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Activity of *Turbinaria Ornata* (Turner) J. Agade Against Blue Tongue Virus (Btv)

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ABSTRACT:-A sensitive and accurate method was developed to test the efficacy of the aqueous extract of marine seaweed, Turbinaria ornata (Turner) J. Agardh against blue tongue virus using the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide(MTT) method. The abundance of this algae on the shores during the month of November and no reports so far ,having antiviral activity in addition to this other solvent extracts were toxic to the cell line hence water was selected as the solvent for extraction. On the other hand dextran sulphate was used a positive control and the bioactive compounds efficacy was compared with it. The optical density of formazan was used to determine cell viability. The IC_{50} values of dextran sulphate and the extracts were found to be nearly similar to those obtained by the plaque reduction method**.**

key words:- *mtt, seaweed, bioactive, aqueous extract, formazan,IC50value ,dextran sulphate*.

I. INTRODUCTION

Plants yield many biomedically useful substances. Apart from land plants, the oceans have also served as a source of a large group of structurally unique natural products of pharmacological significance. Chemical investigations of many benthic marine algae have illustrated that these algae produce a wide variety of structurally unique and biologically active secondary metabolites. There are large numbers of viral pathogens for which no effective chemotherapy currently exists [(1)]. Uniformly accepted standards for *in vitro* susceptibility testing are not available for antiviral drugs. Therapeutic drug level monitoring, commonly available to clinicians for antibacterial therapy, is not a part of routine care because of lack of standardization, uncertain relationship to clinical response, toxicity and cost [(2).] 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. 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 can not afford the cost of long-term treatment. Further, the increased availability and use of antiviral drugs, however, has led to the emergence of drug-resistant viruses, especially in immuno-compromised hosts [(3).] 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 *Turbinaria ornata* (Turner) J. Agardh of Indian coastline was studied for its activity and the aqueous extract was tested. This paper also describes the inhibitory activity of dextran sulphate a standard agent against the viruses mainly RNA.

II. MATERIALS AND METHOD

Turbinaria ornata (Turner) J. Agardh of Phaeophyceae, Phaeophyta, was selected as the experimental algae ,was collected from the rocky shores of of Kanyakumari district (latitude 80° N), Tamil Nadu . The collection was made in the month of November ,authenticated by Dr. R.Thevanathan ,Presidency college, Chennai.

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 four layers of cheese cloth and the filtrate was freeze dried. From the stock solution. test solutions having varying concentrations of the extract residue was used for antiviral studies. BHK cell lines (Baby hamster kidney cell line) were used for the culture obtained from TANUVAS (Tamilnadu University of Veterinary and Animal Sciences), Chennai and maintained in MEM containing 5% foetal calf serum, kept in a walk-in incubator at 37^{o}C. The estimated active antiviral concentrations were subjected to the MTT (3 - (4,5 dimethyl thiazol - 2yl) 2,5 - diphenyl tetrazolium bromide) assay.After estimating the minimal concentration having activity it was subjected to the dye, MTT (3 - (4,5 dimethyl thiazol - 2yl) 2,5 - diphenyl tetrazolium bromide) assay was performed to measure the cell viability and cytotoxicity [(4)] . The tetrazolium salt used in the assay is cleaved by mitochondrial dehydrogenase in viable cells yielding a purple product formazan. The production of formazan is proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. This test is performed to prove the viability of the extract treated cells after subjection to virus and also to find out the

active concentration of the extract as it will have greater number of viable cells.

