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Pharmaceutical Biology of Seaweeds from the Karachi Coast of Pakistan

Muhammad Afzal Rizvi[1] and Mustafa Shameel[2]

[1]Hamdard Institute of Advanced Studies and Research, Hamdard University, Karachi, Pakistan; [2]Department of Botany, University of Karachi, Karachi, Pakistan

Abstract

A variety of marine benthic algae belonging to the Chlorophyta, Phaeophyta, and Rhodophyta were collected from different coastal areas of Karachi (Pakistan), and several biological tests were conducted on their methanol extracts in order to investigate antibacterial, antifungal, phytotoxic, and insecticidal activities. Brown seaweeds showed greater antibacterial activity than the green and red ones. *Botryocladia leptopoda* (J.Ag.) Kylin exhibited the greatest antifungal activity and the least was exhibited by *Codium shameelii* Nizam. The highest phytotoxic activity was displayed by *Ulva intestinalis* L., and *Osmundea pinnatifida* (Huds.) Stack. showed the greatest insecticidal activity as compared to the other investigated species. Furthermore, a number of species were analyzed for their elemental composition with the help of a Perkin-Elmer 3100 atomic absorption spectrometer. Elements such as Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Pb, and Zn were determined quantitatively. Among them, Ca, Cr, and Pb were found to occur in the highest proportion in green seaweeds, Co, Cu, Fe, and Zn in highest proportion in brown seaweeds, and Cd, K, Mg, and Na were in highest proportion in the investigated red seaweeds.

Keywords: Biological testing, crude MeOH extract, elemental analysis, Karachi coast, seaweeds.

Introduction

The sea offers a reservoir of useful seaweeds with biodynamic activities. In recent years, a number of marine plant extracts have exhibited a variety of antimicrobial activities (Baslow, 1969; Naqvi et al., 1980; Rao et al., 1991; Usmani et al. 1991, Alam et al., 1994). Among seaweeds growing at the coast of Karachi, extracts of *Codium dwarkense* Børg. and *Bryopsis plumosa* J. Ag. exhibited antifungal activity (Aliya & Shameel, 1999), the methanol extract of *Caulerpa veravalensis* Thivy et Chauhan showed activity against *Candida tropicalis* Castellani et Berkhout, and that of *Ulva prolifera* (Mull.) J. Ag. displayed activity against *Aspergillus niger* van Tieghem (Anonymous, 1985). Seaweeds concentrate minerals and trace elements from marine water and convert them in organic forms as they grow in a mineral-rich medium (Chapman & Chapman, 1980). The numerous elements coming from the sea are Ca, Cl, Cu, I, Mg, Mn, Na, P, S, and Zn (Jarvis, 1976). They selectively absorb elements like Na, K, Ca, Mg, I, and Br from the seawater and accumulate them in their thalli. The accumulated elements vary from species to species. For example, large quantities of K and I are taken up by many brown seaweeds and Ca and Br by red algae. Marine algae generally contain Na, K, Ca, Mg, and Fe in large quantities, up to 15–25% of dry weight. The inorganic content appears very high when compared with 5–6% in hay or nearly 4% in cereals. Seaweeds are known as alkaline food, as their inorganic components play a very important role in preventing blood acidosis (Kaur, 1997).

The Karachi Coast (100 km) is located on the Arabian Sea. It includes beaches and numerous islands. The coastal waters around Manora, Sandspit, Hawkesbay, Buleji, Paradise Point, Pacha, Nathiagali, and Cape Monze are inhabited by a variety of marine benthic algae (Shameel & Tanaka, 1992). Although a lot of work has been done on their taxonomy and distribution, as well as morpho-ecological and phycochemistry studies (Anand, 1940, 1943; Nizamuddin, 1963, 1964; Saifullah,

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Address correspondence to: Dr. Muhammad Afzal Rizvi, Hamdard Institute of Advanced Studies and Research, Hamdard University, Karachi 74600, Pakistan; E-mail: marizvi_pk@yahoo.com

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1973; Shameel, 1987; Afaq-Husain et al., 1991; Shameel et al., 1996, 2000; Usmanghani & Shameel, 1996; Hameed et al., 2000, 2001), little data are available in the literature related to their bioactivity and elemental composition (Rizvi & Shameel, 2001). Therefore, this work was undertaken to examine specimens from the coast of Karachi, Pakistan.

Materials and Methods

Collection of marine algae

The healthy and mature specimens of different species of marine algae were collected in bulk quantity from sandy bays, large and shallow sand-bottom flats, small and large pools with rocky and sandy bottom at the ledges along various coastal areas of Karachi, Pakistan (e.g., Manora, Buleji, Sandspit, and Paradise Point) during 1997–2001 (Table 1). The sublittoral algae were picked up as drift material. The collected seaweeds were brought to the laboratory, washed immediately with running water to remove epiphytes and attached debris, and then soaked in MeOH for biological testing. A part of the specimens were washed with distilled water for elemental composition. Species were identified by one of us (M.S.), and voucher specimens (KUH-SW) were placed in the Seaweed Herbarium, MAH Qadri Biological Research Centre, University of Karachi.

Antibacterial bioassay

The antibacterial bioassay was performed against a variety of Gram-positive and Gram-negative bacteria using the agar well diffusion technique (Carron et al., 1987). The details of test organisms are given in Table 2. The 6 mg sample of each crude methanol extract was used for this test. All pathogenic microbes were clinical isolates

Table 1. Investigated seaweeds and the area of their collection.

