, Korea) was used for 5 min to grind the dried tissues into a powder, which was then passed through a 500 mesh sieve. To extract methanol-soluble components, 1 L methanol was added for 1 day to 20 g sieved powder. This process was repeated three times, and the combined extracts were evaporated until completely dry. Then distilled water (1 L) was added to the remaining powdered tissue to extract water-soluble components. Stock solutions were prepared by adding 1 mL methanol or distilled water to 100 mg dried extract and filtering through a 0.22 μm filter. Stock

solutions were stored at -20°C until needed (Choi *et al*., 2011).

***Artemia salina* hatching:** Dried brine shrimp eggs (*Artemia salina*; SWORM, Seoul, Korea) were hatched in 0.22 μm filter-sterilized seawater (1 g eggs per L) at 20°C, with mild aeration, under constant illumination (light intensity: 40 μmol/m^2/s). The eggs were incubated in a glass flask (250 mL) with a water height of 20 cm. These hatching conditions correspond to the natural environment of *A. salina*. Shrimp larvae were used for the experimental bioassay 24 h after hatching (Milhem *et al*., 2008). At this stage, nauplii larvae are still nourished by yolk sacs and we supplied no further food during the experimental period. The living larvae are highly mobile and phototaxic, making them easy to collect near a light source. Larvae were collected with a Pasteur pipette and concentrated in a small vial (Zhang *et al*., 2012).

Brine shrimp lethality assay: An assay was conducted to determine the toxic effects of seaweed extracts on *Artemia salina* nauplii larvae using the methods described in Milhem *et al*. (2008) and Vinayak *et al*. (2011), with minor modifications. Briefly, 100 μL aliquots of 0.22 μm filter-sterilized seawater were pipetted into a 24-well plate. Each experimental condition was accompanied by positive and negative controls in separate wells, so that a total of three wells were used per condition. Brine shrimp larvae solution (50 μL, containing ca. 20 larvae) was added to each well along with individual seaweed extracts at varying concentrations (0.25%, 0.5%, and 2.5%). Sterilized seawater was added immediately to bring the final volume of wells to 200 μL. Toxicity was determined after 2, 4 6, 12, 24, and 24 h of exposure by counting the number of survivors under a stereomicroscope (Olympus, Tokyo, Japan) and calculating the mortality rate. Solutions (50, 100, and 500 μg/mL) of potassium dichromate ($K_2Cr_2O_7$; Sigma-Aldrich 207802) were used as a positive control, and 2.5% methanol and 0.22 μm filter-sterilized seawater were used as a vehicle control and a negative control, respectively. The results of these assays are expressed as the means ± SDs from at least three separate experiments.

Mortality was evaluated using the criteria established by Solis *et al*. (1993), with slight modifications. Larvae were considered dead if they did not exhibit any internal or external movement during the observation period. Extracts with a mortality rate below 50% were considered non-larvicidal. Those with a rate higher than 50% but below 75% were considered mildly larvicidal. Those with a rate higher than 75% were considered highly larvicidal, and if 100% of larvae died, the extract was considered extremely larvicidal.

Statistics: Each experiment was repeated at least three times for each independent assay. Mean values of indices were compared to controls using Student's *t*-test.

RESULTS AND DISCUSSION

Larvicidal effects of methanol and potassium dichromate: Methanol at a concentration of 2.5%, used as a vehicle control, caused very little mortality among *Artemia salina* nauplii larvae (Fig. 1). Therefore, methanol seaweed extracts were always added to assay cultures in concentrations that resulted in less than 2.5% methanol. The survival rates in 50, 100, and 500 μg/mL potassium dichromate ($K_2Cr_2O_7$), used as a positive control, were 97.6%, 80.0%, and 60.2% at 12h, and 91.7%, 38.9%, and 5.0% at 24 h, respectively.

Larvicidal effects of methanol extracts of six species of Chlorophyta: The larvicidal effects of methanol extracts of six species of Chlorophyta against *Artemia salina* nauplii larvae are shown in Table 1. The survival rate of *A. salina* exposed to a 2.5% concentration of *Enteromorpha linza* methanol extract was 83.3% at 4 h, 29.4% at 6 h, and 0.0% thereafter; thus, this extract was classified as extremely larvicidal. The survival rates after exposure to 2.5% *Enteromorpha compressa* and *Ulva pertusa* methanol extracts were 4.2% and 1.8% at 48 h, respectively; these extracts were classified as highly larvicidal. The rates for 2.5% *Capsosiphon fulvescens* and *Monostroma nitidum* methanol extracts were 37.0% and 33.3% at 48 h, respectively; these were classified as mildly larvicidal. The rates for 0.25%, 0.5%, and 2.5% concentrations of *Codium fragile* methanol extract were ≥79.4% at 12, 24, and 48 h; this extract was considered non-larvicidal (Table 1).

