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Research Article

Environmentally Friendly Antifouling Metabolites from Red Sea Organisms

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Seventy-one marine organisms representing different classes of marine fauna and flora were collected from the Red Sea. They include sponges, hydrozoan, soft corals, sea cucumber, ascidian, cyanobacteria, and macroalgae. The methanolic extracts were evaluated for their toxicity and settlement inhibition effects by using cultured *Balanus amphitrite*. Thirty-three extracts displayed antifouling effects: four samples were highly potent at 1 μg/mL with a percentage of settlement inhibition above 31%, twenty-two were potent at 10 μg/mL with a percentage of settlement inhibition between 16 and 30%, and seven were active at 10 μg/mL with a percentage of settlement inhibition between 0 and 15%. Two promising extracts were purified by employing several chromatographic techniques, leading to the isolation of 12 known compounds. The isolated compounds were evaluated for their antifouling activities and demonstrated potent antifouling effects with EC_{50} values of less than 10 μg/mL.

1. Introduction

Biofouling occurs due to the adhesion of barnacles, microbial slimes, and macroalgae on wetted marine surfaces [1–3]. These organisms produce adhesive organic materials which enhance the ability of microorganisms (microalgae, protozoa, and bacteria) to settle on the marine surfaces [4–6]. Such biofilms lead to several economic hazards, including increased fuel consumption, ship damage, and immigration of invasive macromolecules [6, 7]. The diverse fouled surfaces become vectors for transporting nonindigenous species when they are moved from one area to another. Then, they can attach to a new wetted surface and form a core for new biofouling, which is responsible for increasing fuel consumption by $56 million annually for the entire fleet of DDG-52 (midsize) crafts [8]. This process also increases the production costs of aquaculture operations by $1.5 to $3 billion annually [9]. Hence, biofouling is a real challenge for both chemistry and marine technology [10, 11].

Blocking marine-immersed surfaces to reduce the attachment of biofouling organisms can be performed by employing synthetic paint-added chemical compounds (biocides) [12, 13]. This has been done by using potent antifouling synthetic organotin compounds such as tributyltin self-polishing copolymer paints (TBT-SPC paints) [14]. Unfortunately, tributyltin-based paints have serious

adverse effects. They cause toxicity to humans, in the form of liver impairment, hypoglycemia, glycosuria, and respiratory disturbances. In experimental animals, they also showed immunosuppressive, endocrinopathic, neurotoxic, hepatotoxic, nephrotoxic, and skin & eye irritation effects. Gonadotoxic, embryotoxic, fetotoxic, and developmental effects were also observed. In addition, they have phytotoxic effects on marine organisms, even at low concentrations [15, 16]. Accordingly, the International Maritime Organization (IMO) stopped the application of organotin compounds as antifoulants, in January 2003. WHO has prohibited the use of organotin biocides since 1990 [17].

The safety profile of natural products has allowed researchers to apply a "back to nature" approach, aimed at developing environmentally friendly natural antifouling paints instead of using synthetics. Several successful examples have resulted from the application of this natural product strategy. Capsaicin, the major bioactive principle of chili pepper, effectively inhibited zebra mussel byssal attachment. It has been used in ship antifouling paints in China after approval from the National Environmental Protection Standard of the People's Republic of China. In the last three decades, marine natural products have gained great attention, as an alternative solution after the ban of organotin derivatives [18].

Several studies have examined antifouling metabolites, which are isolated from marine invertebrates, particularly, sponges and soft corals [19–26]. Both organisms have established an impressive warehouse of chemical defense systems against biofouling. It is interesting that the majority of environmentally friendly antifouling metabolites, based on research done in the last 30 years, belong to the same natural classes: terpenoids (i.e., sesquiterpenes and cembranoid diterpenes), alkaloids, and steroids. On this basis, our sample collection was directed towards the marine organisms which can produce such metabolites [27–29].

The current manuscript focuses on discovering antifouling metabolites among selected Red Sea organisms. Seventy-one marine extracts were screened for their antifouling activity, including sponges, algae, tunicate, sea cucumbers, soft corals, and cyanobacteria. Two of the most promising extracts were purified, which led to the isolation of twelve known metabolites. The isolated compounds were evaluated for their antifouling effects.

2. Materials and Methods

2.1. Collection and Preparation of Samples. Both divers who collected the samples are marine biologists. The divers searched in different sites at depths ranging from 1 to 20 m. After collection of samples by proper methods, including scuba diving, the samples were sorted into separate boxes for each site. Underwater photographs were taken to help in systematic identification and herbarium formation. The collected samples were coded using the standard system indicating the date of sampling, the phylum and order number, etc. A simplified code was later used for the biological activity assays (SH-). Monthly cruises were organized by the team to explore the marine habitats of the north and south coasts of Jeddah city. All diving sites were listed and the corresponding points localized using GPS techniques. Different selected samples were collected from different places along the Red Sea coast. These organisms were taxonomically identified, and their descriptions are listed in Table S1. Figure 1 illustrates the places of collection of the Red Sea samples.

2.1.1. Algal Samples. Specimens of marine algae, belonging to different genera, were collected from the water around the Jeddah coast (Table S1). After collection, the samples were washed with filtered seawater to remove associated debris and large epiphytes. A 10 min 5% ethanol wash was performed to clean the surface of microflorae. The cleaned material was lyophilized, and then the samples were extracted.

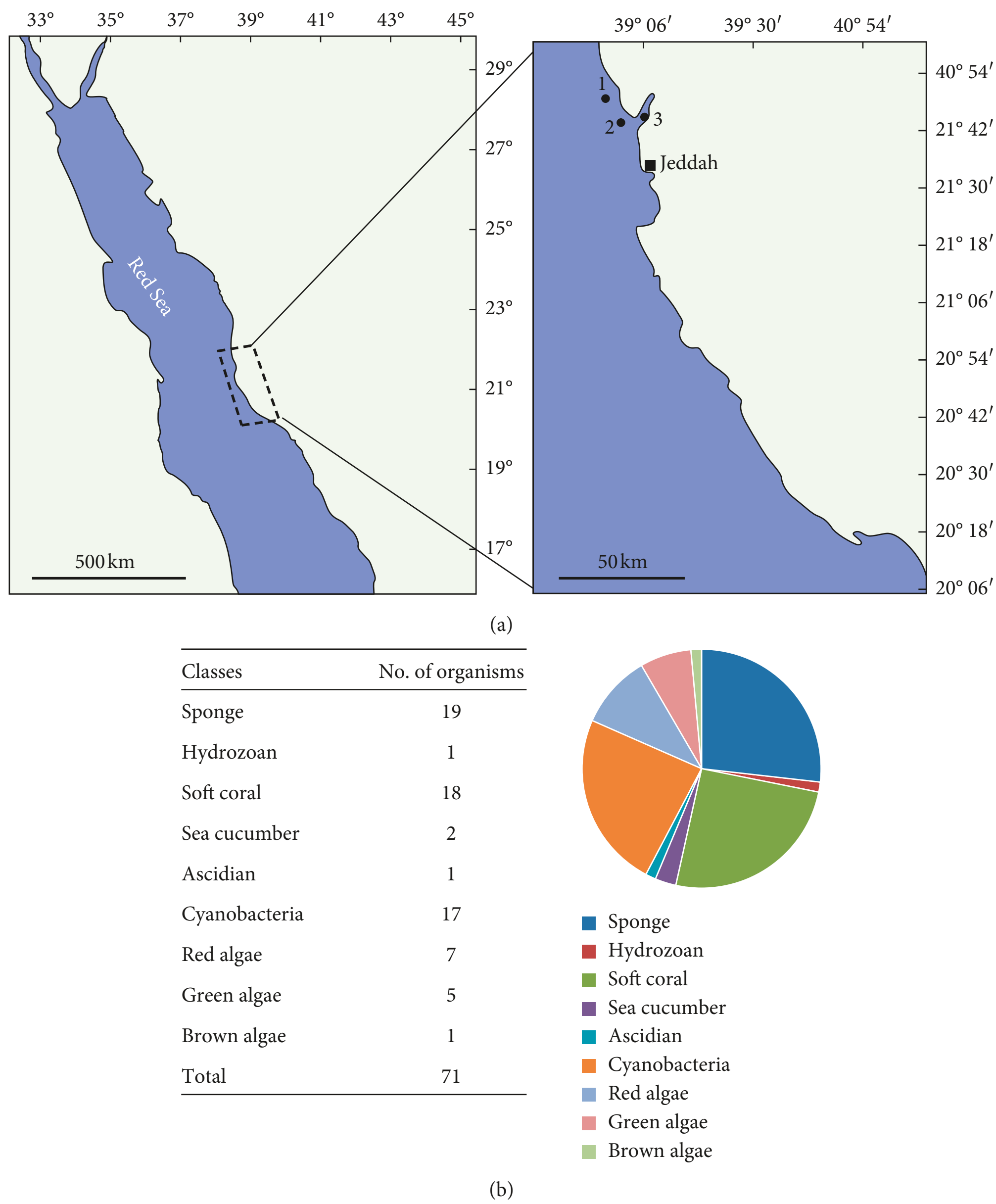
2.1.2. Sponge Samples. The sponge species (Table S1) were collected from different depths and bodies of water around Saudi Arabia, using different techniques such as skin and scuba diving. The sponges were washed with freshwater and transported to the laboratory for lyophilization and extraction.

