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# Suppression of cyst germination success in *Gymnodinium catenatum* and *Ostreopsis* cf. *ovata* by macroalgal extracts from the southern Mediterranean Sea

by

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## Abstract

This study examines the potential inhibitory effects of extracts of seven Mediterranean macroalgae on the germination success of two harmful dinoflagellate cysts, *Gymnodinium catenatum* and *Ostreopsis* cf. *ovata*. The results revealed that aqueous and methanolic extracts of these macroalgae showed varying inhibitory effects on the viability of the cyst germlings, preventing them from dividing and producing motile vegetative cells. Among macroalgae, three *Cystoseira* species (*C. compressa*, *C. barbata*, and *C. crinita*) exhibited the strongest inhibitory effects on the germination success of the two cyst types. The methanolic extracts of these species showed higher inhibitory effects on *O.* cf. *ovata* cysts (% inhibition = 88%–100%) than *G. catenatum* cysts (83%–95%). Based on the median inhibitory concentration ($IC_{50}$), the methanolic extracts of these macroalgae have exhibited stronger inhibitory effects on germling viability ($IC_{50}$ = 0.05–1.5 mg extract $g^{-1}$ sediment) than aqueous extracts ($IC_{50}$ = 0.9–590 mg extract $g^{-1}$ sediment). The study suggests that macroalgal materials, specifically *Cystoseira* species, would be a promising approach to retard the germination success of dinoflagellate cysts in constricted coastal areas, hence limiting the recurrence of harmful algal blooms in the water column.

**Key words:** dinoflagellates cysts, macroalgae, harmful blooms, mitigation

# 1. Introduction

Dinoflagellates are one of the most important microorganisms in the aquatic ecosystems, playing a prominent role as primary producers in the trophic chain, especially in the marine environment (Bravo and Figueroa, 2014; Rukminasari and Tahir, 2021). Nonetheless, many dinoflagellate species form harmful algal blooms (HABs) and produce toxins that endanger aquaculture, fisheries, and public health (Mohamed, 2018; Mohamed & Al-Shehri, 2012). It has been established that over 200 of the more than 2000 species of dinoflagellates can form cysts as a part of their life cycle (Likumahua et al., 2021; Li et al., 2020). These cysts sink into the sea bottom, sustain unfavorable conditions and remain viable in sediments for up to 100 years (Cuellar-Martinez et al., 2023; Figueroa et al., 2007; Miyazono et al., 2012; Vahtera et al., 2014). When environmental conditions are restored, the cysts germinate to form vegetative cells that re-enter the water column, thereby triggering the formation of HABs (Butman et al., 2014).

Cyst germination is regulated by internal factors, including the obligatory dormancy (i.e., maturation period for cysts to germinate), which occurs immediately after cyst formation and can last from 12 hr to 12 months, depending on the species (Su et al., 2016). On the contrary, cyst germination can also be governed by external factors such as temperature, light, oxygen, and salinity (Cuellar-Martinez et al., 2023; Kremp & Anderson, 2000; Zheng et al., 2024). When these external factors are satisfied, temperature is the key determinant of cyst germination (Fischer & Brosnahan, 2022; Vahtera et al., 2014). Hence, dinoflagellate cyst germination is predicted to be enhanced by warming and climate change (Brosnahan et al., 2020).

Therefore, the inhibition of cyst germination in the natural environment would restrict the development of extensive coastal blooms. Most studies conducted on the mitigation of HABs by ecofriendly methods such as macroalgae or their extracts investigated the effects of macroalgal materials only on vegetative cells (Accoroni et al., 2015; Ben Gharbia et al., 2017; Benitt et al., 2022; Wang et al., 2007). Little is known about the inhibitory effects of macroalgae on the cyst germination of dinoflagellate species. The only one study that has addressed the inhibition of dinoflagellate cyst germination by the brown alga (*Turbinaria ornate*) is Mohamed et al. (2022)).

Our previous study recorded intense bloom of *G. catenatum* and *O.* cf. *ovata* in Tajoura coastal waters in September 2023, with a high prevalence of these species' cysts in the surface sediments (Mohamed et al., 2025). Remarkably, an assemblage of seven macroalgae (*Jania rubens*, *Laurencia obtusa*, *Cystoseira compressa, Cystoseira crinita, Cystoseira barbata, Ulva lactuca*, and *Ulva linza*) was found on the Libyan Mediterranean coast, approximately 1 km away from the bloom area (Elfituri et al., 2022); nevertheless, these macroalgae were not found in this bloom area. Therefore, we hypothesized that these macroalgae might have had a role in the impairment of cyst germination and development of this bloom in the non-impacted area, but their absence permitted the bloom formation in the impacted area.

Therefore, this study was conducted to examine the effect of these macroalgae on the germination success of *G. catenatum* and *O.* cf. *ovata* cysts found in surface sediments of the Libyan Mediterranean Sea. This study also examined the use of these macroalgae as an ecofriendly bioagent of controlling HABs formation.

# 2. Materials and methods

## 2.1. Macroalgae and dinoflagellate cysts

Seven macroalgae including *U. lactuca*, *U. linza* (Ulvales, Chlorophyta), *J. rubens* (Corallinales, Rhodophyta), *Laurencia obetosa* (Ceramiales, Rhodophyta), *C. compressa*, *C. crinita*, and *C. barbata* (Fucaales, Phaeophyta) were collected from the Tajoura Mediterranean coast, about 20 km east of Tripoli, Libya (13°34′85″E, 32°89′43″N). The macroalgae were identified with the aid of taxonomic papers (e.g., Ateweberhan & Prud'homme, 2005; Lobban, 2006). First, macroalgal thalli were washed thoroughly with sterile distilled water to remove sand and epiphytes. Next, they were treated with a mixture of 30% ethanol and 1% sodium hypochlorite for 10 min to kill and eliminate attached bacteria and microalgae without damaging the algal cells (Kientz et al., 2011). Then, cleaned macroalgal thalli were dried in an oven at 37°C for 48 hr. Finally, dried thalli were cut into small pieces and ground into a fine powder and stored in darkness until use.

The cysts of *G. catenatum* and *O.* cf. *ovata* used in this study were obtained from surface sediments, which were previously collected from the Tajoura Mediterranean coast, Libya (13°22′00″E, 32°54′00″N), after the collapse of *G. catenatum* and *O.* cf. *ovata* blooms (Mohamed et al., 2024, 2025). Surface sediments were collected by a Van Veen grab (15 cm × 30 cm) and stored in plastic pouches in the dark, at 4°C until processing.

### 2.2. Preparation of dinoflagellate cysts and macroalgal extracts

Dinoflagellate cysts used in the germination experiments were isolated from surface sediments according to the method of Matsuoka and Fukuyo (2000). Briefly, surface sediment samples were resuspended in filtered seawater (0.45 μm-Whatman cellulose filters), sonicated for 5 min, and then passed through 100 μm and 20 μm sieves. The fine particles that passed through the 20 μm sieve, were transferred to a Petri dish and allowed to stand for 20 min to suspend lightweight cysts in the supernatant and settle heavier substances at the dish bottom (Zheng et al., 2024). The supernatant was carefully pipetted into a 10 mL vial as a stock of dinoflagellate cysts. Cyst species were identified according to their morphological characteristics described by Accorni et al. (2015) and Matsuoka et al. (2006). Single cysts of each species were isolated by the capillary pipette method under inverted light microscope according to Hoshaw (1973) and Accoroni et al. (2015), and they were kept separately in 10 mL sealed vials in the dark at 4°C to prevent germination before beginning the experiments. The number of cysts was estimated using a hemocytometer under Zeiss light microscope.

According to Kumar et al. (2013), aqueous extract was prepared by dissolving 5 g of powdered algal thalli in 250 mL of sterile distilled water, heated at 60°C for 30 min, and filtered through Whatman GF/C filter paper. The aqueous extract is adjusted to a final concentration of 20 mg · $mL^{-1}$ with sterile distilled water and then stored at –20°C until use. By following the method of Esquer-Miranda et al. (2016), methanol extracts were prepared by soaking 5 g of milled macroalgal thalli in 250 mL of methanol (95%), sonicated for 15 min, and left at room temperature for 16 hr while stirring. The extracts were centrifuged (5000×*g*, 15 min, 20°C) and filtered through Whatman GF/C filter paper (Whatman no. 4). The filtrate was then dried under vacuum to evaporate the organic solvent. Dried extracts were resuspended in water to a final concentration of 20 mg · $mL^{-1}$ and stored at –20°C until use.

### 2.3. GC-MS analysis of macroalgal extracts

Gas chromatography-mass spectrometry (GC-MS) analysis was used to identify the composition of the macroalgal extracts. GC-MS analysis was performed using GC-MS device (7890A-5975B; Thermo Scientific GC/MS; model ISQ; USA), at Assuit University, with a nonpolar HP-5MS Capillary Standard column (30 mm × 0.25 mm × 0.25 mm). The following was the cycle's parameters: oven program on at 120°C for 5 min, 30°C · $min^{-1}$ rising to 265°C for 25 min, then 50°C · $min^{-1}$ increased to 280°C for 5 min; run duration 48 min; post run 260°C for 2 min; flow program 0.5 mL · $min^{-1}$ for 10.9 min, and then 1 mL · $min^{-1}$ for 30 min. Equilibration time was 0.5 min and the maximum temperature was 280°C. Peak identification of crude algal extracts was performed based on comparing the obtained mass spectra with those available in the NIST mass spectral library.

