Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 7(1); 34-42

ISSN: 0976 822X

Research Article

# Antiviral Activity of Sulfated Polysaccharides Carrageenan from Some Marine Seaweeds

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*Available Online: 26th December, 2015*

**ABSTRACT**

In this study, the antiviral effect of carrageenan sulfated polysaccharides extracted from red alga *Acanthophora specifira* and brown alga *Hydroclathrus clathratus* was investigated against Herpes simplex virus type 1 (HSV-1) and Rift valley fever virus (RVFV). These viruses are serious pathogens to human and animal. Carrageenan content was increased in *Acanthophora specifira* more than *Hydroclathrus clathratus*. *C*arrageenan hot water extract from these algae has shown a considerable antiviral property. Initially, cytotoxicity examination was conducted for crude aqueous Carrageenan. The maximum nontoxic dose (MNTD) treated Vero cells did not show any morphological difference when compared with control. These extracts were tested by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenlytetrezolium bromide] method, cytopathic effect reduction assay, and plaque reduction assay to assess their cytotoxicity and antiviral activity. The antiviral effect was further determined by flow cytometric analysis. The results revealed that most of these extracts inhibited the propagation of HSV-1 and RVFV standard strains with minimal cytotoxicity to the host cells. The inhibition of viral replication was observed by amplify P53 gene (HSV-1) and BCL-2 gene (RVFV) the log 10 reduction in virus titer was observed compared with viral control.

**Keywords**: Antiviral activity, Cytotoxicity, Herpes simplex virus type 1 (HSV-1), Rift valley fever virus (RVFV), Carrageenan, *Acanthophora specifira*, *Hydroclathrus clathratus*

## INTRODUCTION

Seaweeds have long been recognized as rich and valuable natural resources of bioactive compounds because of their various biological properties[1,2]. Since the finding of antimicrobial activities in many species of marine algae and the isolation of some active compounds from them, marine algae have become recognized as potential sources of antibiotic substances[3,4]. New types of antiviral agents from marine natural sources are considered to be most promising, especially those that have high efficacy on resistant mutant viral strains and low toxicity to host. The water-soluble extracts of seaweeds have been shown to exhibit antiviral activity against a wide spectrum of viruses[5]. There are more than 200 species of seaweeds in Hong Kong coastal waters[6], but research on their antiviral activity is very limited[7]. Seaweeds contain many different polysaccharides, which chemical structure relates to the corresponding taxonomic classification of algae and their cell structure[8,9]. Sulfated polysaccharides inhibit activity of many bacterial species as well as viruses[10]. Polysaccharides are the main component of seaweed cell walls[11]. Kraan[12] mentioned that marine algae contain large amounts of polysaccharides, notably cell wall structural, but also mycopolysaccharides and storage polysaccharides[13]. Polysaccharides are polymers of simple sugars (monosaccharides) linked together by glycosidic bonds, and they have numerous commercial applications in products such as stabilisers, thickeners, emulsifiers, food, feed, beverages etc.[14]. The total polysaccharide concentrations in the seaweed species of interest range from 4-76 % of the dry weight. All seaweed species produce at least one type of sulfated polysaccharide[5]. Due to the wide variations in their molecular weights, structural parameters and physiological characteristics, seaweed polysaccharides show diverse bioactivities, such as antiviral[15], antiproliferative or antitumor, anticoagulant, antioxidant, anti-inflammatory and anti-complementary effects[16]. The red and brown algae produce carrageenan, it be defined as a polysaccharide. From a human health perspective, it has been reported that carrageenans have anti-tumour and anti-viral properties[17]. New research on the biocide properties shows that applications of carrageenan gels from *Chondrus crispus* may block the transmission of the HIV virus as well as other STD viruses such as gonorrhoea, genital warts and the herpes simplex virus (HSV)[18]. Viral infections remain a major threat to humans and animals and there is a crucial need for new antiviral agents especially with development of resistant viruses[19]. Herpes simplex virus (HSV) belonging to herpes group and Reft vally fever virus (RVFV) belonging to the Bunyavirus family cause serious diseases to human and animals. Recently, screening assays of antiviral activity of many extracts have led to the identification of a number of polysaccharides[20] and the ditrepenes[21] having potent inhibitory effect against Herpes simplex virus type 1. Herpes virus is a kind of ancient virus that causes different

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types of infections. Approximately 80% of the adult population worldwide are infected with herpes simplex virus (HSV-1) and approximately 20% of them are also infected with HSV type 2 (HSV-2)[22]. The red alga *Acanthophora specifira* has shown antiviral activities[23]. Wang *et al*.[11] mentioned that the brown alga *Hydroclathrus clathratus*, collected from Hong Kong coastal water, exhibited high antiviral activity against HSV with low cytotoxicity to Vero and HEp-2 cells. Carrageenan, had been found to possess antiviral properties attributed to the galactan units in the polysaccharides of both. The specific antiviral activity had been shown against influenza B and mumps virus in embryonated eggs even after 24 h inocubation[24]. The objective of this study was to determine the antiviral activity of carrageenan sulfated polysaccharide extracted from marine seaweeds as red alga *Acanthophora specifira* and brown alga *Hydroclathrus clathratus* against the pathogenic Herpes simplex virus type 1 (HSV-1) and Rift valley fever virus (RVFV).