2.1.3. Coral Samples. Soft corals (Table S1) were collected from different depths and different locations of the Jeddah coast, using different techniques such as skin and scuba diving. The samples were kept in ice boxes and transported to the laboratory for lyophilization and extraction.

2.1.4. Cyanobacteria Samples. Samples were obtained from coral reefs near Jeddah, Saudi Arabia, via scuba diving. They were collected by hand or using metal forceps and placed in nets or Ziploc bags (Table S1). As much as possible, seawater and foreign organisms were manually removed. The samples were immediately immersed in methanol for preservation and initial extraction. A portion of the samples was placed in Nalgene bottles containing a SWBG11 medium for culturing. A small part was also placed in Falcon tubes containing 10 mL RNA later for DNA extraction.

2.2. Identification of the Samples. The sample identification was done based on the morphological observation of the biologist during collection, followed by a deep investigation in the lab by different biologists employing high-resolution microscopes (Table S1). This was done through the cooperation of many marine biologists. There are special methods for identification of the cyanobacteria samples (see supplementary material available here).

2.3. Antifouling Assay. B. amphitrite, attached to bamboo poles, were procured from oyster farms in Lake Hamana, Shizuoka, Japan, and were maintained in an aquarium at 20°C by feeding them *Artemia salina* nauplii. Broods released I-II stage nauplii upon immersion in seawater after being dried overnight. The nauplii thus obtained



Classes	No. of organisms
Sponge	19
Hydrozoan	1
Soft coral	18
Sea cucumber	2
Ascidian	1
Cyanobacteria	17
Red algae	7
Green algae	5
Brown algae	1
Total	71

Figure 1: Places of collection of the marine samples from Saudi Red Sea territorial water. The map indicates the location of collected marine samples; different classes of the collected marine samples. (a) The map indicates the location of collected marine samples. (b) Different classes of the collected marine samples.

were cultured in 80% filtered seawater (filtered seawater diluted to 80% by deionized water) including penicillin G (20 μg/mL, ICN Biochemical) and streptomycin sulfate (30 μg/mL, Wako Pure Chemical Industries, Ltd.) at 25°C, by feeding them the diatom *Chaetoceros gracillis* (about 40×10^4 cells/mL). Larvae reached the cyprid stage in 5 days. The cyprids were collected and then stored at 4°C until use. The test samples were dissolved in ethanol. Aliquots of the solution were supplied to wells of 24-well polystyrene tissue culture plates and air-dried. 2 mL of 80% filtered seawater, and six 2-day-old cyprids were added to each well. Four wells were used for each concentration. The plates were kept in the dark for 48 h at 25°C, and the number of larvae that attached, metamorphosed, died, floated, or did not settle were counted under a microscope. The initial screening assays of the extracts were performed at 10 and 100 μg/mL and then the promising extracts were re-evaluated at 1.0, 10, and 100 μg/mL. For isolated compounds, each concentration was repeated 3 times. The antifouling activity of the isolated compounds after 48 hours was expressed as an EC_{50} value, indicating the concentration that reduces the larval settlement to 50% of the control. The EC_{50} values were calculated by a probit analysis. When a probit analysis could not be adopted, then graphical methods were used to decide the EC_{50} values.

2.4. Extraction and Isolation

2.4.1. Extraction of Samples for Screening. The dried material (5–10 g of each organism) was macerated and exhaustively extracted by MeOH (200 mL × 3). The combined extracts were concentrated under a reduced vacuum with a rotary evaporator until dry.

2.4.2. Isolation of Compounds **1**–**6** *from Sarcophyton glaucum (SH-21).* The fresh soft coral *S. glaucum* (5.0 kg) was minced and exhaustively extracted with a mixture of CH_2Cl_2 : MeOH (2 : 1 v/v, 24 hours for each batch, 22°C, 10 L × 3), and then the combined extracts were concentrated under vacuum and yielded a viscous blackish residue. The residue was partitioned between diethyl ether and water and then the organic layer was dried to give a yellowish material (30.0 g), which was fractionated on normal phase silica gel (NP-silica), eluted stepwise with *n*-hexane containing increasing amounts of diethyl ether, and then increased in polarity with EtOAc. One hundred fractions (F: 1–100) were collected. The fractions were investigated by a TLC pattern using a UV lamp and/or 50% sulfuric acid in methanol as a spraying reagent. The fraction F-3 eluted with *n*-hexane: diethyl ether (19 : 1, 300.0 mg) was purified by preparative TLC using the solvent system *n*-hexane: diethyl ether (19 : 1). The band with R_f = 0.60 (a violet-red color with sulfuric acid-methanol) was purified to yield compound **5** as colorless oil (9.0 mg). The fraction F-13 eluted with *n*-hexane: diethyl ether (9 : 1, 120.0 mg) was purified by preparative TLC using the solvent system n-hexane: diethyl ether (1 : 9), to give a band with R_f = 0.8 (a violet color with sulfuric acid-methanol) was purified to yield give compound **6** as colorless oil (10.0 mg). The fraction F-3 eluted with *n*-hexane: diethyl ether (19 : 1, 300.0 mg) was purified by preparative TLC using the solvent system *n*-hexane: diethyl ether (19 : 1), leading to compound **3** (5.0 mg). The fraction F-13 eluted with *n*-hexane: diethyl ether (4 : 1, 125.0 mg) was purified by preparative TLC using the solvent system n-hexane: diethyl ether (4 : 1), to give compound **4** (4.5 mg). The fraction F-41 eluted with *n*-hexane: EtOAC (9 : 1, 123.0 mg) was purified by preparative TLC using the solvent system *n*-hexane: ethylacetate (4 : 1), leading to compound **2** (12.0 mg). The fraction F-50 eluted with *n*-hexane: EtOAC (4 : 1, 70.0 mg) was purified by RP-18 HPLC (MeOH/H_2O, 65 : 35) to yield compound **1** (3.0 mg).

2.4.3. Characterization of Compounds Isolated from S. glaucum

(1) 7R,8S-Dihydroxydeepoxy-ent-sarcophine **(1)** [30]. Colorless crystals, $[\alpha]_D^{25}$ –125.0 (*c* 0.18, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 5.59 (1H, dd, *J* = 10.2, 5.4 Hz, H-2), 4.95 (1H, d, *J* = 10.2 Hz, H-3), 2.19 (2H, m, H_a-5), 1.97 (1H, m, H_b-5), 2.32 (1H, m, H_b-6), 2.11 (1H, m, H_b-6), 3.50 (1H, dd, *J* = 10.8, 1.2 Hz, H-7), 1.82 (1H, m, H_a-9), 1.76 (1H, m, H_b-9), 1.77 (1H, m, H_a-10), 1.29 (1H, m, H_b-10), 5.01 (1H, dd, *J* = 9.6, 4.8 Hz, H-11), 2.05 (1H, m, H_a-13), 2.00 (1H, m, H_b-13), 2.78 (H, ddd, *J* = 13.2, 5.4, 2.4 Hz, H_a-14), 2.13 (1H, ddd, *J* = 18.0, 9.6, 7.8 Hz, H_b-14), 1.80 (3H, s, H-17), 1.87 (3H, s, H-18), 1. 23 (3H, s, H-19), 1.61 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 175.3 (C, C-16), 163.2 (C, C-1), 144.4 (C, C-4), 134.9 (C, C- 12), 125.2 (CH, C-11), 123.0 (CH, C-15), 121.4 (CH, C-3), 79.5 (CH, C-2), 73.0 (CH, C-7), 75.6 (C, C-8), 56.8 (CH_2, C-9), 37.3 (CH_2, C-13), 35.8 (CH_2, C-5), 35.7 (CH_2, C-14), 27.1 (CH_2, C-6), 24.5 (CH_3, C-19), 23.8 (CH_2, C-10), 16.4 (CH_3, C-20), 15.6 (CH_3, C-18), 9.1 (CH_3, C-17).

(2) ent-Sarcophine **(2)** [31]. White amorphous, mp 134–136°C; $[\alpha]_D^{25}$ –82.0 (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 5.57 (1H, dd, *J* = 10.2, 1.2 Hz, H-2), 5.05 (1H, dd, *J* = 10.2, 1.2 Hz, H-3), 2.37 (2H, m, H_2-5), 1.90 (1H, m, H_a-6), 1.69 (1H, m, H_b-6), 2.68 (1H, dd, *J* = 4.8, 4.2 Hz, H-7), 2.10 (1H, m, H_a-9), 1.10 (1H, m, H_b-9), 2.26 (1H, m, H_a-10), 1.93 (1H, m, H_b-10), 5.17 (1H, dd, *J* = 9.6, 5.4 Hz, H-11), 2.18 (1H, ddd, *J* = 18.0, 12.6, 9.0 Hz, H_a-13), 2.01 (1H, ddd, *J* = 13.2, 10.8, 2.4 Hz, H_b-13), 2.78 (H, ddd, *J* = 13.2, 5.4, 2.4 Hz, H_a-14), 2.37 (1H, ddd, *J* = 18.0, 9.6, 7.8 Hz, H_b-14), 1.85 (3H, s, H-17), 1.89 (3H, s, H-18), 1. 28 (3H, s, H-19), 1.61 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 174.1 (C, C-16), 162.3 (C, C-1), 144.0 (C, C-4), 135.5 (C, C-12), 124.9 (CH, C-11), 122.9 (CH, C-15), 120.6 (CH, C-3), 78.2 (CH, C-2), 61.4 (CH, C-7), 59.9 (C, C-8), 39.0 (CH_2, C-9), 37.4 (CH_2, C-5), 36.4 (CH_2, C-13), 27.6 (CH_2, C-14), 25.2 (CH_2, C-6), 23.3 (CH_2, C-10), 17.1 (CH_3, C-19), 16.1 (CH_3, C-18), 15.4 (CH_3, C-20), 9.0 (CH_3, C-17).

