Marine molluscs as a potential drug cabinet: an overview

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Marine molluscs have emerged as an important source containing numerous unique secondary metabolites which could be used for development of new drugs against many communicable and non-communicable deadly diseases. The current status of biologically active compounds extracted, identified and isolated from marine molluscs and tested for their anti-cancer, anti-inflammatory and anti-microbial activities together with important compounds isolated from them such as Dolastatin 10 & 15, Kahalalide F, Keenamide A, Spisulosine-ES-285 etc. which possess anti-cancer and Ziconotide having anti-inflammatory properties are discussed in this paper.

[Keywords: Marine molluscs, bioactive compounds, drugs]

Introduction

As many as 34 phyla, of the total 36 known animal phyla were reported from marine biosphere against 17 phyla from land. The plant components in sea contain all classes of algae (as phytoplankton and seaweeds), angiosperms (sea grasses and mangroves) and several species of fungus and an array of microbes (bacteria and virus). Marine organisms are used as nutritious foods, animal feed, ornamental and recreational items and also as potential source of marine natural products in health care since ancient times.

Benthos in the sea, live in most harsh and hostile physical environment being exposed to extreme conditions of temperature, pressure, salinity and often hypoxia and anoxia like situations. They are also susceptible to stiff competition for space, food, mate as well as microbial infections. Since there is almost no scope for physical protection to escape from different hazards, they adapt themselves through complex biochemical processes and physiological mechanisms. Synthesis of secondary metabolites is their unique ability that helps them to protect against the ill effects of environmental hazards and microbial infections and many of these are, off late found as promising source to meet the human health care demand.

Exploration and exploitation of sea based resources have witnessed a paradigm shift in recent years. The rapid increase in human population, change in their life style and climate change impacts have propelled the origin and spread of many incurable and fatal diseases like influenza, diabetes, coronary disorder, AIDS and cancer globally. Again, many disease causing pathogens became drug resistant giving rise to their mutant forms. Coupled with such threat perceptions and rapid fall in the availability of land based natural resources, the scientists have shifted their research to marine environment which has been found as the hidden ground for a plethora of bio-molecules which can be used for discovery of new drugs. In fact, over 7000 natural products have been isolated from marine organisms so far and this number is increasing with addition of new products every day. The bioactive compounds having pharmaceutical application were mainly derived from some microbes, seaweeds, ascidians and sponges. In addition, some valuable products from salmon and shark and lysate from the Limulus had also contributed significantly to the pharmaceutical industries. Several products with nutritional and pharmaceutical value such as Poly Unsaturated Fatty Acids (PUFA), β-carotene etc. are now available in the racks of medical shops and some more are in pre-clinical and clinical trials. The recent research findings have further shown that many more species of marine organisms, particularly the molluscs, stand as a prospective source of valuable bioactive compounds with great potential for new leads.

Molluscs constitute an important group of animals in marine hydrosphere representing 23% of all the organisms. Since majority of these live...
in harsh and hostile intertidal rocky shores, mudflats and shelf zone exposed to a series of physical and chemical stresses, they are susceptible to wounds and damages caused by inter-species and intra-species competitions and microbial attacks. These organisms therefore have the capability to adapt themselves and overcome different kinds of stress with the synthesis of secondary metabolites possessing immunological property and anti-microbial activity. A neurotoxin, having analgesic properties making it 1000 times more potent than morphine without any side effects isolated from gastropod Conus magus shows the uniqueness of secondary metabolites extracted from cone shells. Research findings have further delineated the presence of many other secondary metabolites in mollusces having anti-oxidant, anti-inflammatory, anti-microbial and anti-tumour properties. In fact, molluscan origin alkaloids, carotenoids and conotoxins have been tested successfully for their strong bioactive characteristics. However studies pertaining to drug-discovery from them remains under explored.

Present study consists the current status of the exploration and exploitation of bioactive compound source from marine molluscs with due emphasis to Indian coasts. It is hoped that it would provide basic insight in the field of pharmaceutical discoveries from the molluscs from the sea in order to battle with the growing health issues in new millennium.

