OPEN ACCESS Freely available online

Virology & Mycology

Mini Review

Antiviral Activity of a Marine Seaweed *Tubinaria Ornata* (Turner) J. Agardh *(Phaeophyceae)* Against Herpes Simplex Virus Ii (HSV-II)

Poonam Sethi*

Department of Virology and Mycology, Guru Nanak College (Autonomous), Chennai,India

ABSTRACT

Turbinaria ornata marine brown seaweed found along the coastal areas of Rameswaram, Tamil Nadu, and India was screened *in vitro* for antiviral activity against Herpes Simplex Virus-2.Two extracts one aqueous and the other ethanolic (95%) were screened for activity. The extracts were subjected to Pretreatment assay (whereby the plant extract is added before the addition of virus to the cell line) and post treatment assay (whereby the plant extract is added after the addition of virus to the cell line). Abundance of this algae on the shores during the month of November in the coastal waters of Rameswaram was intuited to select this alga, in addition to this the solvent extracts were non cytotoxic to the cell line. On the other hand dextran sulphate was used a positive control and the bioactive compounds efficacy was compared with it.

Keywords: Antiviral; Dextran sulphate; Herpes Simplex virus; Seaweed; Turbinaria; *In vitro*

INTRODUCTION

Plants yield many biomedically useful substances. Apart from land plants, the lotic and lentic sources have also served as an outburst of a large group of structurally unique natural products of significance. Uniformly accepted standards for *in vitro* susceptibility testing are not available for antiviral drugs. Antiviral resistance is another critical aspect of clinical importance. Lastly, antiviral chemotherapy is a reality for only a segment of the world's population because of financial considerations [1].

Hope for millions of individuals in developing world afflicted by severe viral infections, rests with vaccine development, as the practical considerations such as cost are a barrier for access to many currently available and future drugs.

Antiviral drugs available in the market are very expensive and patients with frequent attacks cannot afford the cost of long-term treatment [2]. For these reasons, the search for new, effective and inexpensive antiviral drugs from natural resources continues to go on.

In the search for new antiviral agents, the antiviral activity of Stoechospermun marginatum off Indian coastline was studied for its activity and the aqueous and ethanolic extracts were tested. This paper also describes the inhibitory activity of dextran sulphate a standard agent against the viruses.

Over the past three decades, the importance of marine-derived physiologically active substances effective on mammalian tissues and on pathogenic microbes affecting the mammalian system has received much attention leading to the development of 'marine biomedical research [3].

MATERIALS AND METHODS

Turbinaria ornata (Turner) J. Agardh a genus of *Phaeophyceae*, Phaeophyta, was selected as the experimental algae, collected from the rocky shores of Rameswaram, Tamil Nadu. The collection was made in the month of November 2018, authenticated by Dr. R.Thevanathan Dean of Sciences, Presidency College, and Chennai.

Botanical description of the experimental plant

Plants erect and stiff, 2-20-(30) cm long when reproductive, usually isolated or in small groups, often rusty brown to dark brown; holdfast bearing one (or more) terete erect portion, basally a conical or irregular holdfast with several unbranched or

Correspondence to: Poonam Sethi, Department of Virology and Mycology, Guru Nanak College (Autonomous), Chennai,India, E-mail : poonam123.73@rediffmail.com

Received date: September 29, 2021; **Accepted date:** October 15, 2021; **Published date:** March 1, 2023

Citation: Sethi P (2023) Antiviral Activity of a Marine Seaweed *Tubinaria Ornata* (Turner) J. Agardh *(Phaeophyceae)* Against Herpes Simplex Virus Ii (HSV-II). Virol Myco. 12:250.

dichotomously branched stolons, these often remaining when erect portion torn off, or appearing before erect portion formed.

Juvenile plants with flattened blades can form new plants, become free-floating; larger plants with several orders of branching. Blades peltate, with 'petiole' and double row of stiff spines often with secondary branching from lower adaxial surface of blades; rarely irregularly triangular margin of leaves in apical view; petiole cylindrical near base, becoming traingularly compressed in distal portions; many plants with some leaves having hollow centers that function as floats.

Receptacles developing into tightly branched clusters on adaxial side of leaf petiole near base, mostly cylindrical, to 1.5 cm long, with blunt apices.

Macroscopical characters of the experimental plant

The plant body consisits of branched cylindrical axis and terminal clusters of funnel shaped expanded bodies.the surface of the plant body is smooth and even (Figure 1)

Figure 1: Turbinaria ornata (Turner) J. Agardh.