Marine algae	Code no. used	Area of collection	Date of collection
Chlorophyta			
Bryopsis pennata Lamour.	BP-01	Buleji	07.02.1998
Caulerpa racemosa (Forssk.) J. Ag.	CR-02	Buleji	07.02.1998
Caulerpa scalpelliformis (R. Br.) C. Ag.	CS-03	Paradise Point	25.01.1998
Caulerpa taxifolia (Vahl) C. Ag.	CT-04	Manora	31.01.1999
Codium iyengarii Børg.	CI-05	Buleji	07.01.1998
Codium shameelii Nizam.	CS-06	Paradise Point	25.01.1998
Ulva fasciata Delile	UI-07	Buleji	30.07.2000
Ulva intestinalis L.	UF-08	Sandspit	15.05.1998
Ulva lactuca L.	UL-09	Paradise Point	25.01.1998
Phaeophyta			
Colpomenia sinuosa (Roth) Derb. et Sol.	CS-10	Manora	27.12.1997
Cystoseira indica (Thivy et Doshi) Mairh	CI-11	Buleji	02.03.1998
Dictyota dichotoma var. *intricata* (C. Ag.) Greville	DD-12	Buleji	27.12.1997
Dictyota hauckiana Nizam.	DH-13	Manora	06.02.2001
Iyengaria stellata (Børg.) Børg.	IS-14	Manora	27.12.1997
Padina antillarum (Kütz.) Piccon.	PA -15	Buleji	19.11.1998
Padina pavonica (L.) Thivy in Taylor	PP-16	Paradise Point	25.01.1998
Sargassum boveanum J. Agardh	SB-17	Buleji	16.02.2000
Sargassum ilicifolium (Turn.) C. Ag.	SI-18	Buleji	16.02.2000
Sargassum swartzii (Turn.) C. Ag.	SS-19	Paradise Point	25.01.1998
Sargassum tenerrimum J. Ag.	ST-20	Manora	27.12.1997
Sargassum vulgare C. Ag.	SV-21	Buleji	02.03.1998
Stoechospermum polypodioides (Lamour.) J. Ag.	SP-22	Buleji	20.01.1997
Stypopodium shameelii Nizam. et Aisha	SS-23	Manora	27.12.1997
Rhodophyta			
Botryocladia leptopoda (J. Ag.) Kylin	BL-24	Manora	19.11.1998
Champia compressa Harv.	CC-25	Buleji	30.07.2000
Gracilaria corticata (J. Ag.) J. Ag.	GC-26	Buleji	01.12.1998
Hypnea musciformis (Wulf.) Lamour.	HP-27	Buleji	01.12.1998
Hypnea valentiae (Turn.) Mont.	HV-28	Paradise Point	27.02.1999
Osmundea pinnatifida (Huds.) Stack.	OP-29	Buleji	30.07.2000
Sarconema filiforme (Sond) Kylin.	SF-30	Manora	25.01.1998
Scinaia saifullahii Afaq. et Shameel	SS-31	Buleji	07.02.1998
Solieria robusta (Grev.) Kylin	SR-32	Buleji	24.10.1999

Table 2. Test organisms used for bioassays.

No.	Test organisms	Abbreviation used	Procurement
	Bacteria		
	Gram positive		
1.	*Bacillus subtilis* (Ehrenberg) Cohn	B.s	Department of Microbiology, University of Karachi
2.	*Corynebacterium diptheriae* (Kruse) Lehmann et Neumann	C.d	
3.	*Staphylococcus aureus* Rosenbach	S.a	Liaquat National Hospital
4.	*Streptococcus pyogenes* Rosenbach	S.p	
	Gram negative		
5.	*Pseudomonas aeruginosa* (Schroeter) Migula	P.a	Department of Microbiology, University of Karachi
6.	*Proteus miriablis* Hauser	P.m	
7.	*Klebsiella pneumoniae* (Schroeter) Trevisan	K.p	
8.	*Shigella dysenteriae* (Shig) Castellani et Chalmers	S.d	
9.	*Salmonella typhi* (Schroeter) Warren et Scott	S.t	
	Fungi		
	Human pathogens		
10.	*Aspergillus flavus* Link ex Fr.	A..f	Clinical isolate
11.	*Aspergillus niger* van Tieghem	A..n	Clinical isolate
12.	*Candida albicans* (Robin) Berkhout	C.a	LN Hospital
13.	*Candida glabrata* Saito	C.g	LN Hospital
14.	*Pseudallescheria boydii*	P.b	Department of Microbiology, University of Karachi
15.	*Trichophyton longifusus* Malmsten	T.l	
16.	*Trichophyton schoenleninii* Lebert	T.s	
	Animal pathogens		
17.	*Microspermum canis* Bodin	M.c	Department of Microbiology, University of Karachi
18.	*Trichophyton mentagrophytes* Blanchard	T.m	
19.	*Trichophyton simii*	T.s	
	Plant pathogens		
20.	*Fusarium moniliforme* Shel.	F.m	Department of Botany, University of Karachi
21.	*Fusarium solani* (Mart.) Sacc.	F.s	
22.	*Mucor* sp. Mich. ex Fr.stenni etumann	M.s	
	Common stored grain pests		
23.	*Callosobruchus analis* Fabricius	C.a	MAHQ
24.	*Rhyzopertha dominica* Fabricius	R.d	Biological Research Center,
25.	*Sitophilus oryzae* (L.) Fabricius	S.o	University of Karachi
26.	*Tribolium castaneum* Herbst	T.c	
27.	*Trogoderma granarium* Everts	T.g	

and kindly provided by the Department of Microbiology, University of Karachi, except *Staphylococcus aureus* Rosenbach and *Candida albicans* Robin (Berkhout), which were generously given by Liaquat National Hospital, Karachi. The pure bacterial cultures were inoculated in nutrient broth and incubated at 37°C for 2–8 h until turbidity developed. The turbidity of nutrient broth (NB) in the test tube was compared with the McFarland turbidity standard (Oxoid Uni Path Ltd., Hampshire, UK). Test samples at a concentration of 2 µg/100 ml as well as dimethyl sulfoxide (DMSO) were added in their respective wells (Atta-ur-Rahman et al., 2001). The zones of inhibition were measured in millimeters and compared with a reference antibacterial drug, tetracycline.

