African Journal of Biotechnology Vol. 11(33), pp. 8332-8340, 24 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3982
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Antimicrobial and antiviral activities against Newcastle disease virus (NDV) from marine algae isolated from Qusier and Marsa-Alam Seashore (Red Sea), Egypt

Ibraheem Borie Mohammad Ibraheem[1]*, Neveen Abdel-Raouf[2], Mohamed Sayed Abdel-Hameed[3] and Khaled El-yamany[4]

[1]Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia.
[2]Botany and Microbiology Department, Faculty of Science and Medical Studies, Women Students Medical Studies and Sciences Sections, King Saud University for Girls, Riyadh, Kingdom of Saudi Arabia.
[3] Biology Department, Faculty of Science, Jazan University, Jazan, Kingdom of Saudi Arabia.
[4]Botany Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Accepted 23 March, 2012

Diethyl ether, acetone and ethanol extracts of ten marine macroalgae; two belonging to Chlorophyceae (*Ulva lactuca* and *Caulerpa racemosa*), two belonging to Rhodophyceae (*Acanthophora spicifera* and *Galaxaura elongata*) and six taxa belonging to Phaeophyceae (*Liagora farinosa*, *Cystoseira compressa, Cystoseira myrica*, *Hydroclathrus clathratus, Turbinaria ornata* and *Padina pavonia*) isolated from the inter tidal zone along Qusier Marsa-Alam seashore (Red Sea), Egypt, were evaluated for their antibacterial, antifungal and antiviral activities against 3 Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina maxima*), 3 Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*), one unicellular (*Candida albicans*) and two filamentous fungi (*Aspergillus flavus* and *Fusarium oxysporum*) and against the Newcastle sense Virus (NDV)-(Paramyxoviridae) which is responsible for acute respiratory distress in chicken. Data showed that some extracts recorded strong inhibitory activities than the reference antibiotics, while others were with moderate and/or week inhibitory activities. However, many were without any inhibitory effects. The cytotoxicity effect of the tested algal extracts on chicken embryo showed that both diethyl ether and acetone extracts had toxic effects, but the ethanol extracts had no toxic effect, so that the ethanol extract was considered to be the most suitable for further studies. The antiviral activities of the ethanol extracts against NDV (Newcastle disease virus) showed that seven of the ten tested algal extracts have strong activities against NDV.

Key words: Antimicrobial and antiviral activities, Newcastle disease virus (NDV), marine macroalgae, Qusier and Marsa-Alam, Red Sea, Egypt.

INTRODUCTION

Research activities concerning the investigation of metabolic products of macroalgae were under taken not only for a better understanding of nature, but also to discover metabolites of possible use for humans in different fields of interest. The screening of extracts or isolated compounds from different natural sources is a common way to discover the biological active metabolites. Secondary or primary metabolites from macroalgae or seaweeds may be potential bioactive compounds of interest for the pharmacological industry (Lima-Filho et al., 2002).

Special attention has been reported for antibacterial, antifungal and antiviral activities related to marine algae against pathogens (Deig et al., 1974; Caccamese and Azzolina, 1979; Perez et al., 1990; Ballesteros et al., 1992; Nagayama et al., 2002; Haliki et al., 2005; Fitton

*Corresponding author. E-mail: ibraheemborie@hotmail.com.

2006 Taskin et al., 2007; Zandi et al., 2007; and Salvador et al., 2007).

Some algal substances have bacteriostatic, while others have bactericidal activities. Among the algal substances which have this kind of activity; amino acids, terpenoids, phlorotannins, steroids, phenolic compounds; halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted (Mtolera and Semesi, 1996). There are not enough published data on antimicrobial or antiviral activity of Egyptian Red Sea macroalgae. In this paper, 10 Egyptian marine macroalgae of Red Sea were subjected to *in vitro* studies for this purpose.

MATERIALS AND METHODS

Collection and identification of algal species

The studied algal species collected from the inter-tidal region of Red Sea shores between Quseir and Marsa-Alam. Algal species were identified according to Borgesen (1900, 1931), Borgesen and Fremy (1936), Svedelius (1906), Hamel (1916), Setchell and Gardner (1920), Taylor (1928, 1960), Papenfuss (1940, 1946), Parr (1939), Nasr (1940a, b), Nasr and Aleem (1949), Smith (1944); Levring (1946), Bouk (1965), Scagel (1966) and Bold (1978). Taxonomic classification of the algal species was made and modified according to the system developed by Papenfuss (1955, 1968).

Extraction of selected algal species

Using three different solvent (diethyl ether as non polar solvent, acetone as aprotic polar solvent and ethanol as protic polar solvent), 25 g of dry weight of each algal species were socked in 250 ml of the previous three solvent for 24 h, then filtered and concentrated under reduced pressure by using rotary evaporator.

Test microorganisms

All tested microorganisms were kindly supplied from Biotechnological Research Center, AL-Azhar University (for boys), Cairo, Egypt.

