Marine natural flora: A potent source of anticancer metabolites

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Invertebrates belonging to marine environment along with a growing number of marine organisms including bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes making over 90% of the oceanic biomass are considered novel sources of anticancer molecules production. These marine floras diverse in taxonomy are largely productive, biologically active, and chemically unique moiety and offer a great scope for discovery of new anticancer drugs. The marine floras are rich in medicinally potent chemicals predominantly belonging to polyphenols and sulphated polysaccharides. The chemical and biological diversity of the marine environment is immeasurable, hence represents an extraordinary resource for the discovery of new anticancer drugs. Recent advances in research and technology have significantly contributed in structure elucidation, and organic synthesis of target molecules of therapeutic potential, resulting in the isolation and clinical evaluation of various novel anticancer agents. This review is presented as a brief survey of recent investigations in the marine pharmacology and discovery of new anticancer drugs to provide baseline information for promoting the marine flora-based anticancer research. This review in the current scenario of increasing cancer incidence may provide scientific insights deprived of cheaper, safer, and potent medicines to challenge the dreadful human disease.

[Keywords: Anticancer agents, Marine metabolites, Marine sources, Bioactive compounds]

Introduction

Marine world covers more than 70% surface area on Earth with a huge diversity of millions of multicellular organisms such as plant and animals including billions of unicellular organisms distributed under 100 different phyla. Marine environment provides the organisms to sustain in such a crucial environment with a highly competitive range of living standard resulting in the perfection to survive these organisms through chemical defence. Evolution of millions of years has made the marine world a goldmine of genetic diversity and novel secondary metabolites. Previously systematic chemical and pharmacological investigations of these organisms were not explored to certain extended levels due to several factors such as inaccessibility of their habitats, and very low yield of bioactive metabolites. However, the recent research on marine floras, natural products chemistry, genome mining and bioassays, have led to a huge upsurge in the search for novel biomolecules from this rather underexploited habitat.

Throughout all the generations, cancer is a debilitating disease which has afflicted a noticeable proportion of the entire population of the world. Generally in the form of apoptosis, a balance between proliferation and programmed cell death is maintained under normal circumstances, by tightly regulating both processes. In carcinogenesis process, normal cells are transformed into cancer cells (Fig. 1), certain mutations in DNA may lead to cancer by disrupting the programming regulating processes. In the treatment of various cancers, development of resistance to chemotherapy is considered a major hurdle, considered to be a notable amount of tumors relapses and develops resistance, eventually resulting in multi-drug resistance following exposure to multiple anticancer drugs with prevalent structure and mechanisms of action.

Moreover, noble anticancer molecules act exclusively against tumor cells; however, number of reported chemotherapeutic drugs being used for cancer treatments clinically exhibit severe adverse side effects on human body including bleeding, hair loss, diarrhea, and immunosuppression. Hence, discovery of new class of biologically active natural products and metabolites from marine organisms, animals, and plants with potent antitumor effects without any toxicity on normal cells could be a big
From decades, chemistry of natural products has played a significant role in the treatment and prevention of severe human diseases. Although natural products provide a huge platform on disease remedy they belong to different resources including terrestrial plants and microorganisms, sea macro- and micro-organisms, as well as terrestrial invertebrates and vertebrates. Despite the huge scientific research on terrestrial natural products, the first pioneer work on studying marine natural products began in early nineteen century. While the difficulties of collecting marine samples cannot be underestimated, a huge number of easily accessible marine samples are available simply by shore-wading. During early nineteen century, marine world was not explored well possibly due to the difficulties of isolation and purification of marine natural products with the limited techniques available.

Since the discovery of new and potent therapeutic agents, marine natural products research attracted the world-wide attention of scientific from various disciplines including organic chemistry, bioorganic chemistry, pharmacology and biology. Recently a number of biologically active alkaloids are involved in the clinical trials for the treatment of different human tumors at certain different levels. Origin analysis of drug discovery between 1981 and 2002 confirmed that therapeutic remedies developed from marine natural products alone stand for 28% of all chemical entities introduced at commercial and clinical used level.