### 2.4. Inhibitory effects of macroalgal extracts on dinoflagellates cyst germination

Individual cysts (100 cysts) were inoculated separately into a 50 mL glass beaker containing 5 g of sterilized surface sediments not containing cysts and 20 mL of culture f/2 medium (Guillard, 1978) prepared in filtered (0.2 μm) autoclaved natural Mediterranean seawater. Aqueous or methanol macroalgal extracts were added separately at concentrations of 0, 0. 5, 1, 5 and 10 mg · $g^{-1}$ to glass beakers. The beakers were then incubated at 25°C and 12:12 hr light:dark cycle provided by cool white illumination tubes at 60 μmol · $m^{-2}$ · $s^{-1}$ (Mohamed & Al-Shehri, 2011). Beakers containing cysts, surface sediments, and sterile filtered seawater but without macroalgal extract were used as the control. Both control and treated cyst cultures were made in triplicate. Cyst germination was monitored at 2-day intervals for 30 days. This was carried out by counting ungerminated cysts using a hemocytometer under Zeiss light microscope according to Genovesi et al. (2009). Evaluation of germination will be considered successful if an empty cyst produces a live motile cell (i.e., viable germling) at least once during the experiment. In case, if empty cyst is presented without live cell, it is assumed that the germling cell was died after germination and cyst germination was unsuccessful (Jerney et al., 2019; Lopez et al., 2019; Mohamed et al., 2022). Based on these criteria, the percentage of successful cyst germination was calculated by dividing the number of vials containing living cells by the total number of empty cysts in all vials of each treatment group and multiplying the product by 100.

### 2.5. Statistical analysis

All data were expressed as a mean of three replicates ± standard deviation (SD). Differences in cyst germination between control and treatment groups were first evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to identify which specific group differs significantly from

each other using the software SPSS (version 16.0, *IBM Company*). Differences were significant at $p < 0.05$. Computations of median inhibitory concentration ($IC_{50}$) of a dataset obtained from this study were performed using Finney's method of "probit analysis" and with SPSS computer statistical software. The $IC_{50}$ value is derived by fitting a regression equation arithmetically.

## 3. Results and discussion

The results of experiments investigating the inhibitory effects of Libyan macroalgae on the germination success (i.e., germling viability) of cysts of two harmful dinoflagellates species (*G. catenatum* and *O.cf. ovata*) forming blooms in the Libyan Mediterranean coast are presented in Figs. 1 and 2.

The cysts of *G. catenatum* and *O. cf. ovata* in the control and treatment groups (i.e., with macroalgal extracts) incubated in sterilized seawater at 25°C, passed the dormancy period and underwent excystment as early as the 2nd day (63%) and as late as the 20th day (98%). However, the percentage of successful cyst germination (i.e., empty cysts forming live motile cells) varied significantly between the control and treatment groups ($p < 0.0001$). The highest germination success in the control group (96%) reached on day 20 and remain constant until the end of the germination course (30 days). On the contrary, the highest germination success of cysts incubated with macroalgal extracts was recorded after 20 days and continued for up to 30 days, depending on the macroalgae species and the type of extract (i.e., aquatic/ethanolic extract). Therefore, the cyst germination experiment was terminated on day 30. The germination of *G. catenatum* and *O. cf. ovata* cysts in a short period in natural seawater at optimal temperatures of 23–25°C has also been reported in previous studies, which showed 1 day for *Gymnodinium* cysts (Liu et al., 2020) and 2–5 days for *Ostreopsis* cysts (Accoroni et al., 2014; Bravo et al., 2012).

The addition of aqueous or ethanol extracts of seven seaweeds (*J. rubens*, *L. obtusa*, *C. compressa*, *C. crinita*, *C. barbata*, *U. lactuca*, and *U. linza*) to cultures of *G. catenatum* and *O. cf. ovata* cysts caused varying reduction in the percentage of successful germination (i.e., viable germlings) of these cysts. The percentage of successful germination of the two cyst species decreased significantly ($p < 0.001$) with the increase in the concentration of aqueous or ethanol macroalgal extract (Figs. 1 and 2). The aqueous extracts of four macroalgae (*J. rubens*, *L. obtusa*, *U. lactuca*, and *U. linza*) showed weak inhibitory effects on the germination success of both *G. catenatum* (% germination inhibition = 12%–26%) and *O.* cf. *ovata* cysts (5%–27%); while the aqueous extracts of the three species of *Cystoseira* (*C. compressa*, *C. barbata*, and *C. crinita*) showed moderate inhibition on germination success of *O.* cf. *ovata* cysts (% inhibition = 53%–69%; Fig. 1A) and *G. catenatum* cysts (37%–59%) (Fig. 1B).

On the other hand, methanol extracts of these macroalgae exerted greater inhibitory effects on the cyst germination success compared with aqueous extracts ($p < 0.05$), and these effects varied significantly with the increase of macroalgal extracts ($p < 0.05$). On the species level of *Cystoseira*, the methanol extracts at a concentration of 10 mg · $mL^{-1}$ of the three species of *Cystoseira* (*C. compressa*, *C. barbata*, and *C. crinita*) exhibited the strongest inhibitory effects on the germination success of *G. catenatum cysts* (% inhibition = 83%, 90%, and 95%, respectively; Fig. 2A) and *O. cf. ovata* cysts (88%, 95%, and 100%, respectively; Fig. 2B). Whereas the methanol extracts of the remaining macroalgal species (*J. rubens*, *L. obtusa*, *U. lactuca*, and *U. linza*) at the highest concentration (10 mg · $mL^{-1}$) showed moderate inhibitory effects on the germination success of *G. catenatum* (% inhibition = 69% and 74%, and *O. cf. ovata* cysts (74%–77%, respectively; Figs. 2A and 2B).

Based on the $IC_{50}$ values, the inhibitory effects of methanol extracts of all macroalgae tested were greater than aqueous extracts ($p < 0.05$) on the germination success of *G. catenatum* and *O. cf. ovata* cysts (Tables 1 and 2). The $IC_{50}$ values also varied significantly among macroalgae tested ($p < 0.05$), either for aqueous or methanolic extracts. For aqueous extracts, *C. crinita* had the lowest $IC_{50}$ values (i.e., highest inhibition degree) against *G. catenatum* and *O. cf. ovata* cysts ($IC_{50}$ = 3.9 and 9.9 mg · $g^{-1}$, respectively), followed by *C. compressa* ($IC_{50}$ = 4.4 and 11.7 mg · $g^{-1}$) and *C. barbata* ($IC_{50}$ = 6.6 and 14.8 mg · $g^{-1}$). By contrast, the aqueous extracts of other macroalgae exhibited lower inhibition levels ($IC_{50}$ = 58.6–590 mg · $g^{-1}$) toward the germination success of the two cyst types (Tables 1 and 2).

For the methanol macroalgal extracts, the three *Cystoseira* species also showed the strongest inhibitory effects against the germination success of the two cyst types, with $IC_{50}$ values (0.05–0.23 mg · $g^{-1}$) lower than those reported for aqueous extracts of these macroalgae (Tables 1 and 2). On the contrary, the methanolic extracts of other macroalgal species demonstrated a moderate level of inhibition against cyst germination success (Tables 1 and 2); the order of inhibition efficiency (i.e., low $IC_{50}$ value) was as follows: *J. rubens* (1.1–1.4 mg · $g^{-1}$) > *U. linza* = *U. lactuca* (1.3–1.4 mg · $g^{-1}$) > *L. obtusa* (1.6–2.4 mg · $g^{-1}$). The variation in the inhibition of cyst germination among