Medium used for bacterial maintenance

According to (Wedberg, 1966), nutrient agar medium was used to maintain the tested organisms. The modified Czapek-Doxs medium (Davet and Rouxel, 2000) was used to maintain the tested fungi.

Measurement of microbial inhibition

Antimicrobial activity was conducted according to the agar diffusion assay which performed according to European Pharmacopoe (1997). Measurement of anti-viral activity was done in corporation with the Laboratory of Virology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt. A velogenic viscerotropic Newcastle disease virus (NDV) strain having a titer of $10^{9.1}$ EID50/ml was obtained from the Veterinary Serum Research Institute, Newcastle Disease Unit, Abbasia, Cairo, Egypt. Specific Pathogen free embryonated chicken eggs (SPF-ECE) were purchased from Koum Oshim Farm, Fayoum, Egypt and used to evaluate antiviral activity of tested algal extracts. ECE were inoculated via allantoic sac route on 9 to 11-day old embryos and incubated at 35°C and 70% humidity for 5 days post inoculation. Red blood cells obtained from adult chicks were added to 4% sodium citrate as anticoagulant. The cells were washed three times with normal physiological saline and the packed cells were diluted to 1% for the haemagglutination test.

Experimental solutions

Physiological saline buffer (85% pH 7.2) was prepared by dissolving 8.5 g of sodium chloride in 1000 ml distilled water and sterilized by autoclaving. As regard the antibiotic solutions, crystalline penicillin and streptomycin were used to inhibit bacterial growth in the inoculated samples. Vial of each type was dissolved in 10 ml distilled water and then added in the form of 100 IU crystalline penicillin and 100 µg streptomycin/ 1ml of the inoculums.

Detection of cytotoxicity of tested algal extracts to chicken embryos

Three doses of diethyl ether extract of each tested algae (from ten tested samples) were inoculated into ECE aged 9 to 11-day old embryos via allantoic sac route (2 eggs prepared for each dose). The inoculated eggs were incubated at 37°C and 80% humidity for 7 days with daily candling for death. At the end of incubation period, embryos were harvested and examined for death or embryo lesion. The previous procedures were repeated with the other solvents (acetone and ethyl alcohol) separately.

Propagation of NDV mixed with different algal extract in SPF–ECE

According to Allan et al. (1973), three SPF-ECE were prepared for each algal extract and inoculated via allantoic sac route (9 to 11-day old embryos) by 0.2 ml mixture (containing 0.1 ml NDV + 10 mg ethanol algal extracts). The inoculated eggs were incubated at 37°C and 80% humidity for 5 days. Twice daily candling was performed and mortalities during the first 24 h after inoculation were considered non specific and discarded, while those that survived thereafter were kept at 4°C for 24 h. The allantoic fluids of each group were aseptically harvested and centrifuged at 1500 rpm for 15 min for clarification. The clarified allantoic fluids were stored at -20°C until used for virus titration.

Infectivity titration of NDV in ECE

Ten-fold serial dilution from each harvested allantoic fluid of the ten used samples from 10^{-1} to 10^{-10}, were prepared in sterile saline solution containing antibiotic (each sample of the ten tested extracts was prepared separately).

Four ECE of 9 to 11-day old embryos were prepared for each dilution from 10^{-1} to 10^{-10} and inoculated via allantoic sac route by 0.1 ml / egg. The inoculated eggs were sealed and incubated at 37°C and 80% humidity and candled twice daily for 5 days. Dead embryos within the first 24 h post inoculation were considered non specific death and discarded. Therefore, dead embryos were removed daily, recorded and kept at -4°C until the end of incubation period. At the end of incubation period all the remaining embryos were chilled for 24 h at 4°C. Allantoic fluids were harvested from dead and live embryos, and haemagglutination test was carried out. The value of egg infective dose fifty (EID50) was calculated according to Reed and Muench (1938). Control NDV without solvent extracts was also titrated with same procedure.

Haemagglutination test (HA)

This test was done according to the standard procedure given by Anon (1971) as follows:

Each well of HA micro titer plate was filled with 50 µL saline and 50 µL of the tested sample, then 50 µL of the freshly prepared 1% washed chicken red blood cells (RBCs) were added to each well and the plates were incubated at room temperature for 15 to 30 min. Positive and negative control wells should be included. The plates were examined when RBCs in negative control settled down forming bottom like shape, and positive control showed lattice shape agglutination.

RESULTS AND DISCUSSION

Thirty extracts of 10 marine macroalgae were tested against 3 Gram-positive and 3 Gram-negative bacteria (Table 1). Crude extracts of all investigated macroalgal species except *H. clathratus* and *Padina pavonia* showed inhibitory effects at least against two of the tested bacterial species. In this respect, some extracts have shown high inhibition activities higher than recorded by Penicillin G. Among the active extracts, one extract (diethyl ether of *Galaxaura elongata*) showed specific inhibition against *Bacillus subtilis*, three extracts (diethyl ether and acetone of *Ulva lactuca* and acetone of *Acanthophora spicifera*) showed specific inhibition against *Sarcina maxima*, two extracts (diethyl ether of *A. spicifera* and ethanol of *Liagora farinosa*) showed specific inhibition against *Escherichia coli* and two extracts (diethyl ether of *Ulva lactuca* and *Cystoseira myrica*) showed specific inhibition against *Pseudomonas aeruginosa*. On the other hand, three extracts (ethanol extracts of *Ulva lactuca* and *G. elongate,* and acetone extract of *Caulerpa racemosa*) showed specific inhibition against *P. aeruginosa* resembles to that recorded by Penicillin G.