## MATERIALS AND METHODS

*Algal material*

Specimens of the red alga *Acanthophora specifira* (Vahl) Børgesen and the brown alga *Hydroclathrus clathratus* (C.Agardh) M.A. Howe were collected from El Shoaiba coast, eastern part of Saudi Arabia, Red Sea (20° 48` & 20° 51` N and 39° 24` & 39° 28` E) through the period from summer 2011 to spring 2012. The selected species were identified by[25-28]. The specimens were washed with local sea water from attached epiphytes and other associated organisms. Then, they were gently brushed with tap water. The samples were air-dried at room temperature. The dried samples were grinded into powdered form using an electrical blender prior then kept in plastic bags at dry and cool place until extraction.

*Extraction of carrageenan*

The tested material carrageenan was extracted from the selected seaweeds species as antiviral agents. The dried seaweeds were extracted with hot distilled water, filtered on diatomaceous earth and the filtrate was poured into absolute ethanol with stirring. The precipitate was recovered and washed with 95°C ethanol dehydrated with diethyl ether and dried overnight at 50°C. Stock solutions (10ml) were prepared in PBS (Phosphate buffer saline) buffer then kept in refrigerator until use and were stored at -4°C.

*Viruses and Cell culture*

Vero cells (African green monkey kidney cell line, ATCC CCL81) (GMK) were used for culturing Herpes simplex virus type 1 (HSV-1) and Rift Valley Fever virus (RVFV). All the cells were cultured at 37°C in humidified atmosphere supplied with 5% $CO_2$. The two viruses provided by serum and vaccine laboratory. The viruses titter was estimated from cytopathogenicity according to Reed and Mench[29] dilution method and expressed as 50% infection doses per milliliter ($ID_{50}$ $ml^{-1}$).

*Culture medium*

Eagle's minimum essential medium (EMEM) and Dulbecco's modified eagle medium (DMEM) were supplemented with antibiotics (pencillin 100 µg /ml and streptomycin100 µg /ml) 10% HEPEGS (4-2 hydroxyethyl-1-piperozneethanesulfonic acid) and inactivated FBS (fetal bovine serum) 10% for cell growth and 2% maintenance of cells or virus stock preparation[30].

*Virus titration*

Anti-HSV-1 and Anti-RVFV was evaluated by reduction of the virus titers using GMK cells. GMK cells were seeded in 96 wells culture plates at a density of $5x10^4$ cells/ well. The seeded cells were incubated for 24h, at 37°C in humidified atmosphere containing 5ml / L $CO_2$. A serial dilution of viruses stock were prepared in ten-fold serial dilutions of viruses separately (four well per dilution) and incubated for 5 days. The cytopathic effect and plaque forming unit (PFU) were recorded by inverted microscope. The 50% tissue culture infections doses/ml ($TCID_{50}$10/ml) was calculated using Karber method[31]. Plaques were counted and viral concentrations were calculated as plaque-forming unit (PFU/ml) per milliliter by formula as described by Arnold *et al*.[32]. The virus titer (PFU/ml) = mean number of counted plaques x 1/ (dilution factor) x 1(ml of inoculum).

*Cytotoxicity of test carrageenan using MTT assay*

To evaluate the cytotoxic activities of extract, growth medium was decanted from 96 microtiter plate after confluent sheet of Vero cell were formed, then take 0.2ml of each dilution different extracts [starting $10^0$ (1mg /ml) till $10^{-4}$ dilutions] was tested in three wells and two wells receiving only medium as control. The plate was incubated in $CO_2$ incubator for any physical signs of toxicity[33]. Cytotoxic effect of test carrageenan to Vero cells was carried out using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenlytetrezolium Bromide] assay according to Berridge *et al*.,[34], where test carrageenan extract was sterile filtrated using 0.22 µm syringe filter. Double fold dilutions were prepared by adding equal volumes of the dissolved extract to fresh minimum essential medium supplemented with Earle's salt (E-199). MTT was PBS (Phosphate buffer saline) dissolved as 5 mg/ mL and filtered through 0.22 µm syringe filters. Media present in cell culture plates were discarded and plates were washed with sterile saline three times. Plates were inoculated aseptically with 20 µl/well of the previously prepared MTT solution. Plates were incubated at 37°C for 4 h. MTT solution was discarded and plates were washed with PBS three times. Inverted microscope was used to detect the morphological changes of cells induced by carrageenan. The maximal non-cytotoxic concentration (MNCC) of the extract was determined the shape of cells between the treated cells with that of the untreated cells.

