The Egyptian Journal of Hospital Medicine (Jul. 2016) Vol. 64, Page 430- 435

Screening For Antiviral Activities of Aqueous Extracts of Some Egyptian Seaweeds

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ABSTRACT

Background: aqueous extracts of six species of marine seaweed were studied as antiviral activity on different viruses. **Materials and methods:** these collected from two sites Hurghada at the Red Sea and Al-Agami area in Alexandria Mediterranean Sea Egypt and belonging to the classes Chlorophyta, Phaeophyta and Rhodophyta were assayed for the cytotoxicity and antiviral activity by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenlytetrezolium bromide] and by neutralization methods.

Results: these extracts have antiviral activity to herpes simplex virus types-1 (HSV-1) and type-2 (HSV-2), hepatitis A virus (HAV-H_{10}), and Coxsackie B_4 virus in Vero cells with very low cytotoxicity to the host cells.

Keywords: Antivirus, Marine seaweed, Hurghada, Red Sea, Al-Agami, Alexandria, Egypt.

INTRODUCTION

Seaweeds are the most interesting algal groups because they are considered as a unique source of antimicrobial[1,2], antiviral[3,4], antifungal[5], anti-allergic[6], anticoagulant[7], antitumer[8,9,10,11], antifouling[12] and antioxidant activities[13].

The present study was aimed to examine the antiviral activity of aqueous extracts of six seaweed species that were collected from two locations: Alexandria on the Mediterranean Sea and Hurghada on Red sea shores. The chosen viruses in Vero cell cultures and had cytopathic effects. They were grew herpes simplex virus types 1 (HSV-1), herpes simplex virus types 2 (HSV-2), hepatitis A virus (HAV-H_{10}), and Coxsackie B4 virus.

MATERIALS AND METHODS

Seaweed collection and identification

Six seaweeds species belonging to three algal divisions (Chlorophyta, Phaeophyta and Rhodophyta), including *Sargassum latifolium, Cystoseira myrica, Turbinaria ornate, Jania rubens,* were collected from Hurghada Red Sea, while, *Ulva lactuca* and *Codium tomentosum* were collected seasonally from two sites Hurghada at the Red Sea and Al-Agami area in Alexandria Mediterranean Sea, Egypt from on November 2009 to January 2011 with temperature average from 25°C to 37°C.

Samples were washed in seawater and delivered to the laboratory in plastic bags containing sea water to prevent evaporation, sorted and carefully cleaned from associated biota, then dried at room temperature and ground to a fine powder before performing extraction. Samples were identified according to **Nasr and Aleem**[14,15]. Samples were stored in dry cold place until performing extraction.

Crude extract preparations from seaweed

Sixty gm of each dried seaweed sample were weighed, crushed by an electric blender jar to get the fine powder, and then the powder of dried seaweeds was extracted with 100ml of water in one liter flask for 24h at 45°C with ground stopper. Then the water was filtered through a filter paper. The aqueous extract was evaporated by rotary evaporator and the residues were completely dried to constant weight by placing it in a porcelain dishes inside desiccators with calcium carbonate. Then the powder was stored at -12°C till further uses. Five grams of the residue was dissolved in 100ml of sterile distilled water to make 5% seaweed suspension. They were filtered and then, the filtrate was used for the antimicrobial test as described by **Meisner *et al.***[16].

Determination of extract cytotoxicity

For cytotoxicity assay, aqueous crude extracts were prepared individually from the collected algae. The procedure described by **Van den Berghe *et al.*** was applied[17].

Antiviral activity test

The first step to determine each virus titre and to prepare a dilution that contains

Received: 22/08/2016
Accepted: 30/08/2016

DOI : 10.12816/0029035

between 100-300 TCD50 which is called challenge dose of virus' CDV. This CDV of herpes simplex virus types-1 (HSV-1) and type2 (HSV-2), hepatitis A virus (HAV-H10), and Coxsackie B4 virus were used for antiviral assay. The viruses were obtained from the virology laboratory of Medicine Faculty, Azhar University (Girls Branch). The antiviral effect of these seaweed crude extracts on these viruses was determined activity in *Vero* cell using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenlytetrezolium bromide][18].

RESULTS

Cytotoxicity of seaweed extracts on *Vero* cell cultures

By observing the morphological changes (CPE) of *Vero* cells induced by the water extracts of *Sargassum latifolium*, *Cystoseira myrica*, *Turbinaria ornate*, *Jania rubens*, *Ulva lactuca* and *Codium tomentosum*, the lowest toxicity on *Vero* cells was observed for *Codium tomentosum* with the CC_{50} values equal to 0.05 µg/ml and the highest with *Jania rubens*, *Sargassum latifolium* and *Ulva lactuca* with the CC_{50} values equal to 5 µg/ml (Table-1).

