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Inhibitory effects of halophyte *Sesuvium portulacastrum* on the marine diatom *Skeletonema costatum*

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ABSTRACT

Allelopathy plays an important role in the formation and succession of algal communities; however, there are few studies on the allelopathic effects of terrestrial plants on algal blooms. We investigated the potential allelopathic effects of the halophyte *Sesuvium portulacastrum* against *Skeletonema costatum* (a unicellular marine diatom). In co-culture experiments, time-course growth curves of *S. costatum* showed that algal growth in both the lag and exponential phase was inhibited by *S. portulacastrum*. The inhibitory activity of *S. portulacastrum* was biomass-dependent, suggesting that *S. portulacastrum* can prevent or reduce the magnitude of algal blooms. A water extract and a petroleum ether extract were prepared from *S. portulacastrum.* When treated with either of these extracts, *S. costatum* showed abnormal cell morphology. In addition, both these extracts decreased the maximum cell density and specific growth rate of *S. costatum* in a dose-dependent manner. *S. costatum* was more sensitive to the petroleum ether extract than the water extract of *S. portulacastrum*. GC-MS analysis of the petroleum ether extract revealed 45 lipophilic compounds. Our results suggested that the growth inhibition of *S. costatum* was due to the allelopathic potential of *S. portulacastrum* and that certain allelochemicals influenced the algal cell division.

Keywords: Algal growth, allelopathy, cell division, dose-dependent, extracts, GC-MS, *Sesuvium portulacastrum*, *Skeletonema costatum*

INTRODUCTION

Sesuvium portulacastrum L., a fast growing perennial halophyte (family Aizonaceae) (20), is an edible wild herb. It is also grown as an ornamental groundcover in coastal areas of China. This plant has a remarkable ability to survive under various stress conditions (9,29,33) and can be cultivated in seawater agriculture systems (21). The essential oils extracted from its leaves have antibacterial and antifungal properties (24). However, the allelopathic potential of this plant is largely unexploited.

Harmful algal blooms (HABs) are becoming an increasing problem to human health and environment world wide (16). Recent studies indicate that along with many other biotic and abiotic factors, allelopathy may play an important role in controlling algal blooms, especially in shallow lake and semi-closed sea areas (31). Many potential active

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allelochemicals have been identified (23). However, few have been identified from halophytes, and there is little information about the allelopathic effects of such plants on marine diatoms. In addition, while many studies have focused on the inhibitory effects of allelopathic substances, few have been carried out using *in situ* co-culture conditions (10). During a field study in Yundang Lagoon a highly eutrophic lagoon in centre of Xiamen city, Southeast China, we observed the low algae densities near roots of *S. portulacastrum*. Hence, we evaluated the possible allelopathic potential of *S. portulacastrum* on the target marine diatom *Skeletonema costatum* (cosmopolitan and predominant bloom-forming algal species in coastal marine waters) (28). Outbreaks of *S. costatum* can cause hypoxia and fish mortality and can have other harmful effects on the environment (18).

This study aimed to investigate the allelopathic effects of *S. portulacastrum* on *S. costatum.* We conducted co-culture studies to evaluate (i). the allelopathic effects of *S. portulacastrum* on *S. costatum* in different growth phases (ii). the effects of *S. portulacastrum* at different biomass densities on growth of *S. costatum* under adequate nutrients conditions, (iii). the effects of water- and petroleum ether-extracts from *S. portulacastrum* organs on *S. costatum* in monoculture conditions and (iv). determine the chemical composition of petroleum ether extract by gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Algal culture: The diatom *S. costatum* (YDL1108) was originally isolated from Yundang Lagoon (24°47′14″N, 118°08′23″E). The alga was maintained in f/2 medium (13) in an illuminating incubator under the following conditions: temperature, 20 ± 3°C; photoperiod, 12 h light/ 12 h dark; illumination, 72 µmol photons m^{-2} s^{-1}. The flask was shaken twice a day. The initial pH and salinity of culture medium were adjusted to 8.0 ± 0.02 and 30 psu (defined as practical salinity unit), respectively.

Preparation of *S. portulacastrum*: Individual *S. portulacastrum* plants were collected from Xisha Islands, South China Sea. Shoots with three nodes and two leaf pairs were cut from the maternal plants. Each cutting (fresh weight approx. 5 g and height approx. 10-15 cm) was pre-cultured for root differentiation in enriched Yundang Lagoon seawater (hereafter referred to as enriched seawater) for 1 month before the start of experiments. The concentrations of major nutrients were similar to those found in natural conditions of Yundang Lagoon (NO_3^- 0.5 mg L^{-1}, NH_4^+ 3.5 mg L^{-1} and PO_4^{3-} 0.05 mg L^{-1}), and the concentrations of other major elements (e.g. Ca, Si), trace metals, and vitamins were the same as those in f/2 medium. We confirmed that these nutrient components did not inhibit algal growth in a series of preliminary experiments (data not shown). The initial pH and salinity of the enriched seawater were adjusted to 8.0 ± 0.02 and 30 psu, respectively.