Antifungal bioassay

The fresh algal material (1 kg each) was soaked in MeOH for 7 days at room temperature (Fig. 1). The MeOH extract was filtered through Whatman filter paper and concentrated under reduced pressure at 35°C in a rotary evaporator. The crude gummy methanol extract (24 mg) was dissolved in 1 ml of sterile DMSO serving as stock solution. Sabouraud dextrose agar (SDA; Merck, Darmstadt, Germany) was prepared in screw-capped

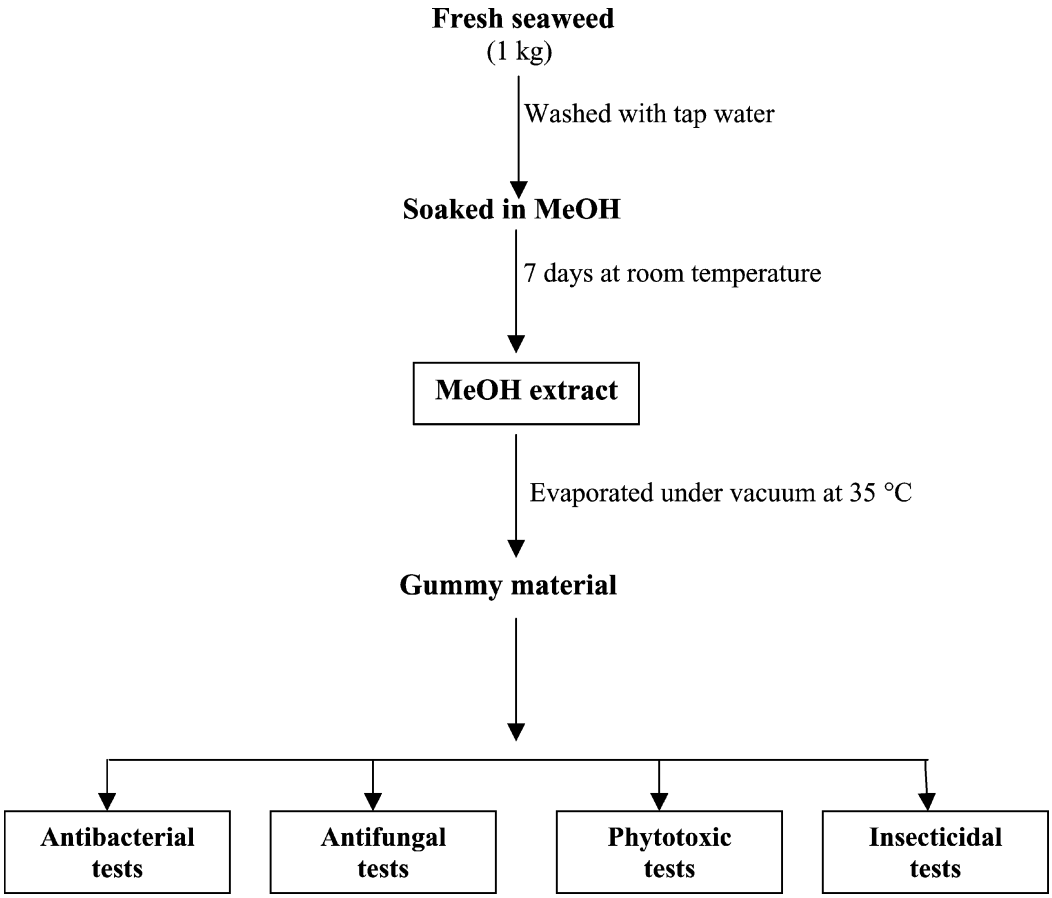

Figure 1. Scheme for algal extraction.

tubes and autoclaved at 121°C for 15 min (Paxton, 1991). Tubes were allowed to cool to 50°C, and nonsolidified SDA medium was mixed with 66.6 µl of stock solution giving a final concentration of 400 µg/ml of SDA. Tubes were then allowed to solidify in a slanting position at room temperature. Each tube was inoculated with a 4-mm-diameter piece of inoculum removed from a 7-day-old culture of fungi. For nonmycelial growth, an agar surface streak was employed. The various fungal organisms used are given in Table 2. The tubes were incubated at 27–29°C for 7–10 days (Atta-ur-Rahman et al., 2001). Growth inhibition was calculated in percentage and compared with standard antibiotic drugs, miconazole and ketoconazole, sometimes amphotericin B and benlate.

Lemna bioassay

This bioassay was used to study the phytotoxic activity of MeOH extracts of the seaweeds on the plant *Lemna aequinoctialis* Welv. The stock solutions were prepared by dissolving 30 mg of the crude extract in 1.5 ml of methanol. Nine flasks (three for each concentration) were inoculated with 1000, 100, and 10 µl of the stock solution for 1000, 100, and 10 µg/ml. The solvent was evaporated overnight under sterile conditions. To each flask, 20 ml of E-medium at pH of 5.5–6.0 was added. Then, 10 plants of *Lemna aequinoctialis* having a rosette of three fronds were added to each flask. Two other flasks were supplemented with solvent and reference plant growth inhibitor as well as promoter serving as negative and positive controls, respectively. For a positive control, paraquat (ICI Pak. Ltd, Karachi, Pakistan) was used. The flasks were plugged with cotton and placed in a growth cabinet for 7 days. On the seventh day, the number of fronds per flask were counted (Atta-ur-Rahman, 1991). Interpretation of the result was made by analyzing growth regulation in percentage, which was calculated with reference to the negative control.

