

*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

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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 weak 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

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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 Freymy (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 soaked 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  $10^{9.1}$  EID<sub>50</sub>/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 (EID<sub>50</sub>) 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  $\mu$ L saline and 50  $\mu$ L of the tested sample, then 50  $\mu$ L 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 antibacterial 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) disorganizing 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           |   |                                     |                                       |   | <i>Klebsiella pneumoniae</i><br>NCIMB 911 |   |
|---|-------------------------------|---|---|-------------------------------------|---------------------------------------|---|---|---|
|   |                               | * <i>Bacillus Subtilis</i><br>NCTC 1040 | <i>Staphylococcus aureus</i><br>NCTC 7447 | <i>Sarcina maxima</i><br>ATCC 33910 | <i>Escherichia coli</i><br>NCTC 10416 | <i>Pseudomonas aeruginosa</i><br>ATCC 10145 |   |   |
| Reference antibiotic Penicillin G (50 µ/disk) |                               | +++                                     | +++                                       | +++                                 | +++                                   | +++   | +++                                       |   |
| Control (Diethyl ether, acetone and ethanol)  |                               | -                                       | -   | -                                   | -                                     | -   | -   |   |
| Algal species and algal extracts              | <i>Ulva lactuca</i>           | Diethyl ether                           | +   | +                                   | ++++                                  | +   | ++++                                      | + |
|   |                               | Acetone                                 | +   | +                                   | ++++                                  | +   | ++  | + |
|   |                               | Ethanol                                 | +   | +                                   | +                                     | +   | +++                                       | + |
|   | <i>Caulerpa racemosa</i>      | Diethyl ether                           | +   | +                                   | +                                     | -   | +   | + |
|   |                               | Acetone                                 | +   | ++                                  | +                                     | -   | +++                                       | - |
|   |                               | Ethanol                                 | -   | +                                   | +                                     | -   | ++  | - |
|   | <i>Galaxaura elongata</i>     | Diethyl ether                           | ++++                                      | -                                   | +                                     | -   | -   | - |
|   |                               | Acetone                                 | -   | -                                   | +                                     | -   | -   | - |
|   |                               | Ethanol                                 | -   | -                                   | +                                     | -   | +++                                       | - |
|   | <i>Acanthophora spicifera</i> | Diethyl ether                           | -   | -                                   | ++                                    | ++++  | -   | - |
|   |                               | Acetone                                 | -   | -                                   | ++++                                  | -   | -   | - |
|   |                               | Ethanol                                 | -   | -                                   | ++                                    | -   | -   | - |
|   | <i>Liagora farinosa</i>       | Diethyl ether                           | -   | -                                   | -                                     | -   | ++  | - |
|   |                               | Acetone                                 | -   | -                                   | -                                     | -   | -   | - |
|   |                               | Ethanol                                 | -   | -                                   | -                                     | ++++  | +   | - |
|   | <i>Cystoseira compressa</i>   | Diethyl ether                           | -   | -                                   | -                                     | -   | -   | - |
|   |                               | Acetone                                 | -   | -                                   | -                                     | -   | ++  | - |
|   |                               | Ethanol                                 | +   | -                                   | -                                     | -   | +   | - |
|   | <i>Cystoseira myrica</i>      | Diethyl ether                           | -   | -                                   | -                                     | -   | ++++                                      | - |
|   |                               | Acetone                                 | -   | -                                   | -                                     | -   | +   | - |
| Ethanol                                       |                               | -                                       | -   | -                                   | -                                     | +   | -   |   |
| <i>Hydroclathrus clathratus</i>               | Diethyl ether                 | -                                       | -   | -                                   | -                                     | -   | -   |   |

