2. LITERATURE OVERVIEW

A brief review related to the work on “Screening of antioxidant, anticancer, antimetastatic and immunomodulatory effects of marine alga in cancer” is presented with the recent findings and advancements in the biochemical and molecular mechanism of carcinogenesis and management of cancer.

2.1 Marine Natural Products

Marine organisms encompass nearly 80% of the world’s flora and fauna and are a prolific source of novel and potentially active biomolecules [93]. These marine forms live in a complex environment and are exposed to radiation, interspecies competition and harsh climatic changes. To survive in such a hostile environment, they produce numerous bioactives called Marine Natural Products (MNPs). MNPs can interact specifically with the mammalian macromolecules and possess unique structure. Thus, the drug likeliness and biological specificity makes the MNPs as lead compounds for novel drug discovery [94], [95].

In the history of MNPs, there is less reference for their utilization as medicine. However, the usage of ocean mud, algal extracts and ointments in the treatment of various diseases have been reported since ages in the Traditional medicine of Chinese and Japanese. In 1900, the first marine drug Kainic acid was commercialized to use as anthelmintic and as insecticide. It was isolated from the marine algae *Dignea simplex* [22]. Later in 1950, two compounds such as spongothymidine and spongouridine were isolated from marine sponge *Cryptotheca crypta* [23].The algal compounds are currently used as antivirals and anticancer drugs [2]. With the recent advancements in the isolation and characterization techniques the efficient utilization of MNPs has increased in the drug industry. In the last three decades large number of MNPs have been isolated and their pharmacological properties were reported. Till date, eight marine based drugs are approved by FDA and EU including Cytrabine (1969) [92], vidarabine

(1976), omega-3-acid ethyl ester (2001), iota- carrageenan, ziconotide (2004) [96], Trabectedin (2007) [97], Eribulin Mesylate (2010) [98] and Brenturimab vedotin (2011) [99] which are employed in the treatment of various human diseases.

Owing to the increase in the isolation and identification of the pharmacological properties of marine based drugs, the global market for marine pharmaceuticals is expected to increase from 563 billion to 5.69 trillion US$ [48].

Various marine products including fish oils, algal polysaccharides, galactans, peptides, steroids, pigments and other MNPs are reported for their varied pharmacological properties [100]. The diverse therapeutic potentials of MNPs are well documented [101], [102].

Among the FDA and EU approved marine based drugs, Iota carrageenan isolated from red marine algae *Euchema* and *Chondrus* is used for treating respiratory infections caused by Rhino viruses [92]. Trabectedin derived from *Ecteinascidia turbinate* is employed in the treatment of ovarian cancer and soft tissue sarcomas [92]. Brenturimab vedotin was derived from the sea mollusk *Dolabella auricularia* and is employed in the treatment of Hodgkin's lymphoma and large cell lymphomas [103]. Omega-3-acid ethyl esters were isolated from fish oil and are widely used in the management of hypertri glyceridemia [104]. Ziconitide was isolated from the venom of marine sponge *Conus magnus*. It is a potent analgesic and is comparatively thousand fold effective than morphine. Hence, it is approved for the management of pain in AIDS and cancer patients [103, 104] (Figure 2.1).