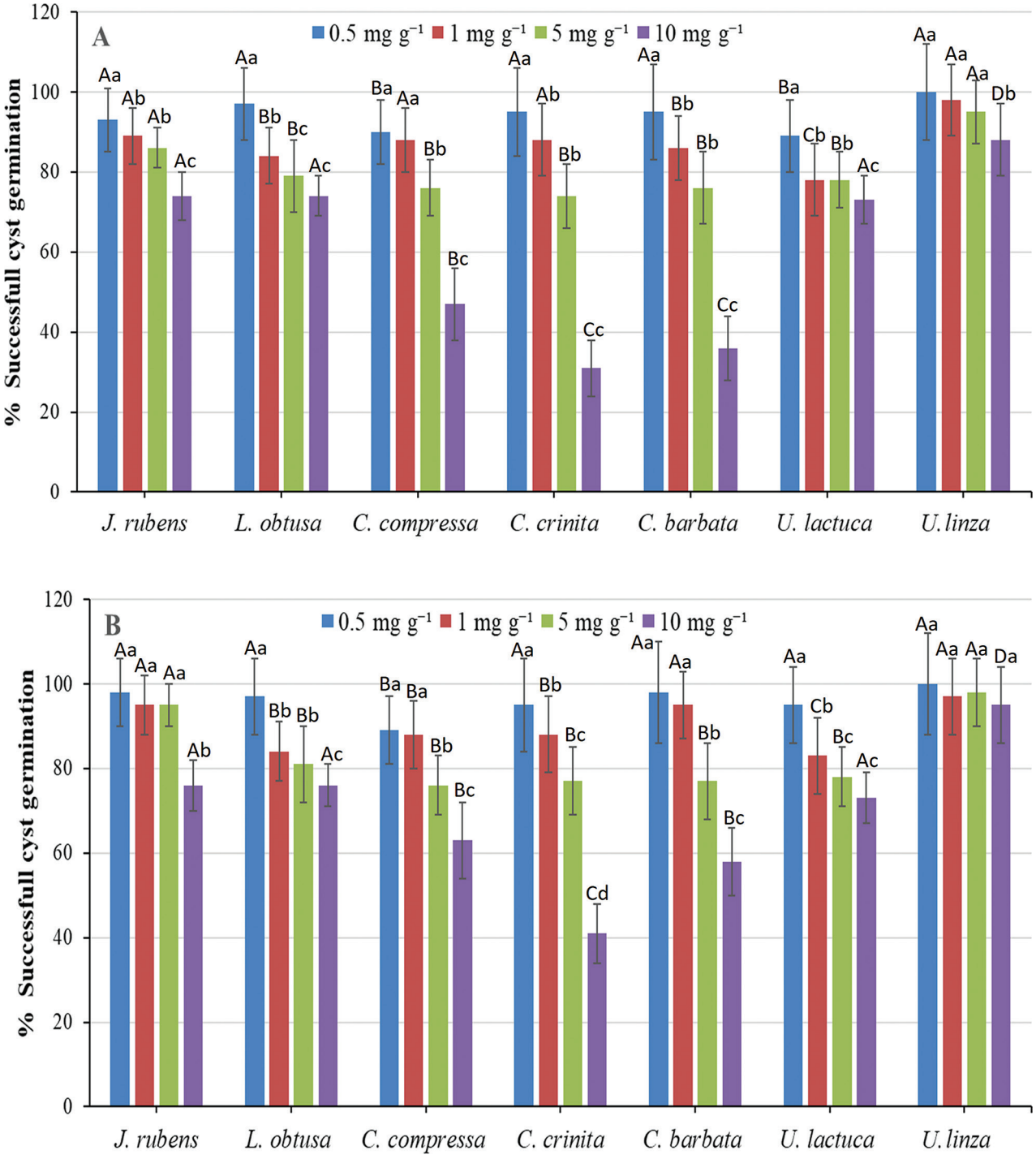


**Figure 1**

Effect of aqueous macroalgal extracts on the success of cyst germination in harmful microalgae: **(A)** *Ostreopsis* cf. *ovata* and **(B)** *Gymnodinium catenatum*. Data points are means ± SD ($n$ = 3). Different uppercase letters indicate statistically significant differences ($p < 0.05$) in the cyst germination among different macroalgae for each extract concentration, whereas different lowercase letters indicate statistically significant differences ($p < 0.05$) in the cyst germination between different extract concentrations for each macroalgal species (i.e., horizontal track). SD, standard deviation.

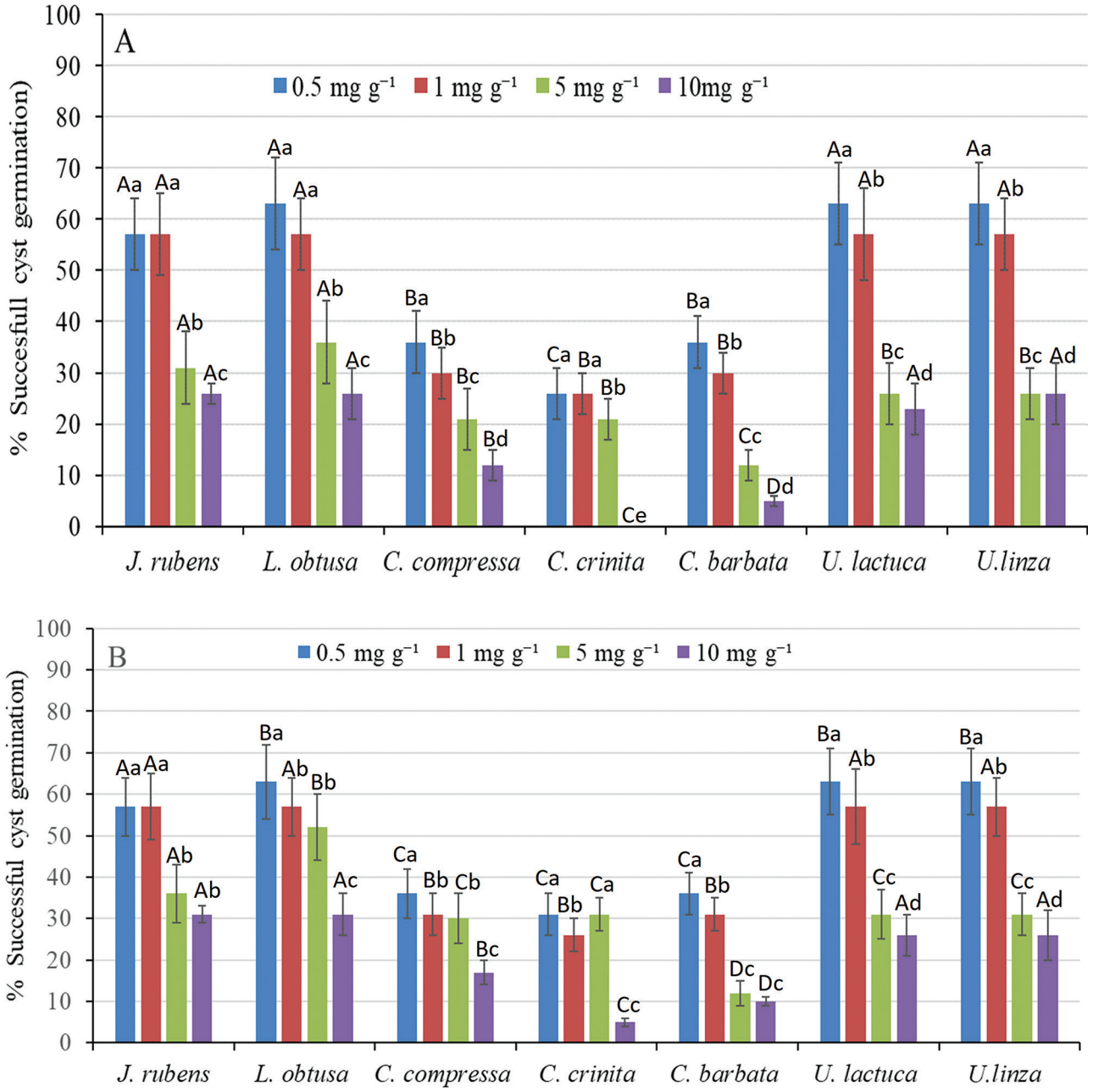


**Figure 2**

Effect of methanol macroalgal extracts on the success of cyst germination in harmful microalgae: **(A)** *Ostreopsis* cf. *ovata* and **(B)** *Gymnodinium catenatum*. Data points are means ± SD ($n$ = 3). Different uppercase letters indicate statistically significant differences ($p < 0.05$) in the cyst germination among different macroalgae for each extract concentration, whereas different lowercase letters indicate statistically significant differences ($p < 0.05$) in the cyst germination between different extract concentrations for each macroalgal species (i.e., horizontal track). SD, standard deviation.

the different macroalgae in this study may be due to the difference in the type and amount of bioactive compounds in the macroalgal extracts, as suggested by earlier research (Ben Gharbia et al., 2017).

The results of this study showed that the macroalgal extracts did not directly affect the germination of dinoflagellate cysts (i.e., excystment), but rather hindered the survival of the germling cells, preventing

**Table 1**

Results of probit analysis used for calculation of $IC_{50}$ (mg · $g^{-1}$ sediments) values of the inhibitory effects of aqueous and methanol extracts of macroalgae on the germination success of *Gymnodinium catenatum* cysts

| Macroalgae | Type of extract | $IC_{50}$ | Regression equation | $R^2$ |
|---|---|---|---|---|
| *Jania rubens* | Aqueous | 206.7 | y = 0.5434x + 3.7036 | 0.8799 |
| | Ethanol | 1.1 | y = 0.7043x + 4.9505 | 0.9537 |
| *Laurencia obtusa* | Aqueous | 58.6 | y = 0.8506x + 3.6622 | 0.7708 |
| | Ethanol | 1.55 | y = 0.7531x + 4.8602 | 0.9932 |
| *Cystoseira compressa* | Aqueous | 37.7 | y = 1.2099x + 4.0006 | 0.9365 |
| | Ethanol | 0.12 | y = 0.5774x + 5.5143 | 0.9484 |
| *Cystoseira crinita* | Aqueous | 9.9 | y = 1.8123x + 3.9096 | 0.9571 |
| | Ethanol | 0.08 | y = 1.0561x + 5.7358 | 0.6061 |
| *Cystoseira barbata* | Aqueous | 14.8 | y = 1.656x + 3.9192 | 0.9581 |
| | Ethanol | 0.23 | y = 0.9758x + 5.5847 | 0.9777 |
| *Ulva lactuca* | Aqueous | 238 | y = 0.4171x + 4.0481 | 0.7512 |
| | Ethanol | 1.28 | y = 0.9013x + 4.9035 | 0.9735 |
| *Ulva linza* | Aqueous | 141 | y = 1.424x + 2.6289 | 0.8886 |
| | Ethanol | 1.33 | y = 0.8443x + 4.8995 | 0.9511 |