Meanwhile, some extracts recorded moderate inhibitory activities; these were acetone extracts of *C. racemosa* against *Staphylococcus aureus*, diethyl ether and ethanol of *A. spicifera* against *S. maxima* and acetone of *Ulva lactuca* and *Cystoseira compressa*, ethanol extracts of *C. racemosa* and diethyl ether of *L. farinosa* against *P. aeruginosa*. Among the crude extracts, some of them exhibited week inhibitory effects; there were all extracts of *U. lactuca,* diethyl ether and acetone of *Caulerpa racemosa* and ethanol of *C. compressa* against *B. subtilis*, all extracts of *U. lactuca,* diethyl ether and ethanol of *Caulerpa racemosa* against *S. aureus,* ethanol of *U. lactuca* and all extracts of *Caulerpa racemosa* and *G. elongata* against *S. maxima,* all extracts of *U. lactuca* and *Turbinaria ornata* against *E. coli*, diethyl ether of *C. racemosa*, ethanol of *L. farinosa* and *C. compressa* and acetone and ethanol of *C. myrica* against *P. aeruginosa*. The other macroalgal extracts were unaffected. The above results indicated that diethyl ether was the most effective solvent for extraction of the bioactive compounds followed by acetone. Furthermore, *U. lactuca* and *C. racemosa* (Chlorophyceae) were the most effective marine algae against tested bacterial species followed by the two red algal species (*G. elongata* and *A. spicifera*). Accordingly, we can suggest that antibacterial activities depend on both algal species and the efficiency of solvent used, which is in accordance with Olessen et al. (1963) and Kamat et al. (1992). The antimicrobial activity shown by *U. lactuca* might be due to acrylic acid commonly found in it. *U. lactuca* commonly known as sea lettuce, has long been used as food and as a traditional medical agent to treat helminthic infections, fever, urinary diseases, dropsy, etc (Chengkui and Junfu, 1984). The presence of active substances in *U. lactuca* is in agreement with that observed by Rao and Pareksh (1981) and Awad (2000).

The antimicrobial activity shown by *C. racemosa* in this study may be attributed to caulerpin or caulerpicin (Doti and Santos, 1966; Paul et al., 1987) or flexin and trifarin (Blackman and Wells, 1976) or by caulerpanyene (Amico et al., 1978). Several studies have found stronger anti-bacterial effects of marine algae on Gram-positive bacteria than on Gram-negative bacteria (Ikigai et al., 1993; Ibraheem, 1995; Nakaamura et al., 1996). However, in the present study, we could not find any remarkable differences in susceptibility, and this was in accordance with Nagayama et al. (2002) who reported that *Campylobacter spp*., which are Gram-negative, were most susceptible among the tested bacteria undergoing brown algal extracts. Schulz et al. (1992), suggested that the antimicrobial activities of crude extracts may result from their interaction with bacterial enzymes and proteins. Other workers (Abdel-Raouf and Ibraheem, 2008) suggested that the inhibitory effects of the purified antibiotic on the bacterial growth could be attributed to one or more of the following actions; (1) the stopping of peptidoglycan step in cell wall synthesis, (2) dis-organizing the structure or inhibiting the function of bacterial cell membrane, (3) inhibition of protein synthesis, (4) affecting the synthesis of DNA or RNA or binding to DNA or RNA so that their messages cannot be read, either case of course can block the growth cells, (5) act as competitive inhibitors (growth factor analogs) which are structurally similar to a bacterial growth factor but do not fulfill its metabolic function in the cell. Some are bacteriostatic, and some are bactericidal.

Thirty crude extracts of 10 marine macroalgae were tested against 3 fungal species. The results of the screening tests are summarized in Table 2. *Candida albicans* was the most susceptible organism, which was strongly inhibited by acetone extract of *U. lactuca*, *C. racemosa* and *L. farinosa*, but exhibited moderate inhibitory activities by diethyl ether and acetone extracts of *C. racemosa* and *C. myrica*. However, 7 extracts (diethyl ether of *U. lactuca*, ethanol of *U. lactuca* and *C.*

Table 1. Antibacterial activities of the investigated diethyl ether, acetone and ethanol extracts of ten marine macroalgal species against six bacterial species on the agar plate by diffusion assay method.

