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ANTI-VIRAL ACTIVITY OF AQUEOUS EXTRACT FROM BROWN SEAWEED, SARGASSUM ILLICIFOLIUM (TURNER) ON THREE DIFFERENT TYPES HERPES SIMPLEX VIRUS

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ABSTRACT:

To evaluate the antiviral activity of aqueous extract of Brown Seaweed, *Sargassum illicifolium* on three types of herpes simplex viruses such as Wild type, Thymidine Kinase-Deficient and Acyclovir and Phosphonoacetic Acid Resistant ones. The anti-viral activity of aqueous extract of Sargassum illicifolium of different concentration such as 5%, 10%, 15%, 20%, 25% and controls both positive and negative were carried out against three types of Herpes Simplex Virus using Plaque Reduction Assay.The results indicated that in the negative control and lower concentrations (5%, 10%, 15%) there was no or least amount of antiviral activity which was followed by that of the positive control. Acyclovir, and the higher concentration of 20% and 25% showed better antiviral activity than that of the patented medicine. This preliminary study indicates that the aqueous extract of Sargassum illicifolium, is indeed endowed with antiviral properties against herpes simplex virus.

Keywords: *Sargassum illicifolium,* Aqueous Extract, Antiviral Activity, Herpes Simplex Virus, Plaque Reduction Assay.

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INTRODUCTION

Algae contain large amounts of cell wall polysaccharides. Several sulfated polysaccharides, such as heparin, dextran sulfate, pentosan polysulfate, mannan sulfate cyclodextrins and others, inhibit the replication of various enveloped viruses, including herpes simplex virus (HSV), human cytomegalovirus (HCMV) and human immunodeficiency virus (HIV)[1]. Although mechanistic studies have generated contradictory results, their mode of action has often been attributed to a blockade of the early stages of the virus replication cycle[2]. These compounds are effective against clinical isolates of HSV-1 and HSV-2 as well as against ACV resistant variants. To develop new antiviral agents from algae, we investigated the anti-HSV-1 activities of aqueous extracts from seaweeds that are widely available in India, Gulf Countries, Korea, Japan and China.

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), also known as Human herpes virus 1 and 2 (HHV-1 and -2), are two members of the herpes virus family, Herpesviridae, that infect humans. Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are ubiquitous and contagious. They can be spread when an infected person is producing and shedding the virus.

Symptoms of herpes simplex virus infection include watery blisters in the skin or mucous membranes of the mouth, lips or genitals. Lesions heal with a scab characteristic of herpetic disease. Sometimes, the viruses cause very mild or atypical symptoms during outbreaks. However, as neurotropic and neuroinvasive

viruses, HSV-1 and -2 persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. After the initial or primary infection, some infected people experience sporadic episodes of viral reactivation or outbreaks. In an outbreak, the virus in a nerve cell becomes active and is transported via the neuron's axon to the skin, where virus replication and shedding occur and cause new sores.

Many natural products can inhibit viruses. Natural marine products contain an abundance of biologically active substances with novel chemical structures and favorable pharmacological activities[3]. New types of antiviral agents from natural source especially those who have high efficacy on resistant mutant viral strains and low toxicity to host, are considered to be the most promising agents. Products from marine organisms show many interesting activities. Their constituents are more novel than those of many rich and valuable natural resources of bioactive compounds because of their various biological properties (Mayer and Lehmann, 2000). Several investigators have described the inhibitory effects of algal extracts and their constituents on the replication of herpes simplex viruses[4-6]. Serkedjieva[7] recently found that a water extract of *Polysiphonia denudata* (Rhodomelaceae) prevents HSV-1 reproduction by inhibiting viral adsorption to susceptible cells as well as viral synthetic stages.

The water-soluble extracts of seaweeds have been shown to exhibit antiviral activity against a wide spectrum of viruses[8]. There are more than 200 species of seaweeds in Indian coastal waters[9], but research on their antiviral activity is very limited[10]. The present paper describes the antiviral activity of aqueous extracts of *Sargassum illicifolium* collected in the coastal regions of Tamil Nadu, India.