Antiviral screening of seaweeds extracts

This method was done to show the effect of the crude seaweed extracts on some viruses before cell penetration. Table (2) demonstrated the algal aqueous crude extract of the six different seaweeds effect on the HAV-H_{10}, Cox-B_4, HSV1 and HSV-2 viruses at non-lethal or toxic dose by MTTs assay. It was noticed that highly significant antiviral activity on all selected viruses with *Ulva lactuca* and then *Turbinaria ornate* with HAV-H_{10} and *Cystoseira myrica* with Cox-B_4, HSV-1 and HSV-2 whilst *Codium tomentosum* and *Jania rubens* were lowest significant.

When compared the anti-viral activities between the seaweed extracts against different viruses (HAV-H_{10}, Cox-B_4, HSV-1 and HSV-2), it was found that *Ulva lactuca* crude extract was the highly active extract followed by *Cystoseira myrica* when compare to the other extracts (Table 2). But at the same time Table (3) showed that the action behaviors of the crude extract of the same algal species act differently when compare between types of virus by increase or decrease the antiviral activities.

DISCUSSION

While researchers trying to found and develop a vaccine or antiviral treatment against the wide world viruses, there are a natural abundant product already present around us. Marine algae have shown their potential activities as important sources of antiviral as well as other bioactive compounds[19,20]. The main objective of this study was to evaluate the ability of different seaweeds from Egyptian coast to inhibit the growth of some clinically important pathogenic viruses.

In the present investigation, the water extracts of seaweeds showed antiviral activity, our findings are consistent with some earlier reports[21]. Who reported that *Polysiphonia denudate* aqueous extraction appears to be an effective technique that inhibited the reproduction of Herpes virus type1 and type 2 in cell cultures (IC_5= 8.7 to 47.7 mg/ml), he also proved that the inhibition affected adsorption, as well as the intra-cellular stages of viral replication.

Also, **Haslin *et al.*** and **Bouhlal *et al.*** mentioned that an aqueous extract from *Rhodophyceae* have an antiviral activity which can inhibits the human immunodeficiency virus (HIV-1) replication at 10 µg/ml water extract seems to be more effective and non-cytotoxic on cell lines than methanolic, dichloromethanlic and chloroforme-methanolic extracts[1,22].

The antiviral activity of *Codium tomentosum* and *Jania rubens* were moderate or low which in contradictory with other studies reported by **Karabay-Yavasoglu *et al.***; **Ismail-Ben Ali *et al.*; Mohy El-Din and El-Ahwany** whose stated that these two species have strong antiviral and antimicrobial activities this difference may be due to the difference in the solvent system[23,24,25].

On the Other Hand, it seems from the present investigations that Egyptian brown marine seaweed water extracts *Cystoseira myrica*, *Sargassum latifolium* and *Turbinaria ornata* gave a good antiviral activity. This result is similar to that found by **Manivannan *et al.*; Sridharan and Dhamotharan and Sethi** whose found that the methanol extract of *Turbinaria conoides* inhibit bacteria and viruses[26,27,28]. In contrast, no significant antiviral activity or cytotoxicity

were observed for the compounds of the cyclohexane extract of brown alga *Turbinaria conoides* which were performed in Crandell-Rees feline kidney (CRFK) cells by a colorimetric formazan-based MTS by **Kumar *et al***.[29].

The algal extracts may have different antiviral activity according to the type of virus. The work of **Chirasuwan *et al.*** showed that 25.1 µg/ml of methanol extract *Spirulina platensis* exhibited 50% reduction with HSV-1[30]. On the other hand, **Corona *et al.*** also found that methanol extract of *Spirulina maxima* exhibited antiviral activity against HSV-2 with EC_{50} 6.9 mg/ml, and IC_{50} (IC_{50}: Minimum concentration required to reduce control virus infection by 50%) 0.13 mg/ml[31]. They suggested that the antiviral activity could be due to highly polar compounds present in methanol extract so there is different antiviral susceptibility for the same algal extracts which agree with our finding.

Also, **Vijayabaskar and Shiyamala** found that *Sargassum wightii* and *Turbinaria ornate* from the Gulf of Mannar Biosphere Reserve gave antibacterial activities against various Gram positive and Gram negative human pathogenic microbes[32]. **Pushparaj *et al.***, **Barot *et al.*** and **Deveau *et al.*** demonstrated that *U. lactuca* methanolic extracts inhibit a variety of clinically relevant human pathogenic bacteria and fungi strains and showed maximum inhibitory from methanol extract than acetone, chloroform, hexane and ethyl acetate solvents[33,34,35]. On the contrary, **Saritha *et al.*** showed that acetone extract was the best in inhibit pathogenic microorganisms[36]. On the other hand, **Mendes *et al.*** investigated *Ulva fasciata* were collected from Rasa beach and Forno beach, Rio de Janeiro, Brazil for having antiviral activity on the replication of human metapneumovirus (HMPV)[37]. But other studies reported that *Codium fragile* had an antiviral activity against HSV-1 and HSV-2[38,39].