Antibiotic reference			Inhibition zones (mm) against					
			****Bacillus Subtilis* NCTC 1040**	***Staphylococcus aureus* NCTC 7447**	***Sarcina maxima* ATCC 33910**	***Escherichia coli NCTC 10416***	***Pseudomonas aeruginosa ATCC 10145***	***Klebsiella pneumonia* NCIMB 911**
Reference antibiotic Penicillin G (50 µ/disk)			+++	+++	+++	+++	+++	+++
Control (Diethyl ether, acetone and ethanol)			-	-	-	-	-	-
Algal species and algal extracts	*Ulva lactuca*	Diethyl ether	+	+	++++	+	++++	+
		Acetone	+	+	++++	+	++	+
		Ethanol	+	+	+	+	+++	+
	Caulerpa racemosa	Diethyl ether	+	+	+	-	+	+
		Acetone	**+**	**++**	**+**	**-**	**+++**	**-**
		Ethanol	-	+	+	-	++	-
	Galaxaura elongata	Diethyl ether	++++	-	+	-	-	-
		Acetone	-	-	+	-	-	-
		Ethanol	-	-	+	-	+++	-
	Acanthophora spicifera	Diethyl ether	-	-	++	++++	-	-
		Acetone	-	-	++++	-	-	-
		Ethanol	-	-	++	-	-	-
	Liagora farinosa	Diethyl ether	-	-	-	-	++	-
		Acetone	-	-	-	-	-	-
		Ethanol	-	-	-	++++	+	-
	Cystoseira compressa	Diethyl ether	-	-	-	-	-	-
		Acetone	-	-	-	-	++	-
		Ethanol	+	-	-	-	+	-
	Cystoseira. myrica	Diethyl ether	-	-	-	-	++++	-
		Acetone	-	-	-	-	+	-
		Ethanol	-	-	-	-	+	-
	Hydroclathrus clathratus	Diethyl ether	-	-	-	-	-	-

	Acetone	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-
	Diethyl ether	-	-	-	+	-	-
Turbinaria ornata	Acetone	-	-	-	+	-	-
	Ethanol	-	-	-	+	-	-
Padina pavonia	Diethyl ether	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-

Inhibition zones: - (no activity); + (3-10 mm); ++ (10-15 mm), +++ (15-20 mm) and ++++ (>20 mm). *Pathogen.

racemosa, acetone of *A. spicifera*, *C. compressa*, *T. ornata* and *Padina pavonia*) showed weak inhibition activity against *C. albicans.* Among the 30 marine algal extracts, only one extract (acetone extract of *U. lactuca*) showed strong inhibitory activity against *Aspergillus flavus.* Furthermore, 4 extracts (diethyl ether and ethanol of *U. lactuca*, diethyl ether of *C. racemosa* and acetone of *L. farinosa*) exhibited weak inhibitory activities against this fungus, which appeared more resistance for other algal extracts. On the other hand, three acetone extracts (*L. farinosa*, *G. elongata* and *Turbinaria ornata*) showed strong inhibitory activity against *Fusarium oxysporum,* which appeared more resistance for other algal extracts except diethyl ether extract of *U. lactuca,* which inhibits this fungus with weak proportion. These beneficial effects of the algal extracts on the investigated fungi could be attributed to the exudates which produced a range of compounds with inhibitor properties (secondary meta-bolites) retarding the growth of other microorganisms and antagonize the infection mechanisms of these organisms. These involved peptides, alkaloids and phenols (Campbell, 1984) and sometimes mono- and divalent cations (Abdel-Rahman et al., 2004). Saffan (2001) reported that the quantitative analysis of some algal exudates revealed the presence of phytohormones, amino acids, total soluble nitrogen and total reducing sugars that might be implicated as allelochemical agents. Extra metabolites of algae may induce specific reactions or modify specific physiological activities either positively or negatively within the microbial pathogen. There appears to be no end to the discoveries of substances produced or liberated by some microalgae. Some workers reported that the analyses of extracellular sub-stances are difficult because of problems involved in separating organic substances one from another or from compounds in the medium (Prescott, 1969). Antibiotics have an important role in modern drug medicine. The philosophy behind their use in the treatment of infection is to kill the invading organisms without harming the host tissues. The development of antiviral drugs along similar lines has not lived up to early expectation because viruses do not show all the qualities of living organisms, However, with the discovery of the first antiviral drugs in 1950 and their first clinical use in 1962, it became clear that antiviral therapy was possible (Bauer, 1985). Many traditional plants have been reported to have strong antiviral activity, and some of them have already been used to treat animals and people who suffer from viral infection (Hudson et al., 1999). One of these organisms is the marine algae, which have been reported to contain antiviral substances (Faulkner, 1986). In the present study, thirty crude extracts (each extracts was used with 3 doses) of 10 marine macroalgae were tested against the Newcastle sense virus (NDV)-(Paramyxoviridae) which is responsible for acute respiratory distress in chicken, and it represents a single-stranded RNA virus that can be successfully propagated in embryonated chicken eggs (ECE).

