Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

A study on anti-proliferative property of some green algae on human cervical cancer cells (SiHa) *in vitro*

Indira Majumder, Sayani Chatterjee and Rita Kundu*

Department of Botany, Centre of Advanced Study, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700019.

Phone no: +9133-2461-5445; Fax no: +9133-2461-4849 Email: kundu_rita@yahoo.co.in

---

Indira Majumder, Sayani Chatterjee and Rita Kundu. 2015. A study on anti-proliferative property of some green algae on human cervical cancer cells (SiHa) *in vitro*. *J. Algal Biomass Utln.* **6(**4): 21-25

Keywords: *Chaetomorpha brachygona*, cytotoxicity, MTT assay, SiHa, Sundarban mangrove ecosystem, T293.

Abstract:

Marine algae are found to contain high amount of nutrients, vitamins (A,B,C,D &E), minerals (Ca, P,Na &K), antioxidants and dietary fibers, that's why they are used as food, fodder and other commercial purposes throughout the world. They are also very rich in novel compounds and can be explored for the development of drugs to combat deadly diseases like cancer , diabetes etc . Recently, several reports were published regarding the cytotoxic and anti-proliferative activities of marine algae . Sundarban mangrove ecosystem (SME) is rich in such algal flora . In the present study, antiproliferative property of four green(Chlorophycean) macro algal species, collected from SME, were evaluated on human cervical cancer cell line, SiHa. SiHa cells responded differentially to different algal extracts . Among the four,chloroform fraction of *Chaetomorpha brachygona* showed the best result, inhibiting the cell growth at a very low concentration (IC_{50} dose: 23.6 µg/ml) in SiHa cells, whereas zero percent cell death was observed in normal human embryonic kidney cells (T293). This indicates selective cytotoxic nature of this extract. We are hopeful that in future, the algae can be utilized in drug development for cancer treatment.

Introduction:

From the ancient times, algae from marine origin were used commercially as food, fodder, dietary supplements and medicine, as they are rich sources of vitamine, minerals, lipids and proteins. Algal phytochemicals exhibit immense structural diversity and heterogenicity (Konig et al, 1994). These diverse groups of chemicals are mainly responsible for protecting the marine algae from many predating animals and adverse marine environment (Hay et al, 1988). Macroalgae were reported to have anti leshmanial (Sabina et al, 2005; Fauladvand et al, 2011), anti-diabetic (Unnikrishnan et al, 2015; Nwosu et al. 2011, Senthilkumar et al, 2013, Hardoko et al, 2014),and anti bacterial (Lima-Filho et al, 2002; Al-Saif et al, 2014) activities.Recent studies are being carried out to evaluate the cytotoxic and anti-proliferative potentiality of macro algal extracts (Mayer et al, 2007; Moo-Puc et al, 2009 and Kwon et al, 2007)). According to NCI, USA, many drugs obtained and purified from macro algal origin were used as potential complementary and alternative medicine to cure cancer. Many of these drugs had undergone clinical tests on various animal systems. Sundarban mangrove ecosystem (SME) of Indian peninsula comprises large number of algal biomass and that huge unused biomass of algae can be explored for anti-cancer drug development.

In this present study, algae collected from SME, India; were tested against SiHa, cervical cancer (adherent) cell line.This pioneer study may lead to open up a new pathway to develop drug against cervical cancer, the deadliest female cancer in Indian population. Further studies are needed to isolate the bioactive compounds present in the extracts.

Materials and method:

Collection and extraction of sample:

Four algae were collected from SME, all of them, are Chlorophycean (green algae). *Rhizoclonium tortuosum* was collected from Sandeshkhali of Sundarban north (22°21·360´ north & 88°52·816´ east). This alga is epiphytic in nature and with distinct characters in its filaments. *Chaetomorpha brachygona* was collected from Sandeshkhali (22°21·471´north & 88°52·792´ east). Another Chlorophycean species of *Chaetomorpha linum* was collected from Jhorkhali fishing area 22º04.97067´ north and 88º66.874299999995´ east) and *Cladophora glomerata* , the green branched alga was collected from Sandeshkhali region (22°21·471´north & 88°52·792´ east).

For identification, the collected algal specimens were preserved in FAA (formaldehyde: acetic acid: alcohol) solution and are maintained with proper accession numbers at CUH.

For the experiment, algal samples were thoroughly washed with water and air dried. After drying the algae were cut into small pieces and extracted with about five times methanol at dark for two nights. The methanolic extract was concentrated about ten times with rotary evaporator (Eyela). The final fractions were made with different solvent systems viz; petroleum-ether, chloroform and methanol-water according to polarity. These fractions were lyophilized and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 µg/µl.

