ANTI-CANCER POTENTIAL OF THE SELECTED RED SEAWEEDS COLLECTED FROM THE SOUTH EAST COAST OF TAMIL NADU, INDIA ON A549 CELL LINE.

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Abstract: The aim of the present investigation was to evaluate the anti-cancer potential of red seaweeds namely *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew, *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville collected from Hare Island (Thoothukudi district), Koothankuzhi (Tirunelveli district) and Kanyakumari (Kanyakumari district) respectively in the south east coast of Tamil Nadu, India for the inhibitory effect against A-549 cell line. The viability percentage of the cell lines were carried out by using Trypan blue dye exclusion method and the cytotoxicity. The methanolic extract of the selected seaweeds has significant cytotoxicity effect on A-549 cell line. Among the three seaweeds studied, *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew showed more effect, followed by *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville in the concentration range between 25 to 200µg/ml of methanolic extract by using MTT assay. From the performed assay, it was noted that methanolic extract of the selected red seaweeds show greater activity on A-549 cell line which can be used as anti-cancer activity.

Keywords: Anti-cancer, A-549 cell line, Methanolic extract, Red seaweeds, Tamil Nadu, India

I. INTRODUCTION

In the past few decades, cancer is the leading cause of death worldwide and it is characterized by uncontrolled growth and spread of abnormal cells. World Health Organization (WHO) reported that there are 7.6 million deaths in the recent years and it is estimated up to 13.1 million deaths in 2030 (Merel *et al.*, 2012). Treatment of cancer varies according to each type, has been facing a serious problems. Many ways in the treatment of cancer have been developed. At present cancer is treated using surgery, radiation and chemotherapy which are associated with various side effects (Garcia *et al.*, 2001; Edy *et al.*, 2012). Even a large number of tumors are insufficiently responsive to cancer therapeutic drugs and radiotherapy. Identification and development of natural products used for cancer prevention have attracted a lot of attention at global level. Herbal extracts with their confirmed potential and less side effects in therapeutics has replaced the synthetically derived drugs in modern allopathic medication system (Sakthivel and Guruvayoorappan, 2012). Traditionally used large number of medicinal plants and plant products has become the possible source of antitumor agents. Traditional healers of different regions in India used a number of medicinal plants for treatment of various cancer types of mouth, bone and skin (Kalaivani and Mathew, 2010). Therefore based on this background, the present study was planned to explore the possible *in vitro* anti-cancer activity of methanolic extracts of the selected red seaweeds namely *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew, *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville collected in the south east coast of Tamil Nadu, India.

II. MATERIALS AND METHODS

2.1. Collection of Materials

The collection of *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew, *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville was made during the low tidal and subtidal regions (up to 1m depth) by hand picking from Hare Island (Thoothukudi district), Koothankuzhi (Tirunelveli district) and Kanyakumari (Kanyakumari district) respectively in the south east coast of Tamil Nadu, India. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution (John Peter Paul, 2010).

2.2. Preparation of extracts

For the preparation of methanolic extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8h separately (Iniya Udhaya and John Peter Paul, 2017).

2.3. Cell lines and culture conditions

A-549 (Human lung cancer) cell line was procured from the National Centre for Cell Science (NCCS), Pune. The cell line were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal Bovine Serum (FBS) and streptomycin and penicillin (100µg/ml and 100IU/ml respectively). Cells were cultured in a 5% CO_2 humidified atmosphere at 37˚C until confluence. All the processes were carried out in a vertical laminar air flow chamber (John Peter Paul, 2014).

2.4. *In vitro* cell viability

2.4.1. Trypan Blue Exclusion Assay

The percentage of viable and non viable cells was determined using trypan blue exclusivity stain. Cell growth and viability was measured by adding 0.4% trypan blue in 0.9% saline to a 50% dilution and cells were counted using haemocytometer. Cells were examined and counted in duplicates under light binocular microscope 100× (Olympus, Japan). Percentage cell viability was calculated by the formula,

$$\text{Cell viability} = \frac{\text{No. of viable cells}}{\text{Total No. of cells}} \times 100$$