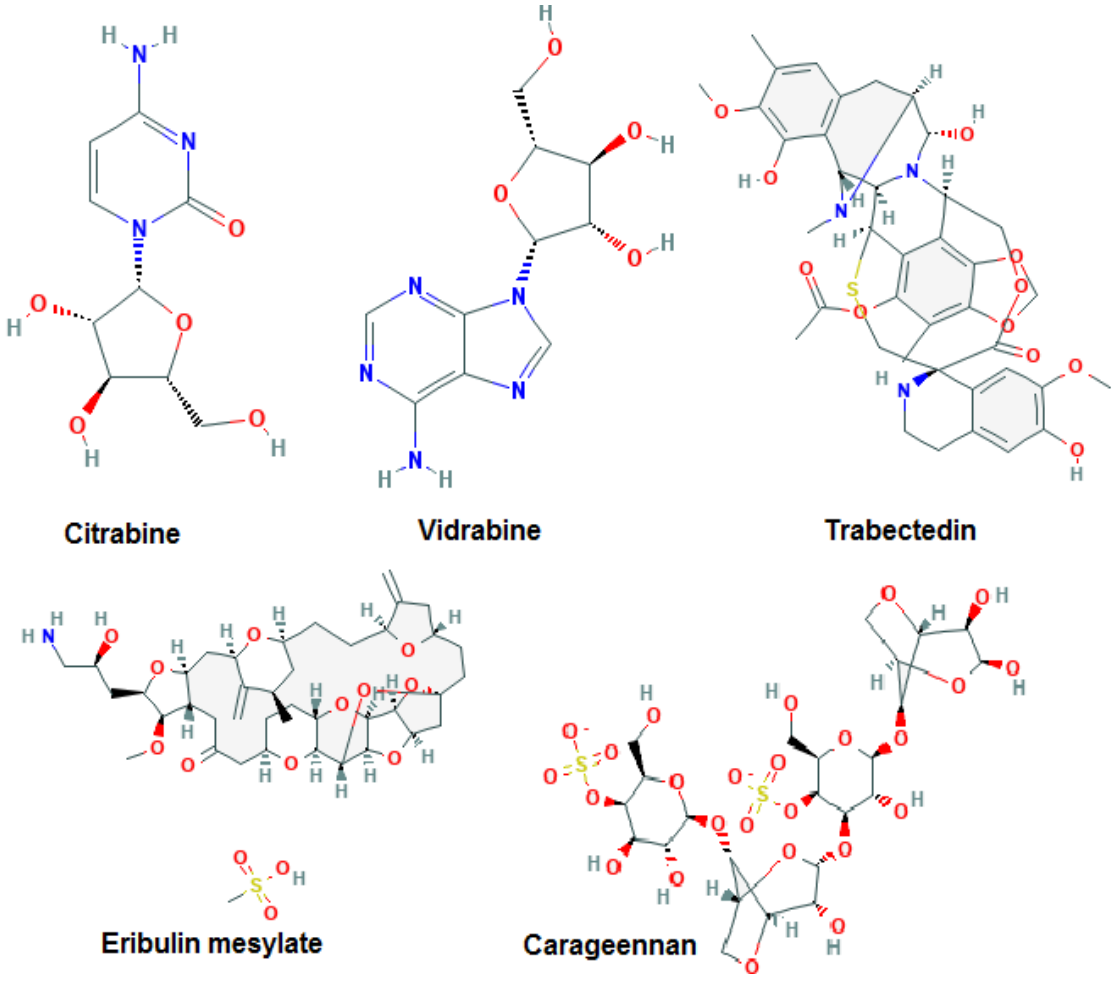

Figure 2.1 Chemical structures of marine based drugs approved by FDA and European Union.

The recent pipeline of marine based drugs include 20 new compounds currently undergoing different phases of the clinical trials [104]. PM-10450, Discodermolide, HT1286, LAF389, Hemiasterlin are marine based drugs derived from sponges [105], [106]. These compounds are undergoing phase I trial in the development of anticancer drugs. They act by interfering with microtubule formation (Figure 2.2).

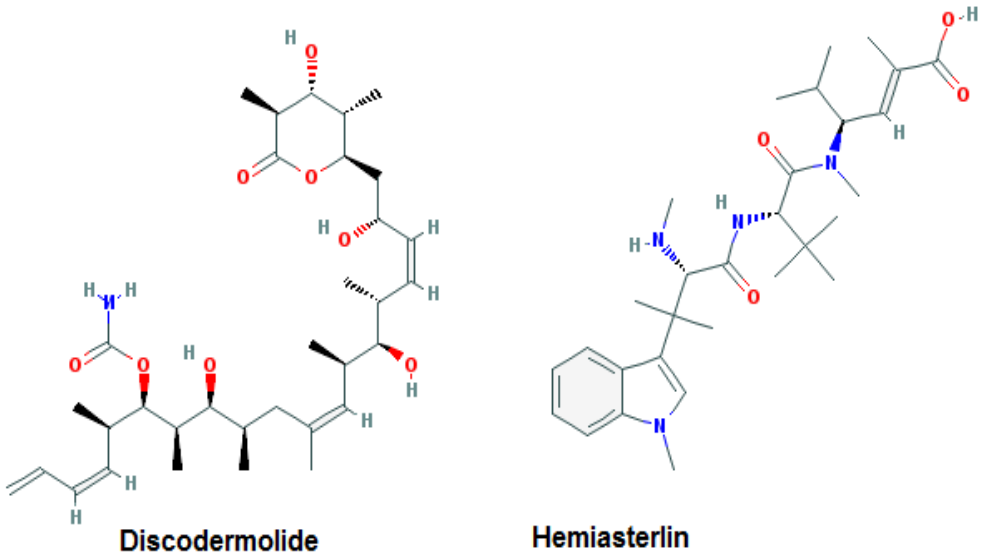

Figure 2.2 Chemical structures of marine based drugs undergoing clinical trials (Phase I)

The compounds Plitidepsin and Gemcitabine are undergoing phase III trial. Plitidepsin is a depsipeptide derived from marine tunicate *Aplidiumalbicans.* inhibits the proliferation of cancer cells through the JNK pathway [107]. The other compound Gemcitabine, is an analogue of Cytrabine. It is a nucleoside that acts as ribonucleotide reductase inhibitor is utilized for the treatment of various types of cancers [108].

Glembatumumab vedotin and Elisidepsin, undergoing phase II trials are isolated from marine mollusks. Glembatumumab vedotin is a peptide which inhibits glycoprotein NMB expressed by cancer cells. The compound is analyzed for the treatment of breast and skin cancers [109]. Elisidepsin is a depsipetide that acts as an antineoplastic agent [110]. Pseudopterosins is a compound undergoing phase II trial. It is a diterpene glycoside isolated from soft corals is analyzed for its wound healing potential and neuromodulatory activity [111] (Figure 2.3).

