INTRODUCTION
CHAPTER 1

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Marine microorganisms represent a potential source for production of biomedically useful compounds active against inflammation, cancer, diabetes etc. The decrease in the rate of discovery of novel drugs from terrestrial source has necessitated the evaluation and establishment of new source of chemically diverse bioactive compounds (Imhoff et al. 2011). The marine habitat has enormous ecological diversity and represents an untapped reservoir of novel bioactive substances. It is recognized that the discovery of marine compounds as therapeutic agents is still in its infancy and is expected that the ocean which remains virgin, underexplored and unexploited will become an invaluable source for tapping bioactive secondary metabolites possessing pharmacodynamic properties, in the future.

Cancer is recognized as the largest single cause of death in both men and women and is considered the scourge of human kind. Over the last decade tremendous advances have been made in understanding cancer biology and cancer genetics (Lowe and Lin 2000).

Apoptosis is an evolutionarily conserved and genetically regulated process that plays an essential role in the development and maintenance of cell homeostasis and is morphologically and biochemically different from necrosis (Hacker 2000). Cells dying by apoptosis display several morphological and biochemical alterations including chromatin condensation, nuclear segmentation, intranucleosomal DNA fragmentation, cytoplasmic vacuolization, cell shrinkage and membrane blebbing with shedding of apoptotic bodies (Wang et al. 2009).
Induction of apoptosis is initiated by variety of signaling mechanisms, predominantly caspases and mitochondrial Bcl-2 family proteins. Caspases exist in cells as inactive proenzymes, with the active tetramer being formed by the removal of the prodomain and cleavage between the large and small subunits (Earnshaw et al. 1999). A central constituent of the apoptotic machinery is a family of cystein-containing, aspartate-specific proteases, termed as caspases (Philchenkov et al. 2004). Caspase activity, either directly or indirectly, is responsible for the cleavage of several intracellular proteins, which are typically proteolysed during apoptosis (Hseu et al. 2004). It has been well described that two major pathways, the death receptor pathway and the mitochondria-dependent pathway, lead to the activation of caspases and consequent apoptosis in mammalian cells (Aggarwal et al. 2004; Bellet et al. 2004).

Activation of Bax, release of cytochrome c into cytosol and induction of caspases are cascade of events leading to apoptosis. Besides unrestrained proliferation of cells, metastasis leads to high rate of lethality.

Cancer invasion due to development of metastasis is the leading cause of death in humans. The prevailing cause of death associated with cancer is the metastasis of cancer cells which fail to respond to various chemo and radio therapies (Tang and Porter 1997). Surgeries and concurrent therapies do not adequately cure or control the development of this disease. Therefore, it has become necessary to search for novel therapeutic drugs from natural sources as an alternative for chemotherapeutic strategies.

The spread of tumors from the primary site to secondary sites is a multistep process which involves invasion, structural changes in extracellular matrix (ECM) and tissue remodeling (Vinodhkumar et al. 2007). ECM degradation by proteolytic
enzymes like matrix metalloproteases (MMPs) such as MMP-2 (gelatinase A) and MMP-9 (gelatinase B), is a crucial step in tumor metastasis and is strongly linked to various types of human cancer (Hung and Chang 2009).

MMP family proteins as well as other genes involved in tumor metastasis have been identified to be regulated by NFκB. NFκB is a transcription factor which plays important role on carcinogenesis and cellular survival processes. They regulate migration and metastasis of cancer cells by upregulating certain chemokine receptor such as CXCR-4 (Yodkeeree et al. 2010). It is noteworthy that natural compounds from marine source, in particular, have been recently recognized as potential source of MMPs and NFκB inhibitors.

In our earlier work we have shown that the ethylacetate extract of *Staphylococcus arlettae* and *Planococcus maritimus* (Krishnaveni and Jayachandran, 2009) isolated from approximately 1000 m deep sea water column near Andaman and Nicobar Islands downregulated the expression of pro inflammatory cytokines and mediators.

We have isolated a battery of marine bacteria from various unexplored sites in Bay of Bengal near Andaman and Nicobar islands with intent to identify bacteria capable of producing bioactive leads active against cancer. *Bacillus pumilus* MB 40 obtained from 1000 m deep water columns produced BEHP that induced apoptosis. The effect of Bis (2-ethylhexyl) phthalate (BEHP) on Human adenocarcinoma, HT-29 cells, Human monocytic carcinoma, K562 cells and human laryngeal cells, HEP-2 was evaluated in detail and the results of our study revealed activation of caspases, upregulation of Bax and down regulation of Bcl-2 and release of cytochrome c. Further cell cycle arrest at sub G0/G1 phase and induction of DNA fragmentation and chromatin condensation were also observed.
Apart from inducing apoptosis in cancer cells, BEHP inhibited the migration and invasion of the all the 3 cell lines. BEHP also down regulated the expression of metastasis associated genes TNF-α, CXCR-4, MMP-2 and MMP-9 besides down regulating the expression of NFκB. This study was undertaken with the following objectives.

**OBJECTIVES:**

- Isolation and identification of bacteria from deep sea water column in Bay of Bengal, capable of producing bioactive leads active against cancer.
- Structural identification of prospective bioactive molecule(s) active against cancer cells.
- Molecular mechanism of action of potential biolead(s) on different apoptotic and metastatic markers.
- *In silico* studies for designing dual target directed drugs that may inhibit Bcl-2 and MMPs.