**Table 2**

Results of probit analysis used for calculation of $IC_{50}$ (mg · $g^{-1}$ sediments) values of the inhibitory effects of aqueous and methanol extracts of macroalgae on the germination success of *Ostreopsis* cf. *ovata* cysts

| Macroalgae | Type of extract | $LC_{50}$ | Regression equation | $R^2$ |
|---|---|---|---|---|
| *Jania rubens* | Aqueous | 89.6 | y = 0.8037x + 3.2067 | 0.7204 |
| | Ethanol | 1.4 | y = 0.5723x + 4.9254 | 0.956 |
| *Laurencia obtusa* | Aqueous | 131.3 | y = 0.7379x + 3.6273 | 0.7205 |
| | Ethanol | 2.4 | y = 0.5341x + 4.7977 | 0.8006 |
| *Cystoseira compressa* | Aqueous | 6.6 | y = 0.6838x + 3.9011 | 0.9513 |
| | Ethanol | 0.05 | y = 0.3645x + 5.4558 | 0.7272 |
| *Cystoseira crinita* | Aqueous | 3.9 | y = 1.2566x + 3.7281 | 0.9018 |
| | Ethanol | 0.06 | y = 0.6381x + 5.597 | 0.4817 |
| *Cystoseira barbata* | Aqueous | 4.4 | y = 1.3949x + 3.3527 | 0.9966 |
| | Ethanol | 0.2 | y = 0.7682x + 5.5592 | 0.9798 |
| *Ulva lactuca* | Aqueous | 94.4 | y = 0.6739x + 3.7685 | 0.7992 |
| | Ethanol | 1.4 | y = 0.7971x + 4.8792 | 0.9878 |
| *Ulva linza* | Aqueous | 590 | y = 0.8067x + 2.5507 | 0.5819 |
| | Ethanol | 1.4 | y = 0.7971x + 4.8792 | 0.9878 |

them from producing motile vegetative cells. Thus, these extracts would hinder cysts' capacity to successfully repopulate new planktonic populations in the aquatic environment. The results corroborate the fact that the viability of germling cells after excystment represents a bottleneck in the bloom initiation (Genovesi et al., 2009; Mardones et al., 2016), and hence the inhibition of germling cell viability of dinoflagellate cysts retards the recurrence of relevant species in the water column. Previous studies have shown that dinoflagellate cysts can germinate (i.e., undergo excystment) but the germling cells would not be able to divide and would die soon under unfavorable abiotic conditions such as temperature, light, and oxygen-availability (Brosnahan et al., 2020; Genovesi et al., 2009; Vahtera et al., 2014). However, Mohamed et al. (2022) investigated the biological interaction between macroalgae and dinoflagellate cyst germination. They demonstrated the inhibitory effects of ethanol extracts of the brown macroalga, *Turbinaria ornata*, on the viability of germlings and formed excystment of some dinoflagellate cysts (e.g., *A. catenella*, *C. polykrikos*, *S. trochoidea*, *P. minimum*, and *D. acuminata* cysts) collected from the surface sediments in the Red Sea. Therefore, the present study is the second to explore the inhibition of germination success of dinoflagellate cysts of harmful species (e.g., *G. catenatum* and *O. cf. ovata*) from the southern Mediterranean Sea.

Additionally, our macroalgal methanol extracts were about 50–200 times more efficient against the germling viability of all cyst species than the aqueous extract. These results supported the fact that alcoholic solvents such as ethanol are superior to water in extracting bioactive compounds from plants (Sultana et al., 2009). This study also revealed that although there was no statistical difference in the susceptibility of germling cells to macroalgal extracts between *G. catenatum* and *O.* cf. *ovata* cysts ($p > 0.05$), *O.* cf. *ovata* germlings were more vulnerable than *G. catenatum* germling. These findings are thus consistent with previous studies demonstrating that the inhibitory effects of macroalgal extracts on microalgal growth differed depending on the target species of microalgae (Mohamed et al., 2022; Zhang et al., 2021).

Such inhibitory effects of macroalgal extracts could be attributed to the active substances found in macroalgae, which may inhibit metabolic processes that govern the division and growth of germling cells formed after cyst excystment (Gémin et al., 2020; Mohamed et al., 2022). In this study, the GC-MS analysis of the extracts of tested macroalgal species revealed the dominance of long chain fatty acid ester, unsaturated fatty acids, alcohols, saturated fatty acids, diisooctyl phthalate ester, and unsaturated aldehydes (Tables S1–S7 and Figures S1-S7 in Supplementary Material). These allelochemicals were previously

isolated from higher plants and macroalgae, and most of them, especially unsaturated fatty acids (e.g., linoleic acid), diisooctyl ester, and polyunsaturated aldehyde, have been confirmed to inhibit the growth of many harmful microalgae such as *Karenia mikimotoi*, *Alexandrium tamarensem Prorocentrum micans*, and *H. akashiwo* (Lenzo et al., 2022; Patil et al., 2024; Pichierri et al., 2016; Wang et al., 2018). Most likely, these allelochemicals might have affected the division and growth of germling cells of *G. catenatum* and *O.* cf. *ovata* cysts. Regarding the algicidal action, it has been stated that macroalgal allelochemicals primarily disrupt photosystem II (PSII) components leading to reduction in photosynthetic pigments, and ultimately inhibit the proliferation of the algal cells (Cheng et al., 2024; Zhang et al., 2019). Simultaneously, allelochemicals cause oxidative stress and formation of excessive reactive oxygen species (ROS), triggering the death of microalgal cells (i.e., cell apoptosis) through the action of caspase-3 and -9 (Wang et al., 2023). However, more research is needed to separate and characterize such active substances produced by these macroalgae in order to potentially use them to control and mitigate HABs in the natural environment.

## 4. Conclusions

This study provided evidence that macroalgae from the Libyan Mediterranean coast, particularly *Cystoseira* species, inhibited the germination success of *G. catenatum* and *O.* cf. *ovata* cysts through suppressing the germling viability after cyst germination. This indicates that these macroalgae could retard the recurrence of blooms of these dinoflagellate species in the water column. The study also showed that methanol extracts of these macroalgae have stronger inhibitory effects on the germination success with lower $IC_{50}$ values (0.05–1.5 mg extract $g^{-1}$ sediment) than aqueous extracts ($IC_{50}$ = 3.9–590 mg extract $g^{-1}$ sediment). Given that cyst germlings are more sensitive toward active substances than mature individuals (Bond et al., 1999; Mohamed et al., 2022), this study suggests that the growth of harmful microalgae should be controlled at the early stages (i.e., germlings) rather than treatment of algal blooms, which may cause algal cell lysis and release massive amounts of algal toxins into the aquatic environment. Since macroalgae or their extracts have been previously reported as ecofriendly materials (Jeong et al., 2000; Wang et al., 2007), the extracts of our macroalgae could be applied to a confined coastal area (i.e., bloom area) to inhibit the growth and division of germling cells after cyst germination, and thus restrict recurrence of HABs in these regions. However, further *in situ* study or mesocosm experiments are needed to determine ecological safety and efficacy under natural conditions. The potential impacts of these macroalgal extracts on other beneficial microalgae and microbial communities should also be examined in future studies.

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## Author contributions

Zakaria Mohamed: conceptualization; methodology; writing-original draft; writing-review and editing. Hana Abohbell: investigation; methodology, formal analysis; writing-original draft. Adel Ben Omran: conceptualization; methodology; writing-review and editing. Tahani Asseri: formal analysis; funding acquisition. Mohamed Hashem: formal analysis; writing-review and editing. Hoida Badr: methodology; validation.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supplementary Material

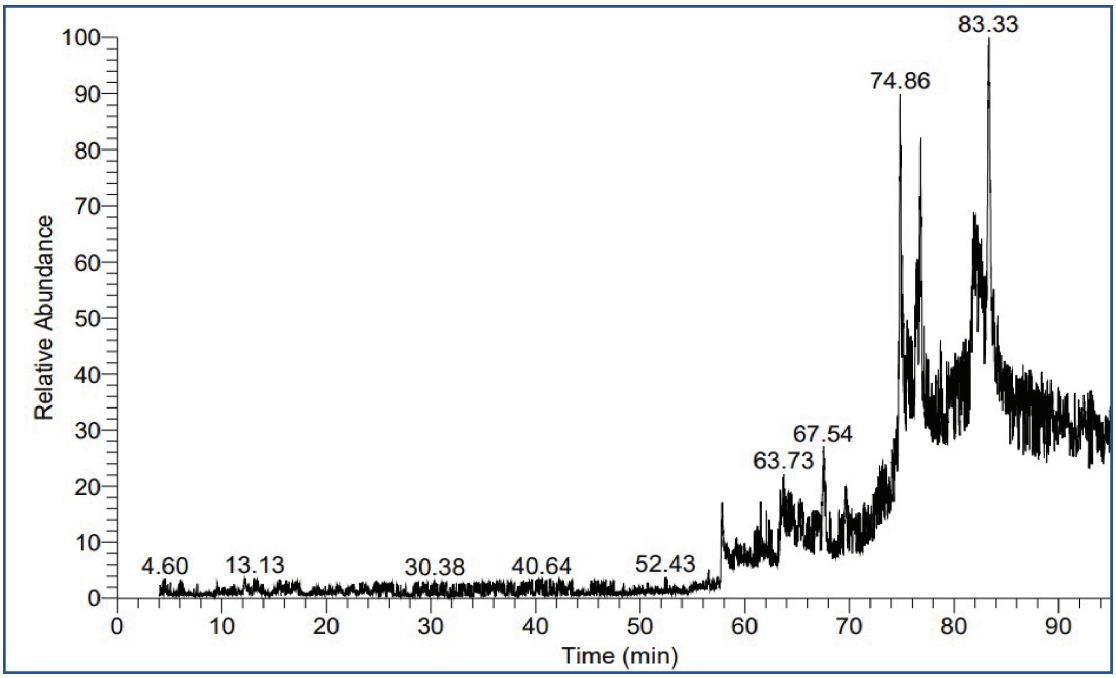


**Figure S1**

GC-MS chromatogram of *Jania rubens* extract. GC-MS, gas chromatography-mass spectrometry.