Marine Mollusca and their significance

Phylum Mollusca were initially divided into 9 different classes of which two have been reported as extinct. Of the seven extant classes, four have been labelled as major and three as minor phyla. The four major classes include Polyclacophora (Chiton, Katharina, Mopalia etc.), Gastropoda (Conch, Cones, Snails, Cypraea sp etc.), Bivalvia (Oysters, Clams, Mussels, Scallops etc.) and Cephalopoda (Squids, Octopuses, Cuttlefish etc). The three minor classes include Monoplacophora (Neopilina), Solenogastres (Neomenia) and Caudofoveata (Chaetoderma, Limifossor). Each of the extant molluscs supports some kinds of economic activity.

Marine molluscs have been used in a variety of ways; the most common being as a source of food, ornaments and production of lime. A few species act as scavengers in cleaning the environment and pollution indicators (Mussel watch programme). Of late, many molluscan species have been identified as superior source of secondary metabolites having wide range of pharmaceutical applications. Some important compounds screened, isolated and tested for their anti-cancer, anti-inflammatory, anti-microbial properties are discussed here.

Anti-cancer compounds

Cancer is one of the very painful and deadly diseases of public concern worldwide. Cases of cancer are on stiff rise causing several deaths every year. The World Health Organization (WHO) has projected the cancer related deaths to about 12 million by 2030, while American Cancer Society has projected 27 million new cancer cases leading to 17.5 million deaths by 2050. Despite tremendous developments in science and technology, no effective medicine could be developed so far for treatment of cancer. Present cancer treatment is limited to surgical intervention that involves removal of infected body part and application of chemotherapeutic drugs and radiation therapy to destroy and inhibit the proliferation of cancer cells. None of these treatments actually cure cancer, but mostly only lengthen the lifespan of a patient. Again all these treatments, especially the chemotherapy and radiation therapy are associated with many life threatening side effects. Therefore stride has been made to trace new compounds to combat cancer. It has been established that natural products are more effective in cancer treatments with minimum side effects. According to WHO 80% of the world's population especially living in developing countries rely on plant-derived medicines. Global statistics further suggests that about 60% of drugs approved for cancer treatment are of natural origin and important among them are doxorubicin, daunomicin, bleomycin, mytomicin-C, vincristine and vinblastine. Oceans are now considered as treasure house of bioactive compounds possessing anti-cancer (anti-oxidant/cytotoxicity/anti-tumour) activity. During the last few decades, nearly 2500 new metabolites with promising anti-tumour property have been isolated and their effectiveness against cancer has been evaluated. The Food and Drug Administration (FDA) and those of the European Agency for the evaluation of medicinal products have listed 113 drugs for cancer treatment during 1940 to 2010, in which only 2.65% are of marine origin (Sawadogo et al., 2013) suggesting the need for more efforts, to utilise the marine resources in future. Marine molluscs stand as prospective candidates offering excellent opportunity to isolate anti-cancer compounds. Some important compounds of molluscan origin having convincing anti-cancer properties are given hereunder.

Dolastatin 10 and Dolastatin 15: The linear peptide Dolastatin 10 (Fig.1) and desipeptide Dolastatin 15 (Fig.2) have been isolated from the
sea hare *Dollabella auricularia* of Indian Ocean. They are found to have promising anti-cancer properties. Dolastatin 10 is a pentapeptide having four structurally unique residues viz. Dolavalin, Dolaisoleucine, Dolaproline and Dolaphenine along with Valine. It has been reported that Dolastine 10 interferes and disrupts cell division by mitosis. Dolastatin 15 on the other hand, is a seven-subunit desipeptide structurally identical to anti-tubulin agent of Dolastatin 10 that could act as anti-mitotic agent. According to Poncet (1999), both Dolastatin 10 and Dolastatin 15 were in preclinical trial to use against breast and liver cancers, solid tumours and some leukaemia. Their significant inhibition property of mitotic cell division suggested that they can effectively target cancer cells.

**Kahalalide F**: Kahalalide F (Fig.3) extracted from Hawaiian mollusca *Elysia rufescens* is found to be good anti-cancer agent. It forms the largest and most active compound among the seven Peptides (six cyclic-A to F and one acyclic-G analogue peptides) isolated from *E. Rufescens*. The Kahalalide F developed by the Spanish biopharmaceutical company Pharmamar is considered as a novel anti-tumour drug candidate, which is in phase II clinical trial. It has shown excellent anti-tumour activity in various solid tumour models including colon, breast, non-small cell lung cancers and certain prostate cancers. Kahalalide F could cause oncosis in cancer cells by lysosomal induction and cell membrane permeabilization. In addition it also inhibits the expression of certain specific genes that are involved in DNA replication and cell proliferation and thus the compound could inhibit tumour spreading and growth.