*Determination of anti-infectivity effect of algal extracts on intracellular virus replication*

HSV-1 and RVFV suspension in EMEM (Eagle MEM growth medium); (0.5 ml of 5 x $10^2$ PFU /ml virus) was mixed with 0.5ml of $10^0$ to $10^{-5}$ concentration of each algal extracts. The mixture was incubated at room temperature for 1hr in sterile screw capped vials. Two types of non-treated viruses infected virus as control sets were done. Two plates seeded with Vero cells were incubated with 0.2 ml / well either a test extracts or control vial. One well /each plates was left neither inoculated with virus none

treated with extracts as well control. Plates were incubated at 37°C in $CO_2$ to allow for virus adsorption. Monolayers were washed twice with HBSS (Hanks' balanced salt solution), overlaid with (2x) EMEM/agarose mixture. The experimental was proceeded as under assay[35].

*Viral DNA or RNA extraction*

HSV-1- DNA and RVFV-RNA was extracted by QIAamp Vired Kit (Cat. No.51304) or (Cat No.52904) from infected Vero cells. The yield and quality were determined spectrophotometry from relative absorbance of RNA and DNA at 260 nm and 280 nm. Determination of the integrity of the purified RNA were by running 5µl from the extract on 1% agarose gel electrophoresis after staining with ethidium.

*Determination of HSV-1 viral DNA expression profile*

PCR was done by PCR stratgene Kit. PCR was performed in 100µl containing 10µg purified DNA, 10µl of 10x buffer, 1.0µl dNTPs (10mM), 2.5µl HSV-1 forward primer (53), 2.5µl HSV-1 reverse primer (53), 0.5 µl stratgene enzyme and completed to 100 ml with nuclease free water then the samples were denatured at 95 °C for 15 min. and subjected to 35 cycles of thermal cycling (T- gradient, Biometra, Germany). Each cycle consisted of denaturation for 1min at 95°C, annealing for 1min at 50 °C and extension for 1 min at 72°C. Samples were three incubated for 10 min at 72°C.

*Determination of RVFV viral RNA expression profile*

RT-PCR was done by using RT-PCR Qiagene Kit (Cat. No. 210212). RT-PCR was performed in 25.0 µl containing 3.0 µg purified KNA, 5.0 µl 5 x buffer, 10µl dNTPs (25mM), 1.0 µl of 25 ng from sense (53) and 1.0 µl of 25 ng from antisense (53). 2.0 µl enzyme mix and completed to 25.0 µl with nuclease free water then the samples were incubated at 50 °C for 60 min, then the samples were denaturized at 95°C for 15 min and subjected to 35 cycles of thermal cycling (T- gradient, Biometra, Germany). Each cycle consisted of denaturation for 1min at 95°C, annealing for 1min at 56 °C and extension for 1min at 72°C. Samples were then incubated for 10min at 72°C.

*Detection of PCR product*

Ten µl of PCR product was mixed with 3µl gel loading buffer (forty gm sucrose were mixed with 50mg bromophenol blue and 0.5 gm SDS, then dissolved in 100ml 0.1M EDTA) and slowly loaded into the slot of the submerged gel also a 100 bp DNA marker (GIBCO,BRL) was loaded along as size detector. The gel was then examined under the ultraviolet and photographed.

## RESULTS

*Carrageenan yield*

The carrageenan content was differed with the type of algae. The red alga *Acanthophora specifira* contained 0.411 g/g Dw (1.644%) of carrageenan from the type L-family more than the brown alga *Hydroclathrus clathratus* 0.044g/g Dw (0.075 % which was from the type k- family as shown in Figure 1.

*The cytotoxicity of carrageenan on Vero cell culture*

Before studying the antiviral effects of carrageenan extracts, it was imperative to determine the maximum no toxic level of carrageenan extracts tested in the study. The maximum non toxic dose (MNTD) was $10^{-2}$ for *Acanthophora specifira* and $10^{-3}$ of *Hydroclathrus cathralus* dilution of 100 µl of different carrageenan extracts with 1.0 ml medium. At MNTD treated Vero cells did not show morphological difference when compared with control as shown in figure (2).