Larvicidal effects of methanol extracts of 18 species of Phaeophyta: The larvicidal effects of methanol extracts of 18 species of Phaeophyta have been examined. Among them, 9 species showed larvicidal activities on *Artemia salina* nauplii larvae (Table 2). Seaweed extracts considered extremely larvicidal included those from *Pachydictyon coriaceum*, *Dictyota dichotoma*, and *Ecklonia kurome*. The survival rates after exposure to 0.5% and 2.5% concentrations of *P. coriaceum* methanol extract were 0.0% at 24 and 48 h, and those for 2.5% *D. dichotoma* and *E. kurome* methanol extracts were 0.0% at 48 h. The rates for 2.5% *Ishige sinicola*, *Saccharina japonica*, and *Undaria pinnatifida* methanol extracts were 48.1%, 46.4%, and 47.2% at 48 h, respectively; these were classified as mildly larvicidal. The rates for 0.25%, 0.5%, and 2.5% *Colpomenia bullosa*, *Costaria costata*, *Ecklonia cava*, *Eisenia bicyclis*, *Hizikia fusiformis*, *Myelophycus simplex*, *Petalonia binghamiae*, *Sargassum micracanthum*, and *Sargassum thunbergii* methanol extracts were ≥69.7% at 12, 24, and 48 h; these were classified as non-larvicidal (data not shown).

Larvicidal effects of methanol extracts of 14 species

Rhodophyta: The larvicidal effects of methanol extracts of 14 species of Rhodophyta have been examined. Among them, 4 species showed larvicidal activities on *Artemia salina* nauplii larvae (Table 3). The survival rate after exposure to 2.5% *Gelidium amansii* methanol extract was 0.0% at 12, 24, and 48 h; this extract was classified as extremely larvicidal. The rate for 2.5% *Chondracanthus intermedia* methanol extract was 5.0% at 48 h; this extract was classified as highly larvicidal. Those for 2.5% *Chondracanthus tenella* and *Gracilaria verrucosa* methanol extracts were 26.6% and 33.3% at 48 h, respectively; these were classified as mildly larvicidal. Those for 0.25%, 0.5%, and 2.5% *Ahnfeltiopsis flabelliformis*, *Chondaria crassicaulis*, *Chondrus ocellatus*, *Corallina pilulifera*, *Grateloupia elliptica*, *Hypnea charoides*, *Lomentaria catenata*, *Meristotheca papulosa*, *Prionitis cornea*, and *Symphyocladia latiuscula* methanol extracts were ≥52.0% at 12, 24, and 48 h; these were classified as non-larvicidal (data not shown).

Larvicidal effects of aqueous extracts of six species of Chlorophyta: The larvicidal effects of aqueous extracts of six species of Chlorophyta have been examined. Among them, 3 species showed larvicidal activities on *Artemia salina* nauplii larvae (Table 4). The survival rates after exposure to 2.5% *Enteromorpha compressa*, *Enteromorpha linza*, and *Ulva pertusa* extracts were 27.3%, 48.1%, and 49.9% at 48 h; these were classified as mildly larvicidal. The rates for 0.25%, 0.5%, and 2.5% *Capsosiphon fulvescens*, *Codium fragile*, and *Monostroma nitidum* extracts were ≥64.8% at 12, 24, and 48 h; these were classified as non-larvicidal (data not shown).

Larvicidal effects of aqueous extracts of 18 species of Phaeophyta: The larvicidal effects of aqueous extracts of 18 species of Phaeophyta have been examined. Among them, 13 species showed larvicidal activities on *Artemia salina* nauplii are shown in Table 5.