Insecticidal assay

This simple test was used to assess the insecticidal activity of each MeOH extract (200 mg) of the seaweeds. The insects were exposed to the test extracts by a direct contact method using filter paper impregnated with test sample (1571.33 µg/cm^2, pyrethroid and permethrin, 1:1). Afterwards, 10 adult insects of different types and of the same age were transferred to Petri dishes. A negative control was treated with solvent for the determination of solvent effects. Another batch supplemented

with reference insecticides (i.e., Mortein Coopex; Reckitt Benckiser Pak. Ltd., Karachi, Pakistan) was used. All these were kept without food for 24 h at 27–30°C. Mortality counts were carried out after a 24 h exposure period (Farhana, 2000).

Ashing and digestion of the seaweeds

The algal material was initially dried under shade at room temperature and later in an oven at 60–80°C for 1 h. It was then powdered through a grinder, 1 g of the ground sample was accurately weighed in a porcelain crucible, and ashed at 500°C in an oven to constant weight for 2 h (Fig. 2). The ash was cooled at room temperature, wet with 10 drops of distilled water, and carefully dissolved in 3 ml of HNO_3 (1:1). The acid solution of the sample was then heated gently on a hot plate at 100–120°C until it was nearly dry. The crucible was returned to a muffle furnace and ashed again for 1 h at 500°C. It was then cooled and dissolved in 10 ml of HCl (1: 1), and the solution was filtered through Whatman filter paper no. 42 (Schleir & Schuell, Dassel, Germany) into a 100 mL volumetric flask. The solution was then diluted to final volume with distilled water, mixed well, and prepared for AAS analysis (Jones, 1984).

Elemental assay

Flame atomic absorption spectrometry (AAS; model 3100, Perkin-Elmer, Norwalk, CT, USA) was performed

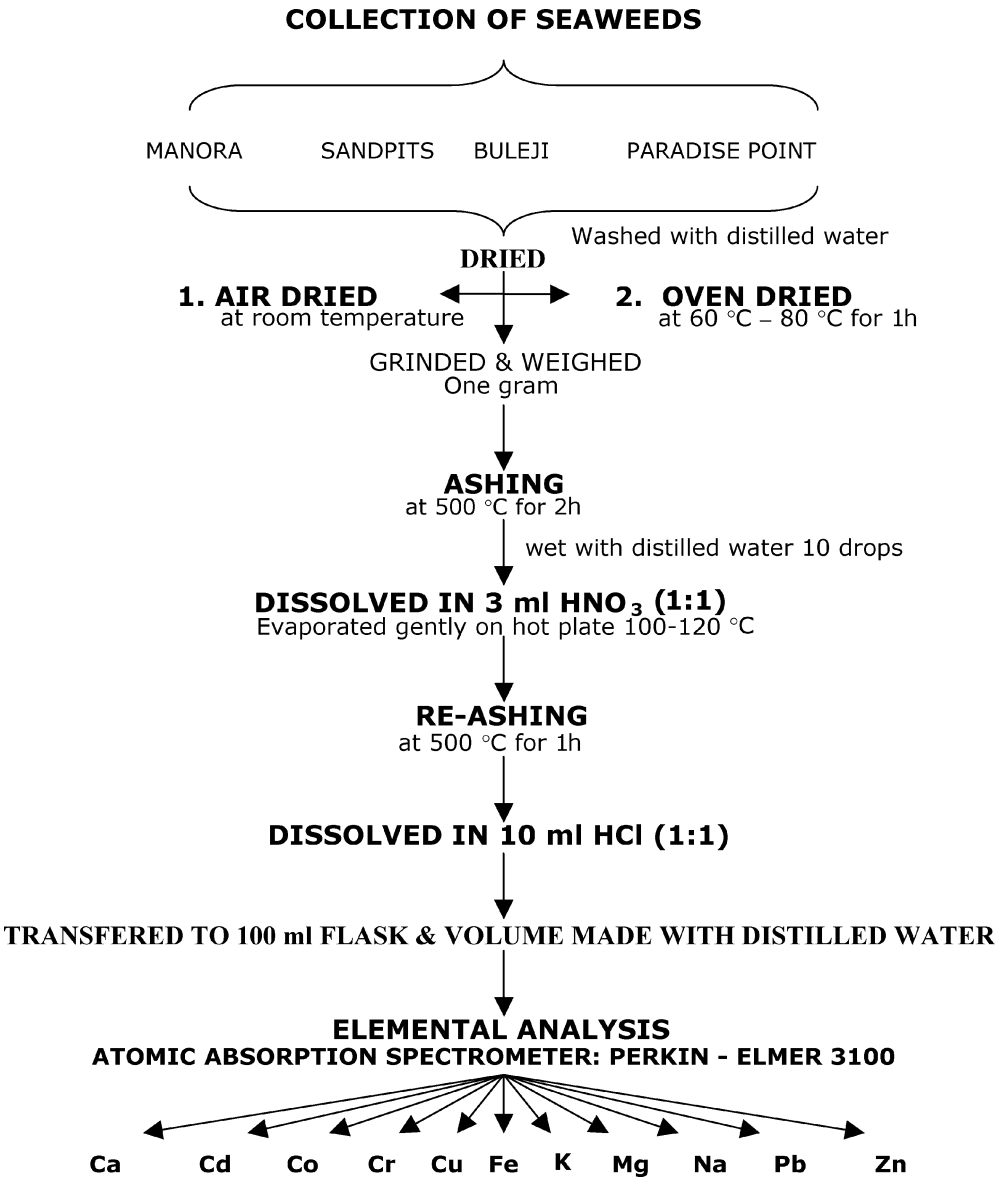

Figure 2. Scheme for ashing and digestion of seaweeds.