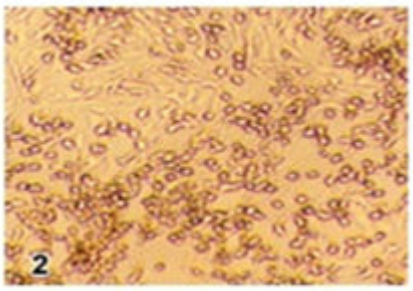

Figure 2: Vero cell line in culture with HSV-2 showing 25% cytopathic effect (CPE).

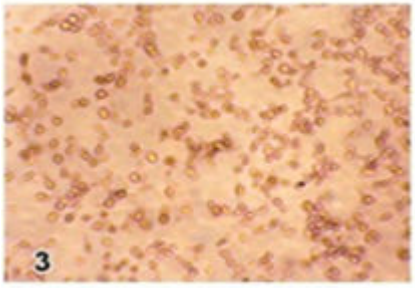

Figure 3: Vero cell line in culture with HSV-2 showing 50% cyto pathic effect (CPE).

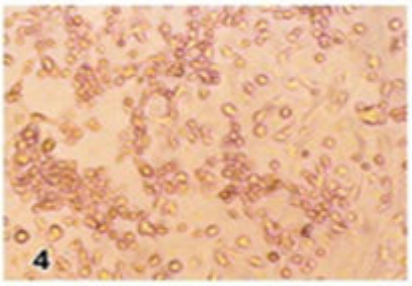

Figure 4: Vero cell line in culture with HSV-2 showing 75% cyto pathic effect (CPE).

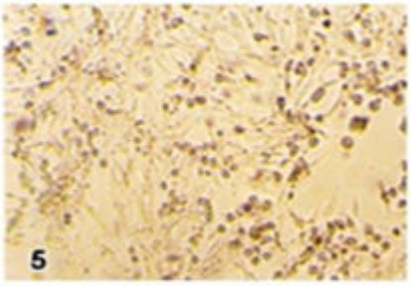

Figure 5: V er o cell line in cultur e with HS V -2 sho wing 100 % cyto pathic effect (CPE).

Figure 6: Vero cell line at cytotoxic concentrations of aqueo us extract residue.

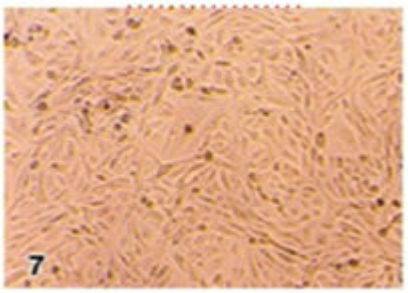

Figure 7: Vero cell line at cytotoxic concentrations of ethanol ic extract residue.

Extract preparation

The extract was prepared by dissolving ten grams of the finely chopped experimental plant in 100.0 mL of double distilled,

millipore filtered water and kept in a shaker. After 48 hrs, it was filtered through cheese cloth and the filtrate was freeze dried-Aqueous extract. Similarly the ethanolic extracts were prepared by dissolving ten grams of the finely chopped experimental plant in 100.0 mL of 90%ethanol. From the stock solution having the residue with distilled water of varying concentrations of the extract residue was used for antiviral studies.

Cell line used for *in vitro* assay

VERO cell lines and the virus were, obtained from KIPM, Kings Institute of Preventive Medicine, Guindy, Chennai and maintained in MEM containing 5% foetal calf serum, kept in a walk-in incubator at 37°C.

Virus

Herpes Simplex Virus HSV-2 [HSV-2/P6/Hep. 2 dt 10/12/01].

Standard strains of HSV-2 P6/ Hep2 dt 10/12/01 of TCID50 107.1 were obtained from the Department of Virology, King Institute of Preventive Medicine, Chennai.Vero cell lines (African green monkey kidney cell line) were used for the culture and routine maintenance of this human pathogenic viruse.

Cytotoxicity assay

As a prerequisite for the studies on the antiviral potential of these extract residues their cytotoxicity was studied on the Vero cell line used for the culture of the (HSV-2) .The non-cytotoxic levels of the different extracts were used for the antiviral assay.

Antiviral assay

Vero cells were seeded onto 96 well plate at a concentration of 1.0 x 105 cells per ml and a volume of 90 µl per well. The minimal active concentrations which had activity against the viruses was selected and subjected to 100 µl of 100 TCID50 of the virus which was added to each well. The plates were incubated at 37°C in a 5% CO_2 atmosphere and observed.

In the Pretreatment assay the extract was added to the cell line before the subjection of the virus [4]. while in post treatment assay the extracts were subjected to the cell line after the addition of the virus.Dextran sulphate, a sulfated polysaccharide was simultaneously used as a positive control in all the experiments because of its known broad spectrum antiviral properties [5].