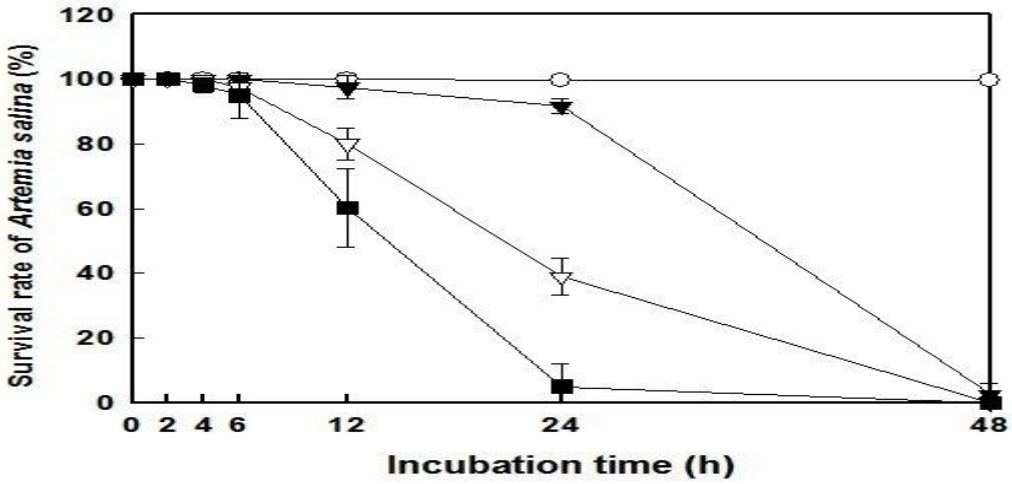

Fig. 1. The survival rate of *Artemia salina* nauplii. Negative control (●), 2.5% methanol as a vehicle control (○), 50(▼), 100 (▽), and 500 µg/mL (■) of potassium dichromate ($K_2Cr_2O_7$; Sigma-Aldrich 207802) as a positive control. Significance was calculated using Student's *t*-test and the threshold was P<0.01.

The survival rate after exposure to 2.5% *Colpomenia bullosa* extract was 0.0% at 24 and 48 h; this extract was classified as extremely larvicidal. The rates for 2.5% *Costaria costata*, *Dictyota dichotoma*, *Ecklonia kurome*, *Eisenia bicyclis*, *Ishige okamurae*, *Ishige sinicola*, and *Sargassum micracanthum* extracts were 17.8%, 22.5%, 15.8%, 5.1%, 18.3%, 24.1%, and 3.1% at 48 h, respectively; these were classified as highly larvicidal. The rates for 2.5% *Ecklonia cava*, *Hizikia fusiformis*, *Myelophycus simplex*, *Sargassum sagamianum*, and *Scytosiphon lomentaria* extracts were 33.8%, 29.4%, 45.6%, 38.6%, and 33.3% at 48 h, respectively; these were classified as mildly larvicidal. The rates for 0.25%, 0.5%, and 2.5% *Pachydictyon coriaceum*, *Petalonia binghamiae*, *Saccharina japonica*, *Sargassum thunbergii*, and *Undaria pinnatifida* extracts were ≥64.8% at 12, 24, and 48 h; these were classified as non-larvicidal (data not shown).

Larvicidal effects of aqueous extract of six species of Rhodophyta: The larvicidal effects of aqueous extracts of six species of Rhodophyta have been examined. Among them, 3 species showed larvicidal activities against *Artemia salina* nauplii larvae (Table 6). The survival rate after exposure to 2.5% *Lomentaria catenata* extract was 0.0% at 24 and 48 h; this extract was classified as extremely larvicidal. The rates for 2.5% *Chondracanthus intermedia* and *Prionitis cornea* extracts were 46.4% and 42.5% at 48 h, respectively; these were classified as mildly larvicidal. The survival rates for 0.25%, 0.5%, and 2.5% *Ahnfeltiopsis flabelliformis*,

Chondracanthus tenella, *Chondaria crassicaulis*, *Chondrus ocellatus*, *Corallina pilulifera*, *Gelidium amansii*, *Gracilaria verrucosa*, *Grateloupia elliptica*, *Hypnea charoides*, *Meristotheca papulosa*, and *Symphyocladia latiuscula* extracts were ≥64.1% at 12, 24, and 48 h; these were classified as non-larvicidal (data not shown).

Marine organisms are increasingly being studied with a view toward isolating biologically active compounds. Seaweeds are considered very attractive subjects for this type of research, due to their huge diversity and their long history of being safely consumed in traditional foods and herbal medicines in Asia (although it must be noted that some macroalgae are toxic).

