Abstract

The marine environment is known to be rich sources of biologically active compounds due to their tremendous biodiversity. A quantitative analysis of marine organisms demonstrated that more than 20,000 compounds have been isolated from marine microorganisms, micro/macro algae and invertebrates. Among marine seaweeds, the members of Phaeophyceae (marine brown algae) have been one of the richest and most promising sources of bioactive primary and secondary metabolites, with a broad range of physiological and biochemical characteristics. Several species of macro algae growing abundantly in the shallow waters of Gulf of Mannar Marine Biosphere Reserve (GoMBR; 8^0 49'–9^0 15' N latitude and 78^0 11'–79^0 30' E Longitude), which is one of the world's richest regions from marine bio diversity perspective and the first marine Biosphere Reserve in South east Asia.

In the present thesis abundantly available fifteen species of seaweeds were collected and washed thoroughly with fresh water and cleaned carefully to remove the extraneous matter. They were shade dried, powdered and extracted with a mixture of dichloro methane and methanol (1:1 ratio). All the crude extracts were evaluated against gram positive and negative bacteria, brine shrimp, and anti-proliferative activities on different cell lines. Based on the preliminary investigations, it was found that the brown seaweed, *Stoechospermum marginatum*, exhibited multiple biological activities. These observations have given me a clue that the secondary metabolites that are produced by *S. marginatum*, may have a beneficial molecule/s, which may possess potent anti-cancerous/anti-proliferative properties. Keeping in view, an extensive study was conducted on the

brown seaweed, *S. marginatum* for isolation of bioactive substances. Bioassay directed fractionation and purification led to the successful isolation of ten bioactive **spatane diterpinoids** from a brown seaweed, *S. marginatum*. These structures were determined by analyzing ^{1}H, ^{13}C NMR and FAB-MS.

The anti-proliferative/cytotoxic activities of these isolated compounds were investigated in different cancer cell lines and found that one of the spatane diterpinoids, **5 (R), 19 - diacetoxy -15,18 (R and S), dihydro spata-13, 16 (E)- diene (DDSD)** has exhibited most significant cytotoxic properties against all the cell lines, *i.e.,* Histiocytic lymphoma (U937), Acute monocytic leukemia (THP-1), Colon adenocarcinoma (COLO205), Promyelocytic leukemia (HL60), Human lung carcinoma (A549), Human breast cancer (MCF7) and mouse melanocarcinoma (B16F10) with IC_{50} values of 5.6, 14.21, 36.15, 40.96, 26.35, 32.34 and 3.45 µg/mL, respectively. It is evident from the results that the mouse melanocarcinoma (B16F10) cells were relatively more sensitive to the isolated compound, DDSD. Interestingly, the DDSD has exhibited fewer symptoms on the human peripheral blood mononuclear cells (PBMC) and mouse fibroblast cell lines (BALB/3T3 and NIH/3T3) with IC_{50} values of 90.18, 69.08 and 59.80 µg/mL, respectively. This data indicated that DDSD may selectively inhibit the viability of mouse melanoma cells, which is a highly desirable property of potential anticancer agents. Light and florescence microscopy studies provide direct evidence that, the compound DDSD exhibited apoptotic activity on several cancerous cell lines.

The molecular mechanism of DDSD mediated apoptosis was also investigated for the first time in *in vitro* and *in vivo* B16F10 melanoma mouse models. Induction of apoptosis in B16F10 cells was

characterized by colony forming inhibition, wound healing inhibition, cell membrane blebbing, chromatin condensation, DNA fragmentation, which leads to cell growth inhibition in a concentration-dependent manner. Data indicate that DDSD induced the generation of ROS, consequentially caused alteration in Bax/Bcl-2 ratio that disrupted the inner mitochondrial transmembrane potential ($\Delta\Psi m$) resulting in cytochrome c redistribution to the cytoplasm and activation of caspase-mediated apoptotic pathway. Flow cytometric analysis clearly indicated that the DDSD inducing phosphatidylserine externalization and mediated "S-phase" arrest in cell cycle. In addition, results also found that DDSD induced apoptosis through deregulating PI3K/AKT signalling pathway. The anti-tumor activity of DDSD was evaluated in C57BL/6 mice bearing B16F10 melanoma. It effectively inhibited tumor growth (volume and weight) in a dose dependent manner, yet without apparent toxic effects. Morphology and apoptotic status of tumor tissues in the treated mice were assessed by microscopy and TUNEL assay, respectively.

It was concluded from the experimental data that Spatane diterpinoid (DDSD) compound exhibits potent anti-proliferative effect in B16F10 cell lines *in vitro*. Present experiments proved that DDSD induced intrinsic mitochondrial apoptosis pathway by generating ROS and inactivation of PI3K/Akt pathway involving modulating expression of Bcl-2 family proteins, leading to apoptosome mediated activation of caspase cascade. Based on *in vivo* antitumor activities against B16F10 subcutaneously grafted murine melanoma, it appears to be a more promising therapeutic compound for melanoma cancer treatment with no side effects. The present *in vitro* and *in vivo* results demonstrated that the DDSD molecule may emerge as efficacious agent for therapeutic use