Table 3. Instrument parameters.

Elements	Symbol	Wavelength (nm)	Slit width (nm)	Sensitivity (mg/l)
Calcium	Ca	422.7	0.7	0.092
Cadmium	Cd	228.8	0.7	0.016
Cobalt	Co	240.7	0.2	0.078
Chromium	Cr	357.9	0.7	0.041
Copper	Cu	324.8	0.7	0.077
Iron	Fe	248.3	0.2	0.039
Potassium	K	766.5	0.7	0.043
Magnesium	Mg	285.2	0.7	0.008
Sodium	Na	589.0	0.2	0.012
Lead	Pb	283.3	0.7	0.079
Zinc	Zn	213.0	0.7	0.018

Recommended flame: air-acetylene.

at Hamdard University, Karachi, for the purpose of estimating Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Pb, and Zn. The various instrument parameters are presented in Table 3. Instructions for instrument setting, calibration, and assay for specific elements as described in the operational manual were strictly followed.

Results and Discussion

The results of antibacterial tests obtained on 14 seaweeds are shown in the Table 4, expressed in terms of zones of inhibition in millimeters. They indicate that 10 algal extracts inhibited the growth of Gram-positive and Gram-negative bacteria. The values higher than 12 were replicated two times, and an average was obtained. Extracts from *Ulva intestinalis*, *Stoechospermum polypodioides*, and *Osmundea pinnatifida* could prevent the growth of only one bacterium, and extracts from *Sargassum ilicifolium* and *Champia compressa* inhibited only two bacteria. Extracts from *Colpomenia sinuosa* and *Iyengaria stellata* showed positive activity against six bacteria including three Gram-positive and three Gram-negative bacterial strains that appeared to be the most active, and the extracts from *Cystoseria indica* exhibited positive activity against four bacteria, indicating that brown seaweeds have more active antibacterial components than do the green and red ones. Extracts from *Codium iyengarii*, *Ulva fasciata*, and *Padina antillarum* showed no inhibition against all nine of the bacteria that were investigated. Similar observations have been made on a variety of algae such as *Ulva compressa*, *Padina gymnospora*, *Sargassum wightii*, and *Gracillaria corticata* that were active against Gram-positive cultures of *Bacillus* (Rao et al., 1991). It has also been reported that extracts from *Gracilaria corticata* and *Padina gymnospora* show no antibacterial activity against *Bacillus megaterium* and *Staphylococcus aureus* Rosenbach (Ahmad & Perveen, 1993).

Twenty-three species of marine benthic algae belonging to the Chlorophyta, Phaeophyta, and Rhodophyta

Table 4. Antibacterial activity of crude methanol extract of different seaweeds shown as zone of inhibition in millimeters.

	Gram positive				Gram negative				
Organisms	B.s	C.d	S.a	S.p	P.a	P.m	K.p	S.d	S.t
Cholorophyta									
Codium iyengarii	x	x	x	x	x	x	x	x	x
Codium shameelii	x	13	12	13	12	x	x	x	x
Ulva fasciata	x	x	x	x	X	x	x	x	x
Ulva intestinalis	x	x	x	x	x	x	x	14	x
Phaeophyta									
Colpomenia sinuosa	17	14	x	17	13	x	14	x	13
Cystoseira indica	x	13	x	14	x	x	16	x	14
Dictyota hauckiana	x	11	x	11	x	x	x	x	x
Iyengaria stellata	14	15	x	14	12	x	14	x	15
Padina antillarum	x	x	x	x	x	x	x	x	x
Sargassum ilicifolium	x	x	x	x	x	x	13	12	x
Stoechospermum polypodioides	x	12	x	x	x	x	x	x	x
Rhodophyta									
Botryocladia leptopoda	x	x	x	x	x	14	x	15	x
Champia compressa	x	x	x	x	x	x	x	x	x
Osmundea pinnatifida	x	x	x	x	x	x	x	13	x
Tetracycline	26.4	26.6	26	29.5	28.8	23	27.25	27	27.4

B.s = *Bacillus subtilis*, C.d = *Corynebacterium diptheriae*, K.p = *Klebsiella pneumoniae*, S.a = *Staphylococcus aureus*, S.d = *Shigella dysenteriae*, S.p = *Streptococcus pyogenes*, S.t = *Salmonella typhi*, P.a = *Pseudomonas aeruginosa*, P.m = *Proteus miriabilis*, x = *no* inhibition. (Concentration of sample 200 µg/100 µl DMSO; values refer to inhibition caused by methanol extract. Standard drug: tetracycline.)

Table 5. Antifungal activity of crude MeOH extract of different seaweeds shown as % inhibition.