The present investigation shows that the aqueous extract possesses a strong antiviral activity specially *Ulva lactuca* and *Cystoseira myrica*. However, the exact mechanism and the compound responsible for the antiviral activities are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and characterization of the compound.

CONCLUSION

Egyptian seaweed is promising source of natural antiviral agents herpes simplex virus types-1 (HSV-1) and type2 (HSV-2), hepatitis A virus (HAV-H10), and Coxsackie B4 virus. Results suggested that the Egyptian marine seaweeds should be considered as biological sources of natural antiviral products for the treatment or control of these viruses. Further work may be performed to evaluate the pure active component and its pharmaceutical application.

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Table (1): Assessment of toxic dose of crude extract from Seaweed algae using *Vero* cell cultures

No.	Species	*Vero* non-toxic dose CC_{50}	*Vero* non-toxic dose (µg/ml)
1	*Jania rubens*	10^{-1}	500
2	*Cystoseira myrica*	10^{-2}	50
3	*Sargassum latifolium*	10^{-1}	500
4	*Turbinaria ornate*	10^{-2}	50
5	*Ulva lactuca*	10^{-1}	500
6	*Codium tomentosum*	10^{-3}	5

Table (2): Antiviral activity of crude seaweed extracts on some viruses.

Sample name		Selected dose	O.D at 560/620nm	*Vero* cell viability	HAV-H10 CDV* cytotoxicity %	Anti-viral effect%
Control(VERO cell line)		None	0.814	100%	------	------
HAV-H10 Virus	Control	$TCD50=10^{-3}$	0.315	38.69	61.31	None
	Jania rubens	10^{-1}	0.340	41.76	58.24	3.07
	Cystoseira myrica	10^{-2}	0.619	76.04	23.96	37.35
	Sargassum latifolium	10^{-1}	0.436	53.56	46.44	14.87
	Turbinaria ornate	10^{-2}	0.625	76.78	23.22	38.08
	Ulva lactuca	10^{-1}	0.728	89.43	10.57	50.74
	Codium tomentosum	10^{-3}	0.321	39.43	60.57	0.74
Cox B 4 Virus	Control	$TCD50=10^{-3}$	0.345	42.38	57.62	None
	Jania rubens	10^{-1}	0.376	46.19	53.81	3.81
	Cystoseira myrica	10^{-2}	0.552	67.81	31.19	25.43
	Sargassum latifolium	10^{-1}	0.475	58.35	41.65	15.97
	Turbinaria ornate	10^{-2}	0.521	64.00	36.00	21.62
	Ulva lactuca	10^{-1}	0.675	82.92	17.07	40.54
	Codium tomentosum	10^{-3}	0.354	43.49	56.51	1.11
HSV-1	Control	$TCD50=10^{-3}$	0.356	43.73	56.27	None
	Jania rubens	10^{-1}	0.396	48.64	51.36	4.91
	Cystoseira myrica	10^{-2}	0.732	89.92	10.08	46.19
	Sargassum latifolium	10^{-1}	0.448	55.03	44.97	11.3
	Turbinaria ornate	10^{-2}	0.713	87.59	12.41	43.86
	Ulva lactuca	10^{-1}	0.765	93.98	6.02	50.25
	Codium tomentosum	10^{-3}	0.368	45.21	54.79	1.47
HSV-2	Control	$TCD50=10^{-3}$	0.334	41.03	58.97	None
	Jania rubens	10^{-1}	0.353	43.37	56.63	2.34
	Cystoseira myrica	10-2	0.616	75.68	24.32	34.64
	Sargassum latifolium	10^{-1}	0.398	48.89	51.11	7.9
	Turbinaria ornate	10^{-2}	0.605	74.32	25.68	33.29
	Ulva lactuca	10^{-1}	0.681	83.66	16.34	42.63
	Codium tomentosum	10^{-3}	0.341	41.89	58.11	0.86

***** Virus strain challenge dose to *Vero* cell culture $TCD_{50;}$ ^TCD_{50}: the concentration that reduced 50% of viable cells tested by MTT method; ** All data in tables were the mean of three repeated tests

Table (3): Comparison between antiviral effects of seaweed crude extract on HAV-H_{10}, CoxB_4, HSV-1 and HSV-2 standard strain.

Sample	Selected dose	HAV-H_{10}	Cox-B4	HSV1	HSV2
Jania rubens	10^{-1}	3.07	3.81	4.91	2.33
Cystoseira myrica	10^{-2}	38.35	26.43	46.19	34.64
Sargassum latifolium	10^{-1}	14.87	15.97	11.30	7.86
Turbinaria ornate	10^{-2}	38.08	21.62	43.86	43.61
Ulva lactuca	10^{-1}	50.74	40.54	50.25	42.63
Codium tomentosum	10^{-3}	0.74	1.11	1.47	0.86