2.5. Cytotoxicity assay

2.5.1. MTT assay (Patel *et al.*, 2009; Raval *et al.*, 2010)

MTT Colorimetric assay is based on the capacity of Mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-Dimethyl Thiazol-2-yl)-2,5-diphenyl Tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. MTT assay was employed to assess cell proliferation. Viable cells were seeded into 96-well microtitre plates at 2×104 cells/well in DME medium supplemented with FBS (Fetal Bovine Serum), 100units/ml penicillin, 100µg/ml streptomycin, and were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. 150µl of cell suspension was cultured with 10µl of various concentrations i.e. 25, 50, 100 and 200µg/ml of the methanolic extract dissolved in DMSO (Dimethyl sulphoxide) as solvent and incubated for 48h. Similar solutions containing the same concentrations of cyclophopamide were also be prepared and served as standard solutions. Control cells were incubated in DME medium only. Wells containing only media were considered as a blank. All cyclophosphamide and methanolic extract dilution doses were tested in triplicates. The cell proliferation is based on the ability of the mitochondrial succinate terazolium reductase system to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) to a blue colored formazan. The test denotes the survival cells after toxic exposure. 10µl of MTT labeling mixture was added and incubated for 4h at 37°C and 6.5% CO_2. After 4h, 100µl of solubilization solution was added in each well. After 48h incubation at 37°C temperature and 5% CO_2, the absorbance of soluble formazan product produced by viable cells was measured at 450nm using ELISA plate reader. Reference wavelength used was 630 nm. Percentage inhibition of the cell proliferation by cyclophosphamide and methanolic extract against all cell lines was calculated using the following formula,

$$\% \text{ Cell survival} = \frac{(At - Ab)}{(Ac - Ab)} \times 100$$

where, At = Absorbance of Test, Ab= Absorbance of Blank (Media), Ac= Absorbance of control (cells)

III. RESULTS AND DISCUSSION

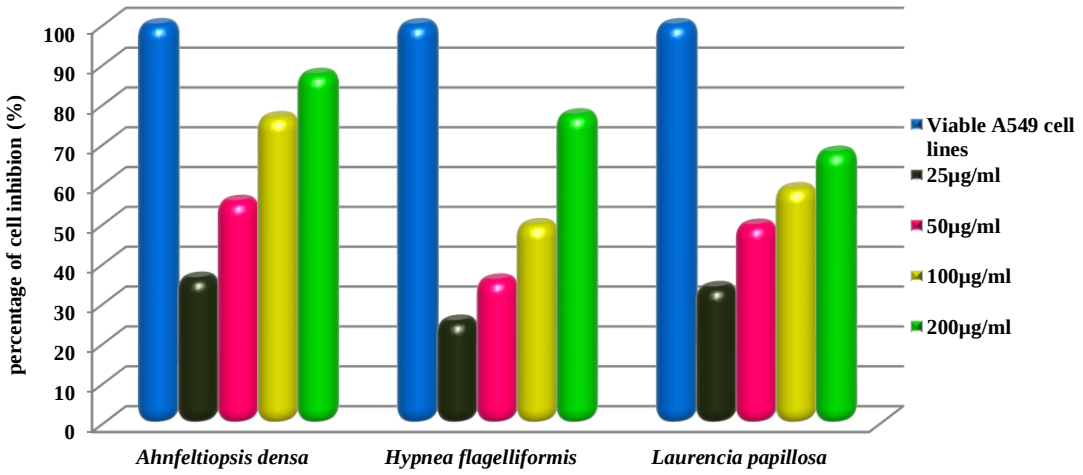
3.1. Viability and characterization of cell lines

Cell line derived from NCCS, Pune were free from any kind of bacterial and fungal contamination. Percentage cell viability of cell lines was carried out by using trypan blue dye exclusion technique. Treatment of different concentrations such as 25µg, 50µg, 100µg and 200µg extracted from the selected seaweeds in the south east coast of Tamil Nadu, India against A-549 showed decrease in percent cell viability, as compared to that of negative control i.e. DME media containing cells. Percentage cell viability of all these cell lines was decreased with increase in concentration of methanolic extract from 25 to 200µg/ml. In overall variation of test samples of methanolic extract from the selected seaweeds showed the best activity in the concentration of 200µg/ml.

Table-1: Anti-cancer activity of the selected red seaweeds on A549 cell line

Methanol extract (µg/ml)	Percentage (%) of inhibition of A549 cell line		
	Ahnfeltiopsis densa	*Hypnea flagelliformis*	*Laurencia papillosa*
Control	-	-	-
25	36.33	25.56	33.98
50	55.63	35.87	49.64
100	76.67	49.79	58.83
200	87.52	77.36	67.98

Figure-1: Anti-cancer activity of the selected red seaweeds on A549ell line



3.2. Cytotoxicity assay

The effect of methanolic extract of the selected red seaweeds on the growth of A-549 cell lines was examined by MTT assay. Percent cell viability and inhibition was also observed for 24 and 48 hrs in the methanol extracts of chosen seaweeds at varying concentrations (25, 50, 100 and 200µg). The control cells were 100% viable. In the case of methanol extract, the viability decreased significantly with increase in concentration. 25µg methanol extract of *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew showed 36.33%, followed by 50µg at 55.63%, 100µg at 76.67% and 200µg at 87.52% (Plate-7). Similarly, as presented in Plate-8, the methanol extracts of *Hypnea flagelliformis* Greville ex J.Ag. showed the inhibitory effects at 25µg (25.56%), 50µg (35.87%), 100µg (49.79%) and 200µg (77.36%). Followed by *Laurencia papillosa* (Forssakal) Greville (Plate-9) was found to inhibit the cell viability at 25µg (33.98%), 50µg (49.64%), 100µg (58.83%) and 200µg (67.98%). The percent of decrease in the cell viability is being indirectly proportional to the concentration of the extracts. The data altogether indicated that methanol extract of the selected seaweeds showed higher activity leading to decrease in percent cell viability of A549 cell lines at 200µg ((Table-1 and Figure-1).