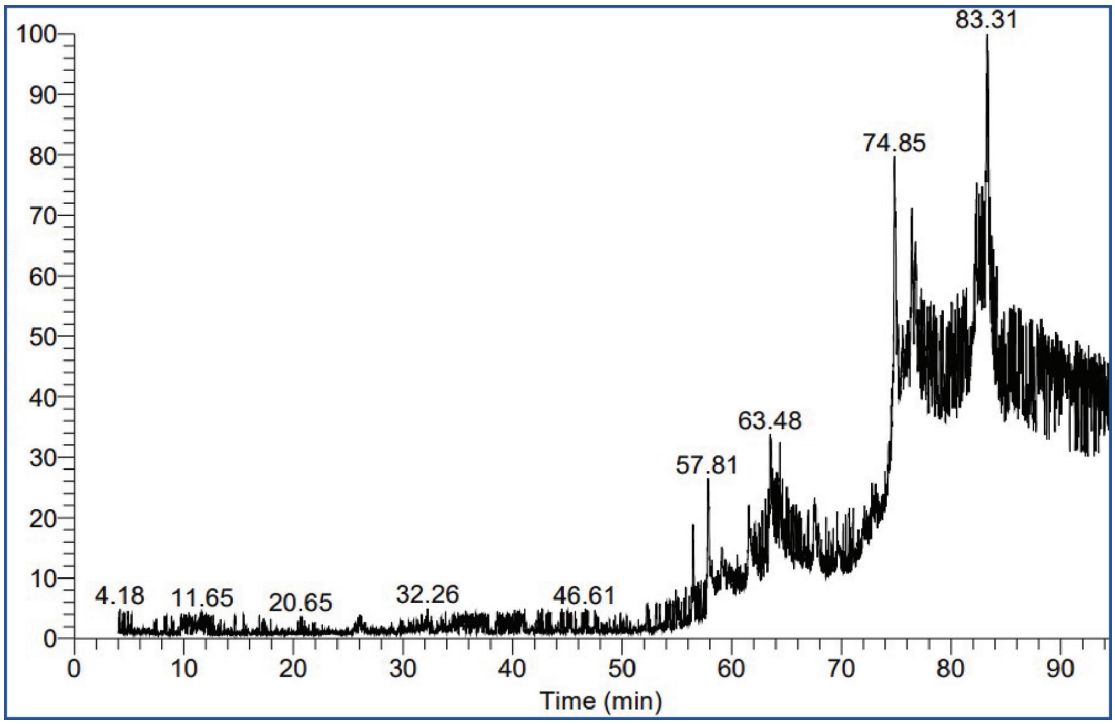


**Figure S2**

GC-MS chromatogram of *Laurencia obtusa* extract. GC-MS, gas chromatography-mass spectrometry.

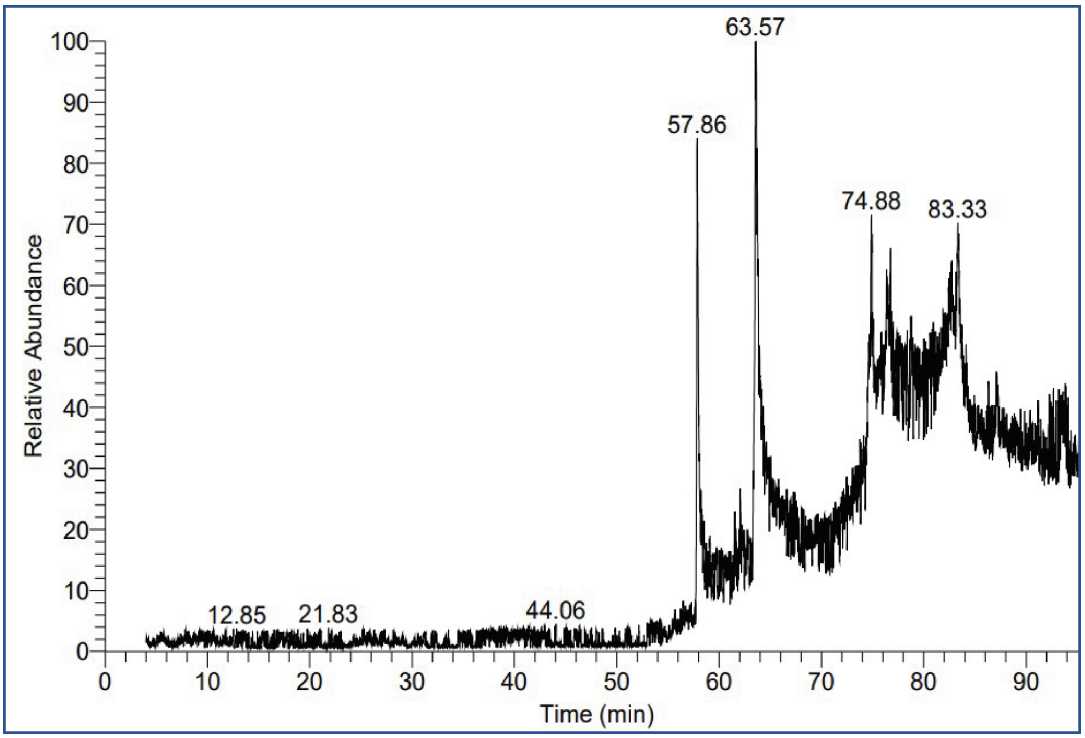


**Figure S3**

GC-MS chromatogram of *Cystoseira compressa* extract. GC-MS, gas chromatography-mass spectrometry.

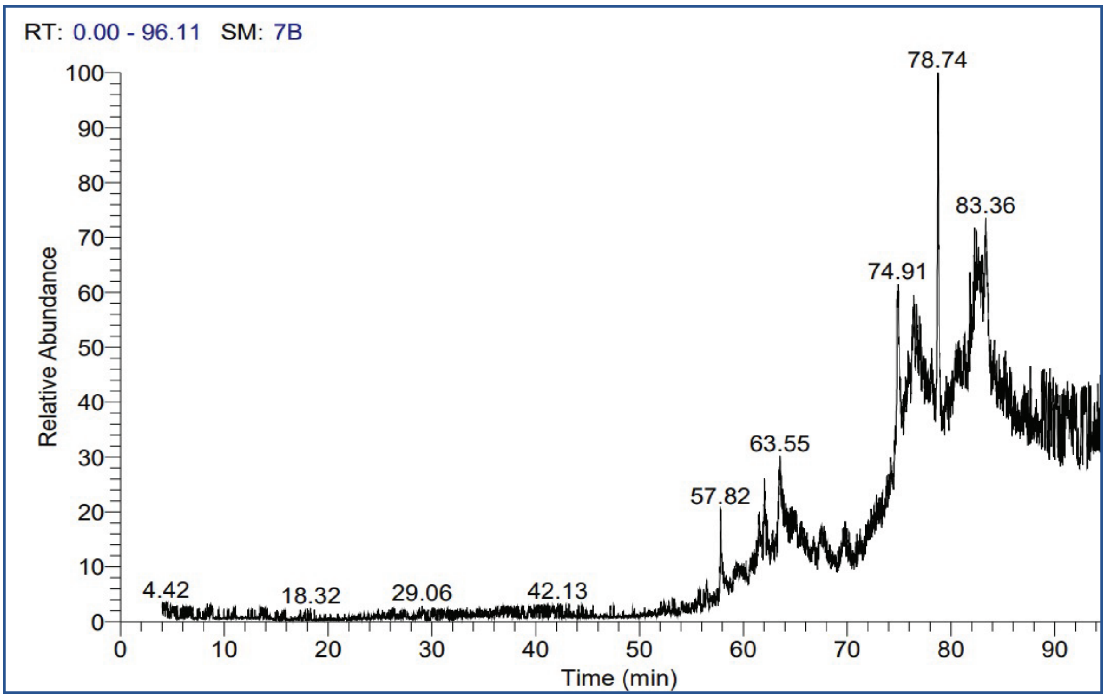


**Figure S4**

GC-MS chromatogram of *Cystoseira crinita* extract. GC-MS, gas chromatography-mass spectrometry.

