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Evaluation of Cytotoxic Activity of *Sargassum* and *Iyengaria sp* on Lung Cancer Cell Line through MTT Assay Test

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Abstract:

***Background**: Bioactive ingredients isolated from algal species are rapidly gaining appreciation and there is an overwhelmingly increasing trend of evaluation of methanolic extracts and bioactive ingredients on different cancer cell lines. **Materials and Methods**: Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, H460 (Lung cancer) cells were used to evaluate anticancer activity of algal extract. **Results**: We were unable to detect significant cytotoxic effects exerted by methanolic extracts of Sargassum and Iyengaria sp on Lung cancer cell line.*

Key words: *Iyengaria*, *Sargassum*, H460, Lung cancer, IME, SME, MTT assay.

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Introduction

It has previously been convincingly revealed that Lophocladines, naphthyridine alkaloids, isolated from the marine red alga *Lophocladia sp* exerted inhibitory effects on NCI-H460 lung cancer cells via flattening of shape and appearance of short neuron-like projections[1]. More interestingly, another subsequent study highlighted role of chamigrane-type sesquiterpenoid, dactylone in regulation of NCI-H460 lung cancer cells. Mechanistically it was shown that dactylone induced apoptosis in cancer cells through a p53 independent mechanism[2]. There is a direct piece of evidence suggesting cytotoxic activity of algal methanolic extracts of *Enteromorpha intestinalis* and *Rizoclonium riparium* in HeLa cancer cells[3]. Recently emerging evidence has started to highlight the fact that fucoxanthin exerted its inhibitory effects on highly metastatic B16-F10 melanoma cells via repression of MMP-9. There are some other proteins reported to be inhibited in fucoxanthin treated B16-F10 melanoma cells including CD44 and CXCR4[4]. There is a rapidly accumulating evidence of role of laminaran isolated from brown alga *Eisenia bicyclis* in suppressing colony formation of human melanoma SK-MEL-28 and colon cancer DLD-1 cells [5].

Cytotoxicity assay Protocol

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, H460 (Lung cancer)cells were cultured in RPMI media, supplemented with 10% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37^oC. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell

culture with the concentration of $4x10^4$cells/ml was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-100ug/ml). After 48 hrs, 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for H460 cells[6]. **The Doxorubicin was used as standaed and showed 0.0404±0.005 IC_{50}±SD value.** The percent inhibition was calculated by using the following formula: % inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100).

Results

	-ve control	Algal concen µg/ml	O.D			average	S.D	% Inhibition			+ve control
	0.066	**100ug/ml**	1.037	0.932	0.988	0.985667	0.052539	36.97161	34.39748	30.15773	1.368
	0.066	**50ug/ml**	1.312	1.133	0.898	1.114333	0.20763	27.5836	26.44795	19.17981	1.315
	0.063	**25ug/ml**	1.374	1.192	1.022	1.196	0.176034	14.71293	9.564669	6.006309	1.514
	0.067	**12.5ug/ml**	1.802	1.286	1.26	1.449333	0.305695	7.596215	5.62776	5.62776	1.41
average	0.0655										1.148
S.D	0.001732										1.563
										average	**1.386333**
										S.D	**0.148524**

MTT assay results for SME *Sargassum* methanolic extract.

	-ve control	Algal concen µg/ml	O.D			average	S.D	% Inhibition			+ve control
	0.066	**100ug/ml**	1.026	1.097	1.104	1.075667	0.043155	27.28076	21.90536	21.37539	1.368
	0.066	**50ug/ml**	1.255	1.163	1.226	1.214667	0.047035	9.943218	16.90852	12.1388	1.315
	0.063	**25ug/ml**	1.305	1.224	1.27	1.266333	0.040624	6.157729	12.29022	8.807571	1.514
	0.067	**12.5ug/ml**	1.324	1.335	1.374	1.344333	0.026274	4.719243	3.886435	0.933754	1.41
average	0.0655										1.148
S.D	0.001732										1.563
										average	**1.386333**
										S.D	**0.148524**

MTT assay results for IME *Iyengaria* methanolic extract.

Discussion

It is interesting to note that different bioactive ingredients isolated from red alga *Laurencia filiformis* considerably re-sensitized resistant cancer cells to chemotherapeutic drugs[7]. Confluence of information verified the fact that sulfated polysaccharides isolated from *Sargassum henslouianum* demonstrated considerably enhanced antitumor activity as evidenced by growth suppression of MKN45 gastric cancer cells[8]. We were unable to detect significant cytotoxic effects exerted by methanolic extracts of *Sargassum* and *Iyengaria sp* on Lung cancer cell line.

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