Antimicrobial activities of three seaweeds extract against some human viral and bacterial pathogens

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Abstract: Microbial infections cause complicated health influences along with bad economic impacts. In the present investigation, three dominant seaweeds namely, Amphiroa anceps, Corallina officinalis and Sargassum filipendula were collected from different Egyptian sites at the Red Sea and Mediterranean Sea during autumn 2019. Organic extracts of the three algae were screened for their antibacterial activity against three pathogenic bacteria Salmonella typhiimurium, Staphylococcus aureus and Escherichia coli, in addition to in vitro antiviral activity against Rotavirus (RV), and Coxsackie virus B3 (CVB3) that cause severe diseases in human. Organic extract of A. anceps, C. officinalis and S. filipendula inhibit E. coli cells by 57.1%, 85.7%, and 91.4%, respectively. The highest level of concentration of the C. officinalis extract (100 µg/mL) inhibits 100% of Staphylococcus aureus cells followed by S. filipendula and A. anceps extract which inhibit 82.5% and 75% of S. aureus. Similarly, the highest concentration of C. officinalis extract inhibits S. typhiimurium by 80%. The extract of A. anceps exhibited a high antiviral effect against RV infection with TI = 22 and virus titers lessened by 2.75 log TCID₅₀ followed by extractions of C. officinalis with TI = 18.3 and virus titers reduced by 2.5 log TCID₅₀. Against CVB3 infection, the extract of A. anceps causes the highest antiviral activity with TI = 15 and reduce the viral titers by 2.5 log $TCID_{50}$, followed by extractions of C. officinalis with TI = 8.8 and inhibition of virus titers by 1.75 log TCID₅₀. Extract of S. filipendula displayed the lowest antiviral effects against RV and CVB3 infection with TI = 2.4 and 1.4, respectively. The obtained results clarified that the extract of three marine seaweeds maintains a potent antimicrobial activity, making them a future promising source of new antimicrobial drugs.

Introduction

Seaweeds, (macroalgae) are a large autotrophic diversified group (Fakayode *et al.*, 2020). They can inhabit the splash zone in the seashore and down for about 30 m depth in the sea where they can obtain enough light for photosynthesis (Mine, 2008). Seaweeds have the capability to produce many unique chemical compounds that displayed many biological activities including antibiotics, antiviral, laxatives, anticoagulants, and anti-ulcer (El-Baroty *et al.*, 2007; Schwartsmann *et al.*, 2001).

Marine macroalgae produce a vast number of multifunction natural secondary metabolites, this is a consequence of continuous exposure to numerous abiotic along with biotic pressures, which enhance organisms to produce such metabolites (Al-Fadhli *et al.*, 2006; Aslam *et al.*, 2020).

Some seaweeds produce a number of interesting chemicals such as halogenated terpenoids which possess antibiotic effect, some red algae are famous for the sulfated polysaccharides like carrageenan and fucoidan which have antiviral and anticancer activities against many human viruses and cancer cells (Smit, 2004). Some seaweed fibers like alginate and laminarin have hypercholesteremic and hypolipidemic effects (Nishide and Uchida, 2003). Oxylipins is a metabolic product of polyunsaturated fatty acids



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(PUFAs) produced by many macroalgae and well known for their anti-inflammatory effect (Brito *et al.*, 2016).

Besides, seaweeds are extensively used in the biosynthesis of nanoparticles using green chemistry technology. Many macroalgae are capable of the efficient bioproduction of nanoparticles with different sizes, shapes, and morphology which are used in different applications including pharmaceutical application especially antimicrobial activity in addition to removal of pollutants (Rezazadeh *et al.*, 2020; Khalafi *et al.*, 2019).

Despite the considerable benefits that bacteria provide in our life, there are some infectious species which counted as less than hundreds and can cause diseases in human, animals, and plants (McFall-Ngai, 2007).

Pathogenic bacteria cause a huge number of global health threats that may be fatal for humans including for instance pneumonia, typhoid fever, diphtheria, tetanus, syphilis, and leprosy (Kim et al., 2020). Staphylococcus aureus is a pathogen responsible for several diseases, including skin infections, toxic shock syndrome, and septic shock. Similarly, Escherichia coli and Salmonella typhimurium are the main causative agents of fatal diseases including uremia, hepatic failure, acute respiratory distress syndrome for E. coli, and typhoid fever for S. typhimurium (Mofeed et al., 2019). In the same line, the human viruses are very small obligate intracellular parasites that differ widely from other microorganisms and cause a number of fatal diseases to humans (Deyab et al., 2020). Coxsackie viruses B, is a prominent member of the (Picornaviridae family) could human such cause many chronic diseases, as cardiomyopathy, exanthema fever, neurological disorders, pancreatitis and severe neonatal diseases (Shaheen et al., 2017).

In the same context, Rotavirus (RV) is an eminent member that belongs to the family of Reoviridae and is regarded as the major reason for infants' diarrhea on a global scale, particularly in developing countries (Yeom et al., 2019). Rotavirus infection regarded to cause 450,000 deaths from 111 million incidences of infantile gastroenteritis annually (World Health Organization, 2013). In a world that suffers from the waning capabilities of antibiotics and the absence of effective antiviral drugs, there is a great need for effective, cheap, and safe alternatives. As a result of that, this work aimed at determining the antimicrobial activities of three marine macroalgae extracts including both the antibacterial activity against S. aureus, E. coli and S. typhimurium and the in vitro antiviral activity against both RV and CVB3 infections.

Materials and Methods

Sampling sites

Seaweed's biomasses were collected from two sites in the Mediterranean Sea, the first at Abu-Qir approximately 20 kilometers east of Alexandria lying longitude between 30°4'E and 30°20'E, and latitude between 31°16'N and 31°28'N, the second at Ras-Elbar City which is bordered by the Damietta Nile branch (N31°30'45" E 31°49'32"). Only one alga was collected from a site at Hurghada city in the Red Sea Governorate of Egypt, stretches for about 36 km (22 miles) along the seashore (N 27°15'28"E 33°48'42").