Since the secondary metabolites from the natural sources were developed in the context of living systems, they are often perceived as biologically more friendly with major similarity to drugs as completely synthetic molecules, leading to be the optimal target molecules for the development of new medical therapies.

There are huge number of instances on recent development and advances in the applications of new technologies to the discovery and development of novel types of drug molecules from marine origin. In this review we have focused on the pharmacologically active marine natural products that have been shown to have biological and therapeutic potential specifically cytotoxicity and anti-cancer activity.

**Updated status of bioactive marine natural products**

Despite the advances in drug design technology, molecular biology and gene therapy, there is still a pressing need on the development of new effective drugs to counteract drug-resistant pathogens, for example, the mycobacterium that causes tuberculosis, or multi-drug resistant cancers, or even severe disease states such as Alzheimer’s which is of great concern as the age demographics of the Western World.
change. In the last five decades, the therapeutic value of marine resources has emerged that has justified the investment of time and money and optimism in this field. In the cancer research therapy, there have been valuable discoveries similarly in the field of inflammatory diseases. Probably the first marine-based drug discovery that was marketed, was in the area of intractable pain management. Previously, few selected compounds including ecteriascin 743, aplidine, didemnin B and dolastatin 10 isolated from marine tunicates and bryozoaos gained significant importance in clinical trials. Didemnin B closely related to aplidine, has previously been in clinical trials and reached as far as phase 2 as had bryostatin 1 before being withdrawn in late nineteen century.

Although a marine sponge metabolite halichondrin B has been used in clinical phase including kahalalide F from marine mollusk followed by series of biologically active compounds from a variety of marine sources, all these compounds have showed potent in vivo anticancer efficacy. Based on the evidences from the mechanisms of action of marine-based secondary metabolites, it is hypothesized that the marine world has become a major source of antimitotic agents. Cancer chemotherapy exploits differences between normal and malignant cells. Although total selectivity between the types of cells is essential, it has not been achieved to the desired levels. The high proliferation rate of cancer cells is one of the target areas when a strike is made on the selection of proper cytotoxic agents. A few selected compounds such as taxol and vincristine which act as antimitotic agents and block mitosis during cell proliferation have become the most important anticaner agents of choice.

These brief perspectives are intended to showcase several marine natural products or derivatives which are advancing through anticancer clinical trials and illustrate the great success of modern academic, industry, and government collaborations. In addition, these examples have been picked, and are generally representative of the importance of microbial processes in the generation of some of the most bioactive and potentially useful marine natural products. In fact, marine organisms including microalgae, cyanobacteria, and heterotrophic bacteria living in association with marine invertebrates such as sponges, tunicates, and soft corals, have been identified, and are suspected to be the strong and true producers of biologically active secondary metabolites from marine origin.

**Sources, collection, screening and supply of marine anticancer agents**

Marine organisms, such as algae, sponges, tunicates and bryozoans have become major elements of choice for the isolation of new anticancer agents derived from marine sources. Recent developments in scuba-diving techniques and deep-water collection techniques have played a pivotal role in the collection programs implemented by academic and pharmaceutical groups. Previously there were several hurdles and limitations on the collection of marine organisms those belonging to intertidal and shallow sub-tidal environments. However, advances in scuba diving have enabled investigators to explore shallow sub-tidal marine environments to an unlimited marine in-depth with no decompression stops. Previously collection of deep marine water used to make by dredging or trawling, and by the use of manned and unmanned subsmeribles, or by remote vehicle operation. Although, cost effectiveness can be obtained by dredging and trawling, major hurdles such as photo-shoot problems, the inability to collect organisms that grow in niches difficult to access; environmental damage and non-selective nature of the sampling have made investigators suffer from several such disadvantages. In addition, high cost efficacy of remote vehicle operations can also adversely affect.

Culture techniques on marine microbes have provided significant output on the production of large quantity of natural products. However, this approach has its own limitations such as difficulties in isolating and culturing marine microbes, the lack of stable production and new unexplored marine micro-floras. Hence, new developments are going on exploring modern techniques in order to exploit the huge range of marine floras with significant outcomes. Researches have confirmed the potential efficacy of marine bacteria to produce the compounds of interest which are not produced by terrestrial sources. In addition, studies on marine-based therapeutic compounds have become important in view of changes in the nature the studies performed with the isolated products.