The cytotoxicity of these 30 algal extracts to chicken embryos as a laboratory host system was detected, and data is presented in Table 3. The data showed clearly that both diethyl ether and acetone extracts of the tested algae are toxic to chicken embryos causing embryo death and presence of petechial hemorrhage all over the body of the embryo (Figure 1), while ethanol extract was not toxic, mainly in the lowest doses (0.05 and 0.01 ml). Therefore, ethanol extract was

Table 2. Antifungal activities of the investigated diethyl ether, acetone and ethanol extracts of ten marine macroalgal species against three fungal species on the agar plate by diffusion assay method.

Antibiotic reference			Inhibition zones (mm)		
			****Candida albicans* IMRU 3669**	***Aspergillus flavus* IMI 111023**	***Fusarium oxysporum***
Reference antibiotic (Amphotericin B 50 µ/disk)			++++	+++	+++
Control (Diethyl ether, acetone and ethanol)			-	-	-
Algal species & Algal extracts	*Ulva lactuca*	Diethyl ether	++	+	+
		Acetone	++++	+++	-
		Ethanol	++	+	-
	Caulerpa racemosa	Diethyl ether	+++	+	-
		Acetone	+++	-	-
		Ethanol	++	-	+
	Galaxaura elongata	Diethyl ether	-	-	-
		Acetone	++++	-	+++
		Ethanol	-	-	-
	Acanthophora spicifera	Diethyl ether	-	-	-
		Acetone	++	-	-
		Ethanol	-	-	-
	Liagora farinosa	Diethyl ether	-	-	-
		Acetone	++++	+	++++
		Ethanol	-	-	-
	Cystoseira compressa	Diethyl ether	-	-	-
		Acetone	++	-	-
		Ethanol	++	-	-
	Cystoseira. myrica	Diethyl ether	+++	-	-
		Acetone	+++	-	-
		Ethanol	-	-	-
	Hydroclathrus clathratus	Diethyl ether	-	-	-
		Acetone	-	-	-
		Ethanol	-	-	-
	Turbinaria ornata	Diethyl ether	-	-	-
		Acetone	++	-	+++
		Ethanol	+++	-	-
	Padina pavonia	Diethyl ether	-	-	-
		Acetone	++	-	-
		Ethanol	-	-	-

Inhibition zones: - (no activity); + (3-10 mm); ++ (10-15 mm), +++ (15-20 mm) and ++++ (>20 mm). Pathogen*

used in a dose of 0.1 ml per egg (1 ml extract represent 100 mg dry algal matter). Evaluation of antiviral activity of ethanol extracts for the ten macro algae against NDV is presented in Table 4. Data showed clearly that 7 out of 10 tested algal species have antiviral activity, resulting in 2 log or more reduction in virus titer. The antiviral activity

Table 3. Cytotoxicity evaluation of ten marine algal extracts on chicken embryos (1 ml extract represent 100 mg dry algal matter).

Dose of extract	Type of solvent (ml crude extract / egg)								
	Diethyl extract			Ethanol extract			Acetone extract		
	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
Ulva lactuca	L	D**	D	L	L	L	L	D	D
Caulerpa racemosa	L	D	D	D	L	D	L	D	D
Galaxaura elongata	L*	D	D	D	L	L	L	D	D
Acanthophora spicifera	L	L*	D	L	L	D	L	D	D
Liagora farinosa	D	D	D	L	L	D	L	D	D**
Cystoseira compressa	L	D	D	L	L	L	L*	D	D
Cystoseira myrica	D	D**	D	L	L	D	L	D	D
Hydroclathrus clathratus	L	D**	D	L	L	L	L*	D	D
Turbinaria ornata	L	D	D	L	L	L	L	D	D
Padina pavonia	L	D	D	L	L	L	L	D	D

L, Live embryo; D, dead embryo; *****embryo lives with stunted (weak) growth; ****e**mbryo die with petechial hemorrhage all over the body.