*Titration of Viruses Seeds*

*Virus infectivity assay*

MTT assay was performed to determine the cytotoxic property of Vero cell lines (Fig. 3). It was found that Vero cells infected with HSV-1 and RVFV isolated and incubated at 37˚C stared to show cytopathic effect (CPE) in the form of cell rounding at 24-48 hrs. It was found that as carrageenan concentrations increases, the number of viable cells decreases. The cytotoxic compounds present in the seaweeds further growth of HSV-1 and RVFV cell lines and decreases the cell viability. The antiviral assay progressed to +3 CPE by fourth day post infection. The virus titer of each HSV-1 and RVFV reached to $10^6$TCID 50/ml.

*Plaque assay of HSV-1 and RVFV*

HSV-1 and RVFV viruses titer were 3x $10^5$ PFU/ml scored at 72 hrs post infection in Vero monolyer cell under agrose overlay as shown in fig. (4).

*Reduction of virus titers*

The mean values of triplicate experiments showed that HSV-1 and RVFV titers were $10^6$ and $10^{5.5}$ respectively in Vero cell cultures. Without, carrageenan (antiviral), which reduced to $10^4$, $10^{3.8}$ (HSV-1) and $10^{3.5}$, $10^{4.1}$ (RVFV) of *Acanthophora specifira* and *Hydroclathrus cathralus* respectively (Tables 1, 2 and 3), the level of reduction was $10^2$, $10^{2.62}$ (HSV-1) and $10^3$, $10^{1.5}$ (RVFV) respectively.

*Antiviral activity and cytotoxicity of carrageenan*

The concentration of carrageenan extracts to produce a 50% reduction of Vero cells culture depending on species of algae. The concentration of carrageenan that kill 50% of Vero cells ($CC_{50}$ ) values as determined by MTT were 72.9 and 100.5 µg/ml for *Acanthophora specifira* and *Hydroclathrus cathralus* respectively (Table 3). The antiviral activity of carrageenan extracted from *Acanthophora specifira* and *Hydroclathrus cathralus* inhibited (HSV-1) and (RVFV) replication and showed higher antiviral activity when it was post incubated with infected Vero cells with (HSV-1) and (RVFV) recording $T_1$ of viruocidal (1.047 and 1.331), $T_1$ of HSV-1 (0.943, 1.005) and $T_1$ of RVFV (1.001, 1.056) respectively. $\log_{10}$ $TCID_{50}$ / 0.1ml reduction in viral titer recording showed significant antiviral activity of marine red algae (*Acanthophora specifira*) compared with marine brown *Hydroclathrus cathralus* (Table 4).

*Confirmation of anti-viral activity using RT- PCR*

*Titration of virus control by RT-PCR*

Serial dilution 10 fold ($10^{-1}$ to $10^{-8}$) from the stock virus was subjected to RT-PCR analysis. HSV-1 and RVFV viruses genome at expected size 390 and 375 bp was visualized by UV irradiation of ethidium bromide- stained gel respectively (Figures 5 and 6).

*Antiviral activity of carrageenan extracts*

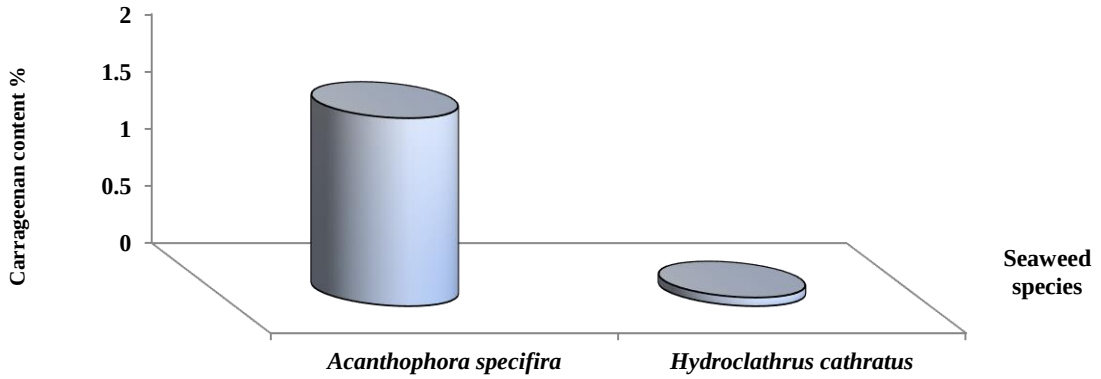


Figure 1: Carrageenan extractive values of *Acanthophora specifira* and *Hydroclathrus cathratus*.