Co-culture of *S. costatum* and *S. portulacastrum*: Co-culture tests were conducted with *S. costatum* in different growth phases: lag phase or exponential growth phase. Nutrients were supplied only at the beginning of the experiment. Each aquarium (30 × 20 × 30 cm) was filled with 15 L enriched seawater 2 d before the start of experiments. We

prepared 9-aquaria (6: for co-culture tests and 3: for *S. costatum* monocultures, which served as the controls). In the co-culture system, *S. portulacastrum* plantlets at moderate biomass (0.5 g dry weight per litre seawater [g DW L^{-1}]) were co-cultured separately with *S. costatum* in the lag growth phase ("L") and the exponential growth phase ("E"), with initial cell density of alga 10×10^3 and 250×10^3 cells ml^{-1}, respectively. Each experimental treatment was replicated thrice. The seawater was continuously aerated and circulated to prevent formation of gradients. All aquaria were placed in a greenhouse under the following growth conditions: temperature, 24/30 °C; photoperiod, 12 h light/12 h dark; illumination, 30 µmol photons m^{-2} s^{-1}. The light, temperature, and pH were optimal for algal growth. The experiment continued until *S. costatum* reached the maximum algal cell density and the population began to decline. We determined chlorophyll *a* (Chl *a*) concentrations and cell density of *S. costatum* each day using the method of Jiang *et al.* (18). Specific growth rates (µ) of *S. costatum* were calculated to determine the rate of cell division (6) as under:

$$\mu\ (d^{-1}) = (ln\ N_t - ln\ N_0) / t;$$

Where, N_t and N_0: Maximum cell density at time t and at the start of the experiment, respectively.

The maximum algal cell density during the experimental periods was normalized and the inhibition ratio was determined (7) as under:

$$\text{Inhibition ratio (\%)} = (1 - N_p/N_c) \times 100;$$

Where, N_p and N_c: Maximum algal cell densities with or without co-cultured *S. portulacastrum,* respectively.

We conducted further co-culture tests with different biomass densities of *S. portulacastrum* and supplied extra f/8 nutrient solution (18.75 mg L^{-1} $NaNO_3$, 1.5 mg L^{-1} $NaH_2PO_4 \cdot 2H_2O$, 2.5 mg L^{-1} $Na_2SiO_3 \cdot 9H_2O$, 0.025 mg L^{-1} vitamin B1, 0.125 mg L^{-1} vitamin B12, and 0.25 mL 1000 × stock solution of f/2 trace metal [4.4 g L^{-1} $Na_2EDTA \cdot 2H_2O$, 3.16 g L^{-1} $FeCl_3.6H_2O$, 12 mg L^{-1} $CoSO_4.7H_2O$, 21 mg L^{-1} $ZnSO_4.7H_2O$, 180 mg L^{-1} $MnCl_2.4H_2O$, 7 mg L^{-1} $CuSO_4.2H_2O$, 7 mg L^{-1} $Na_2MoO_4.2H_2O$]) on alternate days to maintain the nutrient balance and to prevent nutrient deficiency during the experimental period. For these experiments, each aquarium contained 15 L enriched seawater as described above, and was inoculated with *S. costatum* in the exponential growth phase. The initial cell density of alga in the culture medium was 0.2×10^3 cells ml^{-1}. *S. portulacastrum* was separately transplanted into the aquaria at biomass densities of 2 and 5 g FW L^{-1}, which were equal to 0.2 and 0.5 g DW L^{-1}, respectively. A monoculture of *S. costatum* without *S. portulacastrum* served as control. Each experimental treatment was replicated thrice and all other conditions were same as described above. Chl *a* concentration and cell density of *S. costatum* were measured daily. The specific growth rate of the alga and the inhibition ratio of maximum cell density were also determined, and were used to calculate the half maximal inhibitory concentration (IC_{50}) (4).

Preparation of crude extracts of *S. portulacastrum*: *S. portulacastrum* plants were washed with distilled water to remove adhering substances. The leaf, stem and root were then dried in an oven at 40 °C for 96 h, and each tissue was ground separately into a fine powder with a mortar and pestle. The powder was sieved through nylon mesh (96 µm) and ultrasonically extracted in distilled water (100 ml per 20 g plant dry weight) for 1 h and then the mixture was kept in dark at room temperature for 1 d. Each sample was extracted thrice. Total aqueous extracts were filtered (Whatman GF/F) to remove plant debris, and then the filtrates were vacuum-dried and re-suspended in distilled water. The same process was conducted using an organic solvent [100 ml HPLC grade petroleum ether per 20 g plant dry weight] to extract lipophilic compounds from powdered *S. portulacastrum* root and the extracts were resuspended in 100% petroleum ether. The prepared crude extracts were stored at -20 °C.