The media was removed and 25 µL of MTT was added to the confluent monolayer of cells in the plate and incubated at 37°C for 4hours. Hundred microlitres of DMSO was then added and after 15 minutes, the optical densities were measured in an ELISA reader (Multiscan EX, Labsystems) at a test wavelength of 492 nm and a reference wavelength of 620 nm [5] to determine the cellular viability. Difference in the OD values was taken and analysed. The cells not protected by the extract were killed by the virus or did not proliferate, as they produced less formazan thus giving low OD value. Dextran sulphate (DS10000**,** Sigma), a synthetic sulphated polysaccharide was used as a positive control for all the assays .[6]

III. RESULTS AND DISCUSSION

Table 1 and Chart 1 shows the results observed against Blue tongue virus. Aqueous extract residue of *Turbinaria* protected 82% of cells .The concentrations of the algal preparations to be used in antiviral assays were kept within non-cytotoxic range for interference of the antiviral activity. Dextran sulphate, a sulfated polysaccharide was simultaneously used as a positive control in all the experiments because of its known broad spectrum antiviral properties. The aqueous extract exhibited very good activity against BTV as indicated by their very low IC_{50} values 100 µg/mL Nevertheless, the two experimental algae appeared to be effective against blue tongue virus since the IC_{50} values are comparable to that reported by [7].

Activity of this marine seaweed against Blue tongue virus is the first report earlier reports of this alga against NDV-New Castle Disease virus was reported[8]

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TABLES

Table 1Viability of cells on adding aqueous extract of *Turbinaria ornata* [MTT Assay ($OD_{492} – OD_{620}$)] against BTV

ALGAL PREPARATION	OD ($OD_{492} – OD_{620}$) OF WELLS SHOWING CPE			F value	P value
	CC	VC	TC		
***Turbinaria ornata* Aqueous (100 µg/mL)**	**$0.154±0.023^b$**	**$0.147±0.011^b$**	**$0.079±0.023^b$**	**13.71**	**0.0002**

CC – cell controlVC – virus control TC – test compound

The data were subjected to one way ANOVA test followed by Tukey-HSD multiple comparison test. Different alphabets 'a' and 'b' between the cells denote significance at 5% level.

Table 2 Viability of cells on adding dextran sulphate [MTT Assay ($OD_{492} – OD_{620}$)] against BT

VIRUS	OD ($OD_{492} – OD_{620}$) OF WELLS SHOWING CPE			F value	P value
	CC	VC	TC		
BTV	$0.122±0.022^c$	$0.022±0.005^a$	$0.112±0.007^b$	110.108	0.0000

CC – cell controlVC – virus control TC-dextran sulphate

The data were subjected to one way ANOVA test followed by Tukey-HSD multiple comparison test. Different alphabets 'a, 'b' and 'c' between the cells denote significance at 5% level

Chart 1:Viability of the Cells [MTT Assay (OD492 - OD620)] Against BTV in the Presence of Algal extract of Turbinaria

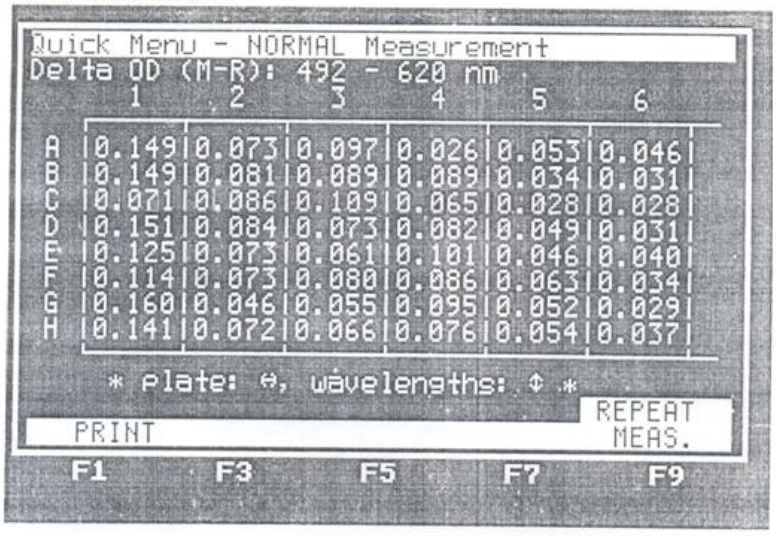

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Chart 2 Viability Of The Cells[MTT Assay(OD_{492}–OD_{620})]Against BTV in the Presence of Dextran Sulphate