**Keenamide A**: Keenamide A is a new cytotoxic cyclic hexapeptide (Fig.4) isolated from the marine mollusc *Pleurobranchus forskalii*. It has exhibited significant activity against the P-388, A-549, MEL-20, and HT-29 tumour cell lines. Thus it could also be a potential anti-cancer biomolecule of molluscan origin.

**Spisulosine ES-285**: The Spanish Pharmamar group has isolated the potent anti-proliferative alkyl amino alcoholic compound (Fig. 5) namely Spisulosine ES-285 from the Arctic surf clam, *Spisula polynyma*. This bioactive compound has intriguing mechanisms of action which is now in the phase I clinical evaluation. The molecular target of this molluscan alkyl amino alcohol compound is Rho (GTP-bp).

**Alkaloids**: Alkaloids such as Lamellarins, a family of hexacyclic pyrrole alkaloids obtained from marine molluscs have shown promising anti-
Figure 4. Keenamide A

**Anti-inflammatory and Analgesic agents**

Inflammation is the complex biological response of vascular system which arises as the end product of oxidative stress. It is associated with over 100 different diseases such as arthritis, asthma, Alzheimer's diseases, allergies, cancer, dermatitis, heart and coronary diseases, Parkinson's diseases etc, which could occur due to physical trauma, nutritional deficiency and environmental pollution. It is usually associated with pains and other sufferings requiring immediate medication. Inflammation control in fact always remains as hot topic in pharmaceutical and medical research. The treatment regimens of an inflammatory disorder include usage of anti-

Figure 5. Spisulosine (ES-285)

*Figure 4. Keenamide A*
Research findings have shown that the venom containing predatory cone snails offer excellent opportunity for obtaining anti-inflammatory ingredients from nature. Conopeptides are small (10–35 residues) neurotoxin products derived from venoms of the Conus sp with solution structures and stabilized by a high density of cysteine residues. According to Olivera (1999), these peptides take part in the defence, prey capture and some other biotic interactions of the concerned organism. Conotoxins of the cone species are neurotoxins of low molecular weight which has pain reducing property. Livett et al. (2004) had described the cone toxins as potential anti-inflammatory natural ingredients stating that the toxins synthesized by several marine cone snails yield numerous structurally and functionally diverse compounds which can provide a wide range of therapeutic applications. They consist of a mixture of peptides of relatively short strings of amino acids and are rich in disulfide bonds. The organisms utilize these compounds in pacifying their prey before immobilizing and killing it. The first marine drug approved in the United States for the treatment of chronic pain in spinal cord injury was of molluscan origin. These toxins act at various locations in the sensory systems that mediate pain, including the periphery is spinal and higher CNS centres. The source of Prialt is marine snails of the genus Conus magus.

Ziconotide (Fig. 6) is a synthetic derivative of short peptide extracted from the venom of two predatory cone snails (Conus geographus & Conus magus) has been approved by the U.S. food and Drug Administration in December 2004, under the brand name “Prialt”. Other compounds from cone snails also are found to have great potential to be highly effective drug candidates against pain. A compound namely AVC1 isolated from Australian cone Conus victoriae proved to be effective in reducing post surgical and neuropathic pains and recovery from nerve injury.

During last decade several toxins have been identified, isolated and tested for their medicinal prospects. Based on their characteristic features these peptides have been classified into several groups. Some of the more important Conopeptides discovered and used in drug discovery exercises are given in Table-1.

The above cited examples and the discussions made thereon clearly show that marine mollusca can be an ideal source of anti-inflammatory and analgesic ingredients in future. The level of research in India in context to exploitation of marine molluscan resource to derive anti-inflammatory and some compounds is mostly limited to screening and isolation of a few peptides. Conotoxins resources of India have not been exploited satisfactorily and therefore it requires vigorous studies to exploit this wonderful peptide resource for societal benefit. Development of a new drug under the brand name Cadalmin-TM from Perna viridis against arthritis can be taken as only landmark achievement till date.