Cervical cancer cell lines SiHa (HPV 16 positive) and T293 cell line (human embryonic kidney) were collected from NCCS, Pune; maintained in Eagle's minimum essential media or MEM (for SiHa) and Dulbecco's Modified Eagle Medium or DMEM (for T293); supplemented with 10% FBS and NEAA at 37°C in a humidified incubator having 5% CO_2.

MTT assay:

SiHa cells (10^4) were seeded on a 96 well plate and incubated for overnight. Cells were treated with different concentrations of algal extracts for 24 hours. Cell viability assay was carried out to evaluate anti-cancerous activity of these algal extracts. Concentrations causing 50% cell death are termed as IC50 doses. In a similar way, T293 cells were seeded in a 96 well plate , grown for overnight, treated with IC 50 doses of the algal extracts and incubated for 24 hours. Positively charged MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) can penetrate viable cells very easily. MTT is reduced by live cells, after getting inside into cell. NADH and NADPH is produced by cell's dehydrogenase enzymes and purple formazan is produced. Whereas the dead cells are unable to reduce the MTT intracellularly. The formazans are solubilised with DMSO and quantified by spectrophotometric means at 570 nm. After treatment viable cell number can be measured by this assay. Inhibition percentage was determined by the following formula:

Percentage of Inhibition was calculated with the formula:

% of inhibition =(Absorbance of control set – Absorbance of treated set / Absorbance of control set) x 100.

Percentage of Viability was calculated with the formula:

% of Viability = 100 - % of inhibition.

Results:

SiHa cells responded differentially to the tested algal fractions. It was observed from the experiment (Fig.1) that the chloroform fractions (CF) were able to inhibit the cell growth at the lower doses, while the petroleum ether (PEF) and methanol-water fractions (MWEF)did not show any significant cell death at the lower doses. Petroleum ether fraction of *Cladophora glomerata* could not induce any cell death , though the methanol-water fraction were able to induce 37% cell death at the highest concentration used in this experiment (900µg/ml). The chloroform fraction from all the algal samples showed higher cell death inducing capability than other fractions. Amongst all the collected algal samples, two species of *Chaetomorpha,* were found to be the most effective, with IC50 doses of 23.6 µg/ml (*C. brachygona*) and 247.3 µg/ml (*C. linum*) respectively and seems to be very promising for new anticancer drug development. According to the US National Cancer Institute, an active extract should have an IC50 dose at ≤ 30 µg/mL (Suffiness et al,1990). In this respect , *C. brachygona* can be considered to show strong cytotoxicity against SiHa cell line, while *C. linum , Cladophora glomerata, R. tortuosum* can be considered to have weak cytotoxicity against human papilloma virus (HPV16) harbouring SiHa cell line. According to their activity the algae can be arranged in an order, as

C. brachygona > *C. linum* > *Cladophora glomerata* > *R. tortuosum* . To evaluate the cytotoxicity of the chloroform extracts on normal cells, T293 cells were treated with the IC50 doses of the extracts. T293 cells treated with the IC_{50} doses of $CHCl_3$ fractions of *Chaetomorpha brachygona* did not show any cell death indicated selective toxicity over the cancer cells (Fig.2). Cells treated with *Chaetomorpha linum* showed 36.34% cell death . Chloroform extract of *Cladophora glomerata* was found to be the least effective as it induced 88.17% cell death in T293 cell line.