(3) Guaiacophene (Guaia-5,7(11)-dien-8-one) **(3)** [32]. Colorless oil, $[\alpha]_D^{25}$–22.0 (*c* 0.10, $CHCl_3$);^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 2.38 (1H, m, H-1), 1.78 (1H, m, Ha-2), 1.70 (2H, m, Hb-2), 1.87 (1H, m, Ha-3), 1.34 (1H, m, Hb-3), 2.54 (1H, sext., *J* = 7.1 Hz, H-4), 6.13 (1H, s, H-6), 2.50 (1H, dd, *J* = 11.4, 6.6 Hz, Ha-9), 2.45 (1H, dd, *J* = 11.4, 7.2 Hz, Hb-9), 2.35 (1H, br. Sep., *J* = 6.8 Hz, H-10), 1.85 (3H, s, H-12), 1.86 (3H, s, H-13), 1. 17 (3H, d, *J* = 6.8 Hz, H-14), 0.93 (3H, d, *J* = 6.8 Hz, H-15); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 207.6 (C, C-8), 151.7 (C, C-5), 137.7 (C, C-7), 137.1 (C, C- 11), 116.9 (CH, C-6), 51.1 (CH_2, C-9), 45.2 (CH, C-1), 40.2 (CH, C-4), 36.7 (CH, C-10), 34.0 (CH_2, C-3), 29.2 (CH_2, C-2), 22.7 (CH_3, C-13), 21.5 (CH_3, C-12), 19.6 (CH_3, C-14), 16.8 (CH_3, C-15).

(4) Gorgosterol **(4)** [33]. White amorphous powder; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 1.10–2.30 (28H, m), 3.53 (1H, dddd, *J* = 10.8, 10.8, 6.6, 4.8 Hz, H-3), 5.37 (1H, br d, *J* = 2.4 Hz, H-6), 0.68 (3H, s, H-18), 1.02 (3H, s, H-19), 0.93 (3H, d, *J* = 6.6 Hz, H-21), 0.19 (1H, ddd, *J* = 11.4, 9.0, 6.0 Hz, H-22), 0.25 (1H, m, H-24), 0.85 (3H, d, *J* = 6.6 Hz, H-26), 0.79 (3H, d, *J* = 6.6 Hz, H-27), 0.85 (3H, d, *J* = 6.6 Hz, H-28), 0.47 (1H, dd, *J* = 9.0, 4.2 Hz, Ha-29), 0.10 (1H, dd, *J* = 9.0, 4.8 Hz, Hb-29), 0.89 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 141.0 (C, C-5), 121.9 (CH, C-6), 72.0 (CH, C-3), 56.9 (CH, C-14), 53.6 (CH, C-17), 51.0 (CH, CH-5), 50.9 (CH, C-9), 43.0 (C, C-13), 42.5 (C, C-4), 39.7 (CH_2, C-12), 37.3 (CH_2, C-1), 36.4 (C, C-10), 35.2 (CH, C-20), 32.2 (CH, C-22), 32.2 (CH, C-25), 31.9 (CH_2, C-7), 31.7 (CH, C-8), 28.8 (CH_2, C-16), 26.0 (C, C-23), 25.0 (CH_2, C-15), 22.4 (CH_3, C-21), 21.9 (CH_3, C-26), 21.8 (CH_3, C-30), 21.6 (CH_3, C-27),

21.1 (CH_2, C-11), 19.6 (CH_3, C-19), 16.0 (CH_3, C-28), 14.7 (CH_2, C-29), 12.0 (CH_3, C-18).

(5) Guaia-5,11-dien **(5)** [32]. Colorless oil, $[\alpha]_D^{25}$ –19.0 (*c* 0.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 2.70 (1H, m, H-1), 1.52 (1H, m, Ha-2), 1.35 (2H, m, Hb-2), 1.50 (1H, m, Ha-3), 1.00 (1H, m, Hb-3), 2.30 (1H, dt, *J* = 13.2, 6.6 Hz, H-4), 5.19 (1H, dd, *J* = 7.2, 3.0 Hz, H-6), 2.62 (1H, m, H-7), 1.60 (1H, m, Ha-8), 1.50 (2H, m, Hb-8), 1.64 (1H, m, Ha-9), 1.20 (1H, m, Hb-9), 1.66 (H, m, H-10), 4.57 (H, brs, H_a-12), 4.56 (H, brs, H_b-12), 1.52 (3H, s, H-13), 0.81 (3H, d, *J* = 6.6 Hz, H-14), 0.64 (3H, d, *J* = 6.6 Hz, H-15); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 150.8 (C, C-5), 147.2 (C, C-11), 122.4 (CH, C-6), 110.8 (CH_2, C- 12), 47.4 (CH, C-7), 45.4 (CH, C-1), 41.0 (CH, C-4), 34.0 (CH_2, C-3), 30.2 (CH_2, C-2), 25.9 (CH_2, C-8), 21.6 (CH_3, C-13), 19.9 (CH_3, C-14), 19.6 (CH_3, C-15), 15.7 (CH_3, C-14).

(6) Calamenene **(6)** [34]. Yellow oil; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 2.26 (1H, m, H-1), 1.98 (1H, m, Ha-2), 1.36 (2H, m, Hb-2), 1.85 (1H, m, Ha-3), 1.65 (1H, m, Hb-3), 2.34 (1H, m, H-4), 7.05 (1H, s, H-5), 7.16 (1H, d, *J* = 7.8 Hz, H-7), 6.99 (1H, d, *J* = 7.8 Hz, H-8), 1.34 (2H, d, *J* = 7.2 Hz, H-11), 2.79 (1H, m, H-12), 0.75 (3H, d, *J* = 7.2 Hz, H-13), 1.03 (3H, d, *J* = 7.2 Hz, H-14), 2.33 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 140.3 (C, C-9), 140.1 (C, C-10), 134.7 (C, C-6), 129.0 (CH, C- 5), 127.0 (CH, C-8), 126.4 (CH, C-7), 43.0 (CH, C-4), 32.5 (CH, C-12), 30.8 (CH, C-1), 30.5 (CH_2, C-2), 22.3 (CH_3, C-11), 21.3 (CH_3, C-14), 21.1 (CH_3, C-15), 17.4 (CH_3, C-13).

2.4.4. Isolation of Compounds **7**–**12** *from Sarcophyton trocheliophorum (SH-13).* Soft coral material was washed with water and dried in the shade at room temperature. The dried material (79.0 g) was exhaustively extracted with equal volumes of $CHCl_3$/MeOH (2 × 6 L, 24 h for each batch) at room temperature. The residue (20.2 g) was partitioned between CH_2Cl_2 and water; the CH_2Cl_2 soluble material was dried to give a brownish oily material (9.2 g) and chromatographed on NP (Merck, 60G) column chromatography employing *n*-hexane/CH_2Cl_2, followed by EtOAc/MeOH mixtures with increasing polarity. Fractions of ~100 mL were collected. TLC was carried out by employing silica gel chromatoplates, appropriate solvent system, and 50% H_2SO_4 in MeOH as spraying reagent. Fractions containing a single compound were combined and further purified by preparative TLC of glass-supported silica gel plates (20 cm × 20 cm) of 250 μm thickness. The fraction eluted with *n*-hexane: methylene chloride (19 : 1, 37.0 mg) was purified by preparative TLC using solvent system *n*-hexane: methylene chloride (9 : 1). The fraction eluted with *n*-hexane (9 : 1, 36.0 mg) was purified by preparative TLC using solvent system *n*-hexane: methylene chloride (4 : 1). The band with R_f = 0.70 (Violet appearance under UV_{254}, and brown color with H_2SO_4-MeOH) was taken to give compound **7** as colorless oil (5.3 mg, 0.0067% dry weight). The fraction eluted with *n*-hexane: methylene chloride (7 : 3, 125.0 mg) was purified by preparative TLC using the solvent system *n*-hexane: diethyl ether (8 : 2), to give two bands. The first band with R_f = 0.71 (violet color with sulfuric acid-methanol) was taken to give colorless oil (16.0 mg) of compound **10**. The fraction eluted with *n*-hexane: EtOAC (9 : 1, 123.0 mg) was purified by preparative TLC using the solvent system *n*-hexane: ethylacetate (8 : 2). The band with R_f = 0.50 (reddish color with sulfuric acid-methanol) was taken to give colorless oil (12.0 mg) of compound **11**. The fraction eluted with *n*-hexane: EtOAC (8 : 2, 70.0 mg) was purified by RP-18 HPLC (MeOH/H_2O, 65 : 35) yielded compound **12** (3.0 mg). The fraction eluted with CH_2Cl_2 : MeOH (9 : 1, 35.0 mg) was purified by preparative TLC using the solvent system *n*-hexane: EtOAc (1 : 4) to give two bands. The first band with R_f = 0.38 (brown color with H_2SO_4-MeOH) was taken to give colorless oil (3.5 mg) of compound **8**. The second band with R_f = 0.35 (brown color with H_2SO_4-MeOH) was taken to give colorless oil (3.6 mg) of compound **9**.