**Anti-microbial properties**

Several infectious diseases in man are caused by microbes and hence efforts have been made to develop anti-bacterial and immunological drugs to cure and prevent the occurrence and spread of such diseases in recent decades. Many of these anti-microbial drugs are produced either using the secondary metabolites isolated from organisms or synthesized in the laboratory taking cues from their biochemical and physiological functions.

The marine environment is rich with varieties of microbes. Hence the biota living there is bound
Table 1. Some analgesic Conopeptides, their sources and biological activity

<table>
<thead>
<tr>
<th>Classification</th>
<th>Conopeptides</th>
<th>Conus Species</th>
<th>Biological activity</th>
<th>Source/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>α- conotoxins</td>
<td>Vcl.1(ACV-1) RglA</td>
<td>C. victoriae C. regius</td>
<td>Targets the nicotinic acetylcholine receptors (nAChR) at nerves and muscles. Inhibitor of N-type Calcium Channel. Analgesic activity in animal pain model when delivered by injection.</td>
<td>68</td>
</tr>
<tr>
<td>μ-Conotoxins</td>
<td>μ GIIMA and GIIB μ PHI A μ TIHA μ-SmlIIA μ O-ConotoxinsMrVIA and MrVIB</td>
<td>C. geographus C. purpurascens C. tulipa C. striatus, C. kinoshitai, C. magus, C. consors, C. catus C. Marmoreus C. gloriamaris</td>
<td>Targets voltage-graded sodium channels in muscles. The mechanism of action is intrathecal and intraneuronal pain.</td>
<td>69</td>
</tr>
<tr>
<td>δ-Conotoxins</td>
<td>GVIIA δ-Conotoxin-GmVIA</td>
<td>Some molluscivorous cone snails</td>
<td>Targets the inactivation of voltage dependent sodium channels and “δ” slows the inactivation of the sodium channel</td>
<td>70</td>
</tr>
<tr>
<td>ω-Conotoxins</td>
<td>MVIIA(Prialt) CVID(AM336)</td>
<td>C. magus C. catus</td>
<td>Affects the calcium channels associated with nerve impulse transmission at the neuromuscular junction. Calcium channels are related to sensitivity to pain.</td>
<td>71</td>
</tr>
<tr>
<td>κ-Conotoxins</td>
<td>kO-conotoxin PVIIA kA- MIVA, SIVA, SIVB, mIVA, SmVB, PIVE, CcTx, and PIVE</td>
<td>Conus purpurascens</td>
<td>Inhibits voltage-graded potassium channels, resulting in tremors. Cardiac reperfusion.</td>
<td>70</td>
</tr>
<tr>
<td>Conantonkins</td>
<td>Contulakin-Gr(CGX-1160 Conantokin-G(CGX-1007)</td>
<td>Conus geographus C. tulipa C. radiatus</td>
<td>NMDA-receptor antagonists,. Targets Epilepsy, pain, stroke, Parkinson’s disease</td>
<td>72</td>
</tr>
<tr>
<td>Chi-conopeptide</td>
<td>MrIA and MrIB(Xen-2174)</td>
<td>Conus marmoreus</td>
<td>Inhibit the norepinephrine transporter</td>
<td>70</td>
</tr>
<tr>
<td>CONTRYPHANS</td>
<td>Contryphan Vn</td>
<td>C. ventricosus C. radiatus C. loroiisi C. amadis</td>
<td>Targets Calcium channels</td>
<td>73</td>
</tr>
</tbody>
</table>

Several compounds such as chlorinated acetylenes, indole alkaloids, glycoproteins and peptides exhibiting anti-microbial activity have been isolated from marine molluscs. Recent observations by Kumaran et al. (2011) delineated that two species of marine molluscs namely *Thais tissotii* and *Babylonia spirata* contain bioactive compounds, possessing strong anti-microbial properties against human pathogens *Klebsilla pneumonia*, *Proteus mirabilis*; fish pathogen *Aeromonas hydrophila*; fungal pathogens *Aspergelas niger*, *Kendida albicans* and against bio-film microbes like *Micrococcus* sp. At the same time Santhi et al. (2011) have observed the presence of secondary metabolites in deep water mollusca *Tonna galea*, that offers promising prospects for anti-microbial activity against the human pathogen *Vibrio cholerrae* and *Aeromonas hydrophila*. They (Santhi et al. 2013) have also reported the presence of anti-bacterial and anti-fungal substances in the benzyl: methanol and methanol extracts of deep water mollusca *Babylonia zeylanica* capable of inhibiting the growth of pathogens like *Salmonella typhie*, *Eschesia coli*, and *Aeromonas hydrophila*.