|                          |               |   |   |   |   |   |   |
|--------------------------|---------------|---|---|---|---|---|---|
|                          | Acetone       | - | - | - | - | - | - |
|                          | Ethanol       | - | - | - | - | - | - |
|                          | Diethyl ether | - | - | - | + | - | - |
| <i>Turbinaria ornata</i> | Acetone       | - | - | - | + | - | - |
|                          | Ethanol       | - | - | - | + | - | - |
| <i>Padina pavonia</i>    | 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)                  |   |                           |
|---|--|---|---------------------------|
|   | * <i>Candida albicans</i><br>IMRU 3669 | <i>Aspergillus flavus</i><br>IMI 111023 | <i>Fusarium oxysporum</i> |
| Reference antibiotic (Amphotericin B 50 µ/disk) | ++++                                   | +++                                     | +++                       |
| Control (Diethyl ether, acetone and ethanol)    | -                                      | -                                       | -                         |
| <i>Ulva lactuca</i>                             | Diethyl ether                          | ++                                      | +                         |
|   | Acetone                                | ++++                                    | +++                       |
|   | Ethanol                                | ++                                      | +                         |
| <i>Caulerpa racemosa</i>                        | Diethyl ether                          | +++                                     | +                         |
|   | Acetone                                | +++                                     | -                         |
|   | Ethanol                                | ++                                      | -                         |
| <i>Galaxaura elongata</i>                       | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | ++++                                    | -                         |
|   | Ethanol                                | -                                       | -                         |
| <i>Acanthophora spicifera</i>                   | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | ++                                      | -                         |
|   | Ethanol                                | -                                       | -                         |
| <i>Liagora farinosa</i>                         | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | ++++                                    | +                         |
|   | Ethanol                                | -                                       | -                         |
| <i>Cystoseira compressa</i>                     | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | ++                                      | -                         |
|   | Ethanol                                | ++                                      | -                         |
| <i>Cystoseira myrica</i>                        | Diethyl ether                          | +++                                     | -                         |
|   | Acetone                                | +++                                     | -                         |
|   | Ethanol                                | -                                       | -                         |
| <i>Hydroclathrus clathratus</i>                 | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | -                                       | -                         |
|   | Ethanol                                | -                                       | -                         |
| <i>Turbinaria ornata</i>                        | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | ++                                      | -                         |
|   | Ethanol                                | +++                                     | -                         |
| <i>Padina pavonia</i>                           | Diethyl ether                          | -                                       | -                         |
|   | Acetone                                | ++                                      | -                         |
|   | Ethanol                                | -                                       | -                         |

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

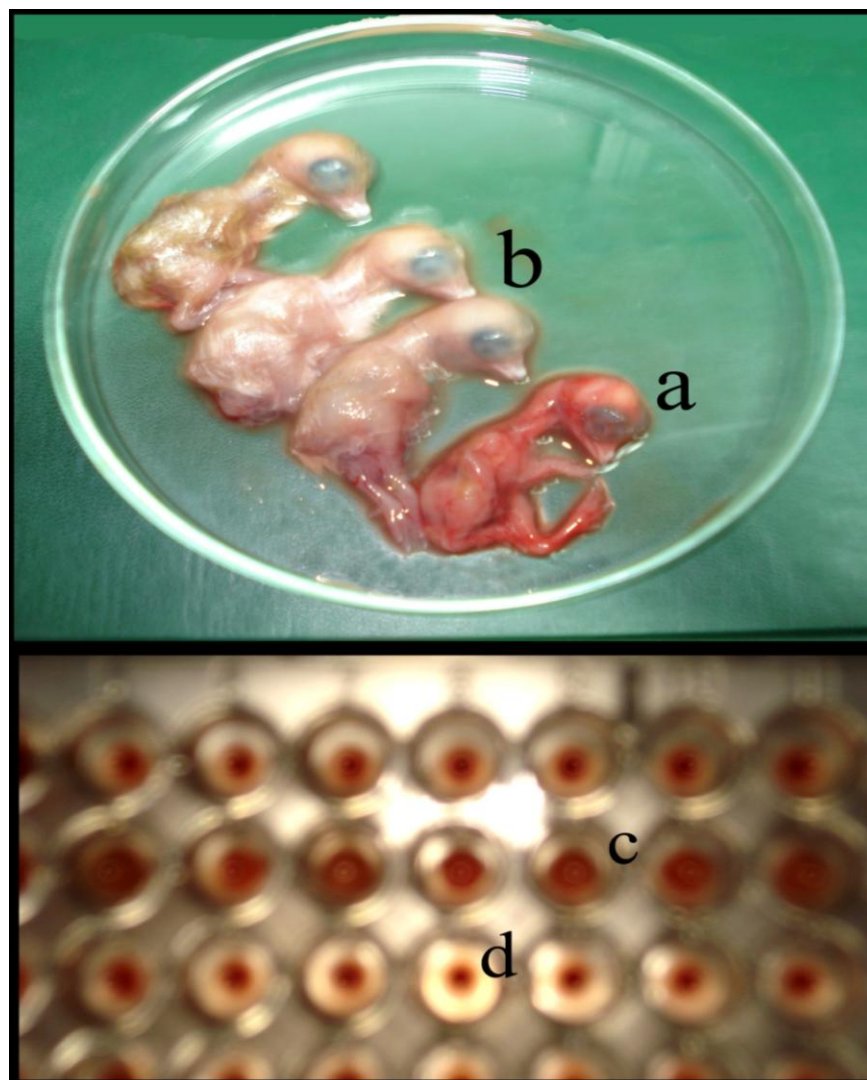
used in a dose of 0.1 ml per egg (1 ml extract represent 100 mg dry algal matter). Evaluation of antiviral activity of ethanol extracts for the ten macro algae against NDV is