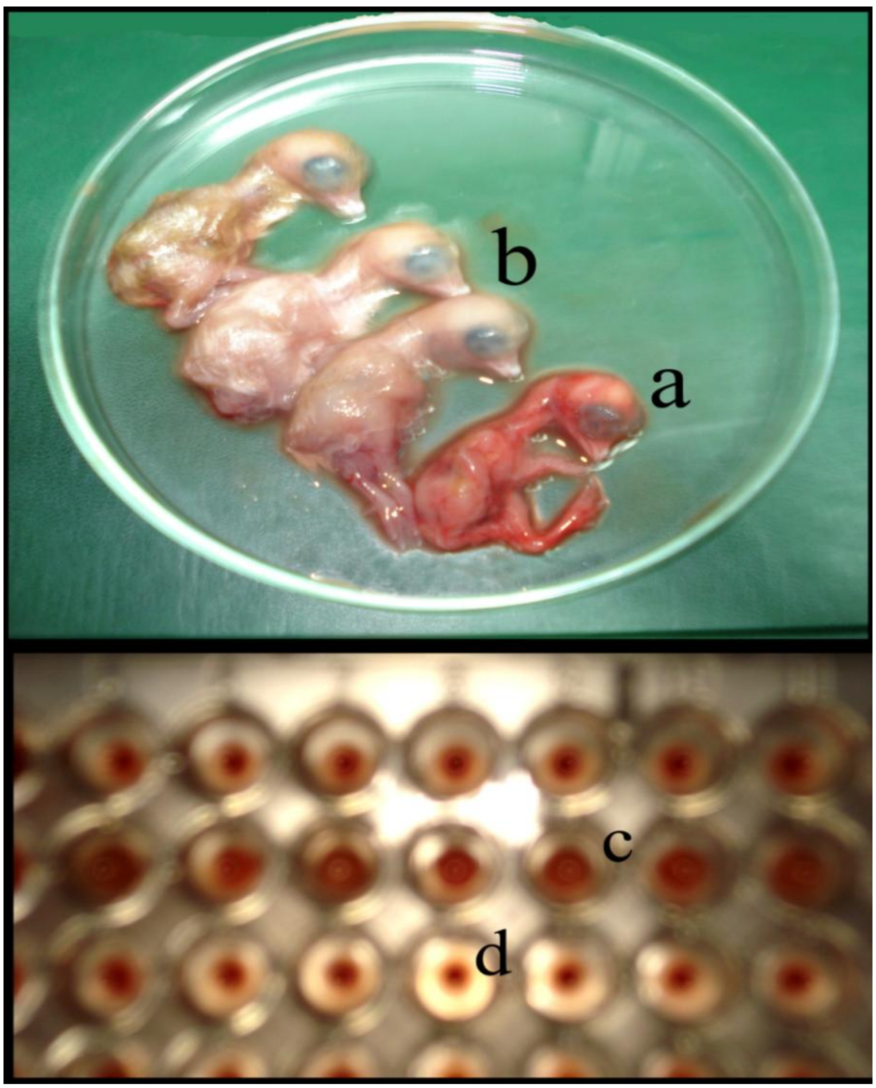

Figure 1. (a) Petechial hemorrhage all over the body of the embryo; (b) normal chicken embryo; (c) lattice shape agglutination; (d) button-like precipitation.

Table 4. Evaluation of 0.1 ml ethanol extracts of ten marine macro algae species in reduction of New Castle Disease Virus (NDV) titer in chicken embryos.

Algal species	Virus titer log 10 EID50*		
	Virus alone	Virus extract mixture	Reduction in virus titer
Ulva lactuca	9.1	6.4	2.7*
Caulerpa racemosa	9.1	6.4	2.7*
Galaxaura elongata	9.1	6.7	2.4*
Acanthophora spicifera	9.1	7.5	1.6
Liagora farinosa	9.1	7.5	1.6
Cystoseira compressa	9.1	6.2	2.9*
Cystoseira myrica	9.1	6.8	2.3*
Hydroclathrus clathratus	9.1	6.4	2.7*
Turbinaria ornate	9.1	8.1	1
Padina pavonia	9.1	6.3	2.8*

*2 log or more means significant reduction in virus titer.

of the tested algae may be attributed to their content of polysaccharides, which block viral adsorption point in the cell membrane of the host cell. This speculation comes in agreement with Baslow (1969), Baba et al. (1988), Mitsuya et al. (1988) and Ueno and Kuno (1987) who suggested that specific carbohydrates in marine red algae have antiviral activity against the infection of DNA and RNA virus. The data obtained therefore proved that some of the marine macro algae have antiviral activity, especially *U. lactuca*, *C. racemosa*, *G. elongata*, *C. compressa*, *C. myrica*, *H. clathratus* and *P. pavonia*.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Deanship of Scientific Research and the Science Research Center in the College of Science, King Saud University, Saudi Arabia, for financially supporting this research effort.

REFERENCES

Abdel-Rahman IS, Sweiha HE, Mankarios AT, Hamoda RA (2004). Activity of some fresh-water and marine algae against tomato mosaic virus infectivity. 1. Effect of culture filtrates and water extracts, petrolium ether, chloroform and methanol extracts of algae on the virus activity *in vitro.* Abstract book of the Third International Conference on Biological Sciences. Fac. Sci. Tanta Univ. pp. 29-30.

Abdel-Raouf N, Ibraheem IBM (2008). Antibiotic activity of two Anabaena species against four fish pathogenic Aeromonas species. Afr. J. Biotechnol. 7(15): 2644-2648.

Allan WH, Lancoster JE, Toth B (1973). Production and use of New Castle disease Vaccines. Fao Rep. P.35, Rome. Italy, 111 spp.

Amico V, Oriente G, Piattelli M, Tringali C (1978). Caulerpenyne, an unusual sesquiterpenoid from the green alga *Caulerpa prolifera*. Tetrahedron Lett. 38: 3593-3596.

Anon A (1971). Methods for examination poultry biologics and for identifying & quantifying a vian pathogens. Natl. Acad. Sci. Washington, D.C. p. 1-184

Baba M, Nakjima M, Schools D, Pauwels R, Balzarini J, De Clercq E (1988). A sulphated oligosaccharides are potent and selective anti-Hiv agents in vitro. Anti-Viral. Res. (9): 335-343.