Growth of *S. costatum* in culture medium supplemented with crude extracts from *S. portulacastrum*

We conducted LAC (liquid culture assays) (14) to investigate the toxic activity of *S. portulacastrum* crude extracts against *S. costatum*. Water extracts (0.5, 1, 3, and 6 g DW L^{-1}), petroleum ether extracts (0.8, 4, and 8 g DW L^{-1}) and solvent controls (distilled water and petroleum ether) were added to f/2 medium. The pH and salinity of medium were adjusted to 8.0 ± 0.02 and 30 psu, respectively. *S. costatum* cells in the exponential growth phase were then inoculated into the aquaria to obtain an initial cell density of 2×10^3 cells ml^{-1}. Each assay consisted of six replicates. The culture conditions were as under: temperature, 20 ± 3°C; photoperiod, 12 h light/12 h dark; illumination, 72 µmol photons m^{-2} s^{-1}. All flasks were shaken manually twice daily. The cell density and specific growth rate of the alga were determined and the IC_{50} of crude extracts was calculated. After 5 d culture, the morphological features of *S. costatum* cells were observed under a fluorescent microscope (Leica DM4500 B) at 1000 × magnification. Cell length, width and size were measured using Image Measurement Software (Image-Pro Express 6.0) and cell biovolume was calculated using the following formula (30):

$$V = \frac{\pi \times (3 \times L - W) \times (W / 2)^2}{3}$$

Where, *V*: Biovolume (μm^3) of the algal cell; *L*: Length (µm) and *W*: Width (µm).

GC-MS analysis of lipophilic compounds: A 1 µl sample of the petroleum ether extract from *S. portulacastrum* root was injected into the GC-MS for analysis of lipophilic chemical constituents. Compounds were quantified by calculating the area under each respective peak in the chromatogram. Analyses were conducted using a Varian CP-3800/320-MS equipped with a DB-5MS capillary column (60 m × 0.25 mm × 0.25 µm). The GC injection port was set to 260 °C, and the transfer line to 280 °C. The column temperature was held at 60 °C for 1 min, then increased to 280 °C at 5 °C min^{-1}, and then kept at 280 °C for 20 min. Helium was used as the carrier gas at a flow rate of 1.5 ml min^{-1}, and samples were injected in the splitless mode. Electron impact mass spectra were measured at 70 eV with a source temperature of 230 °C. A full-scan mass spectrum was

obtained, and tentative identities were assigned to peaks after Kovats Retention Index analysis and comparisons to the National Institute of Standards and Technology mass spectral library.

Statistical analysis: Data were analysed with OriginPro 7.5 and SPSS 13.0. Mean values and standard errors were calculated for each treatment, and differences between treatments and controls were analysed by one-way ANOVA with significance at the P = 0.05 or 0.01 level. The dose-response relationships and multiple comparisons were evaluated using Tukey's tests.

RESULTS AND DISCUSSION

Growth phases

To ensure that inhibitory effects were neither overestimated nor underestimated, we co-cultured *S. costatum* in different growth phases and at different cell densities with various biomass densities of *S. portulacastrum* (27). The time-course growth curves showed that *S. portulacastrum* inhibited the *S. costatum* both in the lag phase and the exponential phase. The growth inhibition was stronger in the lag phase, when the growth of *S. costatum* was completely suppressed as indicated by Chl *a* concentration and algal cell density (Fig. 1a and b). The inhibition ratios of Chl *a* and cell density were 98.6% and 98.5%, respectively, in the lag phase (Fig. 1a and b), but only 22.2% and 15.4%, respectively, in the exponential phase (Fig. 1c). It has been hypothesized that when the effects of temperature, light, and pH of the culture medium are same in the treatment and the control, the differences between them may be attributed to nutrients competition and allelopathy of *S. portulacastrum* (12,19). However, the mechanisms underlying these differences in this study require further research. Our results agreed with the density-dependent phytotoxicity effects reported by Weidenhamer (34), who suggested that plants growing at low densities are exposed to greater amounts of toxin and consequently show greater growth reductions than plants growing at high densities. Therefore, the greater inhibition effect observed in lower cell densities of *S. costatum* was the expected allelopathic effect of *S. portulacastrum*. This suggests that these inhibitory effects are due to allelopathy, rather than competition for resources, (nutrients).

The ability of *S. portulacastrum* to inhibit the growth of *S. costatum* suggests that it has potential as a new type of phytoremediation material to prevent or reduce algal blooms. However, further research is required to clarify whether the growth inhibition is solely due to allelopathy of *S. portulacastrum*, or whether there are combined effects of allelopathy and nutrients competition.