Brine shrimp lethality bioassays have been used to determine the cytotoxic activities of many seaweed species (Sukoso *et al*., 2012; Devi *et al*., 1998; Ara *et al*., 1999; Ayesha *et al*., 2010). Sukoso *et al*. (2012), for instance, showed that extracts of *Porphyra* spp. were cytotoxic in brine shrimp. In another study (Ara *et al*., 1999), 22 ethanol extracts of seaweed species collected from the Karachi coast in Pakistan were investigated for their cytotoxicity in brine shrimp. Only six of these species, the brown algae *Stoechospermum marginatum*, *Sargassum swartzii*, *S. binderi*, *Spatoglossum asperum*, and *Stokeyia indica*, and the green alga *Caulerpa racemosa*, showed significant cytotoxic activity (Ara *et al*., 1999). A third study, also of seaweeds growing near the Karachi coast, used brine shrimp lethality assays to screen ethanol extracts of the seaweeds *Dictyota dichotoma* var. *velutricata*, *D. hauckiana*, *D. indica*, *Iyengaria stellata*, *Jolyna laminarioides*, *Melanothamnus afaqhusainii*, *Sargassum ilicifolium*, *S. lanceolatum* and *Ulva fasciata*. The extracts of eight species (all but *S. ilicifolium*) showed significant cytotoxicity (Ayesha *et al*., 2010). Devi *et al*. (1998) found that *Acanthophora muscoides* and *Microdictyon pseudohapteron* collected from India were toxic to *Artemia* larvae.

In this study, we investigated the larvicidal activity of indigenous Korean seaweeds, which has not been researched until now.

Among the Chlorophyta, we found a 2.5% concentration of *Enteromorpha linza* methanol extract to be extremely larvicidal. In Phaeophyta, 0.5% and 2.5% *Pachydictyon coriaceum* methanol extract and 2.5% *Colpomenia bullosa* extract were also extremely larvicidal, as was a methanol extract of Rhodophyta species *Gelidium amansii* at 2.5%. *Artemia* larvae exposed to these solutions had a 0.0% survival rate after 24 h. However, two of these seaweed species (*Enteromorpha linza* and *Gelidium amansii*) are known to be edible to humans and are commonly used as food and animal feed in Korea and Japan (Kang 1968).

The red alga *Symphyocladia latiuscula* contains high concentrations of bromophenols (Wang *et al*., 2005), some of which can be toxic to bacteria and other living organisms (Hétu *et al*., 1983; Calza *et al*., 2008). Methanol extracts of *S. latiuscula* can also have cytotoxic effects on eukaryotic cells (Choi *et al*., 2011). However, in this study, *Artemia* larvae had a 52.05% survival rate after 48 h in 2.5% *Symphyocladia latiuscula* methanol extract, indicating that this extract is non-larvicidal (data not shown).

It is apparent from these results that seaweed may have different toxicities for humans and brine shrimp larvae. This is important to note as medicinal chemicals are generally tested in animal models, but pharmacokinetics such as absorption, action, and excretion of medicines and chemicals may be drastically different between different animal species (Aubets *et al*., 2006; Christensen *et al*., 1990).

These preliminary results indicate that *Enteromorpha linza* and *Gelidium amansii* may be sources of larvicidal compounds that do not adversely affect humans, and future studies should seek to isolate the compound or compounds responsible for these larvicidal effects.

Table 1. The larvicidal effects of methanol extracts of six species of Chlorophyta on *Artemia salina* nauplii.

Chlorophyta	MeOH extract	Incubation time (h)						
		0	2	4	6	12	24	48
Capsosiphon fulvescens	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	66.1±47.9	95.0±7.1
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.8±3.2	97.8±3.2	95.9±0.5
	2.5%	100.0±0.0	100.0±0.0	64.0±36.2	100.0±0.0	81.3±26.5	44.0±18.0	37.0±7.6
Codium fragile	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.1±8.3
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.8±8.8
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.0±2.8	88.0±17.0	79.4±21.7
Enteromorpha compressa	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±00	100.0±0.0	94.0±1.3
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	90.8±7.2	51.2±57.3	22.6±26.9	4.2±5.9
Enteromorpha linza	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.3±2.4	90.9±1.2
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.5±3.5	86.4±9.1
	2.5%	100.0±0.0	100.0±0.0	83.3±23.6	29.4±7.4	0.0±0.0	0.0±0.0	0.0±0.0

Monostroma	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.8±3.1	94.5±1.7	92.3±1.4
nitidum	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	95.5±6.4	95.5±6.4	95.5±6.4	95.5±6.4
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	98.2±2.6	65.8±43.1	49.2±61.3	33.3±47.1
Ulva pertusa	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.5±79.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.8±3.1	97.8±3.1	97.8±3.1	90.4±4.8
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	90.8±2.1	52.1±24.1	15.1±11.	1.8±2.5

Means ± SD from three independent assays are shown. Significance was calculated using Student's *t*-test with a threshold of P<0.01.