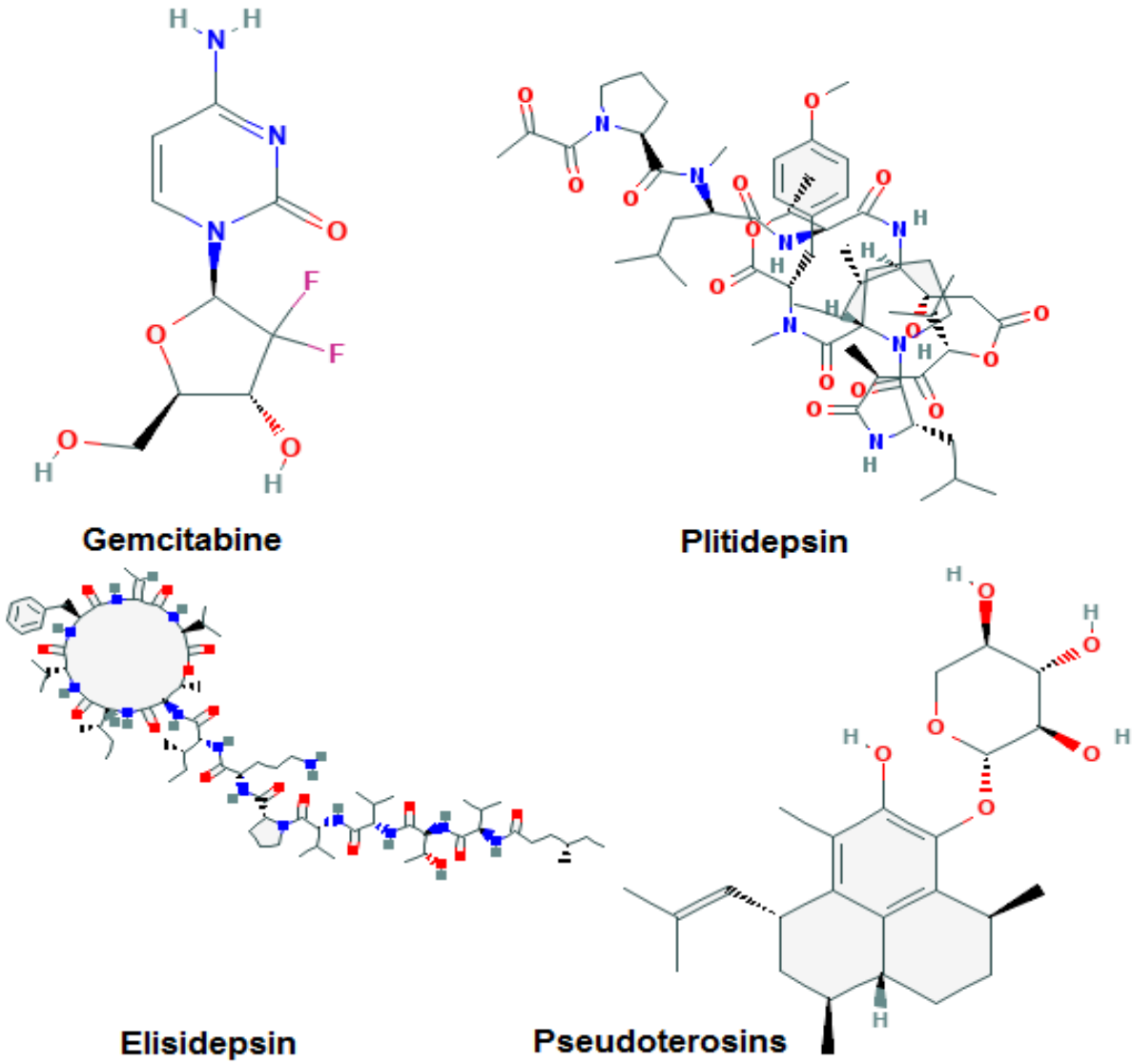

Figure 2.3 Chemical Structures of marine based drugs undergoing clinical trials (Phase III & II)

Based on the current scenario, the current research is focused on identifying, isolating and characterizing bioactives from marine red algae and analyzing their mechanism of action in cancer.

Apart from these, algal extracts are broadly explored for their anticancer activities which can be used alternately to chemotherapeutics. Recent research has identified numerous algal bioactives with good therapeutic potential. They contribute to 9% of the bioactive compounds derived from the marine origin [93].

Palmitic acid from Amphiroa Zonata exhibited antitumor activity in lymphoblastic leukemia cells [112]. Laurenmarianol, thyresenol A & B, isolated from Laurencia species were effective against P388 cells. Bromophenols from *Rhodomelaconfervoides* exhibited antitumor activity against A549 (Adenocarcinoma), Bel7402 (hepato carcinoma), KBC (epithelial tumor) cell lines [113].Among the marine organisms, the marine algae are prolific source of natural products with diverse biological activities. [113], [114].

Biological properties of marine algal compounds and extracts are well documented. Heterofucan SF 1.5V from *S. filipendula* induced mitochondria mediated apoptosis in HeLa cell lines [115]. The red algae *Gracilaria tenxistipitata* induced cell cycle arrest in ca 9-12 oral cancer cell lines [116]. Sargaquinoic acid from *Plocamium corallorhiza* and *P.carnutum* induced apoptosis in MB 231 cell line [117].

The marine algae are the major producers of Oxygen and are rich in nutrients including vitamins, minerals, carbohydrates, fatty acids and pigments [114]. The sulfated polysaccharides exhibited antioxidant, antiviral, anti-inflammatory, immune modulatory properties [117]. Fucoidan from *S. mccluries* effectively suppressed colon cancer cell line proliferation [118]. Ethanolic extracts of *S.wightii* showed anti proliferative activity against AGS, Hela, MCF-7, PCI2 and A549 Cell lines through the induction of apoptosis [119]. The red algae porphyra which contains a mixture of sterols like campestrol, desmosterol, fulosterol, stigmasterol were effective against breast cancer cell proliferation [106]. The chloroform extract of *Hypheamuscifermis* inhibited proliferation of chronic myelolytic leukemia cell line and laryngeal epidermal cell line proliferation and human lung mucoepidermal

cancer cell line [120]. Ethanolic extract from *H. grandifolius* inhibited proliferation of A375, A549 Hep2, HeLa cell lines through the induction of apoptosis [121].

To survive, algae synthesize a wide array of phytocompounds with varied biological properties. This diversity has led to the exploitation of the algae as alternative source of food supplements and for the development of novel drugs [122].