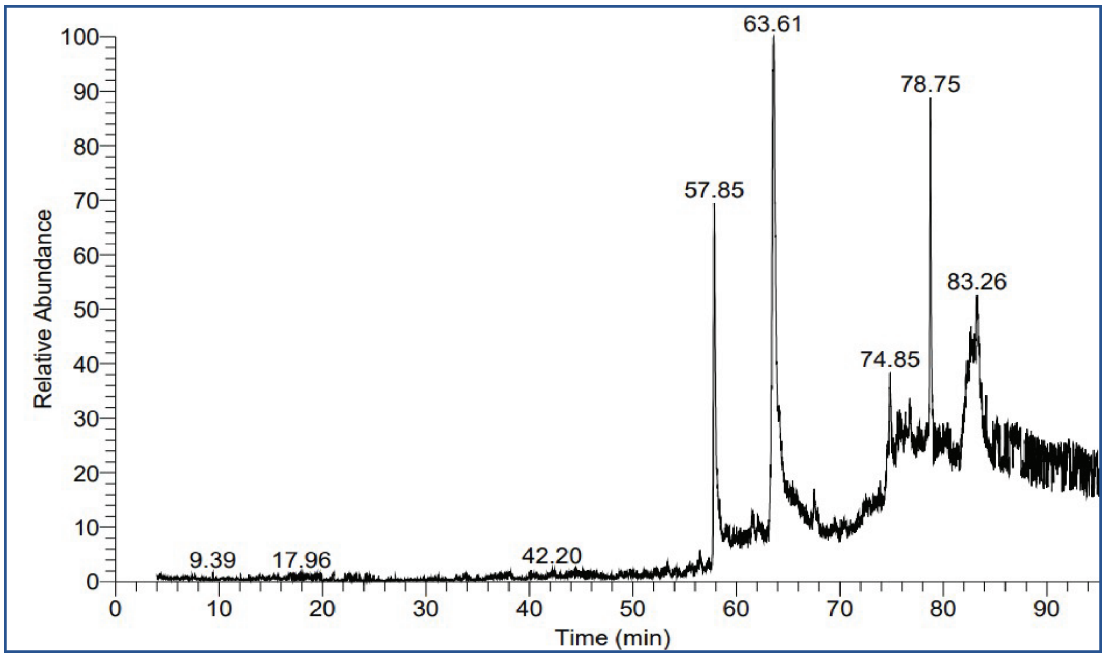


**Figure S5**

GC-MS chromatograms of *Cystoseira barbata* extract. GC-MS, gas chromatography-mass spectrometry.

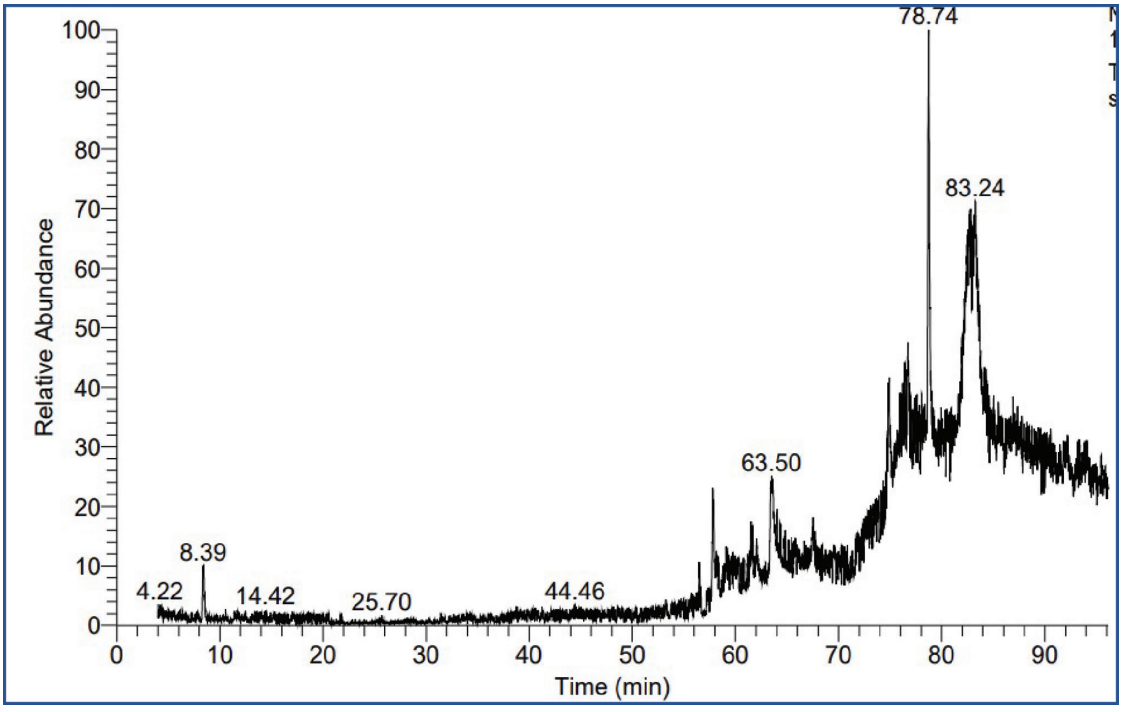


**Figure S6**

GC-MS chromatogram of *Ulva lactuca* extract. GC-MS, gas chromatography-mass spectrometry.

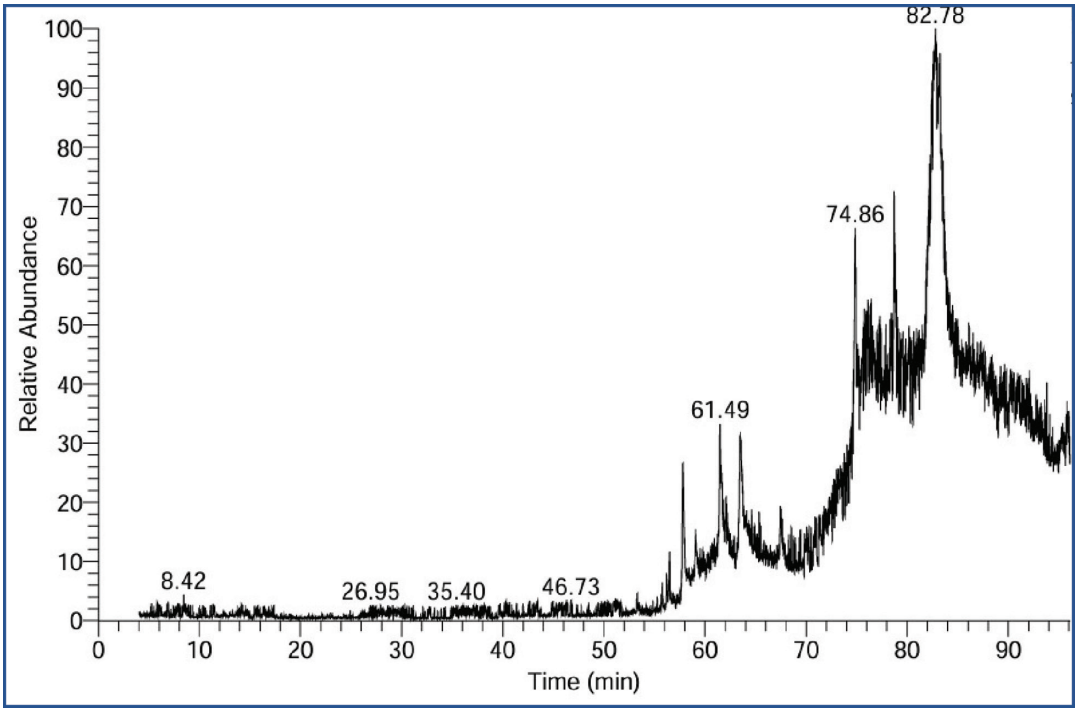


**Figure S7**

GC-MS chromatogram of *Enteromorpha linza* extract. GC-MS, gas chromatography-mass spectrometry.

**Table S1**

Chemical constituents of *Jania rubens* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 14.57 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Unsaturated fatty ester | 12.17 |
| Heptadecanoic acid, heptadecyl ester | Fatty acid ester | 3.26 |
| 17-Octadecynoic acid | Fatty acid alkyne | 3.21 |
| E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate | fatty acid ester | 2.44 |
| Octadecanal, 2-bromo- | Aldehydes | 2.03 |
| Phenacyl 11-octadecenoate | Fatty acid ester | 1.57 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl | Aliphatic hydrocarbon | 1.57 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl- | Aliphatic hydrocarbon | 1.57 |
| n-Hexadecanoic acid (Palmitic acid) | Fatty acid ester | 1.36 |
| 1,3-Dioxolane, 4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-, trans- | Alkenes | 1.8 |
| Stearic acid, 2-phenyl-m-dioxan-5-yl ester, trans- | Fatty acid ester | 0.55 |
| 9,12-Octadecadienoyl chloride, (Z,Z)- | Unsaturated fatty acid chloride | 0.54 |
| 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4, 11)]undecan-1-ol | Alcohols | 0.54 |
| 1,2-15,16-Diepoxyhexadecane | Long-chain fatty epoxide | 0.52 |
| Trans-13-octadecenoic acid | Fatty acid | 0.52 |
| Cis-vaccenic acid | Unsaturated fatty acid | 0.49 |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Unsaturated fatty ester | 7.96 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 2.91 |

(*Continued*)

**Table S1**

Continued

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 1-Octadecyne | Unsaturated hydrocarbon | 1.82 |
| 2,3-Dihydroxypropyl elaidate | Unsaturated fatty ester diol | 1.77 |
| Hexadecanoic acid, 4-nitrophenyl ester | Fatty acid ester | 1.70 |
| [1,1′-Bicyclohexyl]-4-carboxylic acid, 4′-propyl-, 4-fluorophenyl ester | Ester | 3.62 |
| 1-Monolinoleoylglycerol trimethylsilyl ether | Unsaturated fatty ester | 24.32 |
| Hexadecanoic acid, 1-(1-methylethyl)-1,2-ethanediyl ester | Fatty acid ester | 5 |
| Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester | Fatty acid ester | 0.83 |
| n-Propyl 11-octadecenoate | Fatty acid ester | 0.81 |
| 2,2-Dimethyl-6-methylene-1-[3,5-dihyd roxy-1-pentenyl]cyclohexan-1-perhydr ol | Alcohols | 0.80 |
| 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | Fatty acid ester | 0.70 |
| 9,12-Octadecadienoic acid (Z,Z)- | (Linoleic acid) Unsaturated fatty acid | 0.62 |

GC-MS, gas chromatography-mass spectrometry.