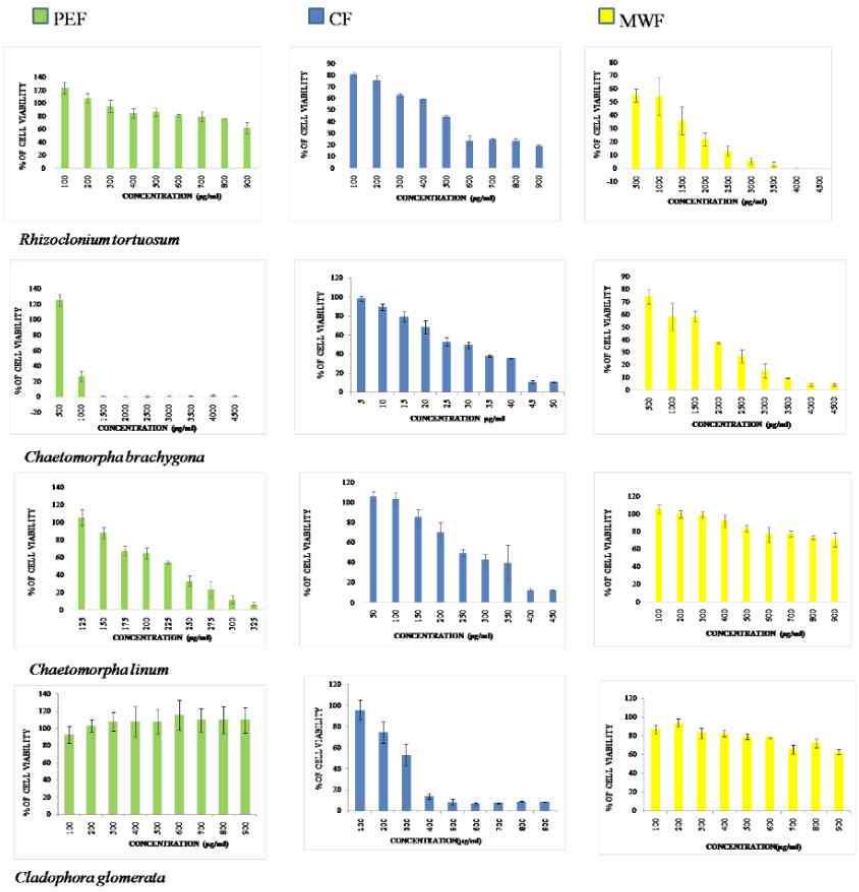

Figure 1. Bar graph showing comparative cell viability percentage of SiHa cells treated with different fractions of green algal extracts namely *Rhizoclonium tortuosum, Chaetomorpha brachygona, C. linum & Cladophora glomerata* as observed by MTT assay. Columns with bars represent average of triplicate ±SD value [PEF- Petrolium Ether fraction, CF-Chloroform fraction, MWF- Methanol water fraction].

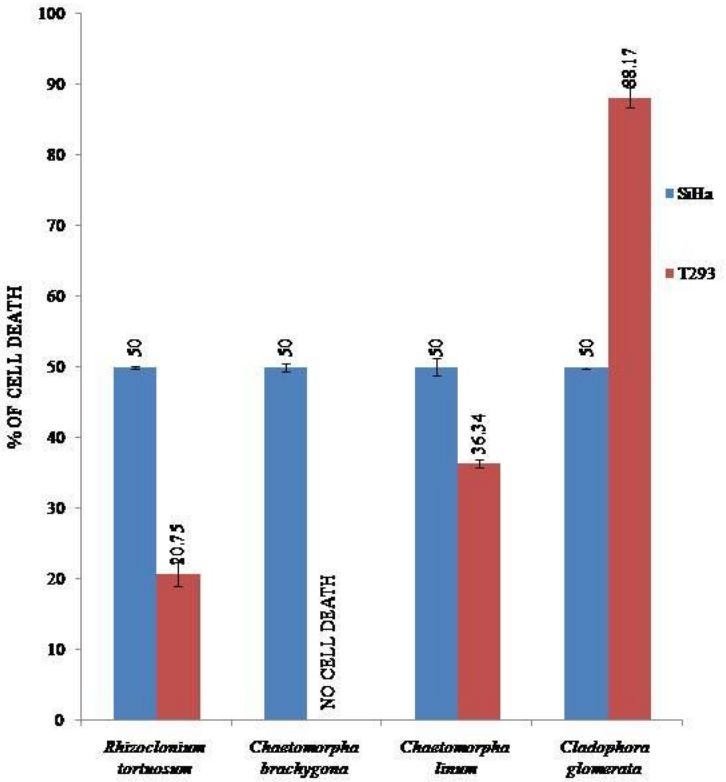

Figure. 2. Bar graph showing comparison of cell viability of SiHa and T293 cells treated with IC_{50} doses (SiHa) of CF of algal extracts. Columns with bars represent average of triplicate ±SD value [CF- Chloroform fraction].

Discussion:

Recent studies regarding cytotoxic efficiencies of various macroalgae and their anti proliferative potential on various cancer cell lines and animal model systems were reported. But this is the first report of these three green algal genera showing cytotoxic effect on human cervical cancer (HPV 16 +ve SiHa) cell line .Salem and his co-worker have (Salem et al, 2011) reported anticancer activity of *Ulva rigida* extracts on Ehrilch Ascites Carcinoma (EAC) cell line. Recently it was reported that *Ulva lactuca* and *Enteromorpha intestinalis* have shown cytotoxicity at a very very low IC_{50} doses against human lung carcinoma A549 cells, human colon carcinoma LS174 cells, chronic myelogeneous leukaemia K562 cells and malignant melanoma Fem-x cells (Kosanic et al, 2015). *Enteromorpha intestinalis* and *Rhizoclonium riparium* were reported to have selective cytotoxic activity tested against human cervical cancer cell line HeLa (Paul et al, 2013). Another green macroalgae *Halimeda macrobola* have shown anti cancerous activity with a low IC_{50} value of 53.80 µg/mL(tested by BSLT method) (Ahmed et al, 2014). One group have reported that two green macroalgae *Udotea conglutinata* and *Udotea flabellum* induced cytotoxicity with very low doses on HeLa and KB cell lines compared with other algal genera (Moo Puc et al, 2009).