Aqueous extract of marine microalgae inhibited antimetastatic activities and colony formation in prostate cancer [123]. Biogenic compounds from marine algae are widely employed as complementary therapeutics in the treatment of cancer [Rita 2016]. Algal phytocompounds are widely reported for various biological activities including anticancer, antimetastatic, antioxidant, anti-inflammatory properties and can function as effective chemopreventive agents [4], [125 – 127].

2.2 Red Algae

Most of the red algae species are edible including *Palmaria palmata*, *Chondru scrispus and Mastocarpus stellatus*. The species belonging to Prophyora are used as food in Japan [Nori], Korea (Gim) and Britain [Laver] [128]. Algal food are rich sources of vitamins and proteins and hence utilized widely [129]. Carrageenan isolated from *Kappaphycus* and *Betaphycus* are used as thickening agents in Yoghurt, Chocolate milk and pudding preparations. Agar is produced from *Gracillaria, Pterocladia and Gelidium* for food and biotechnology purposes [39]. *Calcium carbonate secreted by coralline algae are used in bone*-replacement therapies [130]. Red algae are commercially used in the biomedical pharma and food industries [130]. Red algal extracts from *Gracillaria corticata* and *Gracillaria crassa* exhibited antimicrobial activity [131].

Red algae are broadly classified into 2 classes namely the Florideophycea and Bargiophycea [132]. India has a coastline of 7,500Km harboring rich source of seaweeds. The marine algae of India are highly diversified comprising of 1153 species of which a maximum species belongs to Rhodophyta [133]. In India, the red seaweeds are used for the production of agar and alginates. *Kappaphycus* and *Hypnea*species are cultivated for carrageenan production. The Indian agar industry

depends on *Gracillaria edulis* and *Gelidiella acerosa* for agar production. *Gelidiella* is highly preferred due to the high quality agar it produces. The annual production of agar in India accounts to 50 – 90 tons and costs Rs. 50,000/- ton [133].

The red algae possess phycocyanin and phycoerythrin as the major plant pigments which accounts for its red colour. It contains floridean starch as the main reserve. Apart from this, cellulose, agar, carrageenan and long chain polysaccharides are also present. They grow in the intertidal and sub tidal zones [134].

According to the Algae database, nearly 10,000 species of algae reported are belonging to both the fresh water and marine habitats [135]. Among these, the majority (6500) of the species belong to Rhodophyta (Red algae) [132]. Although the red algae are the predominant seaweed community of India their exploration and commercial utilization is very less. Studies reporting the therapeutic potential of marine red algal metabolites are still in its infancy. Hence, the current research is aimed in screening the phytocompounds and analyzing the pharmacological efficacy of red algae (*Gelidiella acerosa*) in cancer.

2.3 *Gelidiella acerosa*

G. acerosa [Figure 2.4] is an intertidal marine red alga, attached to the rocks at a depth of 0 - 1 meter. It is reported in Asia, Pacific islands and Indian Ocean (Algae Base). *G.acerosa* is red to purple in colour with erect and lateral branches extending from 9 to 10 cm in length.

The alga is exploited worldwide for the production of high quality agar. Studies have shown that *G.acerosa* from the Gulf of Mannar produce superior quality of agar than from other locations [39]. *G.acerosa* was reported to possess high phenolic content which may contribute to its antioxidant and antimicrobial properties [136].

Figure 2.4 The picture of red alga *G.acerosa* collected from Mandapam coast, Tamil Nadu, India for the current study.

The alga is a source of various phytochemicals including alkaloids, tannins, proteins, cardiac glycosides, carbohydrates and lipids [137]. Biogenic silver nano particles synthesized from *G.acerosa* exhibited antifungal activity against *Humicola insolens, Fusarium dimerum, Mucorin dicus* and *Trichoderma reesei* [46]. The presence of terpenoids in the alga which inhibited acetyl choline esterase and butyl choline esterase activities was also reported [45]. The benzene extract of *G.acerosa* scavenged free radicals more effectively and thus revealing the antioxidant nature of the algae [138]. The sulfated polysaccharide fraction of *G.acerosa* exhibited antithrombotic activity in animals [139]. Methanolic extract of *G.acerosa* inhibited tumor growth *in vivo* revealing the anticancer nature of the algae [140]. A similar work [141] also revealed the anticancer activity of the algae against Human Promyelocytic leukemia cell line. *G.acerosa* exhibited antibacterial activity against *vibrio cholera*, *Bacillus subtilis, Proteus mirabilis* and *Streptococcus pneumonia* [142]. The presence of bioactives including sulfated polysaccharides,

eicosane, diisooctyl ester and 9-octadecanoic acid in *G.acerosa* are documented earlier [139].

Methanolic extract of *G.acerosa* protected human PBMCs against TCDD induced cytotoxicity and thus revealing its cytoprotective property [42]. The study identified the presence of caffeic acid, phytol and mannoheptulose in the algal extract which might confer the cytoprotective activity.

Although *G.acerosa* is rich in phytochemical compounds, the isolation and mode of action in cancer was not established till date. Hence, the current study is focused to explore the algae for its potential bioactives and determine their mechanism of action and possible targets in cancer.

2.4 Cancer

Cancer results from uncontrolled cell division [143]. Cancer arises when a cell releases itself from the constraints of cell division and follows its own protocol of proliferation [144]. These results in a tumour or a lump of cells in the tissue where uncontrolled proliferation started - a condition called *in situ* cancer. This tumor can invade to adjacent tissues leading to the condition called Invasive cancer. The invasive tumor sheds its cells which enter the blood stream and forms new tumors at distant sites of the body – a condition called metastasis [144].