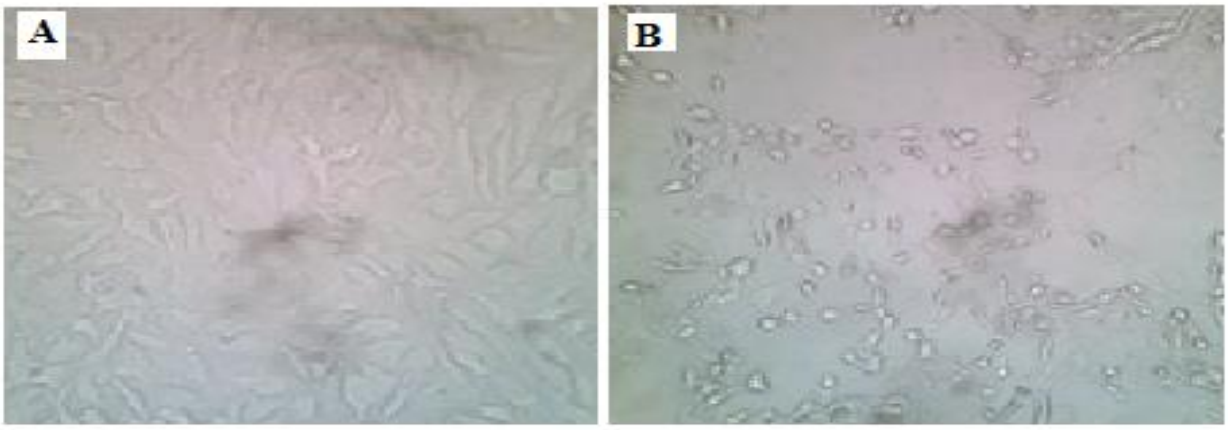


Figure 2: Evaluation of cytotoxic effect of carrageenan extract on vero cell line
A: Cell control, B: Carrageen treated cells

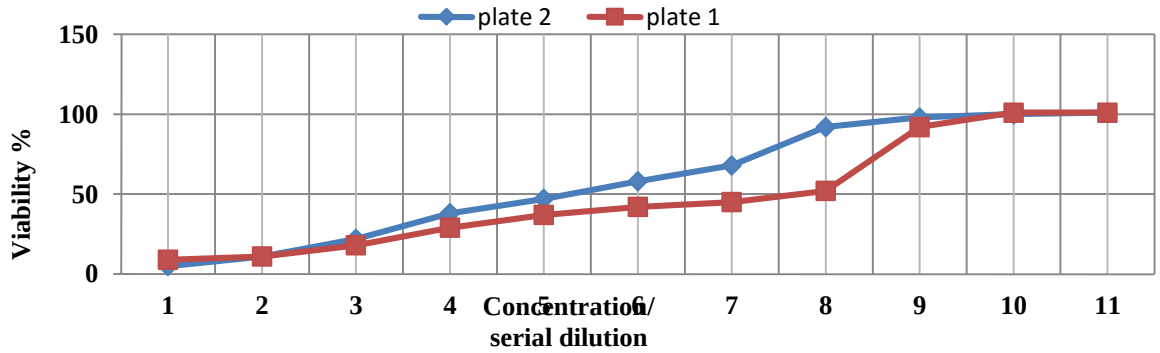


Figure 3: Evaluation of viability % of (Test material) using MTT assay relative to concentration.

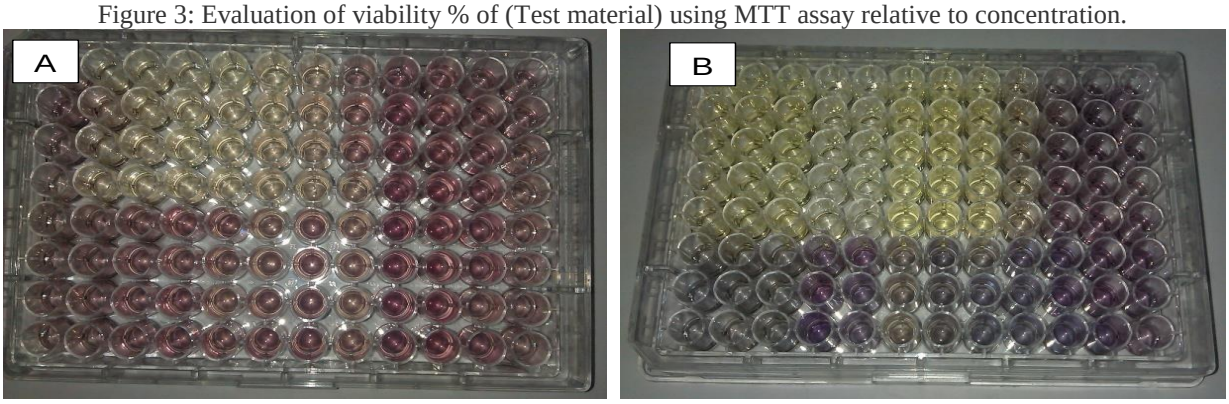


Figure 4: Vero cell monolyer culture treated with HSV-1 (A) and RVFV (B) viruses.