Biomass

To avoid nutrients deficiency, extra nutrients were supplied every other day to the culture medium. The inhibitory effect or the intensity of possible allelopathy of *S. portulacastrum* was investigated by supplying different biomass densities of *S. portulacastrum*. The Chl *a* concentration decreased significantly in cultures containing *S. portulacastrum* biomass densities of 0.2 and 0.5 g DW L^{-1} (Fig. 2a). Compared with

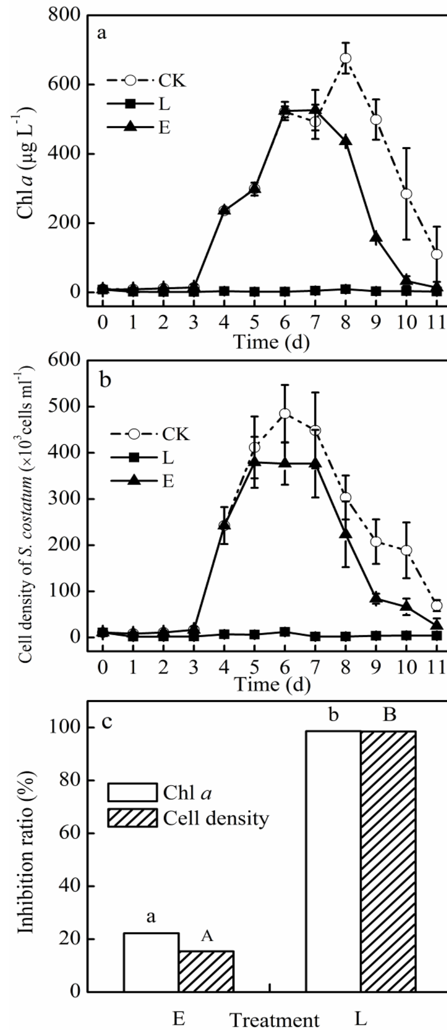

Figure 1. Growth inhibition of *S. costatum* in different phases by *S. portulacastrum* at a biomass density of 0.5 g DW L^{-1}. Treatments "L" and "E" represent *S. costatum* in lag and exponential growth phase, respectively. Algal monoculture served as control (CK). Chl *a* (a) concentration, cell density (b) and inhibition ratio (c) were determined for treatments and controls. Data are mean values (*n*=6) ± SE. Significant differences between treatments and control were tested using one-way ANOVA followed by Tukey's tests (P≤0.05).

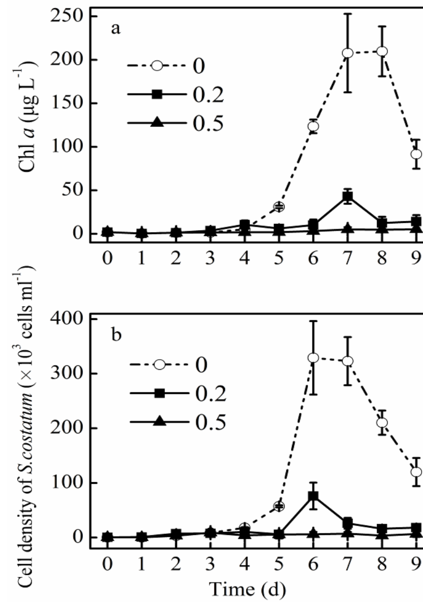

Figure 2. Biomass-dependent inhibitory effects of *S. portulacastrum* on growth of *S. costatum*. Chl *a* (a) concentration and cell density (b) are shown. Biomass densities of *S. portulacastrum* were 0.2 and 0.5 g DW L^{-1}, and algal monoculture of *S. costatum* served as the control. Data are mean values (*n*=6) ± SE.

the control, the maximum cell density and specific growth rate of *S. costatum* in the co-culture system decreased, indicating that *S. portulacastrum* may affect the cell division ability of the alga. The inhibitory effect on growth of *S. costatum* increased with higher biomass density of *S. portulacastrum*. As suggested by Dayan and Duke (3), dose-responsive experiments can provide several key parameters, for example, IC_{50}, which are related to the potency of the tested compounds. In our study, the allelopathic potency of *S. portulacastrum* was determined by calculating IC_{50} values. Data from the control and treatments were subjected separately to four regression analyses for IC_{50}, including the linear, sigmoid, exponential, and polynomial models. On the basis of statistical criteria, the exponential regression model was chosen and the IC_{50} of *S. portulacastrum* was determined to be 0.1 g DW L^{-1} (Table 1). These results support the hypothesis that the observed inhibition of algal growth was due to the allelopathic effects of toxin released from *S. portulacastrum* root, rather than nutrients competition.