Ballesteros E, Martin D, Uriz MJ (1992). Biological activity of extracts from some Mediterranean macrophytes. Bot. Marina, 35: 481-485.

Baslow MH (1969). Marine pharmacology the Williams and Williams Co. Baltimore. p. 56.

Bauer DH (1985). A history of the discovery and clinical application of antiviral drugs. Br. Med. Bull. 41: 309-314.

Blackman AG, Wells RG (1976). Caulerpol, a diterpene alcohol related to vitamin A, from *Caulerpa brownie* (algae). Tetrahedron Lett. 31: 2729-2730.

Bold HC (1978). Introduction to the algae Structure and reproduction. Prentice. Hall. Inc., New-Jersey p. 07632.

Borgesen F, Fremy P (1936). Marine algae from the Canary Islands especially from Teneriffre & Gran. Canaria: I- Chlorophyceae, II-Phaeophyceae, 111- Rhodophyceae, 1V-Cyanophyceae. Det. Kg I. Denske Videnskabernes.

Borgesen F (1900). A contribution to the knowledge of the marine algal vegetation on the coasts of the Danish West-indian Islands. Botan. Tidskrift, 23 Bd. Koebenhavn, p. 49.

Borgesen F (1931). Some Indian green and brown algae especially from the shores of the presidency of Bombay. J. Ind. Bot. Soc. 11: 51-70.

Bouck GB (1965). Fine structure and organelle associations in brown algae. J. Cell. Biol. 26: 513-537.

Caccamese S, Azzolina R (1979). Screening for antimicrobial activities in marine algae from eastern Sicily. Planta Med. 37(4): 333-339.

Campbell JM (1984). Secondary metabolisms and microbial physiology. Adv. Microb. Physiol. 25: 2-57.

Chengkui Z, Junfu Z (1984). Chinses seaweed in herbal medicine. In 11th International Seaweed Symposium (Bird, C.J & Ragan, M.A., editors), Dr. W. Junk Publishers, Dordrccht, Boston, Lancaster. pp. 135-140.

Davet P, Rouxel F (2000). Detection and isolation of soil fungi. Sci. Publishers Inc. p. 188.

Deig EF, Ehresmann DW, Hatch MT, Riedlinger DJ (1974). Inhibition of herpesvirus replication by marine algae extracts. Antimicrob. Agents Chemother. 6(4): 524-525.

Doti MS, Santos GA (1966). Caulerpicin, toxic constituents of *Caulerpa*. Nature 211, 990, through C. A. 65: p. 15966.

European Pharmacopoe (1997). Mikrobiologische Wertbestimmung von Antibiotika, Diffusionsmethode, 3. Ausgabe, Deutscher-Apotheker-Verlag, Stuttgart, Kapitel 2,7,2.

Faulkner DJ (1986). Marine natural Products. Nat. Prod. Rep. (3): 1-33.

Fitton JH (2006). Antiviral properities of marine algae. In: Critchley AT, Ohno M, Largo DB (Eds). World seaweed resources. Windows and Macintosch. ETI Information Services, Workingham, U. K., p. 7.

Haliki A, Denizci AA, Cetingul V (2005). An investigation on antifungal activities of some marine algae (Phaeophyta, Rhodophyta). Eur J. Fish. Aquat. Sci. 22: 13-15.