2.4.5. Characterization of Compounds Isolated from S. trocheliophorum

(1) Trocheliane **(7)** [35]. Gummy material; $[\alpha]_D^{20}$—22.0 (c 0.02, C_6H_6); IR $_{max}$ (film) cm^{-1}: 3050–2700 (CH), 1630, 1620 (C=C), 1510, 925, 740; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 2.01–2.05 (1H, m, H-2), 2.15–2.20 (1H, m, H-3a), 2.10–2.15 (1H, m, H-3b), 4.98 (1H, ddd, *J* = 12.0, 6.0, 1.2 Hz, H-4), 1.98–2.04 (1H, m, H-6a), 1.56–1.58 (1H, m, H-6b), 1.92–1.94 (1H, m, H-7a), 1.32–1.36 (1H, m, H-7b), 5.19 (1H, ddd, *J* = 15.0, 7.2, 1.2 Hz, H-8), 1.64–1.70 (1H, m, H-10a), 1.30–1.33 (1H, m, H-10b), 1.92–1.98 (1H, m, H-11a), 1.76–1.80 (1H, m, H-11b), 5.06 (1H, ddd, *J* = 12.6, 6.6, 1.2 Hz, H-13), 2.01–2.10 (1H, m, H-14a), 1.44–1.48 (1H, m, H-14b), 2.20–2.26 (1H, m, H-15), 0.71 (3H, d, *J* = 6.6 Hz, H-16), 0.99 (3H, d, *J* = 6.6 Hz, H-17), 1.57 (3H, s, H-18), 1.59 (3H, s, H-19), 1.00 (3H, s, H-20), 2.68–2.69 (1H, m, H-21), 1.81–1.86 (1H, m, H-22a), 1.57–1.61 (1H, m, H-22b), 2.12 (1H, d, *J* = 6.6 Hz, H-24a), 2.06 (1H, d, *J* = 6.6 Hz, H-24b), 7.02 (1H, s, H-26), 6.94 (1H, br d, *J* = 7.8 Hz, H-28), 7.11 (1H, br d, *J* = 7.8 Hz H-29), 2.75 (1H, hex, H-31), 1.31–1.39 (1H, m, H-32a), 1.21–1.29 (1H, m, H-32b), 1.26–1.30 (1H, m, H-33a), 1.30–1.32 (1H, m, H-33b), 2.20–2.23 (1H, m, H-36a), 2.16–2.20 (1H, m, H-36b), 1.56 (3H, s, H-37), 4.65 (1H, d, *J* = 2.4 Hz, H-38a), 4.70 (1H, d, *J* = 2.4 Hz, H-38b), 2.32 (3H, s, H-39), 1.26 (3H, d, *J* = 6.6 Hz, H-40), ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C = 39.9 (C-1), 45.9 (C-2), 38.9 (C-3), 125.9 (C-4), 133.9 (C-5), 32.4 (C-6), 30.8 (C-7), 124.0 (C-8), 140.0 (C-9), 28.1 (C-10), 33.9 (C-11), 149.3 (C-12), 121.8 (C-13), 39.4 (C-14), 31.8 (C-15), 17.3 (C-16), 21.3 (C-17), 15.5 (C-18), 15.3 (C-19), 16.6 (C-20), 43.7 (C-21), 21.4 (C-22), 150.3 (C-23), 23.7 (C-24), 139.9 (C-25), 128.7 (C-26), 134.4 (C-27), 126.1 (C-28), 126.7 (C-29), 134.8 (C-30), 32.5 (C-31), 29.6 (C-32), 22.7 (C-33), 128.7 (C-34), 133.4 (C-35), 24.8 (C-36), 18.0 (C-37), 110.1 (C-38), 21.1 (C-39), 22.3 (C-40).

(2) Sarcotrocheldiol A **(8)** [35]. Colorless oil; $[\alpha]_D^{20}$ 62.4 (*c* 0.012, $CHCl_3$); IR $_{max}$ (film) cm^{-1}: 3423 (OH), 3180 (OH), 2937 (C–H), 1645 (C=C), 1378, 1221, 1045; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H = 1.34–1.36 (1H, m, H-1), 4.62 (1H,

dd, $J = 10.8$, 4.8 Hz, H-2), 5.28 (1H, d, $J = 10.8$ Hz, H-3), 2.77 (1H, dd, $J = 12.0$, 10.8 Hz, H-5a), 2.56 (1H, dd, $J = 12.0$, 4.2 Hz, H-5b), 5.87 (1H, $J =$ ddd, 15.6, 10.8, 4.2 Hz, H-6), 5.41 (1H, br d, $J = 15.6$ Hz, H-7), 1.86–1.88 (1H, m, H-9a), 1.54–1.56 (1H, m, H-9b), 1.74–1.76 (1H, m, H-10a), 1.26–1.30 (1H, m, H-10b), 3.48 (1H, d, $J = 9.6$ Hz, H-11), 1.62–1.64 (1H, m, H-13a), 1.35–1.36 (1H, m, H-13b), 2.28–2.32 (1H, m, H-14a), 1.20–1.22 (1H, m, H-14b)1.21–1.23 (1H, m, H-15), 0.88 (3H, d, $J = 6.6$ Hz, H-16), 0.76 (3H, d, $J = 6.6$ Hz, H-17), 1.83 (3H, s, H-18), 1.33 (3H, s, H-19), 1.04 (3H, s, H-20), ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta_C = 45.6$ (CH, C-1), 72.0 (CH, C-2), 124.4 (CH, C-3), 141.1 (C, C-4), 42.5 (CH_2, C-5), 128.2 (CH, C-6), 135.7 (C, C-7), 73.6 (C, C-8), 39.9 (CH_2, C-9), 22.8 (CH_2, C-10), 75.8 (CH_2, C-11), 74.9 (CH_2, C-12), 18.6 (C, C-13), 34.1 (CH, C-14), 29.4 (CH, C-15), 20.7 (CH_3, C-16), 20.5 (CH_3, C-17), 17.6 (CH_3, C-18), 29.3 (CH_3, C-19), 23.8 (CH_3, C-20).

(3) Sarcotrocheldiol B **(9)** [35]. Colorless oil $[\alpha]_D^{20}$ 89.1 (c 0.010, $CHCl_3$); IR $_{max}$ (film) cm^{-1}: 3383 (OH), 3180 (OH), 2937 (C–H), 1645 (C=C), 1378, 1221, 1045; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta_H = 1.29$–1.32 (1H, m, H-1), 4.52 (1H, $J =$ dd, 10.8, 5.4 Hz, H-2), 5.23 (1H, d, $J = 10.8$ Hz, H-3), 2.13–2.15 (1H, m, H-5a), 2.17–2.19 (1H, m, H-5b), 1.88–1.90 (1H, m, H-6a), 2.12–2.15 (1H, m, H-6b), 3.88 (1H, dd, $J = 10.8$, 1.2 Hz, H-7), 2.48–2.51 (1H, m, H-9a), 2.28–2.32 (1H, m, H-9b), 1.95–1.97 (1H, m, H-10a), 1.28–1.30 (1H, m, H-10b), 3.76 (1H, d, $J = 9.6$ Hz, H-11), 1.62–1.64 (1H, m, H-13a), 1.38–1.40 (1H, m, H-13b), 2.26–2.28 (1H, m, H-14a), 1.26–1.28 (1H, m, H-14b), 1.18–1.20 (1H, m, H-15), 0.86 (3H, d, $J = 6.6$ Hz, H-16), 0.71 (3H, d, $J = 6.6$ Hz, H-17), 1.73 (3H, s, H-18), 5.07 (1H, br d, $J = 1.2$ Hz, H-19a), 5.06 (1H, br d, $J = 1.2$ Hz, H-19b), 1.05 (1H, s, H-20), ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta_C = 45.9$ (CH, C-1), 70.4 (CH, C-2), 125.3 (CH, C-3), 137.7 (C, C-4), 36.4 (CH_2, C-5), 30.1 (CH_2, C-6), 67.8 (CH, C-7), 147.5 (C-8), 30.9 (CH_2, C-9), 27.1 (CH_2, C-10), 70.6 (CH, C-11), 74.6 (C, C-12), 18.6 (CH_2, C-13), 33.7 (CH_2, C-14), 29.0 (CH_3, C-15), 20.7 (CH_3, C-16), 20.3 (CH_3, C-17), 14.2 (CH_3, C-18), 112.4 (CH_3, C-19), 23.5 (CH_3, C-20).