Cancer is projected as the major cause of human death globally [60]. Tumors are life threatening as they disrupt the normal function and structure of organs and tissues required for survival [145].

The prevalence of cancer in India was reported by the ICMR. According to the report 1 in 8 Indians are prone to develop the disease in their lifetime. It is stated that 14.5 lakh new cases of cancer are reported in 2016 which is expected to reach 17.5 lakh by 2020. Among the difference types, the breast carcinoma ranks the first with 1.5 lakh new cases [146], followed by the lung carcinoma with 1.15 lakh [147] and cervical cancer with 1 lakh new cases [148]. Further the increasing incidence of rectal and colon cancers are also alarming. Similarly the incidence of

lung cancer are increasing in India among women and that of colon cancer in men [149].

Cancer is a multistep process involving prolonged and complex genetic changes. Two types of genes are involved in the progression of carcinogenesis (namely the proto-oncogenes and tumor suppresser genes [150]. Mutation of these genes causes uncontrolled cell division resulting in cancer [151].

The development of carcinogenesis includes 3 stages: initiation, promotion and progression [153]. Initiation is an irreversible process where spontaneous stable cellular changes occur in a cell thereby predisposing it to neoplastic transformation. Promotion involves the proliferation of the transformed cell under the influence of both intra and extracellular environmental factors [153]. Progression is the final phase which causes an increase in the cancer cell population [152]. Following tumor progression, the neoplastic cells can occur in any part of the body. Based on the site of origin it is classified into several types namely the cancers of the skin or covering of internal organs as carcinoma. Cancers of the occurs in bones, muscle, blood vessel or supportive tissue as Sarcoma, cancers of bone marrow as Leukemia, and those in immune cells as Lymphoma and Myeloma [154]. Cancers of the brain and spinal cord are named as Central nervous system cancer [88].

The cancer cells get detached and invade distant sites and develop secondary tumor by a process termed metastasis [155]. It includes 3 major steps namely invasion of the cells into the blood stream, degradation of the extracellular matrices and colonization at distant sites, to form new tumors [156].

2.5 Molecular mechanism of metastasis

Metastasis or tumor spread from the initial site to distant organ occurs in a number of steps called the “metastatic cascade” [157]. There are physiological barriers at every step which has to be overcome for successful metastasis [158]. The steps of the metastatic cascade include the following

2.5.1 Tumor cell disintegration and EMT.

The epithelial tumor cells undergo change Epithelial Mesenchymal Transition during progression [159]. This involves lack of cell polarity, down regulation of proteins, and variation in cell structure. EMT is mediated through various pathways including the hedgehog [160], Receptor Tyrosine Kinases (RTKs) [161], NFκB pathways [162], WNT [163], NOTCH and signaling molecules like the transforming growth factor β (TGFB) [164]. EMT favours metastasis [165], through release of MMPs, mitogenic and angiogenic factors that favour both migration and angiogenesis [166].

2.5.2 Invasion and cell migration

Invasion depends on the migrating efficiency of cells. Tumor cells move as single cells or in group [167]. Tumors lacking EMT prefer collective migration. Single cells invade by “mesenchymal” or amoeboidal movement [168]. The migration is differentiated on the direction and not by speed. Moreover, non metastatic tumor cells lack polarization whereas, metastatic cells are polarized and migrate towards blood vessels [169].

2.5.3 Anoikis

As tumor cells get detached, they encounter anoikis [170]. This process promotes apoptosis of tumor cells and inhibits metastasis [171]. Inhibition of anoikis is essential for metastasis [172]. Intravasation involves the movement of tumor cells towards the blood vessels with the aid of tumor associated macrophages (TAMs). Similarly tumor cell extravasation involves the migration of tumor cells from vessels into organs. This process is integrin dependent which suppress anoikis [173].

2.5.4 Angiogenesis

The physiological process that involves the creation of blood vessels from already existing vasculature is defined as angiogenesis [174].The initiation of this process is called as angiogenic switch which enables the tumors to grow and spread beyond [175]. Tumor cells induce the surrounding tissues to release chemicals that promote angiogenesis [176]. These adhere to the surrounding mature vessels and promote new vessel formation. Angiogenesis is crucial for cancerous growth and

metastasis [177]. For effective metastasis, tumor cells require nutrients and oxygen for which they develop new blood vessels by tumor angiogenesis. Until tumor angiogenesis is achieved Tumor dormancy results [178]. Hence, current anticancer therapies are targeted in inhibiting establishment of angiogenesis which can hamper both cancerous growth and metastasis [179], [180].

2.5.5 Outgrowth of secondary tumors

The formation of secondary tumors is dependent on various processes. The metastasis is not a random process and is specific to certain sites [181]. For instance, breast cancer commonly metastases to the liver and bones. Hence, a secondary tumor is formed only if the tumor microenvironment of the target tissue or organ is suitable with that of the tumor cell [182]. Further metastatic suppressor genes act on the signaling pathways such as MAP Kinases and RHO to prevent the establishment of secondary tumors [183]. The microRNA miR-335 functions as metastasis suppressor gene by suppressing various pathways thereby hampering metastasis [184], [185].

2.5.6 Metastatic cancer stem cells

Metastasis is reported to arise from cell clones called the “Cancer Stem Cells” (CSCs) [186]. They represent a population of disproportionately proliferating stem cells that have rapidly differentiating and proliferating potential [187]. These CSCs are reported in hematologic malignancies, melanoma and breast, brain, prostate, pancreatic and colon cancers [188], [189].