Table 1. The IC_{50} [a] (g DW L^{-1}) of *S. portulacastrum* and the plant extracts against *S. costatum*

	S. portulacastrum	Water extracts			Petroleum ether extract
		leaf	stem	root	root
IC_{50}	0.10 (0.08-0.13)	0.09 (0.08-0.10)	1.57 (1.41-1.75)	6.97 (6.87-7.11)	5.62 (5.95-6.11)

[a] IC_{50} (50% inhibitory concentration, 95% confidence interval) was determined against the maximum algal cell density using the polynomial and linear regression respectively for the water- and petroleum ether-extracts from plant root and the exponential regression model for the leaf, stem extracts and *S. portulacastrum*.

Algal bioassays with *S. portulacastrum* extracts are important approaches to identify the active compounds (10). In this study, therefore, we compared the toxic effects of both water- and petroleum ether-extracts from *S. portulacastrum* organs. As indicated by maximum cell density (Fig. 3 and 5a), all tested extracts inhibited the algal growth, indicating that potential allelochemicals were distributed throughout the plant. For both types of extracts, the toxic effects were dose-dependent. In the water extracts, the maximum inhibitory activity was found in the leaf (IC_{50} = 0.09 g DW L^{-1}), followed by the stem (IC_{50} = 1.57 g DW L^{-1}) and then the root (IC_{50} = 6.97 g DW L^{-1}). This is similar to the results reported by Dorning and Cipollini (5), who suggested that this may be because the leaf contains higher concentrations of water-soluble active compounds that are then transported to stem and root tissues, or because active chemical compositions differ among the various organs. Moreover, the specific growth rates of *S. costatum* were changed in all treatments of water extracts, suggesting an allelopathic effect on algal cell division. As shown in Figure 4, inhibition of the algal specific growth rate over control increased along with the higher concentrations of leaf and stem extracts. However, the specific growth rate increased slightly under the low concentration (1 g DW L^{-1}) of root extract, with stimulation at 40.40 %, and then decreased significantly under the high concentration (6 g DW L^{-1}).

As discussed by Gross (10), lipophilic complexes released by plant roots, which tend to remain near the donor plant, have been implicated as a strong potential source of

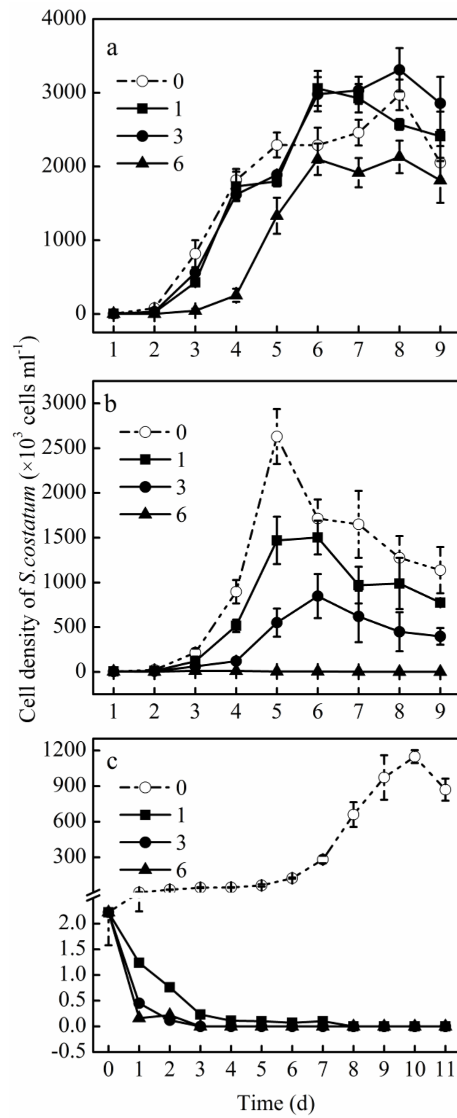

Figure 3. Inhibitory effects of water extracts (0, 1, 3 and 6 g DW L^{-1}) from *S. portulacastrum* organs: (a) root, (b) stem and (c) leaf on *S. costatum*. Data are mean values (*n*=6) ± SE.