MATERIALS AND METHODS

Viruses and Cells

It is Wild type HSV-1, thymidine kinase-deficient (TK-) HSV-1 and acyclovir (ACV) and phosphonoacetic acid (PAA)-resistant APr HSV-1in Vero E6 cells. The infected cultures were frozen and thawed three times, centrifuged at 1650 G for 15 min and then supernatants were stored at -80°C. Vero cells were cultured and maintained in Eagle's minimal essential medium (MEM) supplemented with 5% and 2% calf serum, respectively.

Acyclovir

Acyclovir (ACV) was purchased as tablets from Nippon Wellcome K.K. One tablet (200 mg) was powdered and suspended in distilled water to give an appropriate concentration.

Plaque Reduction Assay

Duplicate cultures of Vero cells in 60 mm plastic dishes were infected with 100 plaque forming units (PFU)/0.2 ml of HSV-1, Apr HSV-1 and TK- HSV-1 for 1 h. The cells were then overlaid with 5 ml of nutrient methylcellulose (0.8%) medium containing the negative and positive controls and different concentrations (5%, 10%, 15%, 20%, 25%) of seaweed-extracts and incubated for 3 days at 37°C. The cells were fixed and stained, and then the number of plaques was counted. The anti-HSV activity of each test sample was determined as the ratio (%) of plaque formation compared with the control as follows:

$$\%\ \text{Plaque formation} = \frac{100 \times \text{Number of plaques (test sample)}}{\text{Number of plaques (control)}}$$

The cytotoxicity of seaweed extracts and their fractions was evaluated as the extent of uninfected cells that were omitted from the surface of stained dishes in the plaque reduction assay (visible cytotoxicity) and compared with that of a control. Results were recorded as follows: (-) no cell detachment, (±) thick coloration of cell layer (less than 10% cell detachment), (+) 10—50% cell detachment, (++) over 50% cell detachment and (+++) complete cell detachment. The cytotoxicity of compounds was determined as the inhibition of Vero cell growth[11].

RESULTS

The results for the effect of the extract on wild type strain of the virus are given in Table-1,2,3. It can be seen that the higher concentrations of the extract are as effective as or more effective than the positive control.

Table -1: Antiviral activity of *Sargassum illicifolium* aqueous extract on Wild Strain of Herpes Simplex Virus with different levels of infection

S. No.	Level of infection in Vero cells	Negative control	5%	10%	15%	Positive control	20%	25%
1	Low level of infection	-	±	+	+	++	+++	+++
2	Medium level of infection	-	±	±	+	++	++	+++
3	High level of infection	-	±	±	±	++	++	+++

In case of Thymidine Kinase Deficient virus the efficacy of the drug increases compared to the wild strain.

Table -2: Antiviral activity of *Sargassum illicifolium* aqueous extract on Thymidine Kinase Deficient Herpes Simplex Virus with different levels of infection

S. No.	Level of infection in Vero cells	Negative control	5%	10%	15%	Positive control	20%	25%
1	*Low level of infection*	-	±	+	+	++	+++	+++
2	*Medium level of infection*	-	±	+	+	++	+++	+++
3	*High level of infection*	-	±	+	+	++	++	+++

Finally in the drug resistant variety of the HSV the seaweed exract was seen to be more effective than the patented medicine, which is a sign of plant medicines being more effective than the pharmaceutical medicine.

Table -3: Antiviral activity of *Sargassum illicifolium* aqueous extract on acyclovir and phosphonoacetic acid resistant strain of Herpes Simplex Virus with different levels of infection

S. No.	Level of infection in Vero cells	Negative control	5%	10%	15%	Positive control	20%	25%
1	*Low level of infection*	-	-	±	±	++	++	+++
2	*Medium level of infection*	-	-	-	±	++	++	+++
3	*High level of infection*	-	-	-	-	++	++	+++

DISCUSSION

Approximately 80% of the adult population worldwide are infected with herpes simplex virus type 1 (HSV-1) and approximately 20% of them are also infected with HSV type 2 (HSV-2)[12]. Until now, a number of nucleoside analogues, especially the guanosine analogue acyclovir, have been developed as anti-herpetic agents. The therapeutic limitation of these nucleoside analogues is that drug resistant strains develop readily through mutations in viral genes for thymidine kinase and/or polymerase. Therefore, the continuous search for new compounds as antiviral agents is urgently needed[13].