2.5.7 Contribution of the microenvironment

Tumor microenvironment refers to the normal cells adjacent to the tumor and tumor stroma [190]. The tumor stroma includes the fibroblasts, immune cells, ECM, blood vessels and lymphatic vessels. Tumor microenvironment is essential for tumorogenesis and tumor progression [191].

Targeting metastasis is a big challenge faced by the biomedical society in the management of cancer [192]. Research is targeted at regulating the stages of

seeding, tumor microenvironment, preventing outgrowth, hampering angiogenesis and other signaling pathways and molecules that are involved in metastasis [192]. A large number of small molecule inhibitors (ERBB2, mevastatin and lovastatin, rottlerin) peptides, plant metabolites and antibodies are currently developed and analyzed for their ability to suppress metastasis [193].

Apart from metastasis, the next major obstacle in the treatment and management of cancer is apoptosis

2.6 Apoptosis

Cancer is defined as a disease resulting from the accumulation of cells that evade apoptosis [113]. Apoptosis maintains homeostasis between cell division and cell death. Inhibition of PCD results in several diseases including cancer [194]. The apoptotic cascade is mediated by the extrinsic or cytosolic pathway which involves the activation of cell death receptors and the intrinsic or mitochondrial pathway which involves the release of cytochrome c. Both these pathways converge to activate the caspases to execute the programmed cell death [195]. The third pathway called endoplasmic reticulum stress-induced pathway also exist.

Apoptosis is a regulatory mechanism in which a cell commits suicide if its damaged DNA is not repaired. Apoptosis removes self-reactive lymphocytes and cells transformed by virus infection. Evasion of apoptosis is a hallmark of carcinogenesis [196].

2.6.1 The mitochondrial pathway

This pathway is regulated by the proteins belonging to the Bcl2 family. These proteins are referred as “regulators of apoptosis” [197]. Increased expression of these proteins is related to cancer, resistance to anticancer drugs and radiation therapy. On the other hand, decreased expression of Bcl2 promotes apoptosis and increases sensitivity to chemotherapy [198]. The Bcl2 family comprises two groups of proteins namely the proapoptotic and anti-apoptotic proteins. Among these, the proapoptotic group includes the Bax, Bad, Bim, Bcl-Xs, Bik, Bid, Bak and Hrk which

favour apoptosis [199]. The members of the anti-apoptotic group namely the Bcl-Xl, Bcl2, Bfl-1, Bcl-W and Mcl-1 interfere with release of cytochrome C and thereby prevent apoptosis [72]. The Bax:Bcl2 ratio determines the progression of apoptosis. If the ratio is above 1, apoptosis is promoted and vice versa [199]. The activation of the intrinsic pathway releases cytochrome c, activates the caspase 9, caspase 3 resulting in apoptosis [200].

Both the apoptotic pathways are regulated by p53, NFKB and PI3K signaling pathways [201]. Defects in NFKB, p53, PI3K/Akt/GSK3B pathways hamper the process of apoptosis leading to imbalanced cell proliferation and survival resulting in cancer [202].

2.6.2 NFKB

NFKB is a vital transcription factor which mediates the expression of nearly 200 genes involved in cell cycle, survival, proliferation, differentiation, migration, adhesion and inflammation [158], [203]. Activation of NFKB confers activation of these genes in cancers including lung cancer [77]. Alternatively, NFKB also suppresses the expression of the tumor suppressor genes especially p53 and PTEN, and thus promoting carcinogenesis [204][205]. The inhibition of NFKB is reported to enhance the efficacy of anticancer drugs. However, NFKB is also reported to favour apoptosis in response to viral infection and certain stimuli through the activation of c-myc, p53, caspase – 1 and interferon-regulated factor [206].

2.6.3 PI3K/Akt/GSK3B signaling cascade

The PI3Ks are lipid kinases which phosphorylate the PIP2 into PIP3 which act as a second messenger and activate several kinases including the protein kinase B/Akt, mTOR, GSK3Band NFKB [72], [206], [207]. Activation of PI3K signaling cascade promotes several cellular processes including cell survival, proliferation, differentiation, cell cycle and apoptosis [208], [209]. In several solid tumors, the constant activation of the cascade is reported to promote evasion of cell cycle surveillance and PCD thus prolonging survival of tumor cells [210]. The regulation

of the key nodes of the PI3K pathway increases the sensitivity to anticancer drugs and promotes apoptosis [124], [125], [211].

2.7 Lung cancer

Lung cancer is the most devastating health problem of undeveloped countries [51]. It is categorized as NSCLC and SCLC. Among these, lung adenocarcinoma constitutes 40% of NSCLC cases [60]. The five year survival rate of NSCLC ranges between 1% to 45%, based on the extent of metastasis. The most common risk factors that contribute to lung cancer include tobacco smoke, environmental carcinogens, radiations and fuel smoke [212]. Molecular profiling of NSCLC revealed that the PI3K/AKT/NFKB cascade is the most commonly activated pathway in NSCLC [81], [80].

The economic burden imposed by the disease is substantial on the human society [212]. The high mortality of the disease is attributed to its late diagnosis and poor 5 year survival rate (17.8%) when compared to other cancers [223], [208].

Lung cancer is reported as the most prevalent fatal malignancy among both men and women worldwide [62]. Based on the incidence it is the leading cause of human deaths accounting for 1.72 million deaths in 2015 [53]. In India, the prevalence of lung cancer is increasing significantly due to the changes in lifestyle and food habits [213]. Molecular profiling studies reported that the PI3K/Akt cascade as the mostly targeted pathway in NSCLC [214].