The carrageenan extract of marine red alga at concentration of 100µg/ ml was added to infect Vero cells with HSV-1 and RVFV viruses. The inhibition of viral replication was observed by amplify P53 gene (HSV-1)

Table 1: Reduction of HSV-1 and RVFV titer treated with carrageenan extracted from *Acanthophora specifira*

| Dilution of carrageenan | HSV-1 | | | | RVFV | | | |
|---|---|---|---|---|---|---|---|---|
| | CPC | No. CPC | ratio | % | CPC | No. CPC | ratio | % |
| $10^{-1}$ | 28 | 0 | 28/28 | 100 | 24 | 0 | 24/24 | 100 |
| $10^{-2}$ | 20 | 0 | 20/20 | 100 | 16 | 0 | 16/16 | 100 |
| $10^{-3}$ | 12 | 0 | 12/12 | 100 | 8 | 1 | 8/9 | 88 |
| $10^{-4}$ | 4 | 4 | 4/4 | 50 | 1 | 8 | 1/9 | 11 |
| $10^{-5}$ | 0 | 12 | 0/12 | 0 | 0 | 15 | 0/15 | 0 |
| $10^{-6}$ | 0 | 20 | 0/20 | 0 | 0 | 23 | 0/23 | 0 |

100- 50/100-50x1=1 ►►HSV-1titer = $10^{-4}$, 88-50/88-11x1=0.49►► RVFV titer =$10^{-3.5}$

Table 2: Reduction of HSV-1 and RVFV titer treated with carrageenan extracted from *Hydroclathrus cathratus*

| Dilution of carrageenan | HSV-1 | | | | RVFV | | | |
|---|---|---|---|---|---|---|---|---|
| | CPC | No. CPC | Ratio | % | CPC | No. CPC | ratio | % |
| $10^{-1}$ | 27 | 0 | 27/27 | 100 | 29 | 0 | 29/29 | 100 |
| $10^{-2}$ | 19 | 0 | 19/19 | 100 | 21 | 0 | 21/21 | 100 |
| $10^{-3}$ | 11 | 1 | 11/12 | 91 | 13 | 0 | 13/13 | 100 |
| $10^{-4}$ | 4 | 6 | 4/10 | 40 | 5 | 4 | 5/9 | 55 |
| $10^{-5}$ | 1 | 12 | 1/3 | 6 | 1 | 11 | 1/12 | 8 |
| $10^{-6}$ | 0 | 20 | 0/20 | 0 | 0 | 19 | 0/19 | 0 |

91- 50/91-40x1=0.8 ►►HSV-1titer = $10^{-3.8}$, 55-50/55-8x1=0.1 ►► RVFV titer =$10^{-4.1}$

Table 3: Cytotoxicity and antiviral of carrageenan extracts determined by MTT method

| Source of carrageenan | $CC_{50}$ | Viruocidial | | HSV-1 | | RVFV | |
|---|---|---|---|---|---|---|---|
| | | $1C_{50}$ | $T_1$ | $1C_{50}$ | $T_1$ | $1C_{50}$ | $T_1$ |
| *Acanthophora specifira* | 75.9 | 72.5 | 1.047 | 80.5 | 0.943 | 75.8 | 1.001 |
| *Hydroclathrus cathralus* | 100.5 | 75.5 | 1.331 | 100.5 | 1.005 | 95.2 | 1.056 |

$CC_{50}$: concentration of carrageenan is cytotoxic to 50% of Vero cells, $1C_{50}$: concentration of carrageenan that inhibits viral infectivity (cytopathic effect) by 50%, $T_1$: therapeutic index= $CC_{50}$ /$1C_{50}$

and BCL-2 gene (RVFV) the log 10 reduction in virus titer was observed compared with viral control (Figures 5).

The Vero cells were treated with carrageenan extract of marine brown alga at concentration of 300µ g/ml for 24h post to cells infection and the inhibition of the HSV-1 and RVFV replication was observed by amplify P53gene (HSV-1) and BCL-2 gene (RVFV). The 3 log10 reduction in virus titer was observed when the extract compared with the viral control (Figure, 6).