**Table S2**

Chemical constituents of *Laurencia obtusa* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 1-Monolinoleoylglycerol trimethylsilyl ether | Unsaturated fatty ester | 26.37 |
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 16.63 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Unsaturated fatty ester | 6.85 |
| 2,3-Dihydroxypropyl elaidate | Unsaturated fatty ester diol | 5.54 |
| 1-Heptadecyne | Unsaturated hydrocarbon | 4 |
| 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | Fatty acid ester | 3.8 |
| E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate | Fatty acid ester | 3.17 |
| Cis-vaccenic acid | Unsaturated fatty acid | 2.19 |
| 1H-Indene, 5-butyl-6-hexyloctahydro- | Esters | 2.13 |
| Trans-13-Octadecenoic acid | Fatty acid | 2.05 |
| Cis-13-Octadecenoic acid | Unsaturated fatty acid | 1.94 |
| Z,E-2-Methyl-3,13-octadecadien-1-ol | Fatty alcohol | 1.82 |
| 9,12-Octadecadienoic acid (Z,Z)- | (Linoleic acid) Unsaturated fatty acid | 1.75 |
| 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- | Unsaturated fatty ester | 1.49 |
| n-Propyl 11-octadecenoate | Fatty acid ester | 1.48 |
| Pentanoic acid, 10-undecenyl ester | Fatty acid ester | 1.43 |
| 2-Piperidinone, N-[4-bromo-n-butyl]- | Cyclic amides | 1.27 |
| 2-Myristynoyl-glycinamide | Unsaturated fatty amide | 1.25 |
| [1,1′-Bicyclohexyl]-4-carboxylic acid, 4′-propyl-, 4-fluorophenyl ester | Ester | 1.09 |
| Pentadecanoic acid, 14-methyl-, methyl ester | Fatty acid ester | 1.08 |
| n-Hexadecanoic acid (Palmitic acid) | Fatty acid ester | 1.03 |
| 5á,7áH,10à-Eudesm-11-en-1à-ol | Sesquiterpenes | 1.02 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 1.01 |
| Dasycarpidan-1-methanol, acetate (ester) | Ester | 0.99 |
| Ethyl iso-allocholate | Sterol | 0.99 |
| 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione | Cyclic amides | 0.93 |

(*Continued*)

**Table S2**

Continued

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 17-Octadecynoic acid | Fatty acid alkyne | 0.84 |
| 2,2-Dimethyl-6-methylene-1-[3,5-dihyd roxy-1-pentenyl]cyclohexan-1-perhydr ol | Alcohols | 0.74 |
| 9-Methyl-Z-10-tetradecen-1-ol acetate | Unsaturated fatty ester | 0.72 |
| Z,Z-3,13-Octadecedien-1-ol | Fatty alcohol | 0.72 |
| E-2-Octadecadecen-1-ol | Fatty alcohol | 0.71 |
| Pregnane-3,11,20,21-tetrol, cyclic 20,21-(butyl boronate), (3à,5á,11á,20R)- | Steroids | 0.67 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl- | Aliphatic hydrocarbon | 0.63 |
| Z-17-Non-adecen-1-ol acetate | Fatty acid ester | 0.58 |
| 9-Octadecenal | Unsaturated fatty aldehyde | 0.56 |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Unsaturated fatty ester | 0.56 |

GC-MS, gas chromatography-mass spectrometry.

**Table S3**

Chemical constituents of *Cystoseira compressa* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 13.13 |
| Cis-vaccenic acid | Unsaturated fatty acid | 12.05 |
| 17-Octadecynoic acid | Fatty acid alkyne | 8.22 |
| n-Hexadecanoic acid (Palmitic acid) | Fatty acid ester | 7.82 |
| 9,12-Octadecadienoic acid (Z,Z)- | (Linoleic acid) Unsaturated fatty acid | 7.07 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 5.66 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Unsaturated fatty ester | 5.41 |
| 1-Monolinoleoylglycerol trimethylsilyl ether | Unsaturated fatty ester | 4.92 |
| 9,17-Octadecadienal, (Z)- | Unsaturated aldehydes | 2.94 |
| Trans-13-Octadecenoic acid | Fatty acid | 2.42 |
| 1H-Indene, 5-butyl-6-hexyloctahydro- | Esters | 2.18 |
| Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate | Fatty acid esters | 2.13 |
| 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | Fatty acid ester | 1.97 |
| Pentanoic acid, 10-undecenyl ester | Fatty acid esters | 1.91 |
| [1,1′-Bicyclohexyl]-4-carboxylic acid, 4′-propyl-, 4-fluorophenyl ester | Ester | 1.89 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl- | Aliphatic hydrocarbon | 1.85 |
| 9-Octadecenoic acid (Z)-, phenylmethyl ester | Unsaturated fatty ester | 1.83 |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Unsaturated fatty ester | 1.63 |
| Ethanol, 2-(9-octadecenyloxy)-, (Z)- | Fatty ether | 1.62 |
| E-10,13,13-Trimethyl-11-tetradecen-1- ol acetate | Fatty acid esters | 1.53 |
| 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsil yl)oxy] methyl]ethyl ester, (Z,Z,Z)- | Unsaturated fatty ester | 1.37 |
| Z,E-2-Methyl-3,13-octadecadien-1-ol | Fatty alcohol | 1.34 |
| 9-Octadecenoic acid (Z)-, methyl ester | Unsaturated fatty ester | 1.27 |
| Cyclohexane, 1,1′-(2-tridecyl-1,3-propanediyl)bis- | Carbohydrates | 1.12 |
| (R)-(-)-(Z)-14-Methyl-8-hexadecen-1- ol | Alcohols | 1.11 |
| E-10-Pentadecenol | Fatty alcohol | 0.98 |

(*Continued*)

**Table S3**

Continued

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 9-Methyl-Z-10-tetradecen-1-ol acetate | Unsaturated fatty ester | 0.81 |
| E-2-Octadecadecen-1-ol | Fatty alcohol | 0.69 |
| 1-Octadecyne | Unsaturated hydrocarbon | 0.69 |
| 13-Octadecenal, (Z)- | Unsaturated aldehydes | 0.68 |
| 9,9-Dimethoxybicyclo[3.3.1]nona-2,4- dione | Cyclic amides | 0.62 |
| 8-Hexadecenal, 14-methyl-, (Z)- | Aldehydes | 0.62 |
| n-Propyl 11-octadecenoate | Fatty acid esters | 0.52 |

GC-MS, gas chromatography-mass spectrometry.

**Table S4**

Chemical constituents of *Cystoseira crinita* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 27.42 |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Unsaturated fatty ester | 9.44 |
| 1,2-Benzenedicarboxylic acid, diisooctyl ester | Ester | 7.70 |
| 1-Monolinoleoylglycerol trimethylsilyl ether | Unsaturated fatty ester | 6.06 |
| 17-Octadecynoic acid | Fatty acid alkyne | 5.56 |
| [1,1′-Bicyclohexyl]-4-carboxylic acid, 4′-propyl-, 4-fluorophenyl ester | Ester | 5.55 |
| 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1[[(trimethylsilyl) oxy]methyl]ethyl ester, (Z,Z,Z)- | Unsaturated fatty ester | 3.33 |
| 9-Octadecenoic acid (Z)-, phenylmethyl ester | Unsaturated fatty ester | 2.97 |
| Trans-13-Octadecenoic acid | Fatty acid | 2.9 |
| 9,12-Octadecadienoic acid (Z,Z) | Unsaturated fatty acid | 2.76 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 2.63 |
| 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | Fatty acid ester | 2.37 |
| n-Hexadecanoic acid (Palmitic acid) | Fatty acid esters | 2.19 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl- | Aliphatic hydrocarbon | 1.75 |
| 9,17-Octadecadienal, (Z)- | Unsaturated aldehydes | 1.68 |
| 12-Methyl-E,E-2,13-octadecadien-1-ol | | 1.68 |
| E-10,13,13-Trimethyl-11-tetradecen-1- ol acetate | Fatty acid esters | 1.68 |
| Octadecanal, 2-bromo- | Aldehydes | 1.64 |
| Z,E-2-Methyl-3,13-octadecadien-1-ol | Fatty alcohol | 1.52 |
| Cis-vaccenic acid | Unsaturated fatty acid | 1.23 |
| 1H-Indene, 5-butyl-6-hexyloctahydro- | Esters | 1.12 |
| cis-13-Octadecenoic acid | Unsaturated fatty acid | 0.97 |
| 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4, 11)]undecan-1-ol | Tricyclic alcohol | 0.96 |
| E-10-Pentadecenol | Fatty alcohol | 0.71 |
| Cyclopentaneundecanoic acid, methyl ester | Fatty acid esters | 0.69 |
| E-2-Octadecadecen-1-ol | Fatty alcohol | 0.68 |
| Z,Z-3,13-Octadecedien-1-ol | Fatty alcohol | 0.59 |
| 1-Hexadecyne | Alkene | 0.58 |
| Cyclohexane, 1,1′-(2-ethyl-1,3-propanediyl)bis- | Aliphatic hydrocarbon | 0.55 |
| Z-8-Methyl-9-tetradecen-1-ol acetate | Fatty acid esters | 0.55 |
| Cyclohexane, 1,1′-(2-methyl-1,3-propanediyl)bis- | Hydrocarbons | 0.50 |

GC-MS, gas chromatography-mass spectrometry.