A number of marine derived compounds have been tested for their biomedicinal and therapeutic potential systematically. One of the major screening systems is carried out by the National Cancer Institute of the USA which tests the target compounds in a panel
selectively against number of cancer or human tumor cell lines18. Alternative strategies employ a more mechanistic-based approach, with systems designed to screen for substances with inhibitory properties towards specific enzymatic reactions. These techniques offer specificity and focus on number of distinct drug targets. Moreover, these techniques allow the screening of crude marine drugs obtained from marine environment, avoiding the confounding effect of toxic compounds19. These screening systems are being adapted for high-throughput screening with an opportunity to readily screen huge number of compounds or crude marine extracts in order to confirm their biomedicinal and therapeutic efficacy.

**Anticancer sources/agents from marine floras**

**Marine bacteria:**

Marine bacteria are the sources of new drug discoveries and therapeutic targets which are being exploited to the certain extended levels. A number of secondary metabolites produced by marine bacteria have shown significant importance in the fields of medicine and pharmaceutical sciences and act potentially as anti-inflammatory agents (pseudopterosins, toptensins, scytomemin, and manoaolide), anticancer agents (bryostatins, discodermolide, eleutherobin, and sarcodictyin), and antibiotics (marinone). Probiosis such as lactic acid bacteria (lactobacilli and bifidobacteria) have significantly contributed to control the growth of pathogenic microorganisms through their versatile ability to produce low molecular weight biologically active antimicrobial peptide and proteins as well as considered the producer of anticancer molecules20-22. The lactobacilli supplementation rich with dietary proportions helps to reduce the induction of colon cancer23. Most of the marine animal phyla produce toxins and some studies shown that these marine toxins may be produced by marine bacteria associated to the animals24. The toxins produced by the marine bacteria of great interest in neurophysiological and neuropharmacological studies. A few bacteria found in *Noctiluca scintillans*, a free-living non-parasitic marine-dwelling species of dinoflagellate, have been found to cause red tides. The bacterial metabolite, macrolactin-A has been found to inhibit B16-F10 murine melanoma cancer cells, mammalian herpes simplex virus (types I and II), and protect T lymphocytes against the replication of human immunodeficiency virus25. Kahalalide F known as depsipeptide and isolated from the marine mollusk *Elysia rubefascens* is believed to be synthesized by microbes associated with the animal. Kahalalide F has been found to induce cytotoxicity and blocks the cell cycle in G1 phase in a p53-independent manner. Kahalalide F has been also found to exhibit antitumor activity against solid tumors with an interesting pattern of selectivity in prostate cancer cell lines26. Moreover, Kahalalide F had presented great ability of anticancer activity and has been found to inhibit breast and colon cancers *in vivo*26. Since only few selected bacteria can be isolated from marine resources under laboratory conditions, there is a growing demand to develop some new and advanced techniques to isolated variety of bacteria from marine resources in order to produce unique and novel types of therapeutic drug molecules to combat against various cancers26.