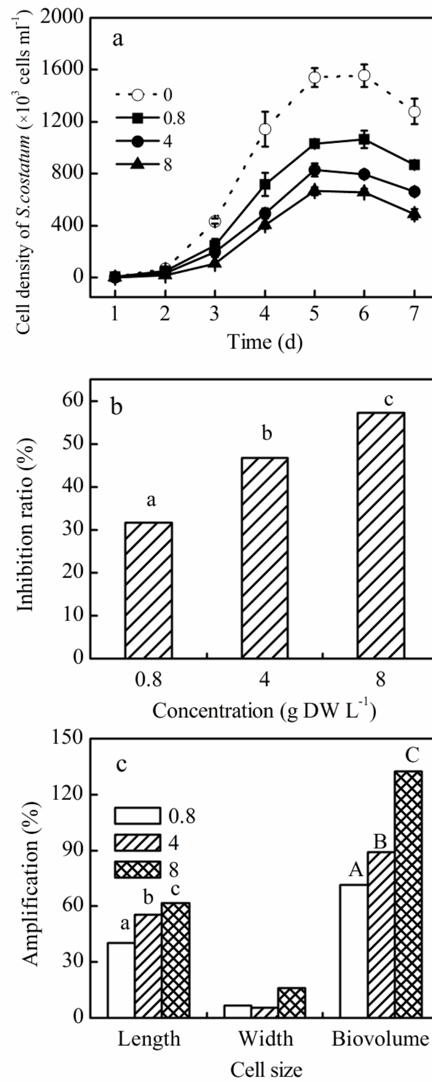

Figure 5. Inhibitory activity of petroleum ether extract from *S. portulacastrum* root against *S. costatum.* (a) Cell density, (b) inhibition ratio (%) and (c) amplification (%) of cell size were determined by comparisons with control. Extract concentrations were 0, 0.8, 4, and 6 g DW L^{-1}. Data are mean values (*n*=6 or 30) ± SE.

allelochemicals in plant defence. Therefore, we extracted the lipophilic compounds from *S. portulacastrum* root, which was directly surrounded by the alga, with petroleum ether. The results showed that growth was more strongly inhibited by the petroleum ether extract, implying that the lipophilic compounds have stronger allelopathic effects than hydrophilic ones. This result is consistent with those reported elsewhere (8,32). Gross and colleagues also reported stronger allelopathic effects of lipophilic extracts. Different allelochemicals act against different targets; hydrophilic compounds are easily released into and transported by water to reach the target organism, whereas lipophilic compounds may act primarily on the cell surface via direct cell-to-cell contact (11).

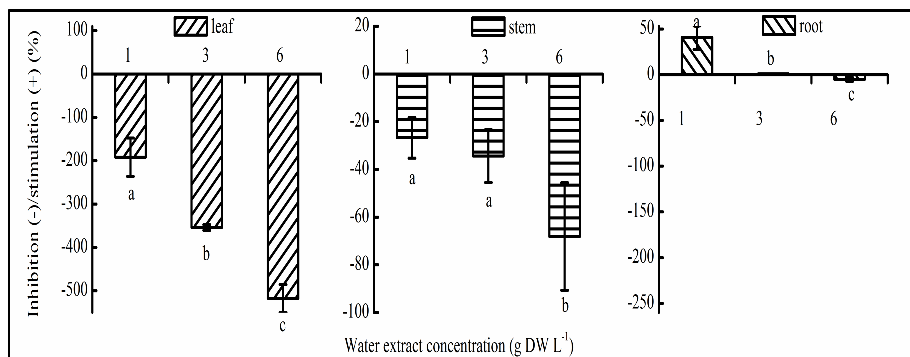

Figure 4. Concentration effects of water extracts from *S. portulacastrum* leaf, stem and root on the specific growth rate of *S. costatum*. Inhibition (-)/stimulation (+) of algal specific growth rate was calculated over the control. Water extract concentrations were 1, 3 and 6 g DW L^{-1}.

Morphological abnormalities

Allelochemicals appear to alter various physiological processes, including cell division, ion and water uptake, phytohormone metabolism, signal transduction and gene expression (1,2,17). The algal cells in the control showed normal morphology, but those treated with any of the *S. portulacastrum* extracts showed plasmolysis and extensive vacuolization (Fig. 6a). After 5 d of culture, algal cells treated with water- and petroleum ether -extracts of *S. portulacastrum* were larger than control cells, e.g., the length and biovolume of cells treated with the water extract increased more than 2-folds than control (Fig. 5c; Fig. 6b, c). There is a negative correlation between the specific growth rate and duration of cell cycle; therefore, a lower specific growth rate indicates a greater cell doubling time (26). Thus, alterations in the specific growth rate will alter the duration of algal cell cycle and the size of algal population. Moreover, the cell size of *S. costatum* continually decreases due to its unusual method of asexual reproduction (25). However, the expanded cells of *S. costatum* treated with plant extracts did not appear to divide into two new daughter cells as normal. Together, these observations suggest that allelochemical(s) from *S. portulacastrum* organs induce the morphological abnormalities in *S. costatum* cells and affected their cell division. Further investigations are required to determine the mechanisms by which *S. portulacastrum* extracts exert these effects.