Collection of algal biomasses

Three commonly flourished seaweed species were handpicked during the mid-autumn at a depth of 0.2 m or less from the rocking of Eastern Harbor water, *Amphiroa anceps* collected from Abo-Qir-Alexandria, *Corallina officinalis* from Ras-Elbar-Damietta, and *Sargassum filipendula* was collected from the seashore of Hurghada-Red Sea, Egypt. The size of the collected living algal biomass samples (fresh weight) followed strictly the rules of the Egyptian Environmental Affairs Agency (EEAA) assigned for bio-conservation of the protected areas. Samples were collected along the intertidal zone using five quadrants (1 mm) according to Londo-scale (Londo, 1984).

Preparation of dry biomass

The algae samples were firstly rinsed several times with saline water and finally with distilled water to remove epiphytes and sediments, then identified to species levels (Brodie *et al.*, 2016; de Clerck and Coppejans, 1996). Seaweeds were dried in shadow, cut into small pieces, macerated, and preserved in a well-closed dark amber-colored container.

Biochemical component of algae

Biochemical analysis was carried out to assess total protein, lipid, and carbohydrate content (% of dry weight), protein was estimated from the elemental nitrogen (N) and the use of nitrogen-protein conversion factor of 6.25 according to Cunniff and Washington (1997). Total carbohydrate was analyzed by using the method of Dubois *et al.* (1956) with the use of glucose as a standard. Lipids were extracted with a chloroform-methanol mixture (2:1 v/v) then were dried over anhydrous sodium sulfate, after which the solvent was removed by heating at 80°C under vacuum (Cunniff and Washington, 1997).

Fourier transforms infrared spectrometry (FTIR) of dry biomass

The FTIR spectroscopic characterization of dry seaweeds was performed using a Mattson 5000 FTIR spectrometer. The solid algal biomasses were compressed with potassium bromide (KBr) and their FTIR spectra were recorded at 25°C in a range of 400–4000/cm.

Extraction of algal biomass

Dried algal biomass (25 g) was packed in a Soxhlet extractor. The solvent mixture methanol and hexane (1:1) was added into the flask and heated. The extraction procedure was repeated until most compounds were completely extracted. The liquid extract was then cooled, decolorized on activated charcoal, and filtered, then concentrated by using a rotary evaporator at 30° C-45°C. The dried remnant was redissolved using 3 mL of dimethyl sulfoxide (DMSO) and stored at 4°C for subsequent use. The previous steps were repeated for each seaweed in addition to a control sample using methanol and hexane only.

GC-MS of crude extracts

The chemical nature of the three crude extracts was detected using Varian Chrompack CP-3800 GC/MS/MS-2000 equipped with split-splitless injector and DB-5.625 GC column (30 m \times 0.25 mm i.d., 0.25 μm -film thickness). Helium was used as a carrier gas with a flow rate of 1 mL/min.

Antiviral activity

The antiviral potent activity of the three algal extracts was tested against simian Rotavirus (RV) and Coxsackie virus B3 (CVB3) in the following manner:

Cell lines and virus titers

African green monkey kidney cells (Vero) and Rhesus monkey kidney cells (MA 104) were used for the CVB3 and RV infections, respectively. Vero cells were enriched in Dulbecco's Modified Eagle Medium (DMEM) (Merck Millipore, Germany), while the MA 104 cells were grown in Eagle's minimum essential medium (EMEM) (Merck Millipore, Germany). As previously described by Devab et al. (2020) 10% heat-inactivated fetal bovine serum (FBS), 100 µg/mL streptomycin, 1% HEPES (4-2-hydroxyethyl-1piperazineethanesulfonic acid), and 100 units/mL penicillin were added to each medium, then they were incubated in 5% CO₂ atmosphere. For the purpose of cytotoxicity and antiviral assays, 2% of fetal bovine serum was added to each medium. For the virus titration, the DMEM and EMEM media were inoculated with CVB3 and activated RV SA-11 with trypsin (10 mg/mL) for 30 min at 37°C, then the virus titers were calculated as TCID₅₀/0.1 mL according to Spearman Kärber formula (Finney, 1978). The virus stocks were preserved at -80°C until further use. Viruses were obtained from the Environmental virology lab (National Research Center, Egypt).

Cytotoxicity assay

A series of algal crude extract concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 μ gml⁻¹) were prepared in DMEM and EMEM media, then the cells were treated with these concentrations. Two control samples were used one containing medium only and the other using media and DMSO. According to Abid *et al.* (2012), the cytotoxic effect of the seaweed extracts was obtained using the MTT colorimetric technique, where absorbance was measured using an ELISA microplate reader at 540 nm. The cytotoxic concentration (CC₅₀) which is the concentration of algal extract that reduces 50% of cell viability comparing to control, was obtained using the following formula [(A-BA) × 100], where A and B refer to of cell control and treated cells, respectively.

Antiviral activity of algal extracts against RV and CVB3

Vero $(5 \times 10^3 \text{ cells/well})$ and MA 104 $(5 \times 10^4 \text{ cells/well})$ cell lines were grown in 96 well microtiter plates for 24 h in a CO₂ incubator at 37°C. The culture media had been withdrawn and three non-toxic concentrations from each seaweed extract were chosen to be tested against viral infections. Inhibition of cytopathic effect of virus was examined daily and estimated by the MTT as mentioned previously. The protection percentage (EC₅₀) was estimated as [(A–B)/ (C–B) × 100], where A, B, and C are the readings of the spectrophotometric absorbance for the extracts with virusinfected cells, virus, and control cells, respectively. The protection percentage (EC₅₀) was considered as the concentration of the extract that protects 50% of treated infected cells, compared to the control cell. Therapeutic indexes (TI) of the seaweed's extracts were estimated by calculating the ratio CC_{50} over EC_{50} .