Table 2. The larvicidal effects of methanol extracts of 9 species of Phaeophyta on *Artemia salina* nauplii.

Phaeophyta	**MeOH**	**Incubation time (h)**						
	extract	0	2	4	6	12	24	48
Dictyota	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.5±17	70.8±17.7
dichotoma	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.7±6.2	27.6±2.2	19.2±3.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	63.8±7.7	36.2±7.7	6.3±8.8	0.0±0.0
Ecklonia kurome	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.7±4.8	96.7±4.7
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.8±0.8	64.9±4.2	53.0±0.8
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	93.75±8.8	68.8±26.5	40.6±22.1	0.0±0.0
Ishige okamurae	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.0±0.7	92.2±4.6	87.6±1.8
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	98.2±2.5	95.8±0.8	64.9±4.2	53.0±0.8
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.9±3.0	37.8±45.6	5.0±7.1	0.0±0.0
Ishige sinicola	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.2±5.4	90.1±7.8
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	96.4±5.1	96.4±5.1	94.5±2.3	92.6±0.4
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	80.6±27.5	75.3±27.9	48.1±52.3
Pachydictyon	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	61.5±37.8	11.8±16.6	2.9±4.16
coriaceum	0.5%	100.0±0.0	100.0±0.0	90.0±14.1	95.7±6.2	22.2±12.9	0.0±0.0	0.0±0.0
	2.5%	100.0±0.0	100.0±0.0	38.3±24.4	44.7±16.0	16.2±11.1	0.0±0.0	0.0±0.0
Saccharina	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	97.8±3.1	97.8±3.1	97.8±3.1	96.0 ±0.5
japonica	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.7±3.2	97.7±3.2	97.7±3.2	97.3±3.2
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.0±11.6	66.2±12.9	46.4±17.2
Sargassum	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.1±2.7	92.3±10.9	75.1±13.5
sagamianum	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.7±1.7	76.9±27.9
	2.5%	100.0±0.0	100.0±0.0	93.7±2.4	70.4±3.3	44.8±23.1	30.4±15.0	0.0±0.0
Scytosiphon	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.3±2.9
lomentaria	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.7±4.7	96.7±4.7	90.0±4.7
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	96.6±4.9	82.3±15.3	49.1±21.5	27.7±8.8
Undaria	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.1±4.2	97.1±4.2
pinnatifida	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	95.5±6.4	95.5±6.4	95.5±6.4	90.5±0.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	93.9±0.8	70.0±28.3	52.8±19.7	47.2±27.5

Means ± SD from three independent assays are shown. Significance was calculated using Student's *t*-test with a threshold of P<0.01.

Table 3. The larvicidal effects of methanol extracts of 4 species of Rhodophyta on *Artemia salina* nauplii.

Rhodophyta	**MeOH**	**Incubation time (h)**						
	extract	**0**	**2**	**4**	**6**	**12**	**24**	**48**
Chondracanthus	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.9±4.4	96.9±4.4	93.8±8.8
intermedia	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±00	100.0±0.0	96.3±5.3	95.0±3.0
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.7±3.2	60.5±49.4	51.2±62.6	5.0±7.0
Chondracanthus	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.7±3.2	95.6±0.3	93.4±3.5
tenella	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.5±6.4	95.5±6.4	79.8±2.9
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	83.3±23.6	29.0±39.8	26.6±36.5
Gelidium	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.6±3.4
amansii	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.0±4.3	78.2±22.2	78.2±22.2	78.2±22.2
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	52.1±67.8	0.0±0.0	0.0±0.0	0.0±0.0
Gracilaria	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.4±7.5
verrucosa	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.4±7.5
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	93.3±9.3	83.3±4.7	60.0±0.0	33.3±9.4

Means ± SD from three independent assays are shown. Significance was calculated using Student's *t*-test with a threshold of P<0.01.

Table 4. The larvicidal effects of aqueous extracts of 3 species of Chlorophyta on *Artemia salina* nauplii.