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 |
| <i>Ulva lactuca</i>             | L  | D** | D   | L               | L   | L   | L               | D   | D   |
| <i>Caulerpa racemosa</i>        | L  | D   | D   | D               | L   | D   | L               | D   | D   |
| <i>Galaxaura elongata</i>       | L*                                       | D   | D   | D               | L   | L   | L               | D   | D   |
| <i>Acanthophora spicifera</i>   | L  | L*  | D   | L               | L   | D   | L               | D   | D   |
| <i>Liagora farinosa</i>         | D  | D   | D   | L               | L   | D   | L               | D   | D** |
| <i>Cystoseira compressa</i>     | L  | D   | D   | L               | L   | L   | L*              | D   | D   |
| <i>Cystoseira myrica</i>        | D  | D** | D   | L               | L   | D   | L               | D   | D   |
| <i>Hydroclathrus clathratus</i> | L  | D** | D   | L               | L   | L   | L*              | D   | D   |
| <i>Turbinaria ornata</i>        | L  | D   | D   | L               | L   | L   | L               | D   | D   |
| <i>Padina pavonia</i>           | L  | D   | D   | L               | L   | L   | L               | D   | D   |

L, Live embryo; D, dead embryo; \*embryo lives with stunted (weak) growth; \*\*embryo 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 <sub>10</sub> EID <sub>50</sub> * |                       |                          |
|---------------------------------|---|-----------------------|--------------------------|
|                                 | Virus alone                                       | Virus extract mixture | Reduction in virus titer |
| <i>Ulva lactuca</i>             | 9.1   | 6.4                   | 2.7*                     |
| <i>Caulerpa racemosa</i>        | 9.1   | 6.4                   | 2.7*                     |
| <i>Galaxaura elongata</i>       | 9.1   | 6.7                   | 2.4*                     |
| <i>Acanthophora spicifera</i>   | 9.1   | 7.5                   | 1.6                      |
| <i>Liagora farinosa</i>         | 9.1   | 7.5                   | 1.6                      |
| <i>Cystoseira compressa</i>     | 9.1   | 6.2                   | 2.9*                     |
| <i>Cystoseira myrica</i>        | 9.1   | 6.8                   | 2.3*                     |
| <i>Hydroclathrus clathratus</i> | 9.1   | 6.4                   | 2.7*                     |
| <i>Turbinaria ornate</i>        | 9.1   | 8.1                   | 1                        |
| <i>Padina pavonia</i>           | 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.

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