Antibacterial activity

The antibacterial inhibition activity of the three crude extracts was tested against three bacterial strains namely, *E. coli* ATCC 8739, *S. typhimurium* ATCC 14028 and *S. aureus* ATCC 6538 (purchased from the Global bioresource center (ATCC, USA)) as follows.

Percent inhibition of bacterial strains

Bacterial strains (24 h aged) were cultivated in tryptic soy broth media TSB (Merck Millipore, Germany) and then incubated in an air incubator at 37°C for 24 h. A volume of 200 µL from each bacterial strain was cultured using the pour plate technique in freshly prepared sterile tryptic soy agar TSA (Merck Millipore, Germany). The plates were then treated with algal extracts using various concentrations (12.5, 25, 50, and 100 µg/mL) with keeping untreated plate as a control. Percent of bacterial inhibition was calculated using the following formula % of inhibition of bacteria = (Cc - Cs/Cc) × 100 Where's: Cc= Control count and the Cs = Count in different concentrations.

Scanning electron microscopy (SEM) of bacteria

The tested bacteria were inoculated in nutrient broth (NB) media (Merck Millipore, Germany) containing algal extract at a final concentration (100 μ g/mL) and incubated at 37°C for 12 h, control bacterial samples were included. After incubation time, the bacteria were centrifuged and fixed using a formalin-glutaraldehyde fixative (4F1G) in 0.1 M phosphate buffer (pH 7.4), then the specimens were furthermore fixed using 1% osmium tetroxide in the same buffer. The specimens were then dehydrated using a series of acetone concentrations. A Polaron E500 sputter coater (Polaron Equipment Ltd., England) was used to coat the dried specimens and coated them with gold-palladium, then specimens examined using a scanning electron microscope (JEOL JSM 35C).

Statistical analysis

All tests were done in triplicates and the results are expressed as a mean \pm standard deviation (SD). Statistical analysis was performed by one-way ANOVA using the SPSS 11.5 for Windows.

Results

Biochemical characterization for tested seaweeds

The biochemical nature of the collected seaweeds is shown in Tab. 1. The protein content ranged between 4.75% and 6.15% of the dry weight, with the highest protein content recorded by *S. filipendula*. Similarly, the highest carbohydrate content was displayed by *S. filipendula* (45%), generally carbohydrate content ranged between 45% and 33.15% of dry weight. Unlike the carbohydrates and proteins, the highest total lipid recorded by *C. officinalis* (8.06%), while the lowest displayed by *A. anceps* (2.65%).

Seaweed	Protein	Lipid	Carbohydrates
Amphiroa anceps	5.60 (±2.83)	2.65 (±0.49)	33.13 (±0.89)
Corallina officinalis	4.75 (±1.68)	8.06 (±0.35)	35.29 (±1.37)
Saroassum filipendula	6.15(+2.71)	3.05(+0.21)	45.00 (+1.24)

Percentage (%) ±SD of biochemical composition of tested seaweeds

FTIR characterization of seaweeds

The FTIR spectrum of A. anceps, C. officinalis and S. filipendula are shown in Figs. 1-3. Tab. 2 clarifies the distinctive absorbed bands. It is worth to mention that the strong absorption bands at 3349, 3447, 3438, 3444, and 3448/cm in different seaweeds are representing the C-H, N-H and O-H stretching, while stretching vibration of CH3 and CH2 groups cause weak band at 2923 and 2900 cm⁻¹. The peak of the weak bands 2500-2524/cm that appeared in A. anceps and C. officinalis may match the C-O stretching band. Meanwhile, the distinctive weak band between 2050/cm and 2113/cm indicative of C = Nwas observed at all seaweeds, but it is absent in S. filipendula. However, the week absorption peaks around 1638 and 1800 cm⁻¹ of three species are due to the C-O stretching and NO asymmetric stretching. Other bands appear in the FTIR profiling of the macroalgal dry biomass indicating the presence of other functional groups like O-H and C-O bending vibration (carboxylic acid), C-F stretching and Si-O in the two red algae.

GC-MS of the seaweed's crude extracts

The chemicals in the three seaweed extracts were analysed by using GC–MS, and the chromatogram of the tested extracts illustrated in Figs. 4–6. A total of 54 various compounds were recognized in the three tested extracts. The recognized compounds with their common name, IUPAC name, molecular formula, retention time, % peak area, chemical group, and bioactivity were clearly shown in Tab. 3. The characterized compounds were classified into 10 chemical groups. The identified chemical groups are Alcohols (5 compounds), aldehydes (5 compounds), amides (1 compound), hydrocarbons (4 compounds), ketones (10 compounds), esters (17 compounds), fatty acids (7 compounds), phenol (2 compounds), terpenes (2 compounds), and steroids (1 compound). A lower number of chemical compounds (12 compounds) were recognised in the crude extract of *A. anceps* than that identified in *S. filipendula* extract (23 compounds).

Antiviral activity

Cytotoxicity against Vero and MA 104 cell lines

Cytotoxicity of the seaweed crude extracts against Vero and MA104 cells was performed using the MTT colorimetric assay. The extracts exhibited less cytotoxicity against Vero cells more than MA104 cells (Tab. 4). Cytotoxicity of the extracts (CC₅₀) against Vero cells ranged between 750 \pm 7.5 and 1293 \pm 12.3 µg/L, while CC₅₀ of the extracts against MA104 ranged between 600 \pm 6.8 and 851 \pm 13.2 µg/L. Meanwhile, the extract of *S. filipendula* showed less cytotoxic effect against Vero and MA 104 cell lines.