**Marine fungi:**

A huge number of marine fungi produce variety of structurally unique and biologically active secondary metabolites. A few selected *Anthracenedione* derivatives isolated from marine mangrove, endophytic fungus *Halorosellinia* sp. and *Guignardia* sp. have shown great ability to act as the potent anticancer agents27. Mathan et al.28 isolated a fungal species *Aspergillus protuberus* from marine sediments of South Indian Coast, which exhibited potent anticancer activity against Hep 2 cell lines. Marine-derived fungi are known to the great sources of structurally novel and biologically active anticancer potent, which have become an attractive target for new molecules in therapeutic drug discovery research29. Recently more interest has been focused on studying biologically active secondary metabolites from higher fungi belonging to basidiomycetes, including endophytic and filamentous fungi from marine habitats. Schiesher et al.29 reported a lignicolous fungus *Leptosphaeria oraeamaris* from marine resources which yielded leptosphaerin. Further, *L. oraeamaris* resulted in the isolation of polyketides, leptosphaerolide, *α*-dihydroquinone derivative, and leptosphaerodione30. Production of marine fungal secondary metabolites might be highly dependent on the culture conditions and the origin of the target fungal strains. As reported previously, growth of these microorganisms on various nutrient limited media may results in the production of novel...
fungal secondary metabolites with maximum yields\textsuperscript{31,32}. Moreover, fungal strains such as Acremonium sp. and Wardomyces anomalus derived from marine environment have also been found to possess potent antioxidant potential which include acrerenin A and xanthone derivative, respectively\textsuperscript{31}. Recently a marine fungal strain Microsporum sp. resulted in the isolation of biologically active molecule neoechinulin A, which has shown cytotoxic effect on human cervical carcinoma HeLa cells and induced apoptosis in HeLa cells by the expressions of p53, p21, Bax, Bcl-2, Caspase 9, and Caspase 3 proteins\textsuperscript{33}. Also it induced apoptosis by down-regulating of Bcl-2 expression, up-regulating of Bax expression, and activating the caspase-3 pathway as confirmed by western blot analysis\textsuperscript{33}. Fueled with these findings, neoechinulin A could be served as a potential target in the field of anticancer drug discovery against human cervical cancer\textsuperscript{33}. Generation of reactive oxygen species and various free radicals such as super-oxide, nitric-oxide, hydroxyl radical, per-oxy radicals, and other reactive oxygen species cause severe chronic diseases including atherosclerosis, dementia, and cancer. Antioxidants delay or prevent oxidative damage and thus they may be useful as therapeutics agents to combat against various cancers.

**Micro algae:**

Micro-algae or blue-green algae (cyanobacteria) are considered to be one of the potential organisms which can be the richest sources of known and novel bioactive compounds including toxins with reported pharmaceutical potential\textsuperscript{34}. Some of the marine cyanobacteria appear to be potential sources for large-scale production of vitamins (B complex, E) of commercial interest. A cyanobacterium Stigonema sp. has been resulted in the isolation of scytonemin, a protein serine/threonine kinase inhibitor\textsuperscript{35}, which is a yellow-green ultraviolet sunscreen pigment, known to be present in the extracellular sheaths of different genera of aquatic and terrestrial blue-green algae. Anastyuk et al.\textsuperscript{36} tested the anticancer potential of a sulfated exopolysaccharide fucoidan isolated from the brown alga Fucus evanescens which was found to exhibit anticancer effect in human malignant melanoma cell lines SK-MEL-28 and SK-MEL-5. Previously a novel fatty alcohol ester, nonyl 8-acetoxy-6-methyloctanoate was isolated from cultured marine diatom, Phaeodactylum tricornutum which was found to possess potent anticancer effect against three different cancer cell lines including human promyelocytic leukemia (HL-60), human lung carcinoma (A549) and mouse melanoma (B16F10)\textsuperscript{37}. Interestingly, nonyl 8-acetoxy-6-methyloctanoate dominantly inhibited the growth of HL-60 cancer cells as confirmed by apoptosis induction through the accumulation of DNA in sub-G1 phase and nuclear condensations in dose-dependent manner\textsuperscript{37}.