Until now, chemically synthesized or modified compounds have been the major source of selective antiviral agents, particularly in the case of antiherpetic compounds. For combating drug resistant viral strain, it has been suggested that the new antiherpes drugs should be non-nucleosides. Thus, many efforts have been carried out to screen for antiviral agents from natural sources. Marine algae have shown their potential as important sources of antiviral as well as other bioactive compounds.

It has been reported that seaweed extracts can be active against various enveloped viruses, including DNA and RNA viruses such as human immunodeficiency virus (HIV), HSV, cytomegalovirus (CMV), RSV and influenza virus. In the present investigation, the best inhibitory effect on HSV was shown by the aqueous extract of *Sargassum illicifolium*. HSV is an enveloped DNA virus while RSV and influenza A are both enveloped RNA viruses. Influenza A contains segmented genome. Therefore, it seems that the extracts from this brown seaweed could exhibit higher antiviral effect on enveloped DNA virus than on RNA virus, and had no effect on RNA virus with segmented genome, e.g., the influenza virus. However it is 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. These findings suggest that there are possible differences in the virus-inhibitory effects of the seaweed extract. These differences may not only depend on the choice of cell lines used in the assay, but also on the seaweed species collected from different habitats, geographical areas.

Many investigators have reported the inhibitory effects of algal extracts and their constituents on the replication of herpes simplex viruses. Anderson *et al.*[14] obtained extracts from 25 marine organisms collected along the Swedish coast and found that the chloroform extract of *Fucus seratus* and the petroleum ether extract of *Laminaria digitata* inhibited plaque formation by HSV-1 at a concentration of 100m g/ml without toxicity. Algae extracts reportedly inhibit plaque formation by herpes virus by through blocking viral adsorption. It was also found that the water extract of *Polysiphonia denudata* reduced HSV-1 reproduction by inhibiting viral adsorption to cells as well as viral synthetic stages. The major constituents of this alga are bromophenols (such as 2,3-dibromo-4,5-dihydroxybenzylalcohol) that are known antioxidants and antimicrobial agents. However, this is the first report to demonstrate the anti-viral activities of this alga. It is shown here that the aqueous extract of *S. illicifolium* had anti-APr HSV-1, TK_ HSV-1 and wild type HSV-1 activities *in vitro* (Table 1-3). The bromophenolic compounds isolated from *S. illicifolium* had anti-viral activity against APr HSV-1 and TK_ HSV-1, as well as wild-type HSV-1 strains and were more effective against the two mutant strains than ACV (positive control). These results support the notion that the seaweed extract exert anti-viral activities through a different mechanism from that of ACV (Table 3).

As can be found in the literature, a number of polyanionic substances or water-soluble sulfated polysaccharides are potential antiviral agents. It is commonly suggested that these negatively charged molecules disturb the proliferation cycle of enveloped viruses at the early stage, therefore inhibiting the viral infection. However, Nakashima *et al.* (1987)[15] found that a sulfated seaweed polysaccharide selectively inhibited reverse transcriptase (RT) enzyme of human immunodeficiency virus (HIV) and its replication *in vitro*. Other studies showed that sulfated polysaccharides could prevent viral protein synthesis or were capable of blocking various steps during the life cycle of HSV. The pleiotropic modes of antiviral actions of sulfated polysaccharides make it less likely for the virus to develop resistant mutants. The carbohydrates contained in seaweed could be extracted by water, diluted acid and alkali. In the present experiment, different concentrations of *S. illicifolium* were tested. They all showed antiviral activities against HSV-1 and HSV-2 and the largest portion, higher concentrations of 20% and 25%, has the most potential for developing into antiviral agent. Some seaweed polysaccharides, e.g., dextran sulfate and heparin, have been shown to inhibit

the replication of various enveloped viruses, including HSV and HIV[16].

The modes of action of most of these polysaccharides have often been attributed to a blockade of the early stages of the virus replication cycle[17]. The polysaccharides isolated from *S. illicifolium* could have the same anti-HSV action as those shown by dextran sulfate, but more details of their antiviral mechanism are still being evaluated.

CONCLUSION

In conclusion, the *S. illicifolium* extract and its constituents (bromophenols) might provide a basis for developing new anti-HSV-1 agents that would be effective against ACV-resistant strains.

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