	Human pathogens							Animal pathogens			Plant pathogens		
Name of objects	A.f	A.n	C.a	C.g	P.b	T.l	T.s	M.c	T.m	T.s	F.m	F.s	M.s
Chlorophyta													
Caulerpa racemosa	–	–	7.9	x	63	–	40	33	30	55.5	–	50	–
Caulerpa taxifolia	–	–	x	x	56	–	50	13.3	30	54	–	55	–
Codium iyengarii	–	46	x	x	54	–	50	50	x	50	–	56.3	–
Codium shameelii	x	–	x	x	–	x	–	x	–	–	–	x	–
Ulva fasciata	x	12.22	49.7	x	–	–	–	38.33	24.32	–	–	55.71	20
Ulva lactuca	x	50	x	x	40	–	26	33.2	x	x	–	4.2	–
Phaeophyta													
Colpomenia sinuosa	x	x	x	x	–	x	–	00	–	–	–	x	–
Cystoseria indica	x	–	x	x	–	26	–	28	–	–	x	x	–
Dictyota hauckiana	x	x	x	x	x	x	x	x	x	x	50	x	–
Iyengaria stellata	x	–	x	x	–	43	–	60	–	–	x	x	–
Padina antillarum	x	–	x	x	60	x	40	16	11	50	x	51.6	–
Sargassum boveanum	–	x	3.43	40	–	–	–	61.66	36.48	–	–	x	8.00
Sargassum ilicifolium	–	3.33	52.68	x	x	–	–	5.00	63.51	x	–	44.28	4.00
Sargassum vulgare	–	56	x	x	50	–	5.7	50	x	45	–	4.2	–
Stoechospermum polypodioides	–	–	x	x	30	–	60	50	46	64	–	58.3	–
Rhodophyta													
Botryocladia leptopoda	–	–	x	x	52	–	60	50	55	60	66.6	60	–
Champia compressa	–	6.66	1.94	46.6	–	–	–	43.33	39.18	–	–	60	2.66
Gracilaria corticata	–	50	x	x	16	x	14.2	16	x	x	–	14.2	–
Hypnea musciformis	–	–	37.16	x	36	x	30	43	58.91	46	–	70	6.66
Hypnea valentiae	–	–	x	x	50	–	40	50	55	40	x	58.3	x
Osmundea pinnatifida	–	23.3	3.43	30	–	–	–	x	8.10	–	–	28.57	x
Sarconema filiforme	–	–	x	x	56	x	64	50	57	56	x	60	x
Solieria robusta	–	–	x	x	27.2	x	10	16	x	28.8	–	40	x
Standard drugs													
Miconazole	–	100	110.8	110.8	100	70	95	98.4	100	100	100	73.25	100
Ketoconazole	–	–	–	–	90	–	100	–	–	90	–	–	–
Amphotericin-B	20	–	–	–	–	–	–	–	–	–	–	–	–
Benlate	x	–	–	–	–	–	–	–	–	–	–	100	–

A.f = *Aspergillus flavus*, A.s = *Aspergillus niger*, C.a = *Candida albicans*, C.g = *Candida glaberata*, F.m = *Fusarium moniliforme*, F.s = *Fusarium solani*, M.c = *Microspermum canis*, M.s = *Mucor* sp., P.b = *Pseudallescheria boydii*, T.l = *Trichophyton longifusus*, T,m = *Trichophyton mentagrophytes*, T.s = *Trichophyton schoenleninii*, T.s = *Tricophyton simii*, – = not tested, x = no inhibition. (Concentration of sample 400 µg/ml of medium; values refer to % inhibition; incubation temperature 27–29°C; incubation time: 7–10 days.)

were tested against seven species of human, three species of animal, and three species of plant pathogens for *in vitro* fungicidal activity (Table 5). Treatments were replicated two times, and an average value was obtained. Only five species of marine algae exhibited moderate activity (45–50%). Extracts from *Botryocladia leptopoda* appeared to be the most active, whereas extracts from *Codium shameelii* were the least active. Extracts from *Codium iyengarii* displayed good activity, and in other observations this extract showed significant antifungal activity against a variety of pathogens (Ali et al., 2000). The crude methanol extract of *Stoechospermum polypodioides* has been found to inhibit the growth of *Micrococcus pyrogenes* Lehman et Neumann var. *aureus* Hucker (Anonymous, 2000). The ethanol extracts derived from seven seaweed species showed no detectable antifungal activity against *Epidermophyton floccose* (Harz) Langeron et Milochevitch, *Microsporum canis* Bodin, and *Trichophyton rubrum* (Castellani) Sabouraud (Alam et al., 1994). It appears that different seaweed extracts behave variably against different fungal species.

The results of the *Lemna* bioassay of methanol extracts of the investigated seaweeds at concentrations of 10, 100, and 1000 µg/ml are presented in Table 6, and an average phytotoxic activity of the three mentioned concentrations is graphically expressed in Figure 3. The extracts from all the seaweeds inhibited the growth of *Lemna aequinoctialis*. The extract from *Ulva intestinalis* displayed the highest activity, followed by the extract from *Champia compressa*. The extracts from

Table 6. Phytotoxic activity of seaweeds in % inhibition.

Name of alga	Concentration of sample		
	1000 µg/ml	100 µg/ml	10 µg/ml
Cholorophyta			
Codium shameelii	22.23	− 5.88	− 13.33
Ulva intestinalis	63.6	95.0	22.2
Ulva fasciata	9.09	8.33	11.11
Phaeophyta			
Colpomenia sinuosa	29.41	7.14	31.57
Cystoseira indica	23.52	14.28	31.57
Dictyota hauckiana	16.6	29.4	6.6
Iyengaria stellata	23.52	− 21.22	47.36
Sargassum boveanum	9.09	8.08	− 22
Rhodophyta			
Botryocladia leptopoda	29.41	− 14.2	10.52
Champia compressa	63.63	41.66	− 11.11
Hypnea musciformis	45.4	16.6	0.0
Osmundea pinnatifida	0.0	0.0	11.11

Standard drug: paraquat; growth period: 7 days.

Table 7. Insecticidal test for seaweeds in % mortality.