A number of cyanobacteria including Calothrix isolates have been found to inhibit the growth of a chloroquine-resistant strain of the malarial parasite, Plasmodium falciparum, as well as inhibit human HeLa cancer cells dose-dependently\textsuperscript{38}. Apratoxin A, a cyanobacterial secondary metabolite isolated from Lyngbya boultonii, known as a potent cytotoxic marine natural product has been found to exhibit cytotoxicity to adenocarcinoma cell line\textsuperscript{39}. Recently a marine compound coibamide A, isolated from Leptolyngbya has shown significant cytotoxicity against NCIH460 lung and mouse neuro-2a cells\textsuperscript{40}. A number of cyanobacterial secondary metabolites exhibit cytotoxicity which is a common mechanism of their anticancer mode of action\textsuperscript{41}. An edible brown seaweed, Cladosiphon okamuranus naturally found and cultivated in Okinawa, Japan has been resulted in the isolation of a biologically active exopolysaccharide fucoidan, being used as an additive to health foods, drinks and cosmetics. This compound has been found to induce apoptosis in human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia (ATL) cells\textsuperscript{42} and apoptosis of U937 cells via caspase-3 and -7 activation-dependent pathways\textsuperscript{43}, confirming its therapeutic potential in cancer patients. It has been well hypothesized that marine algal-based sulfated polysaccharides have been found to be potent antioxidant molecules, preventing body damage caused by oxidative stress, and contributed significantly in carcinogenesis\textsuperscript{44}. Based on the information and scientific literature available on marine algae-based sulfated polysaccharides, it is confirmed that marine algal polysaccharides can be of great medical and pharmaceutical importance to reduce the incidences of cancer formation in human\textsuperscript{44}.

**Marine actinomycetes:**

Since last few decades on drug discovery, terrestrial actinomycetes have provided major pharmaceutical resources for the development of lead
antibiotics and related bioactive secondary metabolites. However, marine actinomycetes have received only very recent attraction on drug discovery development. Recently, very highly complex marine metabolite guttingimycin, a trioxacarcin derivative was isolated from *Streptomyces* species in Mexico which further resulted in the isolation of new trioxacarcins D–F, in addition to the known trioxacarcins A–C.

Among the antibiotic-producing microbes, marine actinomycetes within the family Micromonosporaceae are very promising. These microbes are found to be potent sources of anticancer agents that target proteasome function and their industrial potential is validated by several pharmaceuticals. A novel bioactive depsipeptide, thiocoraline isolated from a marine microorganism *Micromonospora marina* has been found to inhibit RNA synthesis which is also selectively cytotoxic against lung and colon cancer cell lines as well as melanoma. In addition, thiocoraline has also been found to inhibit preferential antiproliferative effects in colon cancer cell lines with defective p53 systems. Kalinovskaya et al. isolated a novel actinobacterium of *Citricoccus* genus, (KMM 3890) from a bottom sediment sample collected from the Sakhalin shallow environment, and it was found that this actinobacterium resulted in the isolation of complexly structured compound cyclic siderophore nocardamine which exhibited potent inhibitory effects in various tumor cell lines including T-47D, SK-Mel-5, SK-Mel-28 and PRMI-7951. This was the first reported study of the genus *Citricoccus* on the isolation of anticancer compound nocardamine with confirmed antitumor activity. The chemical structures of some selected and major anticancer compounds have been given in Fig. 2.

### Anticancer mode of action of marine bioactive compounds

A plethora of scientific reports have claimed the pharmacological potential of biologically active compounds or phytochemicals, especially with increasing search of new and highly effective candidates in anticancer therapy. Bioactive chemicals as biological targets of interest have been found to be involved with inflammatory process and oncogenic transformation in mammalian cells which include alteration of cell cycle control, evasion of apoptosis,
angiogenesis and metastases\textsuperscript{48}. In addition, epidemiological studies have suggested that the daily intake of certain biological compounds can reduce the incidence of several types of cancers\textsuperscript{49}. Hence, uptake of dietary marine phytochemicals in chemoprevention may immerse as one of the most promising approaches with reduced risk of cancer development. Moreover, marine-based phytochemicals also act synergistically with other chemo-preventive agents to overcome the resistance development in cancer cells, leading to utilization of the lowest amount of cancer drugs with increased anticancer efficacy\textsuperscript{50}. As per the literature survey, approximately 80% of the world’s inhabitants rely on traditional plant derived medicines for their primary health care\textsuperscript{51}.