From the study it was observed that Sundarban Mangrove Ecosystem, a rich source of biodiversity, has several macroalgae growing luxuriously in the intertidal regions of riverbanks, inlets and fish-ponds. Members of Chlorophyceae and Rhodophyceae were found to grow mostly as macroalgal clusters. Chloroform fraction of *Chaetomorpha brachygona* was found to be most effective and seems to be very promising than any other algae reported, as it shows selective cytotoxicity against the cancer cells. Further study will be conducted to study the death mechanism involved and isolation and identification of active phytochemicals present in the extract.

Acknowledgement:

The authors wish to thank WB-DST (sanc. No- 778/(sanc.)/ ST/P/S&T/9G-14/2012) for the financial assistance. We would also like to thank Department of Botany, Centre of Advanced Study, University of Calcutta for supporting the research.

References:

Al-Saif S. S. A. Abdel-Raouf N. El-WazananiH. A. Aref I. A. 2014 Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. *Saudi J Bio. Sci* **21**:57–64.

Fouladvand M. Barazesh A. Farokhzad F. Malekizadeh H. Sartavi K. 2011 Evaluation of *in vitro* anti-Leishmanial activity of some brown, green and red algae from the Persian Gulf. *Eur. Rev. Med Pharmaco*l Sci **15**: 597-600.

Hardoko, Siratantri T. Eveline Yogabuana M. Olivia S. 2014 An *In Vitro* Study of Antidiabetic Activity of *Sargassum Duplicatum* and *Turbinaria Decurens* Seaweed. *Int Jour Pharma Sci Inven***. 3(2):** 13-18.

Hay M. E. Duffy J. E. Fenical W. Gustafson K. 1988 Chemical defense in the seaweed *Dictyopteris delicatula:* differential effects against reef fishes and amphipods. *Mar Ecol Prog Ser* **48**: 185-192.

Konig G.M. Wright A.D. Sticher O. Anghofer C.K. Pezutto J.M. 1994 Biological activities of selected marine natural products. *Planta Med*. **60**:532–537.

Kosanić M. Ranković B. Stanojković T. 2015 Biological activities of two macroalgae from Adriatic coast of Montenegro. *Saud J. f Biol Sci* **22(4):**390-397. doi:10.1016/j.sjbs.2014.11.004.

Kwon M.J. & Nam T.J. 2007 A polysaccharide of the marine alga *Capsosiphon f ulvescens* induces apoptosis in AGS gastric cancer cells via an IGF-IR-mediated PI3K/Akt pathway. *Cell Biol Int.* **31(8)**:768-75 DOI: 10.1016/j.cellbio..01.010.

Lima-Filho J. V. M. Carvalho A. F.F.U. Freitas S. M. Melo V. M. M. 2002 Antibacterial Activity of Extracts of Six Macroalgae from the Northeastern Brazilian Coast. Braz J Microbiol. **33**:311-313.

Mayer A.M.S. & Lehmann V.K.B. 2001. Marine Pharmacology in 1999: Antitumor and Cytotoxic Compounds., *Anticancer Res.* **21**: 2489-2500.

Moo-Puc R. Robledo D. Freile-Pelegrín Y. 2009 *In vitro* cytotoxic and antiproliferative activities of marine macroalgae from Yucatán, Mexico. *Cienc Mar.* **35(4**): 345–358.

Nwosu F. Morris J. Lund V. A. Stewart D. Ross H.A. McDougall G.J. 2011 Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chem.* **126 (2011):**1006–1012.

Paul S. & Kundu R. 2013 Antiproliferative activity of methanolic extracts from two green algae, *Enteromorpha intestinalis* and *Rizoclonium riparium* on HeLa cells. *DARU J Pharm Sci.* **21**:72. doi:10.1186/2008-2231-21-72.

Sabina H. & Aliya R. 2011 Bioactive assessment of selected marine red algae against *Leishmania major* and chemical constituents of *Osmundea pinnatifida. Pak J Bot*. **43(6**): 3053-3056.

Salem, T. A. & Ibrahim A. M. 2011. Anticancer activity of Egyptian marine alga *Ulva rigida*. *Int J Health Sci.* ***5*(2 Suppl 1**):6–8.

Senthilkumar P. Sudha S. Prakash S. 2014 Antidiabetic activity of aqueous extract of *Padina boergesenii* in Streptozotocin-induced diabetic rats. *Int J Pharm Pharm Sci*. **6(5)**: 418-422

Suffness M. & Pezzuto J.M. 1990 Assays related to cancer drug discovery. In: HOSTETTMANN K (Ed), Methods in plant biochemistry: assays for bioactivity. London: Academic Press. **71**–133.

Unnikrishnan P.S. Suthindhiran K. Jayasri M.A. 2015 Antidiabetic potential of marine algae by inhibiting key metabolic enzymes. *Front Life Sci.* 8(2) : 148-159