Name of alga	Control	C.a	R.d	S.o	T.c	T.g
Chlorophyta						
Ulva fasciata	100	x	x	x	x	10
Ulva intestinalis	100	x	x	x	20	x
Phaeophyta						
Sargassum boveanum	100	20	x	x	x	20
Sargassum ilicifolium	100	x	x	x	x	10
Rhodophyta						
Botryocladia leptopoda	100	x	x	x	x	x
Champia compressa	100	10	x	x	x	x
Hypnea musciformis	100	x	x	x	x	x
Osmundea pinnatifida	100	20	20	x	x	20

C.a = *Callosobruchus analis*, R.d = *Rhizopertha dominica*, S.o = *Sitophilus oryzae*, T.c = *Tribolium castaneum*, T.g = *Trogoderma granarium*, x = no activity. Standard drug: pyrethroids (Coopex 50%); incubation temperature: 37°C; humidity: 50%; sample solvent, MeOH: sample concentration: 392.83 µg/cm^2.

Codium shameelii and *Sargassum boveanum* exhibited poor activity, and in another observation, the ethanol extract from *S. tenerrimum* showed 100% inhibition of the fronds at a concentration of 500 µg/ml (Ali et al., 2000). It appears that different species of the same genus act variably. Probably they accumulate different natural products, which may be responsible for phytotoxic activity. As compared to the extracts from green seaweeds displaying great fluctuations in their activity, the extracts from the investigated brown seaweeds exhibited uniformity in their phytotoxic activities.

For insecticidal bioassays, eight marine algae were chosen, and their crude extracts were tested against five different insects, *Callosobruchus analis* Fabricius, *Rhyzopertha dominica* Fabricius, *Sitophilus oryzae* L. Fabricius, *Tribolium castaneum* Herbst, and *Trogoderma granarium* Everts (Table 7). Treatments were replicated two times, and an average value was obtained. *Ulva intestinalis, Sargassum boveanum*, and *Osmundea pinnatifida* exhibited moderate activity, whereas *Ulva fasciata*, *Sargassum ilicifolium*, and *Champia compressa* showed a very poor activity against these insects. *Botryocladia lepdopoda* and *Hypnea musciformis* displayed no activity. *Osmundea pinnatifida* appeared to be the most active seaweed: it was found to contain a large variety of natural products (Ali et al., 2000), which might be responsible for such activity.

Twenty-six species of seaweeds were analyzed for the composition of 11 elements (Table 8). Among these elements, Ca, Fe, K, Mg, and Na were found in large amounts (on the average 2411.38–76714.25 ppm), Cr, Cu, Pb, and Zn were present in small amounts (on the average 5.88–53.28 ppm), and Cd and Cr were detected in extremely small amounts (on the average 1.61–5.13 ppm). Iron has been found in large quantity, Cr

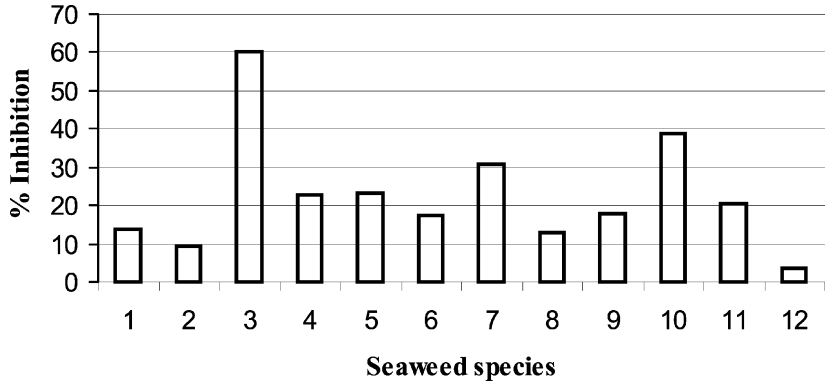

Figure 3. Average phytotoxic activity: **1** = *Codium shameelii*, **2** = *Ulva fasciata*, **3** = *Ulva intestinalis*, **4** = *Colpomenia sinuosa*, **5** = *Cystoseira indica*, **6** = *Dictyota hauckiana*, **7** = *Iyengaria stellata*, **8** = *Sargassum boveanum*, **9** = *Botryocladia leptopoda*, **10** = *Champia compressa*, **11** = *Hypnea musciformis*, **12** = *Osmundea pinnatifida*.

Table 8. Elemental composition in seaweeds from the Karachi coast.