Based on the previous and recent researches on pharmacokinetics, a number of molecular targets have been defined through which marine secondary metabolites evoke their anticancer effects. These targets include induction of apoptotic proteins (caspases and bax), protein kinases (PKA, PKC, MAPK and TYK2), anti-apoptotic proteins (bc12, TRAF1 and survivin), growth factors (TNF, EGF, FGF and PDGF), transcription factors (Ap1, NF-\textsuperscript{k}, Nrf2 and p53), cell adhesion molecules (ICAM-1 and VCAM) and cell cycle proteins (Cyclin D, CDK1, CDK2, p27 and p21). Moreover, interference of biologically active marine secondary metabolites has also been shown with multiple cell signaling pathways\textsuperscript{52}. A marine derived fungus \textit{Aspergillus fumigatus} resulted in the isolation of a marine secondary metabolite fumigaclavine C which exhibited remarkable anticancer potential against MCF-7 human cancer cells and induced apoptosis in

Fig. 3-Mode of action of Fumigaclavine C-induced apoptosis in MCF-7 breast cancer cells through the mitochondrial pathway (Updated from Li et al., 2013).
MCF-7 cells via PI3/Akt and NF-kB signaling which lead to the activation of the mitochondrial cell death pathway. Correlation studies revealed the fact that fumigaclavine C also exerted potent anti-proliferative effect. Another marine metabolite, neoechinulin A isolated from marine-derived fungus Microsporum sp., found to exhibit cytotoxic effect on human cervical carcinoma HeLa cells and induced apoptosis in HeLa cells by the expressions of p53, p21, Bax, Bcl-2, Caspase 9, and Caspase 3 proteins. This confirmed that fumigaclavine C confer potent therapeutic efficacy for using in medicine industry in the treatment of human breast cancer. A schematic diagram of the generalized anticancer mechanisms of phytochemicals has been illustrated in Fig. 3.

**Future trends**

The development of a successful pharmaceutical and medicine requires that attention should also be given to delivery of the drug as well as supply. Since surrounding tumor tissues display sensitivity to the fatal effects of treatment anticancer molecule, it makes a great hurdle to overcome solid tumors on the anti-proliferative efficacy of anticancer drugs. This has made limitations on the use of high concentrations of test compounds. Hence, there is a great need to develop alternative tools to facilitate the specific targeting of different tumors. Polymer therapeutics not only offers improved pharmacokinetic properties, but also provides better targeting of tumor tissues with higher selectivity. The basis for this better targeting and selectivity operates by what is known as the “enhanced permeability and retention” effect (EPR) which has successfully led to use of higher concentrations of treatment anticancer drugs within the tumor in vivo.

Polymer therapeutics can be considered an ideal alternative in order to enhance the value of marine toxins. Anticancer molecules such as halichondrin B have already been well established by in vivo trials as effective agents which can be transferred intravenously to remote sites within the test animal and exhibit inhibitory effect on the growth of variety of human tumors. Significant strategies which can increase pharmacokinetic efficacy, reduce required plasma concentrations and increase selectivity can only be considered advantageous. A huge amount of research and world-wide focus is being made in order to develop new therapeutic tools of medicinal significance based on the halichondrin skeleton. A synthesized amino derivative of the halichondrin skeleton has been converted into a polymeric form and is currently being given trials to confirm its anticancer potential against various tumor cell lines.

**Conclusion**

Environmental abuse, increasing global warming, and malnutrition have a great contribution in the increase of cancer incidences. The American Cancer Society has declared that the global burden is expected to grow as 27 million new cancer cases and 17.5 million cancer deaths simply due to the growth and aging of the population by 2050. Moreover, nature has been instrumental as a source of therapeutics. Since the oceans cover more than 70% of the earth surface and the marine environment is highly diverse, it is very much likely that marine organisms would be a wonderful source of biologically active anticancer molecules in future research. Over the past decade, several new therapeutic agents derived from marine sources have entered preclinical and clinical trials. Natural derivatives play an important role to prevent the cancer incidences since synthetic drug formulations cause various harmful side effects to humans. Of the anticancer compounds isolated so far, the marine algae contribute 65.63%, the mangroves 28.12%, and the bacteria 6.25%. Owing to a diverse chemical ecology, the marine organisms especially marine flora have a great promise for providing potent, cheaper, and safer anticancer drugs, and deserve an extensive investigation.

**References**

SHUKLA & KIM: A POTENT SOURCE OF ANTICANCER METABOLITES


induces apoptosis of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. 