Antiviral activity of the extracts against CVB3 and RV

In the present work, we have assessed the antiviral effect of seaweed extracts on both the attachment and penetration



FIGURE 1. FTIR spectra of A. anceps.



FIGURE 2. FTIR spectra of C. officinalis.



FIGURE 3. FTIR spectra of S. filipendula.

steps of viruses to the host cells. The crude extracts of seaweeds displayed good antiviral activity against CVB3 (Tab. 5 and Fig. 7). Where, the extract of *A. anceps* revealed the highest antiviral effect against CVB3 with TI = 15 and it reduces viral titers by 2.5 log TCID₅₀, while the extract of *S. filipendula* showed the lowest antiviral effect against CVB3 with TI = 1.4 and decrease in viral titers by 0.75 log TCID₅₀. Additionally, the seaweed crude extracts showed high to moderate antiviral activity against RV (Tab. 5 and Fig. 13). The highest antiviral effect against the RV was exhibited by *A. anceps* extract with TI = 22 and reduction in viral titers by 2.75 log TCID₅₀, followed by the extract of *C. officinalis* with TI = 17 and reduction in viral titer by 2.5 log TCID₅₀. The lowest antiviral effect against RV was

displayed also by *S. filipendula* extract with TI = 2.4 and reduce viral titers by 1.0 log TCID₅₀.

Antibacterial activity

Inhibition of bacterial growth

The inhibition percentage displayed by the crude extracts of the three seaweeds against *S. aureus* (Gram-positive), *E. coli* (Gram-negative), and *S. typhimurium* (Gram-negative) were illustrated in Figs. 8–10. The IC₅₀ value is the concentration of the seaweed extract which causes inhibition to 50% of treated bacterial cells compared to control. It is noticeable that there were differences in antibacterial inhibition activities of the crude extracts of the three seaweeds, where each extract showed a varying degree of inhibition against

TABLE 2

Compound	Functional Groups	Amphiroa anceps		Corallina officinalis		Sargassum filipendula	
		Wn/cm	IE	Wn/cm	IE	Wn/cm	IE
Polysaccharides Amino acids	N-H Stretching O-H Stretching	3438	S	3444	S	3448	S
Aliphatic Compounds	CH ₃ and CH ₂ stretching	2923	W	2900	W	2923	W
phosphine	C-O Stretching band, P-H stretching	2524	М	2523	W	-	
Nitrile	cyanide stretch	2083	W	2100	W	-	
Ester, Pectin	asymmetric Stretching vibration of COO- (Uronic acid)	1799	W	1800	W	1638	W
Lignin	C=C Stretching	1508	W	-		-	
Cutin	C–O Stretching O–H bending	_		1411	S	1421	W
Cellulose, Carbohydrates	C–F Stretching Si–O	-		-		1081	W
Starch and poly saccharides	S=O Stretching (sulfonides)	1028	М	1034	М	-	
Glucose, Galactose	Out of plane C-H bending	876	S	876	S	877	W
Sulphates	C-S Stretching C=S Stretching (sulfides)	718	М	718	М	714	W

FTIR absorption frequencies (cm⁻¹), intensity estimation and functional group of collected seaweeds

Note: (Wn, wavenumbers; IE, intensity estimation; S, strong; M, medium; W, weak).



FIGURE 4. Chromatogram of A. anceps crude extract.

tested bacterial strains with different extracts concentrations. The highest concentration of crude extracts (100 μ g/mL) causes mainly the maximum antibacterial inhibition activity towards the tested strains. Extract of *S. filipendula* showed the highest values of antibacterial activity towards *E. coli* with 91.4% inhibition followed by *C. officinalis* with 85.7% inhibition, while the extract of *A. anceps* displayed only

57.1% inhibition against *E. coil* cells. The results showed that the extract of *C. officinalis* inhibited 100% of *S. aureus* cells at 100 μ g/mL concentration followed by *S. filipendula* (82.5%) and *A. anceps* (75%). Again, the extract of *C. officinalis* causes the highest inhibition (80%) against *S. typhimurium*, followed by both *S. filipendula* (65%) and *A. anceps* (60%).





FIGURE 6. Chromatogram of S. filipendula crude extract.