Alga*	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Na	Pb	Zn	Average
C	**36669.75**	**1.38**	**4.39**	**7.60**	**11.89**	**2827.7**	**50457.87**	**10753.06**	**70200.87**	**17.60**	**35.26**	**15,544.42**
BP-01	80800	3.15	8.05	9.925	12.9	3795	10855	6660	28535	43.87	37.425	11888.21
CR-02	70300	2.2	6.8	12.525	11.25	2542.5	19625	764.5	155950	23.67	21.725	22660.01
CS- 03	80750	BDL	BDL	BDL	7.75	8427.5	3900	3725	1909	BDL	37	8977.84
CT-04	14755	0.975	4.0	10.425	8.5	2840	15810	6870	110400	19.1	25.05	13703.91
CI- 05	14730	1.925	9.55	2.825	5.65	862.5	231700	9605	169350	26.07	18.25	38755.61
CS-06	18733	BDL	BDL	BDL	28	1076.6	70633	8100	52833	BDL	42	13767.78
UI- 07	4745	0.5	3.675	23.325	14.0	2695	18590	13400	17470	19.6	81.55	5185.69
UL-09	8545	2.3	3.05	1.85	7.125	382.5	32550	36900	25160	8.55	19.15	9416.32
P	**30857.12**	**1.18**	**7.03**	**1.91**	**13.87**	**3410.62**	**75704.78**	**10073.87**	**26266.16**	**10.40**	**96.09**	**12,276.43**
CS-10	43943.33	2.33	16.65	BDL	19.93	4064.33	125736.66	7156.66	29216.66	15.93	123.1	19117.87
CI-11	19050	3.95	5.125	4.7	8.125	249	118125	9425	80562.5	8.1	33.625	11015.96
DD-12	21080	BDL	13.4	BDL	29.6	1220	5400	7120	10800	4	213.6	4170.96
IS-14	16810	1.05	14.5	BDL	15.3	767	269000	6060	56520	4.8	64.1	31750.61
PA-15	46950	2.875	6.375	16.15	12.375	3105	26620	24500	20530	15.25	44.475	11072.95
PP-16	24900	BDL	BDL	BDL	12.5	5000	47075	11425	1999	BDL	76	5961.36
SS-19	87950	BDL	BDL	BDL	1.65	391.5	76820	7565	37740	40.7	16.85	19138.7
ST-20	13605	0.2	9.9	BDL	12.2	3465	39350	10000	19500	6.5	118.2	7824.27
SV-21	16055	1.2	7.0	0.18	8.6	1740	60666	14166	9902.2	6.8	274.8	9347.98
SP-22	18835	0.7	4.4	BDL	10.5	1570	50750	6450	8902.5	9.6	41.3	7870.36
SS-23	30250	0.7	BDL	BDL	21.8	15945	13210	6945	13255	2.75	50.95	7243.74
R	**2713.14**	**2.27**	**6.23**	**5.87**	**9.86**	**995.82**	**82442.85**	**13206.07**	**133675.71**	**12.29**	**28.50**	**20,645.31**
BL-24	9055	2.9	8.05	3.4	13.6	499.5	65925	27040	202375	7.2	26.375	27723.27
GC-26	11725	1.875	5.55	7.1	8.375	1105	114750	4580	26290	13.32	35.3	14411.04
HM-27	7977.5	1.975	5.1	2.325	6.675	230.5	62125	4930	129687.5	3.85	15.675	11791.24
HV-28	10350	2.7	7.75	10.625	11.625	1825	112937.5	15260	154187.5	14.1	29.925	26785.24
SF-30	7447.5	2.55	7.45	5.425	12.7	340.75	136375	19820	220187.5	16.05	20.775	34930.56
SS-31	8350	1.15	2.85	4.55	4.2	1070	14925	12350	18690	13.45	47.875	1703.44
SR-32	34087	2.8	6.9	7.7	11.9	1900	70062.5	8462.5	184312.5	18.1	23.6	27172.40
Average amount	**26746.67**	**1.61**	**5.88**	**5.13**	**11.87**	**2411.38**	**69535.17**	**11344.34**	**76714.25**	**13.43**	**53.28**	

*Code numbers under the column of "Alga" refer to the taxonomic names of algae given in Table I, B.D.L. = below detection level, *C* = Chlorophyta, *P* = Phaeophyta, *R* = Rhodophyta.

and Zn in medium quantity, and Co in small quantity in several brown seaweeds of the Saronic Gulf, Greece (Kanias et al., 1991). The intestinal absorption of seaweed minerals (Ca, Fe, and Zn) exhibited an interesting result when observed *in vivo* using a perfused intestinal loop in the rat (Bougle et al., 1996). The average quantity of Na was found to be the highest among investigated algae (76714.25 ppm) followed by K (69535.17 ppm) and Ca (26746.67 ppm). The average amounts of Co (5.88 ppm) and Cr (5.13 ppm) were quite low; Cd was detected in the lowest quantity (1.61 ppm). However, Cd was present in smallest amount in Chlorophyta (1.38) as compared to Phaeophyta (1.18) as well as Rhodophyta (2.27 ppm). It was also detected in very small quantity in several other species of green, brown, and red seaweeds (Hasni & Sarwar, 1985; Rizvi & Shameel, 2001). In general, Ca, Cr, and Pb were found to occur in highest proportion in green seaweeds, Co, Cu, Fe, and Zn in highest proportion in brown seaweeds, and Cd, K, Mg, and Na in highest proportion in the investigated red seaweeds. When collectively considered, the average quantity of the detected 11 elements was highest (20,645.31 ppm) in the red seaweeds and lowest (12,276.43 ppm) in the brown seaweeds, and the average amount of green seaweeds was in-between (15,544.42). In many studies, the quantity of different essential elements was higher in red seaweeds as compared to other groups (Ganesan et al., 1991; Munda & Hudnick, 1991; Rajendran et al., 1993).

Kanias et al. (1991) determined trace elements in the dry matter of brown algae of the Saronic Gulf. Some species of marine algae from the coast of Goa, India, have been analyzed for Co, Cu, Fe, Mn, Ni, Pb, and Zn (Forsk) C. Ag. (Hoppe & Levring, 1982). *Ulva lactuca* (Forsk) C. Ag. from China and Southeast Asia, has been reported to be rich in iron (Ahmad et al., 1989). The edible green alga *Codium intricatum* (Mosure-miru) was found to contain a considerable quantity of iodine (0.13–0.16% of the dry weight), and red algae such as *Gelidium* and *Grateloupia* contained a medium amount (Chapman & Champan, 1980). The trace metal distribution in seaweeds of the Indian Coast has also been well documented. Metal concentration in the seaweeds were in the order Fe > Mn > Zn > Cu with the exception of a few seaweeds (e.g., *Ulva reticulata*, *Sargassum wightii Grev.*, and *Sarconema* sp.), which concentrated more Zn than Mn. Seaweeds from other tropical areas exhibit a similar trend. It seems that the tropical seaweeds tend to accumulate more Fe than Mn, Zn, and Cu (Ganesan et al., 1991).

This type of research work is to be continued in order to know the proportion of those elements in algal thalli that are beneficial for the human body, such as Ca, Fe, I, K, Na, and Zn, and also to investigate the distribution of various elements in different thallus parts of marine benthic algae for the sake of comparison. Some seaweeds are used as food because they are not poisonous, usually have soft tissues, and as such have many indirect medicinal effects.

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