**Table S5**

Chemical constituents of *Cystoseira barbata* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 24.81 |
| Cis-vaccenic acid | Unsaturated fatty acid | 15.73 |
| 1,2-Benzenedicarboxylic acid, diisooctyl ester | Ester | 9.47 |
| l-(+)-Ascorbic acid 2,6 dihexadecanoate | Fatty acid esters | 9.24 |
| 1-Monolinoleoylglycerol trimethylsilyl ether | Unsaturated fatty ester | 5.31 |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Unsaturated fatty ester | 4.04 |
| Trans-13-Octadecenoic acid | Fatty acid | 4.01 |
| E-10,13,13-Trimethyl-11-tetradecen-1- ol acetate | Fatty acid esters | 3.91 |
| n-Propyl 11-octadecenoate | Fatty acid esters | 3.53 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 3.15 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Unsaturated fatty ester | 3.12 |
| 17-Octadecynoic acid | Fatty acid alkyne | 2.79 |
| 2,2-Dimethyl-6-methylene-1-[3,5-dihyd roxy-1-pentenyl]cyclohexan-1-perhydr ol | Alcohols | 1.83 |
| Oleic anhydride | Unsaturated fatty acid | 1.34 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl- | Aliphatic hydrocarbon | 1.25 |
| 9,12-Octadecadienoyl chloride, (Z,Z)- | Unsaturated fatty acid chloride | 1.16 |
| Z,E-2-Methyl-3,13-octadecadien-1-ol | Fatty alcohol | 0.82 |
| (R)-(-)-(Z)-14-Methyl-8-hexadecen-1- ol | Alcohols | 0.78 |
| 1H-Indene, 5-butyl-6-hexyloctahydro- | Esters | 0.78 |
| 9-Octadecenoic acid (Z)-, phenylmethyl ester | Unsaturated fatty ester | 0.77 |
| Spirost-8-en-11-one, 3-hydroxy-, (3á,5à,14á,20á,22á,25R)- | Sterol | 0.73 |
| 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsil yl)oxy]methyl] ethyl ester, (Z,Z,Z)- | Unsaturated fatty ester | 0.63 |
| 9-Octadecenoic acid (Z)-, 9-octadecenyl ester, (Z)- | Unsaturated fatty ester | 0.40 |
| 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | Fatty acid ester | 0.40 |

GC-MS, gas chromatography-mass spectrometry.

**Table S6**

Chemical constituents of *Ulva lactuca* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 24.65 |
| 2,2-Dimethyl-6-methylene-1-[3,5-dihyd roxy-1-pentenyl]cyclohexan-1-perhydr ol | Alcohols | 10.84 |
| 17-Octadecynoic acid | Fatty acid alkyne | 8.93 |
| 1-Monolinoleoylglycerol trimethylsilyl ether | Unsaturated fatty ester | 5.85 |
| n-Propyl 11-octadecenoate | Fatty acid esters | 5.38 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 3.89 |
| Trans-13-Octadecenoic acid | Fatty acid | 3.51 |
| 1,2-Benzenedicarboxylic acid, diisooctyl ester | Ester | 3.42 |
| (R)-(-)-(Z)-14-Methyl-8-hexadecen-1- ol | Alcohols | 2.74 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl | Aliphatic hydrocarbon | 2.45 |
| Cis-vaccenic acid | Unsaturated fatty acid | 2.4 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Unsaturated fatty ester | 2.05 |

(*Continued*)

**Table S6**

Continued

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| Cyclohexane, 1,1′-(2-methyl-1,3-propanediyl)bis- | Hydrocarbons | 1.98 |
| 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- | Fatty acid ester | 1.81 |
| Ethanol, 2-(9-octadecenyloxy)-, (Z)- | Fatty ether | 1.72 |
| 1-Cyclohexylnonene | Aliphatic hydrocarbons | 1.33 |
| E-10,13,13-Trimethyl-11-tetradecen-1- ol acetate | Fatty acid esters | 1.32 |
| 9,12-Octadecadienoic acid (Z,Z)- | (Linoleic acid) Unsaturated fatty acid | 1.29 |
| Pentanoic acid, 10-undecenyl ester | Fatty acid esters | 1.27 |
| 1H-Indene, 5-butyl-6-hexyloctahydro- | Esters | 1.18 |
| 9,12-Octadecadienoyl chloride, (Z,Z)- | Unsaturated fatty acid chloride | 1.06 |
| 9,17-Octadecadienal, (Z)- | Unsaturated aldehydes | 1.04 |
| 1,14-Tetradecanediol | Long-chain fatty alcohol | 0.99 |
| 13-Oxabicyclo[10.1.0]tridecane | Aliphatic hydrocarbons | 0.91 |
| 9-Eicosyne | Alkynes | 0.9 |
| E-2-Methyl-3-tetradecen-1-ol acetate | Unsaturated fatty ester | 0.86 |
| Undecanoic acid, 10-bromo- | Bromo fatty acid | 0.83 |
| Cyclohexane, 1,4-dimethyl-2-octadecyl- | Aliphatic hydrocarbons | 0.82 |
| n-Hexadecanoic acid (Palmitic acid) | Fatty acid esters | 0.81 |
| Methyl 8-methyl-non-anoate | Fatty acid esters | 0.75 |
| cis-9,10-Epoxyoctadecan-1-ol | Epoxy fatty alcohol | 0.7 |
| Ethanone, 1-(2-furanyl)- | Heterocyclic ketone | 0.6 |
| Z,E-2-Methyl-3,13-octadecadien-1-ol | Fatty alcohol | 0.57 |
| Cyclopentane, 1,1′-[3-(2-cyclopentylethyl)-1,5-pentan ediyl]bis- | Aliphatic hydrocarbon | 0.55 |
| 9-Octadecenoic acid (Z)-, phenylmethyl ester | Unsaturated fatty ester | 0.53 |

GC-MS, gas chromatography-mass spectrometry.

**Table S7**

Chemical constituents of *Enteromorpha linza* extract as investigated by GC-MS

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | Long chain fatty ester | 24.21 |
| 17-Octadecynoic acid | Fatty acid alkyne | 8.24 |
| Cyclohexane, 1,1′-dodecylidenebis[4-methyl- | Aliphatic hydrocarbon | 7.41 |
| 1H-Indene, 5-butyl-6-hexyloctahydro- | Esters | 5.58 |
| Cis-vaccenic acid | Unsaturated fatty acid | 6.92 |
| 9,12-Octadecadienoic acid (Z,Z)- | (Linoleic acid) Unsaturated fatty acid | 5.04 |
| 2-Myristynoyl-glycinamide | Unsaturated fatty amide | 4.94 |
| [1,1′-Bicyclohexyl]-4-carboxylic acid, 4′-propyl-, 4-fluorophenyl ester | Ester | 4.55 |
| n-Propyl 11-octadecenoate | Fatty acid esters | 4.37 |
| 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Unsaturated fatty ester | 3.85 |
| Trans-13-Octadecenoic acid | Fatty acid | 3.01 |
| Z,E-2-Methyl-3,13-octadecadien-1-ol | Fatty alcohol | 2.62 |
| 2-Pentadecyn-1-ol | Unsaturated fatty alcohol | 2.4 |

(*Continued*)

**Table S7**

Continued

| Chemical name | Nature compound | Mass distribution (%) |
|---|---|---|
| 9-Octadecenoic acid (Z)-, phenylmethyl ester | Unsaturated fatty ester | 1.65 |
| Cyclohexane, 1,1′-(2-methyl-1,3-propanediyl)bis | Aliphatic hydrocarbons | 1.62 |
| n-Hexadecanoic acid (Palmitic acid) | Fatty acid | 1.61 |
| Pentanoic acid, 10-undecenyl ester | Fatty acid esters | 1.46 |
| Oleic acid (cis-9-octadecenoic) | Unsaturated fatty acid | 1.39 |
| Tricyclo[5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramet hyl | Tricyclic hydroxyl ketone | 1.21 |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Unsaturated fatty ester | 1.01 |
| Palmitic acid vinyl ester | Fatty acid esters | 0.93 |
| 2-Piperidinone, N-[4-bromo-n-butyl]- | Cyclic amides | 0.91 |
| cis-13-Octadecenoic acid | Unsaturated fatty acid | 0.76 |
| E-10,13,13-Trimethyl-11-tetradecen-1- ol acetate | Fatty acid ester | 0.69 |
| Cyclopentaneundecanoic acid, methyl ester | Fatty acid ester | 0.67 |
| 2,2-Dimethyl-6-methylene-1-[3,5-dihyd roxy-1-pentenyl]cyclohexan-1-perhydr ol | Alcohols | 0.64 |
| 13-Octadecenal, (Z)- | Unsaturated aldehydes | 0.61 |
| (R)-(-)-(Z)-14-Methyl-8-hexadecen-1 ol | Alcohols | 0.57 |
| Z,Z-3,13-Octadecedien-1-ol | Fatty alcohol | 0.57 |
| Z-10-Pentadecen-1-ol | Unsaturated fatty alcohols | 0.55 |

GC-MS, gas chromatography-mass spectrometry.