(4) Deoxosarcophine **(10)** [36]. Colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta_H = 5.54$ (1H, brd, $J = 9$ Hz, H-2), 5.23 (1H, brd, $J = 9$ Hz, H-3), 2.35 (1H, m, H-4), 1.90 (1H, m, Ha-5), 1.63 (1H, m, Hb-5), 2.7 (H, t, $J = 7.2$ Hz, H-6), 2.10 (1H, m, Ha-8), 1.00 (1H, m, Hb-8), 2.25 (1H, m, Ha-9), 1.9 (1H, m, Hb-9), 5.10 (1H, dd, $J = 6$, 4.8 Hz, H-10), 2.55 (1H, m, Ha-12), 1.91 (1H, m, Hb-12), 1.66 (1H, m, H-13), 4.50 (2H, m, H-16), 1.65 (3H, s, H3-17), 1.61 (3H, s, H3-18), 1.27 (3H, s, H3-19), 1.83 (3H, br s, H-20); ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta_C = 128.0$ (C, C-14), 83.7 (CH, C-1), 126.3 (CH, C-2), 139.5 (C, C-3), 38.0 (CH_2, C-4), 25.3 (CH_2, C-5), 62.0 (CH, C-7), 60.0 (C, C-7), 23.5 (CH_2, C-8), 39.9 (CH_2, C-9), 123.6 (CH, C-10), 136.8 (C, C-11), 36.9 (CH_2, C-13), 26.1 (CH_2, C-14), 131.4 (C, C-15), 78.3 (CH_2, C-16), 10.2 (CH_3, C-17), 15.1 (CH_3, C-18), 16.9 (CH_3, C-19), 15.6 (CH_3, C-20).

(5) Sarcotrocheliol **(11)** [37]. Colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta_H = 1.32$ (H, m, H-1), 4.53 (1H, dd, $J = 10.2$, 5.4 Hz, H-2), 5.27 (1H, d, $J = 10.2$ Hz, H-3), 2.19 (1H, m, Ha-5), 1.97 (1H, m, Hb-5), 2.32 (1H, m, Ha-6), 2.11 (1H, m, Hb-6), 5.00 (1H, dd, $J = 10.2$, 5.4 Hz, H-7), 2.40 (1H, m, Ha-9), 1.97 (1H, m, Hb-9), 1.77 (1H, m, Ha-10), 1.29 (1H, m, Hb-10), 3.88 (1H, d, $J = 9.6$ Hz, H-11), 1.60 (1H, m, Ha-13), 1.40 (1H, m, Hb-13), 2.36 (1H, m, Ha-14), 1.22 (1H, m, Hb-14), 1.18 (1H, m, H-15), 0.88 (3H, d, $J = 6.6$ Hz, H-16), 0.73 (3H, d, $J = 6.6$ Hz, H-17), 1.65 (3H, s, H-18), 1.61 (3H, s, H-19) 1.02 (3H, s, H-20) : ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta_C = 46.6$ (CH, C-1), 71.1 (CH, C-2), 125.4 (CH, C-3), 138.6 (C, C-4), 39.9 (CH_2, C-5), 25.2 (CH_2, C-6), 124.1 (CH, C-7), 136.0 (C, C-8), 33.7 (CH_2, C-9), 18.7 (CH_2, C-10), 31.9 (CH_2, C-11), 35.2 (CH_2, C-12), 75.0 (C, C-13), 71.9 (C, C-14), 29.0 (CH, C-15), 20.8 (CH_3, C-16), 20.3 (CH_3, C-17), 24.2 (CH_3, C-18), 17.3 (CH_3, C-19), 15.1 (CH_3, C-20).

(6) Sarcotrocheliol Acetate **(12)** [37]. Colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta_H = 1.26$ (1H, m, H-1), 4.52 (1H, dd, $J = 10.8$, 5.4 Hz, H-2), 5.50 (1H, d, $J = 10.8$ Hz, H-3), 2.15 (1H, m, H-5_a), 2.10 (1H, m, H-5_b), 2.30 (1H, m, H-6_a), 2.07 (1H, m, H-6_b), 5.05 (1H, dd, $J = 10.2$, 4.8 Hz, H-7), 1.97 (1H, m, H-9_a), 1.63 (1H, m, H-9_b), 1.66 (1H, m, H-10_a), 1.50 (1H, m, H-10_b), 5.37 (1H, d, $J = 10.2$ Hz, H-11), 1.51 (1H, m, H-13_a), 1.23 (1H, m, H-13_b), 1.77 (1H, m, H-14_a), 1.28 (1H, m, H-14_b), 1.17 (1H, m, H-15), 0.69 (3H, d, $J = 6.6$ Hz, H-16), 0.82 (3H, d, $J = 6.6$ Hz, H-17), 1.62 (3H, s, H-18), 1.56 (3H, s, H-19), 1.04 (3H, s, H-20), 2.06 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta_C = 171.0$ (C, C=O), 139.0 (C, C-4), 135.0 (C, C-8), 125.3 (CH, C-3), 124.5 (CH, C-7), 73.7 (C, C-12), 73.5 (CH, C-11), 71.4 (CH, C-2), 46.5 (CH, C-1), 39.8 (CH_2, C-5), 34.4 (CH_2, C-9), 34.3 (CH_2, C-14), 29.0 (CH_2, C-10), 29.0 (CH, C-15), 25.4 (CH_3, C-20), 25.3 (CH_2, C-6), 20.7 (CH_3, C-17), 20.3 (CH_3, C-16), 19.0 (CH_2, C-14), 17.0 (CH_3, C-19), 15.0 (CH_3, C-18), 21.3 (CH_3, CH_3CO).

3. Results and Discussion

Extracts of seventy-one marine organisms, representing seven different classes of marine fauna and flora, were assessed for their antifouling activity. These organisms were taxonomically identified, and their descriptions are listed in Table S1. Figure 1 illustrates the places of collection of the Red Sea samples. The samples were dried by standard methods and extracted with methanol. The extracts were divided into two batches and evaluated for their antifouling effects. The antifouling effects are listed in Tables S2, 1 and 2. The first screening batch is illustrated in Figure S1, and the second batch is illustrated in Figures S2–S5. Analysis of the antifouling activity indicated that the value settlement rates and mortality range from 0–100%.

Thirty-four samples are considered to be active (Figure S6). These samples consist of five classes: sponges, algae, tunicate, soft corals, and cyanobacteria. Four samples were highly potent at 1 μg/mL (Figure S7) with a percentage of settlement inhibition lower than 31%; thirteen samples were potent at 10 μg/mL with a percentage of settlement inhibition between 16 and 30%, and nineteen samples were potent at 10 μg/mL with a percentage of settlement inhibition between 0 and 15%. Potent antifouling activities were exhibited by *Siphonochalina siphonella*, *Sarcophyton*

Table 1: Antifouling activity of the promising marine organisms.

Sample no.	Classes	Name of species	Settlement rate[1]	Mortality[2]
SH-03	Sponges	*Siphonochalina siphonella*	0	0
SH-07		*Hyrtios erectus*	29	0
SH-26		*Halichlona* sp. 2	15	0
SH-29		*Hyrtios* sp.	0	40
SH-36		*Dysideid sponge*	9	36
SH-40		*Callyspongia sp.*	12	10
SH-41		*Callyspongia sp.*	29	16
SH-04	Soft corals	*Sinularia polydactyla*	40	0
SH-11		*Cespitularia sp.*	0	0
SH-13		*Sarcophyton trocheliophorum*	0	0
SH-21		*Sarcophyton glaucum*	0	4
SH-23		*Dendronephytia sp.*	25	0
SH-24		*Sinularia leptoclades*	7	0
SH-54		*Sinularia* sp. 1	23	0
SH-55		*Sinularia* sp. 2	0	0
SH-56		*Lobophyton* sp.	19	0
SH-58		Soft coral K	22	0
SH-60		*Dendronephytia*	18	0
SH-61		*Sponge N*	16	0
SH-62		*Strenophyta*	24	0
SH-35	Sea firs	*Sertularia* sp.	9	2
SH-42	Tunicates	*Ascidian sp.*	28	0
SH-46	Cyanobacteria	*Symploca* sp.	1	88
SH-47		Blue-green algae	9	0
SH-48		Blue-green algae	2	90
SH-49		Blue-green algae	0	49
SH-50		Blue-green algae	20	67
SH-52		Blue-green algae	21	25
SH-65		Blue-green algae	6	0
SH-66		Blue-green algae	9	18
SH-67		Blue-green algae	19	79
SH-34	Algae	*Halimeda tuna*	12	15
SH-37		*Laurencia* sp.	25	17
Control[3]	—	—	92	2

[1]% settlement rates: 48 hr exposing the larvae to extracts at a concentration of 10 μg/mL. [2]% mortality rates: 120 hr exposing the larvae to extracts at a concentration of 10 μg/mL. [3]Control (no samples) of 4 times repeated.