Blueprint of the antiviral assay of extracts

CC	CC	100 µg (BV)	100 µg (BV)	150 µg (BV)	150 µg (BV)		
SC	SC	100 µg (AV)	100 µg (AV)	150 µg (AV)	150 µg (AV)		
Neat	Neat	250 µg (BV)	250 µg (BV)	200 µg (BV)	200 µg (BV)		
VC	VC	250 µg (AV)	250 µg (AV)	200 µg (AV)	200 µg (AV)		

Where,

CC-Cell Control

SC-Solvent Control

Neat -1000 µg of extract (drug)

VC-Virus Control

BV-Drug added before the addition of virus (pre-treatment assay)

AC-Drug added after the addition of virus (post-treatment assay)

RESULTS

The various concentrations of the extracts ranging from 1 µg, 10 µg, 100 µg and 500 µg upto 1000 µg,were subjected to cytotoxicity studies on the vero cell line which acts as an host for the virus HSV-2 [6,7].

Table 1: Cytotoxicity of the preparations of *Turbinaria Ornata* on vero cell line.

S. No.	Algal extract	Cytotoxic concentration µg mL-1
1.	Turbinaria ornata	>1000 µg
	Aqueous extract	>500 µg
	Ethanolic extract	
2	Dextran sulphate (positive control)	>500 µg

After the cytotoxicity testing the Optical Density value were measured in an ELISA plate.

Table 2: Cytotoxicity of extracts and dextran sulphate.

Vero cell line	OD (Mean ± S.E.)		F value	P value
	CC	TC		
Aqueous Extract	0.011.87 .007	0.120 00.13		0.075
Ethanolic Extract	0.145 0.010	1.310 90.13 012.		0.202
Dextran Sulphate	0.140 0.010	1.310 10.14 012.		0.202

TC-test compound, CC-cell control

After the cytotoxicity testing IC 50 value of the virus was detected in 96 well titre plate to estimate the virulence of the virus.

Table 3: IC50 Values for the extract residues of *Turbinaria ornata* against HSV-2.

S. No.	Algal extract	IC50 µg/mL	Concentration of the virus		
			1 TCID50	10TCID50	100 TCID50
a.	Turbinaria ornata	100	A	A	A
b.	Aqueous	250	PA	NA	NA
	Ethanolic				
	Dextran Sulphate	50	A	A	A

A-Active, NA-Not active, PA-Partially Active

The aqueous extract residues of the algae exhibited good activity against HSV-2 virus. The IC50 values for the aqueous extract residue of Turbinaria ornata was 100 µg/mL that of ethanolic was 250 µg/mL Nevertheless, the two fractions of the experimental algae appeared to be effective.

The observations made against HSV-2 indicated that the alga was effective against the virus, irrespective of the nature of the extract used. Dextran sulphate, a sulfated polysaccharide had IC50 value of 50 µg/mL. After the cytotoxicity testing the Optical Density value were measured in an ELISA plate.

Table 4: Cytotoxicity of extracts and dextran sulphate.

Vero cell line	OD (Mean ± S.E.)		F value	P value
	CC	TC		
Aqueous Extract	0.130±0.007	0.144±0.014	2.10	0.075
Ethanolic Extract	0.145 ± 0.010	0.187±0.012	2.40	0.202
Dextran Sulphate	0.140 ± 0.010	0.121±0.012	1.31	0.202

TC-test compound, CC-cell control

CONCLUSION

Both the aqueous and ethanolic extracts were found to be active against HSV-2.Further research has to be carried out on isolation of bioactive compounds from the crude extracts.

REFERENCES

1. De Clercq E. Antiviral drugs in current clinical use. J Clin Virol. 2004;30:115–133.
2. Dejonghe P, Parkinson B. Benefits and costs of Vaccination. Vaccine. 1992;10:936.
3. Hodinka RL. What clinicians need to know about antiviral drugs and viral resistance. Infect Dis Clin North Am. 1997;11(45):945.
4. Kodama E, Shigeta S, Suzuki T, De clercq. EApplication of a gastric cancer cell line (MKN –28) for anti-adenovirus screening using the XTT method. Antiviral Res. 1996;1:159-164.
5. Mc Clure M, Whitby C, Goderham A, Radshaw R, Weber D, Cook R, et al. Dextran sulphate and fucoidan are potent inhibitors of HIV infection in vitro. Antiviral Chem. 1991;2:149-156.
6. Palomino SS, Abad MJ. Bedoya LM lScreening of South American plants against huma immunodeficiency virus: preliminary fractionation of aqueous extract from Baccharis. Biol Pharm Bull. 2005;25:1147-1150.
7. Antimicrobial and antiviral activities against Newcastle disease virus (NDV) from marine algae isolated from Qusier and Marsa-Alam Seashore (Red Sea) Egypt. Afr J Biotechnol. 2012;11(33):8332-8340.