The search for anticancer agents from natural sources has been successful worldwide. Active constituents have been isolated and nowadays are used to treat human tumors (Jing *et al.,* 2007). The ethnopharmacological knowledge is helpful to lead the search for plants with potential cytotoxic activity (Thi-Shan *et al.,* 2008). Melanoma is the most lethal and aggressive form of skin cancer. Available treatment for metastatic melanoma is still poor in overall response and survival. New treatment options as single or in combinations with the use of natural phytocompounds or herbal medicines may provide breakthrough results in the treatment of malignant melanoma. In this context methanolic extract from the selected red seaweeds especially *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew, *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville collected in the south east coast of Tamil Nadu, India may be used as a drug to inhibit the tumor cells. Therefore, it is concluded from cell viability and MTT assay that methanolic extract exhibited very good source for anti-cancer activity.

IV. CONCLUSION

The present work confirms that methanolic extract of the selected red seaweeds namely *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew, *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville collected in the south east coast of Tamil Nadu, India. Methanolic extract of the red seaweeds presented potent inhibitive effects on A-549. Among the three seaweeds studied, *Ahnfeltiopsis densa* (J.Ag.) Silva et Decew showed more effect, followed by *Hypnea flagelliformis* Greville ex J.Ag. and *Laurencia papillosa* (Forssakal) Greville in the

concentration range between 25 to 200µg/ml of methanolic extract by using MTT assay. From the performed assay, it was concluded that methanolic extract of the selected red seaweeds show greater activity on A-549 cell line which can be used as anti-cancer activity.

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VI. REFERENCES

[1] Merel, K., Stephen, J., David, K. and Helen, M. 2012. Socioeconomic Impact of Cancer in Member Countries of the Association of Southeast Asian Nations (ASEAN): the action Study Protocol. Asian Pac J Cancer Prev., 13**:**421-425.

[2] Garcia, F., Reyes, F. and Sanchez, P. 2002. The marine pharmacy, New antitumoral compounds from the sea. Pharmaceutical News, 9:495-501.

[3] Edy, M., Adam, H. and Anindyajati, N. 2012. Natural products for cancer targeted therapy: Citrus flavonoids as potent chemopreventive agents. Asian Pac J Cancer Prev., 13:427-36.

[4] Sakthivel, K.M. and Guruvayoorppan, C. 2012. Biophytum sensitivum: ancient medicine and modern targets. J Adv Pharm Technol Res, 3:83-91.

[5] Kalaivani, T. and Mathew, L. 2010. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. ex Delile, an Indian medicinal tree. Food Chem Toxicol., 48:298-305.

[6] John Peter Paul, J. 2012. Phytochemical studies on Turbinaria ornata (Turner) J.AG. American Journal of PharmTech Research, 2(6):1-8.

[7] Iniya Udhaya, C. and John Peter Paul, J. (2017). Screening of anti-oxidant activity of methanolic extract of Gracilaria fergusonii J.Ag. (Red seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. Indo American Journal of Pharmaceutical Sciences, 4(9):2724-2727.

[8] John Peter Paul, J. 2014. Anti-cancer potential of fucoidan extracted from *Padina distromatica* Hauck (Brown seaweed) from Hare Island, Thoothukudi, Tamil Nadu, India. Asian Journal of Pharmaceutical Science and Technology, 4(4):217-221.

[9] Patel, S., Gheewala, N., Suthar, A. and Shah, A. 2009. *In vitro* Cytotoxicity activity of *Solanum nigrum e*xtract against HELA cell line and VERO cell line. International Journal of Pharmacy and Pharmaceutical science, 1(1):38-46.

[10] Raval, B.P., Shah, T.G., Patel, J.D., Patel, B.A. and Patel, R.K. 2010. Potent anticancer activity of *Nigella Sativa* seeds. Scholars Research Library, 2(1):52-56.

[11] Jing. S., Bao, R.L., Wen, J.H., Li, Y. and Xiao, P.Q. 2007. *In vitro* anticancer activity of aqueous extracts and ethanol extracts of fifteen traditional Chinese medicine on human digestive tumor cell line. Phytother Res., 21:1102.

[12] Thi-Shan, D., Fu-Sheng, J., Ni-Pi, C., Gui-Yuan, L. and Cheng, G. 2008. Isolation and identification of an antitumor promoter component from leaves of *Impatiens balsamia*. Molecules, 113:220.