Table 2: Statistical analysis of the bioactive marine organisms.

Marine classes	Marine classes	Tested sample	Active sample[1]
Algae	13	13	1
Sponges	19	19	3
Soft corals	18	18	8
Cyanobacteria	17	17	3
Others	4	4	1
Total	71	71	16

[1]Those which showed under 20% larval settlement rates and low toxicity at 20 μg/well (<20% lethality).

glaucum, *Sinularia leptoclades*, and *Hyrtios* species. They showed 90% settlement inhibition against barnacle cyprids' larvae at 10 μg/mL. In particular, *Hyrtios* sp. showed less toxicity to barnacle larva even at 100 μg/mL, which suggests environmentally benign and potent antifouling compounds (Table 1).

The chromatographic investigation of the extracts indicated that the diversity of the metabolites was distributed among the soft coral, sponges, and algal samples. Five samples were selected for further investigation: *Sarcophyton glaucum*, *Sarcophyton trocheliophorum*, *Hyrtios* sp., *Laurencia obtuse*, and *Siphonochalina siphonella*. Based on the combination of their antifouling results and their historical productivity recorded in the literature, genus *Sarcophyton* are recognized as a rich source of macrocyclic cembrane-type diterpenoids and biscembranoids. To date, more than 300 natural cembranoid derivatives have been reported. Cembrane-type diterpenoids are a large family with diverse functions that are obtained from both terrestrial and marine organisms. They usually exhibit cyclic ether, lactone, or furan moieties linked to the cembrane framework. The cembrane derivatives play an important role from a biomedical perspective [38–44].

Hyrtios sp. showed promising antifouling activities, and their reported chemical profile indicated that they are a rich source of bioactive compounds such as sesterterpenes [45], sesquiterpenes [46], and macrolides. In addition to these metabolites, they produce indole and β-carboline alkaloids [47, 48]. Unfortunately, the quantity of the collected sample was not sufficient for chemical investigation, even after several attempts to collect the same *Hyrtios* sp. Finally, the

Figure 2: Compounds isolated from *Sarcophyton glaucum* **1**–**6**.

antifouling activities of the remaining two samples (*Laurencia obtusa* and *Siphonochalina siphonella*) have been previously reported [49].

In the current study, processing of two soft coral samples (*Sarcophyton glaucum* (SH-21) and *Sarcophyton trocheliophorum* (SH-13)) was also presented. Investigation of *S. glaucum* (SH-21) has been done by employing different chromatographic techniques and led to isolation of 7*R*,8*S*-dihydroxydeepoxy-ent-sarcophine (**1**), *ent*-sarcophine (**2**), guaiacophine (guaia-5,7(11)-dien-8-one) (**3**), gorgosten-5(E)-3*β*-ol (**4**), guaia-5,11-dien (**5**), and calamenene (**6**) (Figure 2). *S. trocheliophorum* (SH-13) was fractionated and led to isolation of trocheliane (**7**), sarcotrocheldiol A (**8**) and sarcotrocheldiol B (**9**), deoxosarcophine (**10**), sarcotrocheliol (**11**), and sarcotrocheliol acetate (**12**) (Figure 3). All isolated compounds (**1**–**12**) [30–37] have been evaluated for their antifouling activities, and the results are shown in Figure 4.

It is important to emphasize that the isolated compounds showed antifouling effects which registered at 48 and 120 hours, which gives us a better idea of their toxicity. All the compounds, except **4**, **6**, and **10**–**12**, showed EC_{50} values more than 10 *μ*g/mL. The EC_{50} values of the promising compounds (**4**, **6**, and **10**–**12**) were in the 1.1–2.9 *μ*g/mL range at 48 hours, while their EC_{50} values were in the 1.24–10 *μ*g/mL range at 120 hours, as illustrated in Figure 4. Gorgosterol (**4**) showed EC_{50} values of 1.69 *μ*g/mL at 48 hours and 10 *μ*g/mL at 120 hours, respectively. It is a C-30 steroid, which is isolated amongst the metabolites from *Sarcophyton glaucum*. Its antifouling activity is more potent than the similar reported compounds. It is important to mention here that an isolated steroid from the gorgonian coral *Subergorgia suberosa* inhibited the settlement of *Balanus neritina* larvae with EC_{50} values of 6.25 and 7.8 *μ*g/mL, respectively, and $LD_{50} > 250$ *μ*g/mL [50].

Calamenene (**6**) showed EC_{50} values of 2.79 *μ*g/mL at 48 hours and 9.35 *μ*g/mL at 120 hours, respectively. It is sesquiterpenoidal in nature. The obtained antifouling results of compound **6** are in good agreement with the reported activity of sesquiterpenes [23]. Compound (**6**) could be different in functionality from the reported antifouling sesquiterpenes while still keeping its terpenoidal scaffold. Chamigrene sesquiterpene is one of well-known potent antifouling metabolites, which was isolated from the red alga *Laurencia elata*; unfortunately, a leak in the supply prevented its development to biopaints. Moreover, avarol and avarone are sesquiterpenoidal derivatives which were isolated from *Dysidea avara*. They exhibited antifouling against cyprids of the barnacle *Balanus amphitrite*, with ED_{50} values of 0.65 and 3.41 mg/mL, respectively, but their toxicity was low (LD_{50} 13.3 and 27.2 mg/mL, respectively, against cyprids and 1.58 and 25.2 mg/mL, respectively, against nauplii) [23].

The compounds deoxosarcophine (**10**), sarcophytolol (**11**), and sarcotrocheliol acetate (**12**) showed EC_{50} values of 1.6, 1.12, and 2.9 *μ*g/mL at 48 hours and 1.8, 1.24, and 2.78 *μ*g/mL at 120 hours, respectively. These compounds are macrocyclic cembranoid in nature. Our results are closely similar to the previously published cembranoidal derivatives. For instance, pukalide, a furanocembranoid diterpene reported originally from the soft coral *Sinularia abrupta* and also from the gorgonian *Leptogorgia virgulata*, displayed potent inhibition towards the larval settlement of *Balanus amphitrite* larvae with $EC_{50} = 19$ ng/mL [50–52]. The cembranoid alcohols, sinulariols J, P, and Y, isolated from the soft coral *Sinularia rigida* showed potent antifouling effects against the larval settlement of *B. amphitrite* and *Bugula neritina* larvae with $EC_{50} < 14.03$ *μ*g/mL [53]. Pavidolides C and D, cembranoids with unusual C-5-C-9 and C-3-C-7 linkages, respectively, reported from the soft coral *Sinularia pavida*, have been shown to inhibit settlement of *B. amphitrite* larvae with ED_{50} values of 4.32 and 2.12 *μ*g/mL, respectively, and low cytotoxicity ($LD_{50} > 50$ *μ*g/mL) [54]. Fortunately, our findings show that deoxosarcophine (**10**) and sarcophytolol (**11**) are more potent than the aforementioned published compounds. The presence of some functionality such as epoxide, lactone, and a macrocyclic ring may play an important role in their effects.

Figure 3: Compounds isolated from *Sarcophyton trocheliophorum* **7**–**12**.

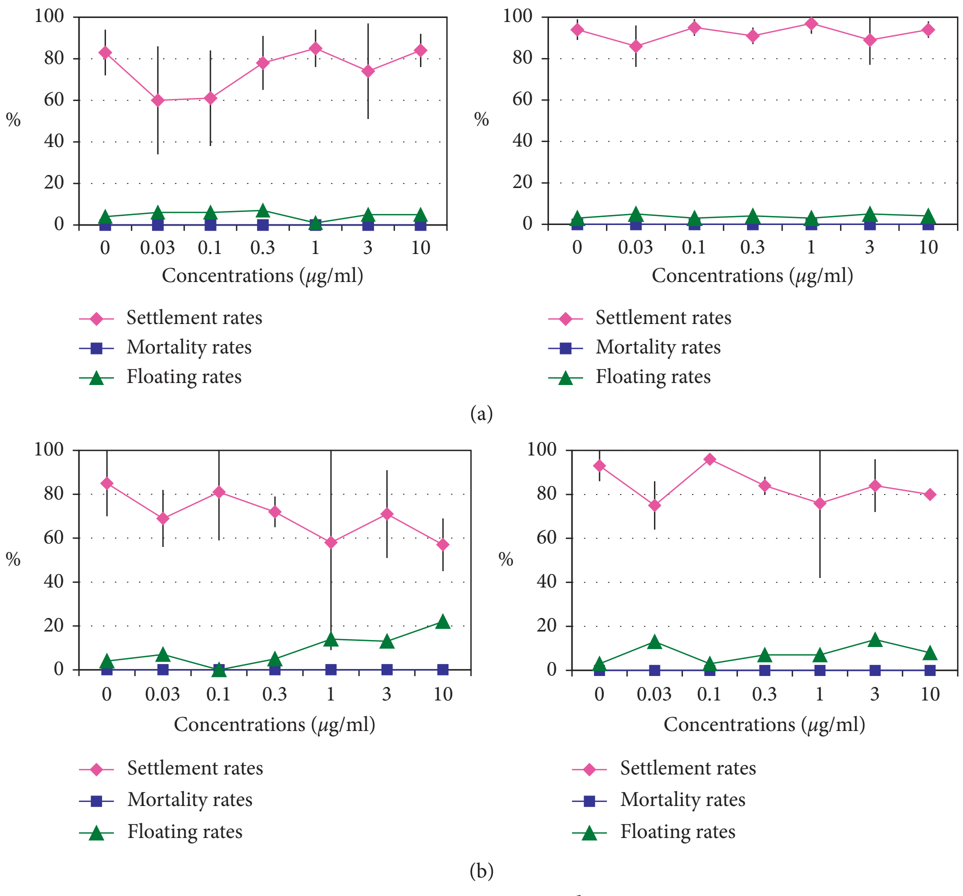


Figure 4: Continued.