2.7.1 The PI3K signaling cascade

The PI3K signaling pathway is the prime regulator of cell growth, survival, proliferation, apoptosis and motility [215]. Constitutive activation of the cascade results in prolonged survival and uncontrolled proliferation of tumor cells in human cancer [216]. Recent studies identified the PI3K and/ or its components to be commonly mutated which, contributes to the aberrant expression of the PI3K/ AKT/ GSK3β/ NFKβ pathway in cancer [215]. Hence, the cell survival pathway genes became the most commonly targeted candidates in anticancer therapy [217].

Together, the constitutive expression of PI3K contributes to multidrug resistance, resulting in poor prognosis [218].

Development of small molecule inhibitors to target the PI3K cascade is an effective way of treating cancer [219]. A novel group of drugs namely PI3K inhibitors were developed which inhibited the PI3K or its components [214], [223]. Wortmannin and LY294002, the first generation small molecule inhibitors of PI3K were discontinued as they were not safe in animals [220]. Based on these, various PI3K inhibitors are developed by the pharmaceutical companies and academic institutions. This list includes the Umralisib, Buparlisib, Serabelisib, IP1-549, Dactolisib, SF-253, GDC-0326 and Alpelisib which are in different phases of clinical trials [211], [220]. The approval of Idelalisib, the first oral PI3K inhibitor by FDA has triggered the search for novel and specific PI3K inhibitors without toxic effects [221], [222].

The activation of PI3K cascade is associated with resistance to chemotherapy and targeted therapy. Inhibition of the cell survival pathway is suggested to sensitize the cancer cells to chemotherapy and enhance the sensitivity to chemotherapy [223]. For the effective development of potential PI3K cascade inhibitors, identification of response biomarkers, optimizing the dosage levels are the major criteria to be investigated [211], [135], [224].

2.8 Chemoprevention

The poor survival rate of lung cancer is highly discouraging. Although enough advancement in the screening, diagnosis and treatment of lung cancer has been achieved, lung cancer is ranked as the prominent cause of cancer-related deaths globally [62]. In order to enhance the quality of life in lung cancer patients an understanding of the molecular events behind lung cancer, knowledge of biomarkers and development of novel therapeutic approaches like chemoprevention is of crucial need [225]. Early detection and effective chemoprevention are the crucial need for decreasing the mortality and morbidity of lung cancer [225].

2.8.1 Mechanism of chemoprevention

As lung cancer is observed to evolve over 20 – 30 years requiring the long term usage of preventive drugs which may result in intolerable side effects [226]. Hence, natural products, dietary component of plant kingdom, crude extracts of medicinal plants and fruits are more preferred candidates for chemoprevention than synthetic derivatives [227],[228]. Chemoprevention was effective in the management of various cancers [229], [230].

Cancer chemoprevention refers to the use of agents which may be chemicals / plant extracts / dietary components to reverse, inhibit, or prevent carcinogenesis [231], [232]. Chemoprevention trials are based on the hypothesis that an intervention of the molecular mechanism of carcinogenesis may inhibit both cancer development and decrease its incidence [233]. Hence, chemoprevention aims to identify the agents that can inhibit the progression towards cancer to develop potential biomarkers in response to treatment [234]. There are 3 strategies in chemoprevention including primary prevention, secondary prevention and tertiary prevention which are aimed to prevent relapse in patients who had cancers [235].

2.8.2 Phytochemicals as Chemo preventive agents

A large volume of natural and synthetic compounds are shown to exhibit chemopreventive activity. They include retinoids, carotenes, tocopherols, NSAIDS, Selenium, N-acetyl cysteine [236], [237]. Use of retinoids was promising in chemoprevention of lung cancer[238]. But they were highly toxic [239] and chemoprevention aims to develop molecules / agents with high therapeutic index for the treatment of lung cancer [240], [241]. Although multiple agents have been analyzed still a potential or effective chemopreventive agent(s) have not been identified or successfully developed in the treatment of lung cancer [238].

2.9 Molecular Docking

Computational Biology and bioinformatics are valuable techniques for the discovery and designing of new drug molecules [242]. Rational Drug Design is a novel tool for identifying new drugs based on the information about the biological

targets [243]. Molecular docking is a computational tool employed in the planning and designing of new drugs. The method predicts the binding mode and affinity between ligand(s) and target receptor and is useful for the drug recovery process which reduces the cost of drug discovery [244].

Molecular docking is widely employed in the drug industry to identify the lead compound and screen large number of biologically active compounds [244].The methodology includes three steps: pose prediction, virtual screening and estimation of binding affinity [245]. It generates information regarding the sequence of molecular events, mode of ligand receptor interaction and the interactions that stabilize the ligand receptor complex [246]. The selection of the best interaction is based on the binding energy and rank of the ligand [247].

High-throughput virtual screening is a computational tool developed for accessing novel drug like molecules and is employed in drug discovery [248]. Docking has enabled the identification of targets for various drugs [249] and to predict the possible toxicity in drug usage by ADMET analysis [250]. Computational studies are employed to determine the anticancer efficiency of drugs [251], [40] to compare antiproliferative activity of new drug molecules with standard drugs [252] for analyzing the antidiabetic activity of natural compounds [253], [254] antimalarial drugs [255], [256] and many more.