**DISCUSSION**

Many studies have been carried to screen for antiviral agents from natural sources. Marine algae have shown their potential as important and natural sources of antiviral as well as other bioactive compound[36,37]. The seaweed polysaccharides are the most abundant polysaccharides in marine plants, and usually possess the special characteristics of high sulfation and carboxylation[38]. Carrageenan, a sulfated polysaccharide, might inhibit viral infection via direct actions on the virus surface by its negative charge[38]. In the present study, we focused on carrageenan extract from red alga *Acanthophora specifira* and brown alga *Hydroclathrus clathratus* to assess its antiviral activities. The carrageenan extractive values from *Acanthophora specifira* were more than *Hydroclathrus clathratus*. The carrageenan extracted from *Acanthophora specifira* belongs to lambda carrageenan (ι- family) whereas that extracted from *Hydroclathrus clathratus* was from Kappa carrageenan (κ- family). There are at least 15 different carrageenan structures[39]. The most industrially relevant carrageenans are κ, ι and λ forms. The major source of κ-carrageenan is the red seaweed *Kappaphycus alvarezii*[40]. Lambda-carrageenan is obtained from species of the *Gigartina* and *Chondrus* genera[41]. The antiviral assay in this investigation was showed against herpes simplex and Rift valley fever virus effect. Herpes simplex virus type 1 (HSV-1) is a neurotropic human pathogen capable of infection and spread in a variety of cells while Rift valley fever virus (RVFV) cause serious diseases to human and animals. It exhibited low cytotoxicity to Vero cells as found by Zhu[42]. The hot water extraction and the antiviral activities were in agreement with Hui *et al.*[43]. They isolated polysaccharides from the brown seaweed *Hydroclathrus clathratus*. They mentioned that heating always promoted the reactions and it was the best way to extract seaweed polysaccharides for their antiviral activities. The increase in yield that was associated with a drop in their bioactivity may be due to the change in the properties of the polysaccharide such as solubility, structure and viscosity and so on. Wang *et al.*[11] also confirmed that the extracts of *Hydroclathrus clathratus* and *Lobophora variegata* showed more potential anti-

Table 4: Reduction of virus titer treated with carrageenan extracts on Vero cells cultures by $TICD_{50}$/0.1 determination.

| Source of carrageenan | HSV-1 titer | | Reduction of virus titer | RVFV titer | | Reduction of virus titer |
|---|---|---|---|---|---|---|
| | Non-treated | Treated | | Non- treated | treated | |
| *Acanthophora specifira* | $10^{-6}$ | $10^{-4}$ | $10^{2}$ | $10^{5.5}$ | $10^{-3.5}$ | $10^{3}$ |
| *Hydroclathrus cathralus* | $10^{-6}$ | $10^{-3.8}$ | $10^{2.62}$ | $10^{5.5}$ | $10^{-4.1}$ | $10^{1.5}$ |

$TICD_{50}$ = 50% tissue culture infectious dose

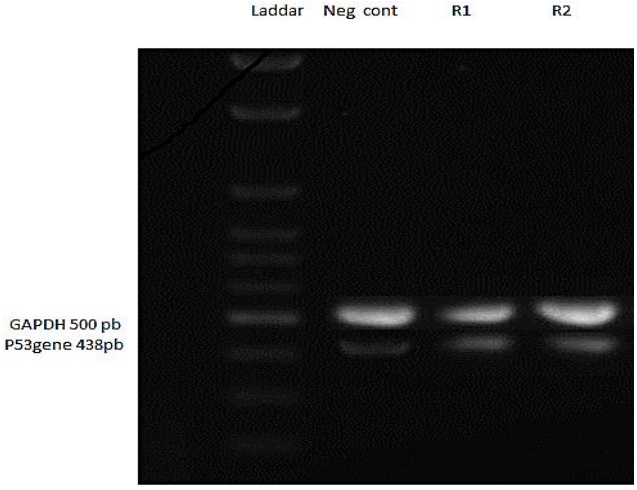


Figure 5: Detection of P53 gene expression post treatment with 2 different natural products compared to non-treated cells. Internal GAPDH control is involved

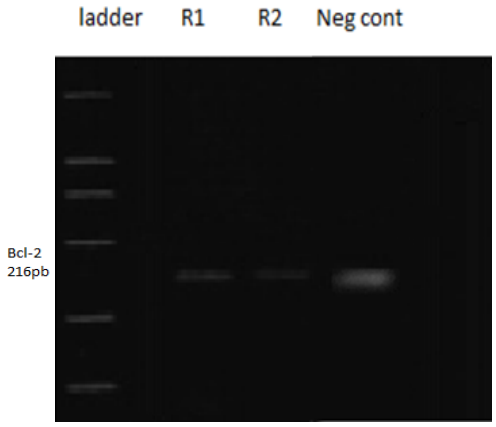


Figure 6: Detection of BCL-2 gene expression post treatment of cells with R1 and R2 compared to non-treated cells