TABLE 3

Bioactive compounds present in the extracts of the tested seaweeds

No.	Amphiroa anceps-Compounds	C.F.	R.T.	P.A.%	C.G.	Bioactivity
1	Benzoic acid, hydrazide	C ₇ H ₈ N ₂ O	30.4	8.1	Aldehyde	-
2	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	21.9	2.8	Ketone	-
3	Dodecyl acrylate	$C_{15}H_{28}O_2$	33	2.2	Ketone	-
4	Nonadecane	$C_{19}H_{40}$	33.9	5.7	Hydrocarbon	-
5	Tetradecanoic acid	$C_{14}H_{28}O_2$	34.1	4.6	Fatty acid	Antiprotozoal & Antimicrobial
6	Carbonic acid, butyl undec-10-enyl ester	$C_{16}H_{30}O_3$	31.4	2.24	Ester	
7	6,10,14-Trimethylpentadecan-2-one (Phytone)	$C_{18}H_{36}O$	35.9	1	Ketone	Antimicrobial
8	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}$	36.1	3.4	Ketone	Antimicrobial
9	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	36.9	29.5	Ester	Antimicrobial
10	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	55.4	7.8	Fatty acid	Anti-tumor & Immunostimulant
11	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	61.1	30.4	Ester	-
12	Cholesterol	$C_{27}H_{46}O$	64.9	2	Steroid	_
No.	Corallina officinalis-Compounds	C.F.	R.T.	P.A.%	C.G.	Bioactivity
1	Benzoic acid, hydrazide	$C_7H_8N_2O$	1.1	1.5	Aldehyde	-
2	Octadecanoic acid, 1-[(tetradecyloxy)carbonyl]pentadecyl ester	$C_{48}H_{94}O_4$	4.9	1.7	Ester	-
3	Nonanal	$C_9H_{18}O$	20.3	2.2	Aldehyde	Antifungal
4	Thiosemicarbazide	CH ₅ N ₃ S	23.8	1.13	Amide	Anticancer, Anti- protozoa & Antibacterial
5	Sulfurous acid, butyl undecyl ester	$C_{15}H_{32}O_3S$	25.6	2.1	Ester	-
6	2,6-Difluorobenzoic acid, tridec-2-ynyl ester	$C_{20}H_{26}F_2O_2$	26.1	5	Ester	-
7	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	27	1.76	Phenol	-
8	4,4,7a-Trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (Dihydroactinolide)	$C_{11}H_{16}O_2$	32	2.26	Ketone	Antiviral
9	Tetradecanoic acid	$C_{14}H_{28}O_2$	33.8	9.1	Fatty acid	Anti-protozoa & Antimicrobial
10	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	34.6	3.1	Alcohol	-
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	35.1	3	Terpene	Antimicrobial and Source of vitamin K
12	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (DIISOBUTYL PHTHALATE)	$C_{16}H_{22}O_4$	35.4	6.1	Ester	-
13	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}$	35.8	8.1	Ketone	-
14	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	36.8	1.7	Ester	Antimicrobial
15	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl) ethyl]pentyl ester	C ₁₉ H ₂₅ NO ₅	39.5	3.1	Ester	_
16	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	40	3	Hydrocarbon	-
17	1-Nonadecanol	$C_{19}H_{40}O$	41.5	3.7	Alcohol	Antibacterial & Antimicrobial
18	Methyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate (Methyl arachidonate)	$C_{21}H_{34}O_2$	45.9	2.6	Ester	Antimicrobial
19	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	60.2	38.2	Ester	-

(Continued)

SEAWEEDS ANTIMICROBIAL ACTIVITY

Table 3 (continued).

No.	Sargassum filipendula-Compound	C.F.	R.T.	P.A.%	C.G.	Bioactivity
1	Acetic acid, 2-(2-acetoxy-2,5,5,8a-tetramethyldecalin-1-yl)-	$C_{18}H_{30}O_4$	14.98	3.8	Aldehyde	-
2	Oleic Acid	$C_{18}H_{34}O_2$	18.12	1.07	Fatty acid	Anticancer
3	Ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-	$C_{13}H_{20}O_{3}$	18.37	0.46	Fatty acid	-
4	Dihydroxanthin	$C_{17}H_{24}O_5$	18.84	0.3	Ketone	-
5	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	19.4	3.07	Alcohol	-
6	Cholestan-3-ol, 2-methylene-, (3β,5α)-	C ₂₈ H ₄₈ O	19.53	4	Terpene	-
7	13-Heptadecyn-1-ol	C ₁₇ H ₃₂ O	19.84	5.5	Alcohol	-
8	1-Heptatriacotanol	C37H76O	20.58	1.38	Alcohol	-
9	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	20.93	3.8	Ester	-
10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	21.78	0.76	Fatty acid	Antitumor & Antiviral
11	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	24.21	0.46	Ester	-
12	Octadecanal, 2-bromo-	C ₁₈ H ₃₅ BrO	24.5	32.5	Hydrocarbon	-
13	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	25.1	0.92	Fatty acid	Anticancer
14	Docosahexaenoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{98}O_6$	27.15	0.61	Ester	-
15	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	27.59	12.2	Ester	-
16	Retinoic acid, methyl ester	$C_{21}H_{30}O_2$	27.95	3.3	Ester	Antitumor
17	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy)-3- [(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-deca	$C_{24}H_{32}O_8$	28.49	2.3	Ketone	-
18	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5- trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	28.62	8	Aldehyde	-
19	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	28.77	2	Ester	-
20	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 4,9,9a-tris (acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,	$C_{28}H_{36}O_{11}$	31.26	1.4	Ketone	-
21	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	$C_9H_9F_3O_2$	31.53		Phenol	-
22	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9a-(acetyloxy)- 1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9-trihyd	$C_{22}H_{30}O_7$	32.77	-	Ketone	-
23	Acetic acid, 3-hydroxy-4,4,10,13-tetramethyl-7-oxo- 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclo	$C_{23}H_{34}O_4$	37.42	-	Hydrocarbon	-

Note: (C.F., Chemical formula; R.T., Retention time(min); P.A%, percent peak area; Bioactivity, Biological activity).

TABLE 4

Extracts of Seaweeds	CC ₅₀	, (μg/mL)
	Vero Cells	MA 104 Cells
Amphiroa anceps	780 ± 8.7	670 ± 9.2
Corallina officinalis	750 ± 7.5	600 ± 6.8
Sargassum filipendula	1293 ± 12.3	851 ± 13.2

Cytotoxicity \pm SD of Seaweeds extracts against Vero and MA 104 cells

Scanning electron microscopy of the bacteria

As a result of the bactericidal activity of the three seaweeds crude extracts, the morphology of the tested bacterial cells was compared to control after treating the bacterial cells with 100 μ g/mL of each extract for 12 h using scanning electron microscopy. The cytomorphology of *E. coli* control

was apparently normal rods with a well-defined rigid and smooth surface, while the treated *E. coli* cell consisted of most degraded cells which appeared shrunk, ragged, wrinkled, several dents and holes were also distinguished on the surface of the cells, in addition to that, some cells were appeared empty with hollow ends (Fig. 11).