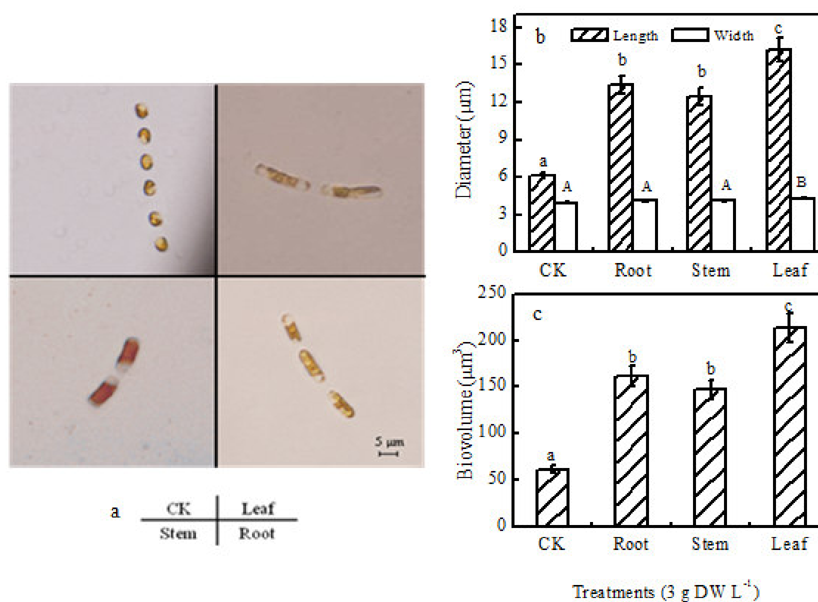

Figure 6. Morphological changes and size of *S. costatum* cells treated with water extracts from *S. portulacastrum* (concentration of organ extract, 3 g DW L^{-1}). *S. costatum* grown in a monoculture served as control (CK). After 5 d of culture, phenotypic changes were observed under a microscope. Micrographs showing phenotypic changes (a), cell size (b), and algal biovolume (c). Data are mean values (n=30) ± SE. Significant differences between treatments and control were determined by one-way ANOVA followed by Tukey's tests ($P \leq 0.05$).

Identification of lipophilic compounds

Bioassay-directed chemical analysis is an important approach to identify the substances that contribute to the observed experimental effects. Such analyses have become much simpler with the advent of modern analytical equipment, such as GC/MS, LC/MS, and LC/MS/NMR (15). Since the petroleum ether extract from *S. portulacastrum* root showed strong inhibitory effects in bioassay, we identified components in this extract by GC-MS analysis. As shown in Table 2, we identified 45 lipophilic compounds, most of which were first reported in *S. portulacastrum*. The major components were fatty acids and their derivatives, terpenoids, phenolic and heterocyclic compounds, the most abundant were: 9-octadecenamide (21.174%), (z)-26-nor-5-cholesten-3-beta-ol-25-one (17.949%) and n-hexadecanoic acid (10.737%). Miscellaneous compounds found at low levels included uridine, 3'-O-methyl-,1,1-dichloropropene and 1-piperazineethanol. The chemicals responsible for allelopathic interactions in aquatic ecosystems can be broadly divided on the basis of chemical structure into seven categories; fatty acids, phenolic compounds, terpenoids, polyethers, oligopeptides, polysaccharides, and miscellaneous compounds (23). Some of the compounds or structural analogues found

Table 2. Identification of chemical constituents in the petroleum ether extract* from *S. portulacastrum* roots