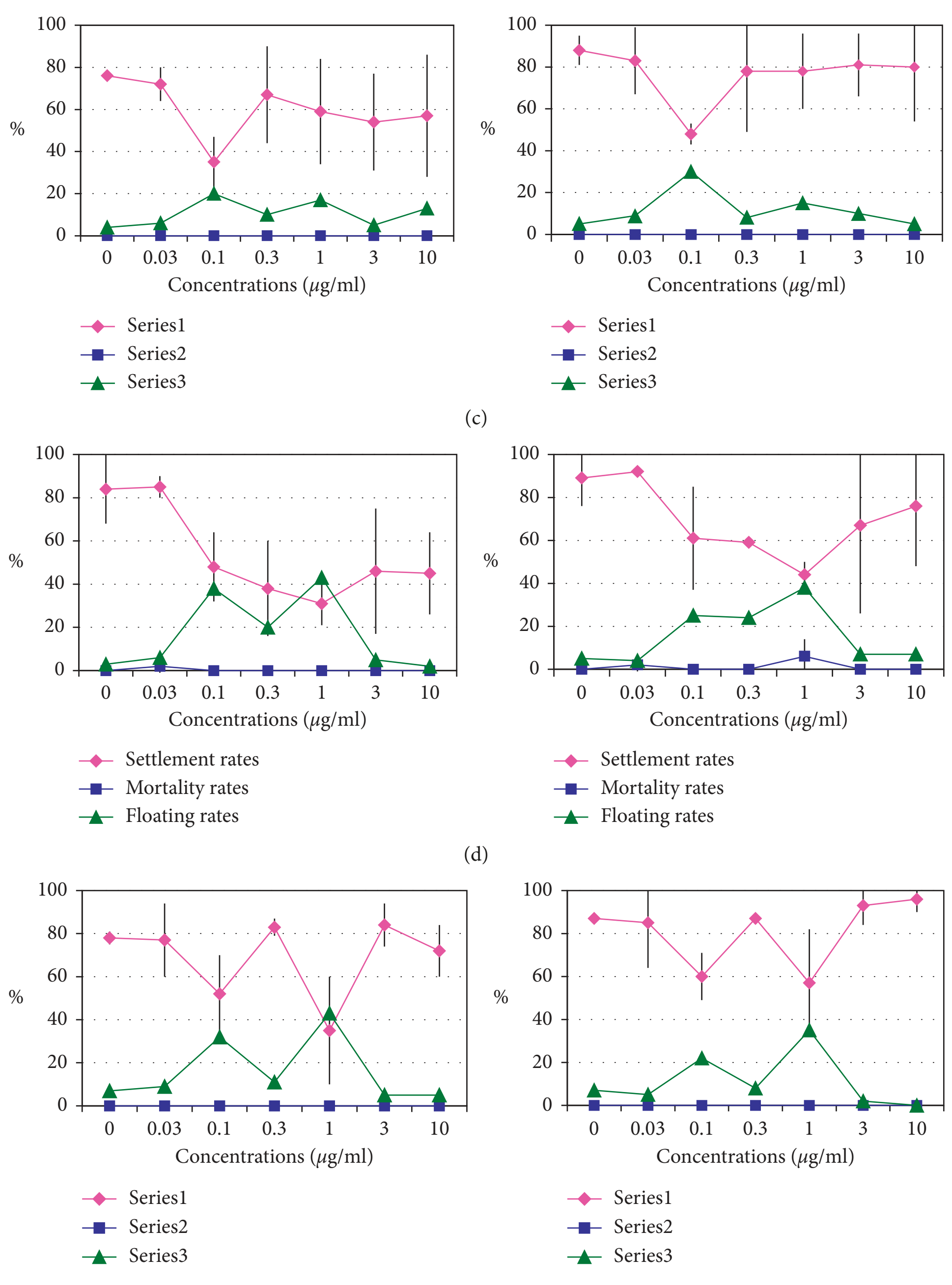
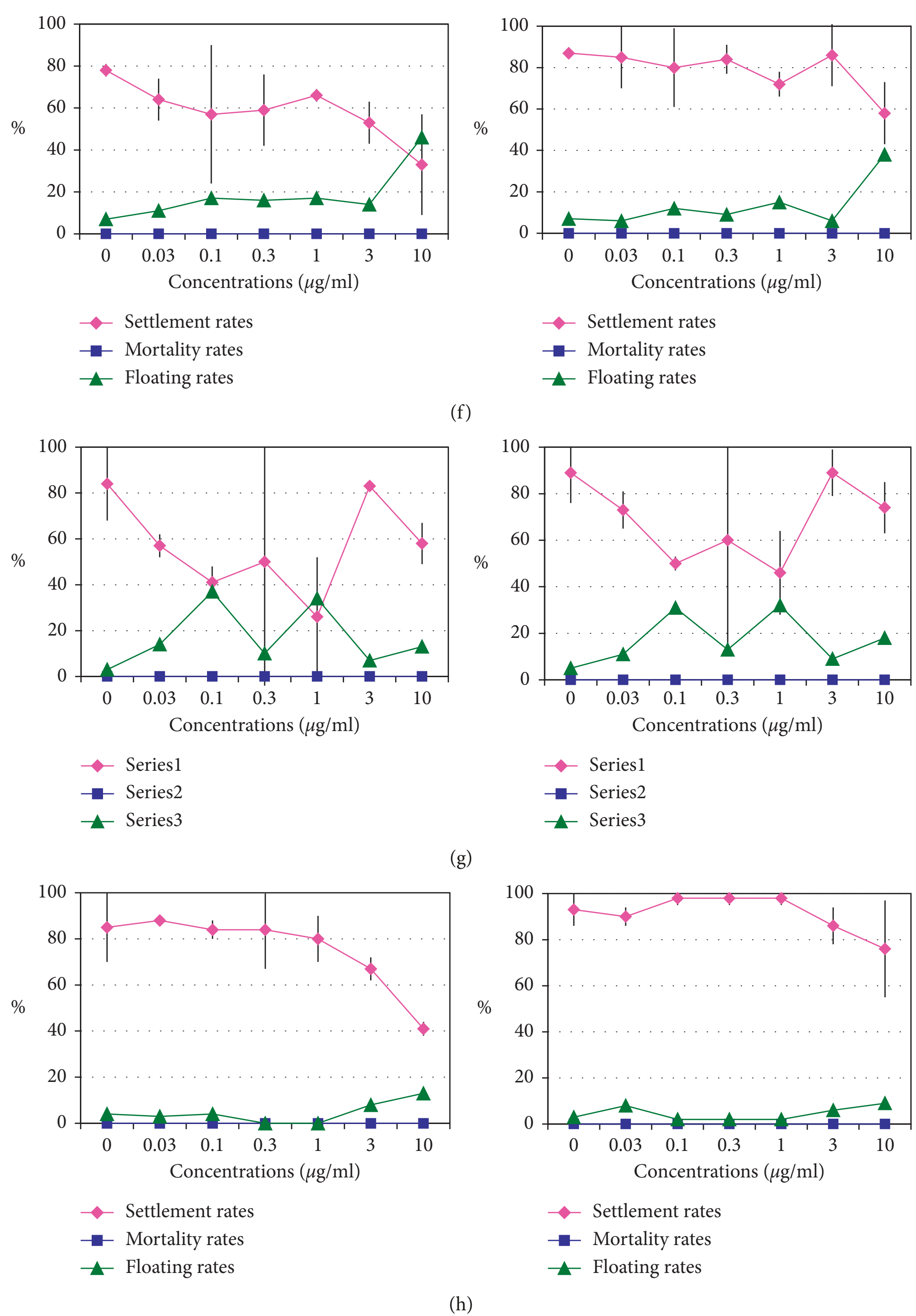


Figure 4: Continued.