TABLE 5

Antiviral activity of Seaweeds extracts against CVB3 and RV determined by MTT and Kärber Method

Extracts of Seaweeds	CV	B3	RV (SA-11)		
	EC ₅₀ ^a	TI ^b	EC ₅₀	TI	
Amphiroa anceps	52	15	30.5	22	
Corallina officinalis	85.2	8.8	32.7	18.3	
Sargassum filipendula	924	1.4	353.3	2.4	

Note: ^aConcentration of extract that inhibits viral infectivity (Cytopathic Effect) by 50%. ^bTherapeutic index = CC_{50}/EC_{50} . Values are given as the mean from triplicate experiments.



FIGURE 7. Inhibitory potential of seaweeds extracts on both CVB3 and RV infections.



FIGURE 8. The inhibition of E. coli growth comparing to control via different extracts.



FIGURE 9. The inhibition of S. aureus growth comparing to control via different extracts.



FIGURE 10. The inhibition of S. typhimurium growth comparing to control via different extracts.

The cytomorphology of S. aureus control apparently indicated the presence of normal straight spherical cells and adhering together by the smooth surface, the extracts influenced the treated bacterial cells to be malformed, enlarged with tiny holes and dents on the cell surfaces, for most of the bacterial population, and few viable cells with a rigid outline and smooth surface remained (Fig. 12). However, the control cells of S. typhimurium were rod-like bacilli with a smooth surface and rigid well-defined shape, unlike the treated cells which appeared elongated, enlarged, and malformed, most of the cell population consisted of most degraded cells which appeared ragged, wrinkled, and shrunk (Fig. 13).

Discussion

Algae specially macroalgae have been a valuable source for a group of valuable metabolites such as vitamins, enzymes, proteins, carotenoids, polysaccharides, lipids, sterols. antibiotics in addition to a lot of fine chemicals (Devab et al., 2020; Mofeed et al., 2019). In this regard, many secondary and primary metabolites produced by macroalgae (Seaweeds) organisms may be of importance in pharmaceutical industries (Mofeed et al., 2019). The present work represents a comprehensive study that focused on searching for new therapeutic alternatives that displayed antimicrobial (antiviral and antibacterial) activities. To achieve this goal, the crude extract of three seaweeds species namely A. anceps, C. officinalis and S. filipendula were assayed in vitro for their antiviral activity against two human viruses (Rotavirus and Coxsackie virus B3), and also for their antibacterial properties against three pathogenic bacteria (S. typhimurium, S. aureus



FIGURE 11. SEM micrographs of *E. coli* cytomorphology before (control) and after treatment with the extracts.

and *E. coli*). The identity of the collected seaweeds in the present study was done by morphological characterization and confirmed by microscopic examination of its edges. In this regard, three seaweeds were collected from different habitats two were Rhodophyta (*A. anceps* and *C. officinalis*) while *S. filipendula* belonging to Phaeophyta. The present investigation paid great attention to the biochemical characterization of collecting seaweeds; a high diversity of the biochemical nature of seaweeds paves the path to explore a variety of compounds in their body composition.

Generally, carbohydrates represent the maior biochemical component in the three seaweeds, this may be due to higher pyrocollodion content in their cell walls (Ismail and Mohamed, 2017; Mendis and Kim, 2011). The changes in the biochemical content may be spatial or temporal but dominantly attributed to water quality (Dave and Parekh, 1975). In the present study, the FTIR results of the crude seaweeds indicated the presence of various chemical functional groups which hinted the presence of many metabolic active products, these results come inconsistency with previous work by Demir et al. (2015), Guerrero et al. (2013), Moustafa et al. (2018) and Singh et al. (2016). The GC-MS analysis of the crude extract of different seaweeds showed the presence of chemical substances with previously known antimicrobial activity effective. Molecules such as tetradecanoic acid, 6,10,14trimethylpentadecan-2-one (Phytone), pentadecanoic acid, 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione,

14-methyl-methyl thiosemicarbazide, ester, nonanal, 1-nonadecanol and methyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14tetraenoate (methyl arachidonate) are known for their antimicrobial activities (Ding et al., 2014; Ertas et al., 2015; Temiz et al., 2015). Dihydroactinolide (4,4,7atrimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one), and n-hexadecanoic acid are compounds known for their antiviral activity found in C. officinalis and S. filipendula crude extracts, respectively (Librán-Pérez et al., 2019; Shilova et al., 2012). The fast development of microbial resistance encouraged researchers to innovate a variety of antibiotics and novel drugs, especially those involved in the treatment of infectious diseases. The cited results revealed that, the three crude extracts displayed a high antibacterial effective against S. aureus, S. typhimurium and E. coli, these results come inconsistency with that recorded by Abdel-Khalig et al. (2014), and Taskin et al. (2007) who noted the antimicrobial inhibiting activity of crude extracts of some seaweeds against a lot of pathogenic bacteria especially S. aureus and E. coli. Despite Many efforts to prevent and/or control RV and CVB3 infections, these infections still affect millions of children worldwide particularly in developing countries (Kalsi and Etzler, 2000). In this line, the present investigation represents an attempt to study the antiviral activity of extracts of three seaweeds against Coxsackie virus B3 and simian Rotavirus (RV). The cycle of viral replication implicates series of steps; attachment, penetration, and replication of the genetic materials, then assembly, followed



FIGURE 12. SEM micrographs of *S. aureus* cytomorphology before (control) and after treatment with the extracts.

by liberation from host cells. These steps can be used as targets of anti-Coxsackie virus B3 and anti-rotavirus agents (Shaheen *et al.*, 2015).