Retention time (min)	Name	MW	CAS NO.	Formula	Conc. (%)
42.469	9-Octadecenamide,(z)-	281	301-02-0	$C_{18}H_{35}NO$	21.174
60.684	26-nor-5-Cholesten-3.beta.-ol-25-one	386	7494-34-0	$C_{26}H_{42}O_2$	17.949
35.205	n-Hexadecanoic acid	256	57-10-3	$C_{16}H_{32}O_2$	10.737
45.132	Di-n-octyl phthalate	390	117-84-0	$C_{24}H_{38}O_4$	6.86
39.332	Chroman-4-one o-(3-diethylamino-propyl)-oxime	276	None	$C_{16}H_{24}NO_2$	6.812
42.856	1-Butanamine,N,N-dipropyl-	157	444-71-7	$C_{10}H_{23}N$	5.809
61.118	Cholestan-3-ol	388	27409-41-2	$C_{27}H_{48}O$	5.106
63.091	Cholest-7-en-3-0l,(3.beta.)-	386	6036-58-4	$C_{27}H_{46}O$	3.411
54.817	_beta.-sitosterol acetate	456	915-05-9	$C_{31}H_{52}O_2$	2.87
38.964	Octadecanoic acid	284	57-11-4	$C_{18}H_{36}O_2$	2.345
42.336	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	222	None	$C_{15}H_{26}O$	1.791
55.091	17-(2-Hydroxy-1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradcahydro-1H-cyclopenta(a)phenanthrene	442	None	$C_{30}H_{50}O_2$	1.677
18.782	Nonanoic acid	158	112-05-0	$C_9H_{18}HO_2$	1.35
38.605	1,1-Dichloropropene	110	563-58-6	$C_{14}H_4Cl_2$	1.278
59.909	1-Triacontanol	438	593-50-0	$C_{30}H_{62}O$	1.219
57.131	Stigmasta-5,22-dien-3-ol,acetate,(3.beta.)-	454	4651-48-3	$C_{31}H_{50}O_2$	1.049
55.903	N-.alpha.-Benzoyl-l-arginine	278	154-92-7	$C_{13}H_{18}N_4O_3$	0.842
43.281	Phenol,2,2'-methylenebis(6-1,1-dimethylethyl)-4-methyl-	340	119-47-1	$C_{23}H_{32}O_2$	0.803
44.811	Phenol,2,4-bis(1-methyl-1-phenylethyl)-	330	2772-45-4	$C_{24}H_{26}O$	0.7
50.375	Acetic acid,octadecyl ester	312	822-23-1	$C_{20}H_{40}O_2$	0.671
30.964	Tetradecanoic acid	228	544-63-8	$C_{14}H_{28}O_2$	0.495
56.233	Pregnane-3,11,20,21-tetrol,cyclic20,21-butyl.boronate), (3.alpha.,5.beta.,11.beta.,20R)	418	55556-74-6	$C_{25}H_{43}BO_4$	0.484
40.041	1-Piperazineethanol	130	103-76-4	$C_6H_{14}N_2O$	0.405
62.638	Cholestan-3-one	386	15600-08-5	$C_{27}H_{46}O$	0.399
44.197	Hexadecanoic acid,(2,2-dimethyl-1,3-dioxolan-4-yl)methyl ester	370	18418-21-8	$C_{22}H_{42}O_4$	0.381
32.797	2-Pentadecanone,6-10,14-trimethyl-	268	502-69-2	$C_{18}H_{36}O$	0.359
34.213	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	82304-66-3	$C_{17}H_{24}O_3$	0.356
39.058	7-Methyl-Z-teradecen-1-ol acetate	268	None	$C_{17}H_{32}O_2$	0.297
33.071	Pentadecanoic acid	242	1002-84-2	$C_{15}H_{30}O_2$	0.271
41.751	2-Ethylhexyl trans-4-methoxycinnamate	290	83834-59-7	$C_{18}H_{26}O_3$	0.264
24.305	2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethylethyl)-	220	719-22-2	$C_{14}H_{20}O_2$	0.237
32.325	7-Methyl-octadecan-7-ol	284	None	$C_{19}H_{40}O$	0.211
41.599	2,6-Pyridinedicarboxylic acid	167	499-83-2	$C_7H_5NO_4$	0.21
38.237	Dotriacontane	450	544-85-4	$C_{32}H_{66}$	0.197
37.529	Oleanitrile	263	none	$C_{18}H_{33}N$	0.194
21.35	Cyclopropanetetradecanoic acid,2-octyl-,methyl ester	394	52355-42-7	$C_{26}H_{50}O_2$	0.15
33.996	1,3,2-Dioxaborolane,4,4'-(1,4-butanediyl)bis(2-ethyl-	254	74793-62-7	$C_{12}H_{24}B_2O_4$	0.11
41.911	Decane,5-ethyl-5-methyl-	184	17312-74-2	$C_{13}H_{28}$	0.104

26.392	Dodecanoic acid	200	143-07-7	$C_{12}H_{24}O_2$	0.084
23.786	5,9-Undecadien-2-one,6,10-dimethyl,(E)-	194	3796-70-1	$C_{13}H_{22}O$	0.082
32.391	Isopropyl Myristate	270	110-27-0	$C_{17}H_{34}O_2$	0.068
31.201	3,5-Di(tert-butyl)-4-hydroxybenzaldehyde	234	1620-98-0	$C_{15}H_{22}O_2$	0.067
24.079	Uridine,3'-O-methyl-	258	60038-59-1	$C_{10}H_{14}N_2O_6$	0.06
30.36	Phenol,2-(1-phenylethyl)-	198	4237-44-9	$C_{14}H_{14}O$	0.043
22.332	Pregnan-17,21-diol-20-one,3,9-epoxy-3-O-methyl-11-thiocyano-	477	102263-11-6	$C_{25}H_{35}NO_6S$	0.038

in our study have shown negative allelopathic effects on phytoplankton growth in other studies (22,23,35), suggesting that these components may also have negative allelopathic effects on the growth of *S. costatum.* However, our bioassay-directed screening was only a preliminary step to determine the specific allelopathic compounds. Further research is required to fully characterize the roles of the putative allelochemicals.

CONCLUSIONS

Our results suggested that *S. portulacastrum* has negative allelopathic effects on the marine diatom *S. costatum,* and that this halophyte contains certain allelochemicals that inhibited the algal growth. Therefore, it is possible that the halophyte *S. portulacastrum* could play a role in preventing or reducing blooms of this marine diatom. Further research is required to fully characterize the bioactive allelochemicals and to clarify their modes of action and their roles in allelopathy.

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