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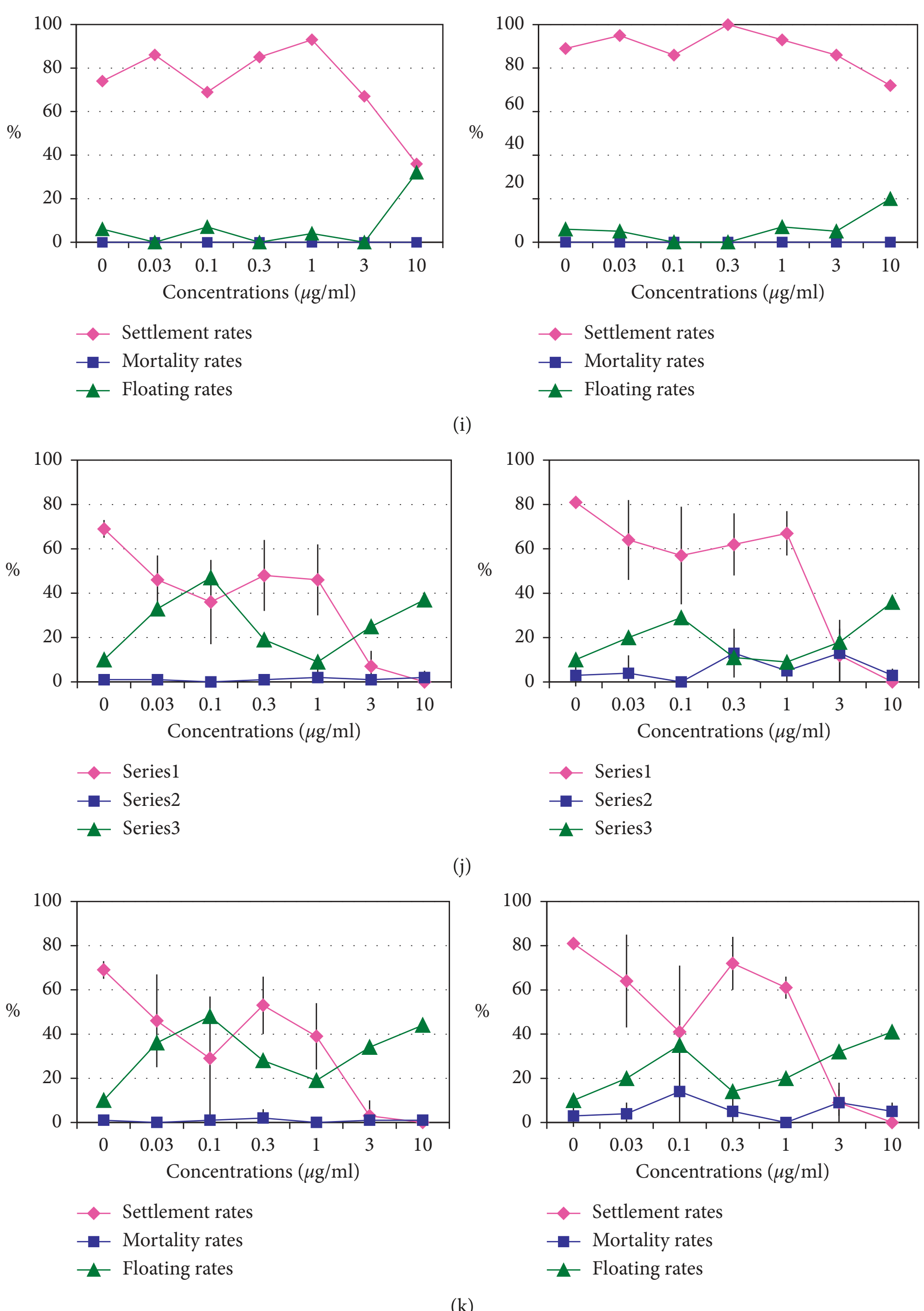

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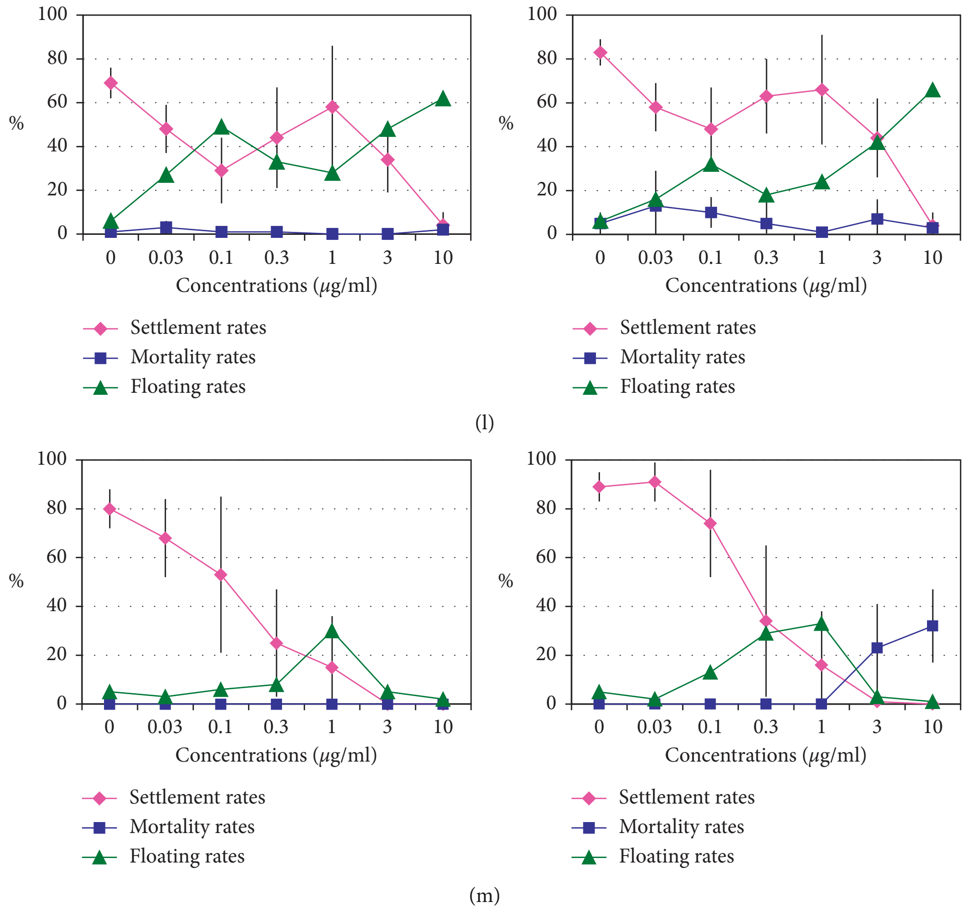

Figure 4: Antifouling activity of isolated compounds **1–12**.

Moreover, their potency is comparable to that of 12-hydroxyisolaurene, which was reported in our previous paper on antifouling metabolites from Red Sea organisms [41]. A recent review listed ten additional antifouling cembranoids against barnacle larvae [55].

Several studies have investigated the antifouling metabolites isolated from marine invertebrates, particularly, sponges and soft corals. Both organisms have established an impressive warehouse of chemical defense systems against biofouling. It was interesting that the majority of friendly antifouling metabolites, identified in the last 30 years, belong to several natural classes: terpenoids (i.e., sesquiterpenes and cembranoidal diterpenes), alkaloids, and steroids [19, 27, 28]. Accordingly, our sample collection was directed towards the marine organisms which can produce such metabolites. It is wealthy that these samples, collected based on the previous reported data of the chemical and biological diversity, led to our obtained results.

4. Conclusion

Seventy-one marine samples, including sponges, algae, tunicate, sea cucumber, and soft corals, were collected and extracted with methanol. Thirty-three samples showed antifouling activity; four were highly potent at 1 μg/mL. Two promising extracts were purified by employing several chromatographic techniques, which led to the isolation of 12 compounds. They were proven to possess potent antifouling activity with EC_{50} values less than 10 μg/mL.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Supplementary Materials

Taxonomical identification of cyanobacteria samples. The Red Sea cyanobacterial samples were collected from different

places by using scuba at a depth of 5–10 m near Jeddah, Saudi Arabia. Foreign particles were removed by hand, and seawater was squeezed out from the samples before storing in MeOH for transportation. A small portion of this material was also preserved in 10 mL of RNAlater (Ambion) solution for genetic analysis. The 16S rRNA gene sequence (GenBank KT380828) of this material is currently available online. (*Supplementary Materials*)

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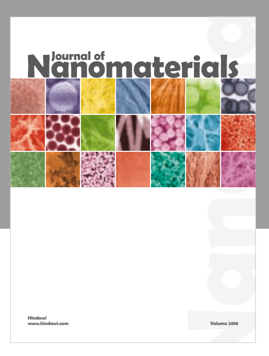

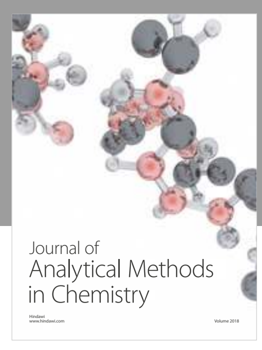

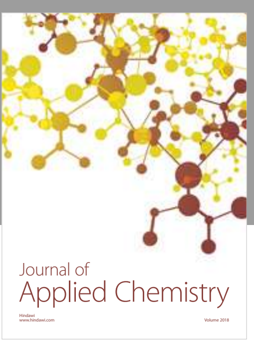

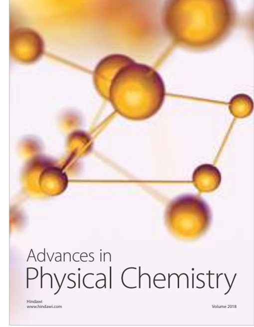

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