In this work, we determined the impact of tested extracts on the attachment and penetration steps. All seaweeds extract exhibited a low cytotoxic effect against Vero and MA 104 cell lines, and these results are in accordance with that reported by Morán-Santibañez *et al.* (2018) who find that, CC_{50} of the *Ulva intestinalis* extract was more than 1500 µg/mL. Extracts of *A. anceps* and *C. officinalis* displayed a high antiviral effect on RV with TI values 22, 18.3 and cause high inhibition in virus titers with 45.8 and 41.6%, respectively. In line with that, the extracts of *A. anceps* and *C. officinalis* showed lower anti-viral activities against CVB3 with TI values of 15 and 8.8, and inhibition of virus titers with 41.6 and 29.1%, respectively. In a previous study, Soares *et al.* (2012) demonstrated that seaweed extract has antiviral activities against the herpes simplex virus (HSV), also Shi *et al.* (2017) and Morán-Santibañez *et al.* (2018) described that, the algal secondary metabolites have been tested as antiviral agents as a prophylactic strategy before viral infection and also as an effective treatment after infection to avoid infection dissemination. There are limited previous studies about the antiviral activities of seaweed extracts against both RV and CVB3, therefore these results may be considered as a pioneer record of the antiviral impact of Seaweed extracts towards CVB3 and RV.



Control

Corallina officinalis

anceps

Sargassum filipendula

FIGURE 13. SEM micrographs of S. typhimurium cytomorphology before (control) and after treatment with the extracts.

Conclusion

In conclusion, the present results proved that the extract of three weeds namely A. anceps, C. officinalis and S. filipendula possess an effective antibacterial activity against S. typhimurium, S. aureus and E. coli pathogenic bacteria and showed antiviral activity against CVB3 and RV infections and can be used as pretreatment for their infections by attaching to the capsids to prevent the connection with the cell receptors and thereby stop their penetration into host cells. The promising results together with the simple and cost-effective extraction method make the tested seaweeds as an opportune source of antiviral and antibacterial drugs, but more in vivo studies are needed to evaluate the antimicrobial activities of these seaweeds.

Availability of Data and Materials: All data generated or analyzed during this study are included in the manuscript.

Authors' Contribution: The authors confirm contribution to the paper as follows: Design and study conception: JM and MD. Draft manuscript preparation: JM and EE-B. Data collection and supervising the practical work: AM. Writing some parts of the article, revise the final version and submit it for publication: MM. Writing some parts of the article, revise the final version: SN. Morphological and molecular identification as well as cultivation of the microbe: EE-B. All authors reviewed the results and approved the final version of the manuscript.

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References

- Abdel-Khaliq A, Hassan H, Rateb ME, Hammouda O, Science G (2014). Antimicrobial activity of three Ulva species collected from some Egyptian Mediterranean seashores. International Journal of Engineering Research 2: 648-669.
- Abid N, Rouis Z, Lassoued M, Sfar S, Aouni M (2012). Assessment of the cytotoxic effect and in vitro evaluation of the antienteroviral activities of plants rich in flavonoids. Journal of Applied Pharmaceutical Science 2: 74.

- Al-Fadhli A, Wahidulla S, D'souza L (2006). Glycolipids from the red alga Chondria armata (Kutz.) Okamura. Glycobiology 16: 902–915.
- Aslam A, Fazal T, Zaman Q, Shan A, Rehman F, Iqbal J, Rashid N, Rehman M (2020). Biorefinery of microalgae for nonfuel products. *Microalgae Cultivation for Biofuels Production*. pp. 197–209. Academic Press.
- Brodie J, Wilbraham J, Pottas J, Guiry MD (2016). A revised checklist of the seaweeds of Britain. *Journal of the Marine Biological Association of the United Kingdom* **96**: 1005.
- Brito T, Barros F, Silva R, Júnior G, Júnior J, Franco Á, de Paula R (2016). Sulfated polysaccharide from the marine algae Hypnea musciformis inhibits TNBS-induced intestinal damage in rats. *Carbohydrate Polymers* 151: 957–964.
- Cunniff P, Washington D (1997). Official methods of analysis of AOAC International. *Journal of AOAC International* **80**: 127A.
- Dave M, Parekh R (1975). Protein content of green seaweeds from Saurashtra coast. *Salt Research India* 11: 41–44.
- de Clerck O, Coppejans E (1996). *Marina Algae of the Jubail Marine Wildlife Sanctuary*, Saudi Arabia. Citeseer.
- Demir P, Onde S, Severcan F (2015). Phylogeny of cultivated and wild wheat species using ATR-FTIR spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 135: 757–763.
- Deyab M, Mofeed J, El-Bilawy E, Ward F (2020). Antiviral activity of five filamentous cyanobacteria against coxsackievirus B3 and rotavirus. *Archives of Microbiology* **202**: 213–223.
- Ding H, Zhu T, Yin X, Liu J, Zhang L, et al. (2014). Pyrrolidine dithiocarbamate protects pancreatic β-cells from oxidative damage through regulation of FoxO1 activity in type 2 diabetes rats. *Acta Biochimica et Biophysica Sinica* **46**: 582–589.
- Dubois M, Gilles K, Hamilton J, Roberts P, Smith F (1956). Phenol sulphuric acid method for carbohydrate determination. Annalen der Chemie 28: 350–359.
- El-Baroty G, Moussa M, Shallan M, Ali M, Sabh A, Shalaby E (2007). Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: *Asparagopsis taxiformis* (Delile) Trevisan. *Journal of Applied Sciences Research* 3: 1825–1834.
- Ertas A, Yilmaz MA, Firat M (2015). Chemical profile by LC-MS/MS, GC/MS and antioxidant activities of the essential oils and crude extracts of two *Euphorbia* species. *Natural Product Research* **29**: 529–534.
- Fakayode OA, Aboagarib EAA, Zhou C, Ma H (2020). Co-pyrolysis of lignocellulosic and macroalgae biomasses for the production of biochar-A review. *Bioresource Technology* 297: 122408.
- Finney D (1978). Statistical method in biological assay. vol. 3. New York: Macmillan Publishing Co., Inc.
- Guerrero P, Garrido T, Leceta I, de La Caba K (2013). Films based on proteins and polysaccharides: Preparation and physicalchemical characterization. *European Polymer Journal* 49: 3713–3721.
- Ismail M, Mohamed S (2017). Differentiation between some Ulva spp. by morphological, genetic and biochemical analyses. Вавиловский журнал генетики и селекции 21: 360–367.
- Kalsi G, Etzler ME (2000). Localization of a Nod factor-binding protein in legume roots and factors influencing its distribution and expression. *Plant Physiology* **124**: 1039–1048.
- Khalafi T, Buazar F, Ghanemi K (2019). Phycosynthesis and enhanced photocatalytic activity of zinc oxide nanoparticles toward organosulfur pollutants. *Scientific Reports* **9**: 1–10.