Hamel G (1916). Chlorophycees des cotes francaise. Rev. Algol. (5): 381-430.
Hudson JB, Kim JH, Lee MK, Dewreede RE, Hong YK (1999). Antiviral compounds in extracts of Korean seaweeds; evidence for multible activities. J. Appl. Phycol. (10): 427-434.
Ibraheem IBM (1995). Phytochemical studies on some common algae of El-Sukhna and Abu-Qir Gulf. M.Sc. Thesis, Al-Azhar Univ., Fac. Sci. Cairo, Egypt.
Ikigai H, Nakae T, Hara Y, Shimamura T (1993). Bactericidal catechins damage the lipid bilayer. Biochimica et Biophysica Acta. 1147: 132-136.
Kamat SY, Wahidulla S, D Souza L, Naik CG, Ambiye V, Bhakuni DS, Goel AK, Garg HS (1992). Bioactivity of marine organisms. VI. Antiviral evaluation of marine algal extracts from the Indian coast. Bot. Marina, 35: 161-164.
Levring T (1946). A list of marine algae from Australia and Tasmania sartryck, meddelanden from Goteborgs Nataniska. Tragard. 16: 215-227.
Lima-Filho JVM, Carvalho AFFU, Freitas SM, Melo VMM (2002). Antibacterial activity of extract of six macroalgae from the northeastern Brazilian coast. Braz. J. Microbiol. 33: 3311-313.
Mitsuya HDJ, Looney S, Kuno R, Ueno F, Wong-Staal F, Broder S (1988). Dextran sulphate suppression of viruses in the HIV family: inhibition of virion binding to CD4 and cells. Science, 240: 646-649.
Mtolera MSP, Semesi AK (1996). Antimicrobial activity of extracts from six green algae from Tanzania. Curr. Trends Mar. Bot. Res. East Afr. Reg. pp. 211-217.
Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T (2002). Bactericidal activity of phlorotannins from the brown alga Ecklonia kurome. J. Antimicrob. Chemother. 50: 889-893.
Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T (2002). Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*, J. Antimicrob*.* Chemo. 50: 889-893.
Nakaamura T, Nagayama K, Uchida K, Tanaka R (1996). Antioxidant activity of phlorotannins isolated from the brown alga *Eisenia bicyclis*. Fish. Sci. 62: 923-926.
Nasr AH (1940a). A repot on some marine algae collected from the vicinity of Alexandria. Fouad I Institute, Hydrobiol. Fisheries, 36: 1-33.
Nasr AH (1940b). A study of the occurrence of some marine algae on the Egyptian Mediterranean coast. FouadI Hydrobiol. Fish. 2(37): 1-9.
Nasr AH, Aleem AA (1949). Ecological studies of some marine algae from Alexandria. Hydrobiologia, 1: 251-281.
Olessen PE, Maretzki A, Almodovar LA (1963). An investigation of antimicrobial substances from marine algae. Bot. Marina. 6: 226-232.
Papenfuss GF (1940). Notes on south African Marine Algae. Chlorophyceae, Phaeophyceae, and Rhodophyceae. Botaniska Notiser. Lun. pp. 200-226.
Papenfuss GF (1946). Proposed names for the phyla of algae. Bullet. Torrey Bot. Culb. 73(3): 217-218.
Papenfuss GF (1955). Classification of the algae. In A century of Progress in the natural Sciences 1853-1953. Calif. Acad. Sci. San Francisco, pp. 115-224.
Papenfuss GF (1968). A history, Catalogue and bibliography of Red Sea Benthic Algae. Israel J. Bot. 17: 1-118.
Parr AE (1939). Quantitative observations on the pelagic Sargassum vegetation of the western North Atlantic, etc. Bull. Bingham Oceangr., Coll., Peabody Mus. Nat. 6: 1-94d.
Paul VJ, Van-Alstyne KL (1987). The metabolism of diterpenoid from green seaweeds. J. Exp. Mar. Biol. Ecol. 119(1): p. 15.
Perez RM, Avila JG, Perez S, Martinez A, Martinez G (1990). Antimicrobial activity of some American algae. J. Ethnopharmacol. 29(1): 111-116.
Prescott GW (1969). The algae. A review. Michigan State Univ., Butler & Tanner LTD, Frome and London. Great Br. p. 355.
Rao PS, Parekh KS (1981). Antibacterial activity by Indian algal extracts. Botanica*.* Marina. 24: 577-582.
Reed IJ, Muench H (1938). Simple methods of estimating fifty percent end points. Am. J. Hyg. 27: 493-497.
Saffan SE (2001). Allelopathic effects of cyanobacterial exudates on some metabolic activities of *Cynara cardunculus* seeds during germination*.* Egypt. J. Biotecnol. 10: 157-178.
Salvador N, Gomez-Garreta A, Lave L, Ribera MA (2007). Antimicrobial activity of Iberian macroalgae*.* Sci. Mar. 71m: 101-113.
Scagel F (1966). The phaeophyceae in perepective oceanogr. Mar. Biol. Ann. Rev. 4: 123-194.
Schulz J, Hunter M, Appel H (1992). Antimicrobial activity of polyphenols mediates plant-herbivore interactions. In Plant Polyphenols Hemingway RW and Lakes PE, Eds. pp. 621-637
Setchell WA, Gardner NL (1920). The marine algae of the pacific coast of North America. Part 11. Chlorophyceae. Univ. Calif. Publicat. Bot. 2: 139-374.
Smith GM (1944). Marine algae of the Monterey Peninsula. Reprinted from Madrono. 7(7): 226-231.
Svedelius N (1906). Ueber die Algenovegetation eines Ceylonischen Korallenriffes, etc., Bot. Stud. Till. F.R. Jjellman, Upsala. pp. 184-220.
Taskin E, Ozturk M, Taskin E, Kurt O (2007). Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr. J. Biotechnol. 6(24): 2746-2751.
Taylor WR (1928). The marine algae of Florida with special reference to the dry Tortugas Pap. Tortugas Lab. of the Carnegie inst. Washington, 25: 1-219.
Taylor WR (1960). Marine algae of the eastern tropical and subtropical coast of the Americas. Univ. Michigan press, Ann. Arbor. p. 870.
Ueno R, Kuno S (1987). Dextran sulphate, a potent anti-HIV in vitro having synergism with zidovudine. Lancet. 1: 1379.
Wedberg SE (1966). Introduction to microbiology. Reinhold Publishing Corporation. New York, p. 426.
Zandi K, Fouladvan M, Pakdel P, Sartavi K (2007). Evaluation of *in vitro* antiviral activity of a brown alga (*Cystoseira myrica*) from the Persian Gulf against herpes simplex virus type 1. Afr. J. Biotechnol. 6(22): 2511-2514.