The development of a drug involves the selection of a ligand, screening it for various possibilities, preparation of the protein (target) and finally determine the interaction of the ligand with the target. These steps are carried out with the aid of drug development softwares [257]. In the past twenty years several docking tools are developed which have revolutionized the field of drug development. These include FlexX [258], GOLD[259] , Auto Dock [260], FRED [261], DOCK[262], Surflex[263], Glide [264], AutoDock Vina [265], MOE-Dock [266], rDock [267], UCSF Dock [268]. These softwares allow the determination of the binding energy, identification of binding sites, interacting residues, bond lengths, bond angles, stability of interaction which are crucial for effective drug design and development [269]. Among these the SYBL X 1.3 Surflex docking suite is used in pharma industries for molecular modeling and determination of structure related activity.

2.10 Animal models in drug research.

Cell-based analysis provides limited information about the absorption, distribution, metabolism and toxicity of compounds that are screened [270]. To overcome this limitation, murine models are employed in drug screening. But owing to the cost, availability and maintenance of animals, the ability of compounds to pass through the blood brain barrier and immunological response induced, an alternate model was always searched for [271 – 273]. This has led to the emergence of Zebrafish as an alternate to murine models.

2.10.1 Zebrafish as animal model

Zebrafish (*Danio rerio*) a fresh water fish that belongs to *Cyprinidae* family is commonly found in the Ganges basin of India [274]. It possesses various characteristics that has made it an attractive model for animal studies. These include the small size with the adult reaching 2.5 – 4 cm in length which minimizes the space constraint in growing the fish and is easy to maintain hence cost effective [275]. Zebrafish has high fecundity and thus produce 250 -300 eggs/mating/week. Moreover, the fertilization and embryo development are ex-utero which has enabled the study of pathological and physiological mechanisms in developmental biology by live cell imaging [276], [277]. Hence, Zebrafish became a preferred model to study human diseases [278].

Further, Zebrafish possess 70% genetic similarity to humans and 82% orthohomology to human disease related genes [279]. This has led to the development of human disease models in Zebrafish which enables the analysis of molecular pathways and screening novel therapeutic compounds. Also the external fertilization of Zebrafish embryos has enabled their exploitation in *in vitro* fertilization studies [280]. The generation time is very short (3-5 months) when compared to murine models. All these features have made the use of Zebrafish as a potential alternate to murine models [281].

Zebrafish embryos are widely employed in High throughput drug screening (HTS). The embryos hatch externally at 28°C in 48 hours post fertilization (hpf) and survive

as free living animals. Drugs / compounds to be screened are diluted in the fish water which is taken by the embryo through the chorion and enters its circulation [282].

The cellular and molecular mechanisms involved in drug detoxification are similar in Zebrafish and mammals, hence, the Zebrafish has evolved as a valuable tool in drug toxicity analysis [283]. As innate immunity is active and adaptive immunity is not developed, immune response is not triggered till 6 weeks of post fertilization in Zebrafish. These features favour the utilization of Zebrafish embryos in genetic manipulation, ribonucleoprotein microinjection, morpholino development, and cancer cell xenotransplantation [284], [285]. The molecular pathways and events in carcinogenesis are similar in Zebrafish and mammals. This has caused the emergence of xenografted embryos where human cancer cells are implanted in Zebrafish embryos and observed for proliferation, angiogenesis and metastasis [286].

As the embryos are transparent, it enables the study of organ development, angiogenesis and drug distribution. The European Food Safety Administration has declared that Zebrafish embryos (5dpf) experience less pain, stress and harm and compile with the 3Rs (replace, reduction and refinement) for human animal research [273].

Similarly, several cancer models are developed in adult Zebrafish [287]. These include the exposure to carcinogens and injection of human cancer cells into adult fish for development of tumors [288]. Hence, Zebrafish has emerged as a valuable comprehensive alternate animal model for analyzing lung [287], colon [289], ovarian [290], skin [292], breast [293], prostate [294] and pancreatic [295] tumors.

2.11 Tissue chip technology

The process of drug development is time consuming and expensive [296]. Although several drugs that reach clinical trials, had produced promising results under *in vitro* and *in vivo* conditions, they are not successful in human trials [297]. Recent estimates showed that 90% of lead compounds failed in clinical trials, out of that 30% failed in toxicity analysis [297] and nearly 60% failed due to lack of

efficacy [298]. A recent report by phMRA (Pharmaceutical Research and Manufacturers of America) stated that the drug industry spends 2.6 billion US$ to develop a new drug which also consumes a duration of 10 years. Although cell culture studies are fast, economic and reliable they are only 2D models which do not report about the physiological response of human systems to drug exposure. To overcome these drawbacks, a novel technique that mimics the Human organ system was looked for which led to the development of the Tissue chip or Micro Physiological System (MPS).

The Zebrafish Tissue-Chip, enables the development of organs (heart, liver, skeletal muscle and brain) in a closed circulatory system with physiological hormonal balance. The Tissue-Chip is a miniature of the whole animal system[299]. Also, it enables the efficient screening of novel drugs within limited duration (48 hours), requires less quantity of compounds, economic and non-laborious [300]. The Tissue-chip, enables to carryout ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity), drug screening and determination of false positives in a single step [300]. The current study used the Zebrafish based tissue chip developed by Pentagrit, India for determining the safety of algal extract in animals.

The plant kingdom especially the marine algae are a predominant source of conventional and biologically active compounds widely used in the treatment of various diseases since ancient times [301]. Apart from this marine compounds serve as lead compounds for the discovery of new drugs [302], [291]. Since the plant extracts have relatively low side effects they are an attractive area in the development of chemo preventive drugs [206]. Based on these reports, the current study is hypothesized to screen the marine red alga *G.acerosa* for its anticancer, antioxidant and anti-inflammatory efficiencies in cancer under *in silico*, *in vitro* and *in vivo* conditions.