Chlorophyta	Water extract	Incubation time (h)						
		0	2	4	6	12	24	48
Enteromorpha compressa	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.9±4.4
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.1±4.2	91.5±4.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	96.3±5.2	83.5±13.3	67.3±4.3	27.3±1.9
Enteromorpha linza	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.5±0.9	73.3±12.8
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.2±5.4	89.0±6.1
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.7±3.2	77.7±19.8	48.1±9.1	48.1±9.1
Ulva pertusa	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.7±11.8	86.7±4.7
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.5±3.5	87.5±17.7	73.3±18.8
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	95.7±6.2	75.9±8.9	67.9±10.0	49.9±15.3

Means ± SD from three independent assays are shown. Significance was calculated using Student's t-test with a threshold of P<0.01.

Table 5. The larvicidal effects of aqueous extract of 13 species of Phaeophyta on *Artemia salina* nauplii.

Rhaeophyta	Water extract	Incubation time (h)						
		0	2	4	6	12	24	48
Colpomenia bullosa	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.7±2.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.5±10.6	84.7±13.7
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	94.7±7.5	50.2±11.5	0.0±0.0	0.0±0.0
Costaria costata	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.5±6.4	92.5±2.3	80.5±11.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.9±3.6	65.0±10.3
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	85.0±21.2	51.1±12.7	28.0±9.9	17.8±10.2
Dictyota dichotoma	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.8±5.9	95.8±5.9
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.4±3.7
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	75.0±35.4	51.1±33.8	38.6±16.1	22.5±12.5
Ecklonia cava	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	96.2±5.4	96.2±5.4	96.2±5.4	91.2±1.6
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.3±0.0	90.0±4.7
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	87.5±17.7	67.5±24.8	41.9±2.7	33.8±5.3
Ecklonia kurome	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.9±4.4
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.1±4.2	90.6±3.3
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	71.1±40.9	50.0±18.6	23.7±3.7	15.8±7.4
Eisenia bicyclis	0.25%	100.0±0.0	100.0±0.0	90.3±0.4	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.9±3.0	94.4±2.1
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	97.5±3.5	94.9±0.2	35.7±13.2	5.1±0.2
Hizikia fusiformis	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.9±7.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	98.0±2.8	95.2±1.1	84.4±6.2	78.9±1.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	88.2±16.6	70.6±0.0	67.7±4.2	29.4±8.3
Ishige okamurae	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.1±11.8
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	96.9±4.4	93.5±0.3	77.3±5.6	77.3±5.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	84.2±22.3	58.3±11.8	26.7±9.4	18.3±2.4
Ishige sinicola	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.2±2.4
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.7±4.7	84.6±2.8	81.3±1.9
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	90.9±12.9	60.2±46.6	33.2±21.3	24.1±14.7
Myelophycus simplex	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.4±5.1
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.0±2.8	96.0±5.7	94.2±3.1
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	71.9±18.5	63.1±31.0	45.6±6.2
Sargassum micracanthum	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.6±7.6
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.7±11.8	86.1±19.7	81.1±12.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.2±25.3	13.4±1.3	3.1±4.4
Sargassum sagamianum	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.1±0.3
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.8±3.1	91.5±12.1
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	88.6±16.1	75.4±23.0	40.9±12.9	38.6±16.1
Scytosiphon lomentaria	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.3±2.4	92.9±0.6	89.4±0.9
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.8±3.0	95.7±6.5
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	88.6±16.1	76.0±23.9	39.1±10.3	33.3±8.5

Means ± SD from three independent assays are shown. Significance was calculated using Student's t-test with a threshold of P<0.01.

Table 6. The larvicidal effects of aqueous extracts of 3species of Rhodophyta on *Artemia salina* nauplii.

Rhodophyta	Water extract	Incubation time (h)						
		0	2	4	6	12	24	48
Chondracanthus intermedia	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.2±1.2	90.8±5.9
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.3±15.2	74.4±24.4
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.6±13.3	67.4±15.8	46.4±30.3
Lomentaria catenata	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	97.1±4.2	94.1±8.3	87.0±1.8	77.5±1.5
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	65.6±41.3	61.0±47.7
	2.5%	100.0±0.0	100.0±0.0	62.3±14.7	62.8±5.6	27.7±10.8	0.0±0.0	0.0±0.0
Prionitis cornea	0.25%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.4±3.0
	0.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.5±7.9	86.1±19.6	83.3±23.6
	2.5%	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.6±8.8	62.1±12.4	42.5±22.4

Means ± SD from three independent assays are shown. Significance was calculated using Student's t-test with a threshold of P<0.01.

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