HSV activities than the extracts of the other tested seaweeds. Damonte *et al*.[44] reported that the water extract of red seaweed showed high inhibitory effect on RSV in HeLa cells and influenza A virus in MDCK cells with very low EC50 values. Our study showed that the antiviral activity of carrageenan on HSV-1 and RVFV as evaluated by plaque assay reduction showed inhibition of HSV and RVFV. This is in agreement with Luescher-Mattli[18] who explained that carrageenan inhibition of herpes simplex virus and HIV-1 infectivity were demonstrated as about a thousand-fold higher than the IC50s observed for genital HPVs *in vitro*. The biological activity results of this study were determined by subjecting incubated cultured cell to MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenlytetrezolium Bromide) colorimetric assay. The tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity[45]. MTT is reduced in metabolically active cells to yield an insoluble purple formazon product. Our results demonstrated that the level of reduction with carrageenan from *Acanthophora specifira* was $10^{2}$, $10^{2.62}$ (HSV-1) more than carrageenan from *Hydroclathrus cathralus* $10^{3}$, $10^{1.5}$ (RVFV). The concentration of carrageenan extracts to produce a 50% reduction of Vero cells culture depends on species of algae. Antiviral activity of carrageenan extracted from marine red algae inhibited (HSV-1) and (RVFV) replication showed higher antiviral activity when it was post incubated with infected Vero cells with (HSV-1) and (RVFV). Damonte *et al*.[46] showed that the direct virucidal actions of carrageenan may be due to the formation of a stable virion–carrageenan complex where binding is not reversible. Hence, the sites on the viral envelope required for virus attachment to host cells are occupied by the sulfated polysaccharide, which renders the virus unable to complete the subsequent infection process. Luescher-Mattli[18] mentioned that carrageenan inhibition of herpes simplex virus and HIV-1 infectivity were demonstrated as about a thousand-fold higher than the IC50s observed for genital HPVs *in vitro*. In general, carrageenan polysaccharides have direct virucidal actions on some viruses to block the virus infection. This virucidal activity is important for marine polysaccharides since it is associated with the augmentation of antiviral activities *in vivo*[47]. Carrageenan had been found to possess antiviral properties attributed to the galactan units in the polysaccharides[24]. The modes of antiviral action of most sulfated seaweed polysaccharides have often been attributed to a blockade of the early stages of the virus replication cycle[48]. Nakashima *et al*.[49] found that a sulfated polysaccharide selectively inhibited reverse transcriptase (RT) enzyme of human immunodeficiency virus (HIV) and its replication in vitro. González *et al*.[50] showed that sulfated polysaccharides could prevent viral protein synthesis or were capable of blocking various steps during the life cycle of HSV and make it less likely for the virus to develop resistant mutants[51]. This study revealed that the inhibition of viral replication by Carrageenan was mediated by amplification of P53 gene (HSV-1) and BCL-2 gene (RVFV). The log 10 reduction in virus titer was

observed compared with viral control. Marine polysaccharides, especially the seaweed polysaccharides, can interfere with virus replication enzymes[52] or other potential targets within the host cells[53], so as to inhibit the virus transcription and replication processes. Wang *et al*.[38] showed that the marine polysaccharides, especially the low molecular weight marine oligosaccharides, cannot only block the virus invasion process, but also inhibit the virus transcription and replication processes after internalization into host cells. The inhibition of virus transcription and replication may be related to the direct interference of viral replication enzymes or on other intracellular targets by marine polysaccharides. González *et al*.[51] used methionine-labeled virions to analyze the internalization of HSV-1 into host cells, and found that carrageenan polysaccharides have no inhibitory effect on virus adsorption or virus entry, but the subsequent protein synthesis of virus is declined. The sulfated polysaccharides inhibit the activity of purified reverse transcriptase and RNase H, which are essential for retrovirus replication[54].

## CONCLUSION

This study has shown that the natural product carrageenan as polysaccharides from red alga *Acanthophora specifira* and brown alga *Hydroclathrus clathratus* extracts can inhibit both herpes simplex (HSV-1) and rift valley fever virus (RVFV) and had low cytotoxicity to Vero cells. These results need further investigations for characterization of active compounds and their specific mechanisms of viral inhibition.

## ACKNOWLEDGMENT

We thanks Prof. Khalid El-Dougdoug and Dr. Afaf Elgharib, Department of Microbiology, Faculty of Agriculture, Ain Shams University for great assistance, helpful.

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