- Kim Y, Gu C, Kim HU, Lee SY (2020). Current status of pan-genome analysis for pathogenic bacteria. *Current Opinion in Biotechnology* 63: 54–62.
- Librán-Pérez M, Pereiro P, Figueras A, Novoa B (2019). Antiviral activity of palmitic acid via autophagic flux inhibition in zebrafish (*Danio rerio*). *Fish and Shellfish Immunology* **95**: 595–605.
- Londo G (1984). The decimal scale for relevés of permanent quadrats. Sampling methods and taxon analysis in vegetation science: releve surveys, 'Vegetationsaufnahmen', floristic analysis of plant communities. No. 4, pp. 45–49. Junk.
- Mendis E, Kim SK (2011). Present and future prospects of seaweeds in developing functional foods. *Advances in Food and Nutrition Research* **64**: 1–15.
- McFall-Ngai, M (2007). Care for the community. *Nature* **445**: 153–153.
- Mine I (2008). Biological Interactions during the life history of seaweed–A microscopic review. *Kuroshio Science* 2: 35–40.
- Mofeed J, Sabry A, Deyab M (2019). Evaluation of Biochemical composition and bioactivities of two Ulva species (Ulva lactuca and Ulva fasciata): A comparative study. *Bioscience Research* **16**: 3801–3811.
- Morán-Santibañez K, Peña-Hernández MA, Cruz-Suárez LE, Ricque-Marie D, Skouta R et al. (2018). Virucidal and synergistic activity of polyphenol-rich extracts of seaweeds against measles virus. *Viruses* **10**: 465.
- Moustafa M, Taha T, Elnouby M, Abu-Saied M, Shati A et al. (2018). Feasible design for electricity generation from *Chlorella vulgaris* using convenient photosynthetic conditions. *Biocell* **42**: 7.
- Nishide E, Uchida H, (2003). Effects of Ulva powder on the ingestion and excretion of cholesterol in rats. *The 17th International Seaweed Symposium*, pp. 165–168. Oxford Press.
- Rezazadeh H, Buazar F, Matroodi, S (2020). Synergistic effects of combinatorial chitosan and polyphenol biomolecules on enhanced antibacterial activity of biofunctionalaized silver nanoparticles. *Scientific Reports* 10: 1–13.
- Schwartsmann G, Brondani da Rocha A, Berlinck RG, Jimeno J (2001). Marine organisms as a source of new anticancer agents. *Lancet Oncology* 2: 221–225.
- Shaheen M, Borsanyiova M, Mostafa S, Chawla-Sarkar M, Bopegamage S, El-Esnawy N (2015). In vitro effect of Dodonaea viscosa extracts on the replication of coxackievirus B3 (Nancy) and rotavirus (SA-11). Journal of Microbiology and Antimicrobial Agents 1: 47–54.
- Shaheen M, Borsanyiova M, Mostafa S, Sarkar M, Bopegamage S (2017). In vitro and in vivo evaluation of Bauhinia variegata extracts to prevent coxsackievirus B3 infection. Journal of Proteomics & Bioinformatics 10: 3.
- Shi Q, Wang A, Lu Z, Qin C, Hu J, Yin J (2017). Overview on the antiviral activities and mechanisms of marine polysaccharides from seaweeds. *Carbohydrate Research* **453**: 1–9.
- Shilova I, Semenov A, Kuvacheva N, Suslov N, Mustafin R (2012). Isolation, identification, and nootropic activity of compounds in *Alfredia cernua* chloroform extract. *Pharmaceutical Chemistry Journal* 46: 363–368.
- Singh RK, Kukrety A, Sharma OP, Baranwal S, Atray N, Ray SS (2016). Study of a novel phenolic-ester as antioxidant additive in lube, biodiesel and blended diesel. *Journal of Industrial Engineering Chemistry* 37: 27–31.
- Smit A (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology* 16: 245–262.

- Soares AR, Robaina M, Mendes GS, Silva TS, Gestinari L et al. (2012). Antiviral activity of extracts from Brazilian seaweeds against herpes simplex virus. *Revista Brasileira de Farmacognosia* **22**: 714–723.
- Taskin E, Ozturk M, Kurt O (2007). Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African Journal of Biotechnology* **6**: 2746–2751.
- Temiz C, Kalemci S, Micili SC, Tekmen I, Yildiz G et al. (2015). The effect of methyl palmitate on treatment of

experimental asthma. *Journal of Pakistan Medical Association* **65**: 632–636.

- World Health Organization (2013). Rotavirus vaccines: WHO position paper—January 2013. Weekly Epidemiological Record=Relevé épidémiologique hebdomadaire 88: 49-64.
- Yeom JS, Park JS, Kim YS, Kim RB, Choi DS et al. (2019). Neonatal seizures and white matter injury: Role of rotavirus infection and probiotics. *Brain Development* **41**: 19–28.