Bacterial and viral infections are directly linked with the immune efficiency of the susceptible organism. Anti-microbial peptides (AMPs) therefore represent the most universal immune effectors. The review of Li et al. (2013) have shown that several bivalves including *Mytilus galloprovincialis*, *M. edulis*, *M. trossolus*, *Crassostrea virginica*, *Ruditapes philippinarum* and gastropods like *Biomphalaria glabrata*, *Haliotis discus hannai*, *H. discus discus*, *H. laevigata* form important source of AMPs. As many as 69 different types of AMPs have so far been isolated from two major groups of molluscs (Table. 2) and this number can increase if
Table 2. Number of extant species of Molluscs and AMPs reported from them

<table>
<thead>
<tr>
<th>Class</th>
<th>Estimated number of species</th>
<th>Number of reported AMPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyplacophors</td>
<td>900 - 1,000</td>
<td>0</td>
</tr>
<tr>
<td>Gastropods</td>
<td>70,000-103,000</td>
<td>8</td>
</tr>
<tr>
<td>Cephalopods</td>
<td>730-900</td>
<td>0</td>
</tr>
<tr>
<td>Bivalves</td>
<td>12,000-20,000</td>
<td>61</td>
</tr>
<tr>
<td>Scaphopods</td>
<td>400-500</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: Li et al.84 (2011)

extensive research will extend to other groups.

The above cited examples explain that marine molluscs could be an important source of antibacterial substances and immunological peptides for development of new generation of therapeutic agents to battle with several infectious diseases.

**Cardiovascular protective compounds:**

Cardiovascular Diseases (CVDs) are non-communicable diseases which affect the heart, blood vessels (arteries and veins) and blood circulation. These diseases constitute one of the major causes of human death and disability in the world today. The report of WHO has estimated that human deaths due to CVDs was at 17.3 million per year in 2008 sharing about 30% of total deaths caused by various diseases. It has been shown that over 80% of CVD deaths take place in low and middle income countries and death rates due to CVD in high income countries are reasonably low. The status report of WHO “Global atlas on cardiovascular disease prevention and control” published on 19 September 2011 projected a sharp increase in human death reaching to 23.6 million per year by 2030 due to CVDs. Owing to such increasing incidences of mortality and disability by CVDs, considerable emphasis has been laid to discover and manufacture cardiovascular protective compounds with a higher potency.

With application of modern technological skill, efforts have been made to locate new cardio protective agents from marine organisms including marine molluscs. For instance, Sherief et al.87 (2004) have studied the cardio protective effects of cuttlefish (Sepia pharaonis) liver oil in isoproterenol administrating on rats. In another study, Sarvanan and Sanmugam88 (2010) have reported the presence of low molecular weight glycosaminoglycan in Amussium pleuronectus (Linn, 1758) that afforded considerable protection to the heart tissues challenged by cardio toxicity induced by isoproterenol. Two selective potassium channel blockers, namely κM-conotoxin RIIJ and κM-RIIIK has been purified from the venom of a gastropod Conus radiatus. The cardio-protective effects of κM-conotoxin RIIJ and κM-RIIIK were assessed in a male Wister rat’s heart model of ischemia/reperfusion. KM-RIIIK found to reduce the infarct size of the risk zone drastically, although it (κM-RIIJ) did not exert any apparent cardio protective action.89 These instance and presence of metabolites in cones having affinity to specific ion channels as seen in cone toxins suggests their effectiveness towards their therapeutics uses controlling the cardiovascular diseases.

**Conclusion**

Among different forms of life, molluscs form a dominant group which provides lots of socioeconomic benefits, serving as source of food, ornaments and home decorative items and shells as raw materials for lime production. In addition, several species have anti-microbial, anti-oxidant, anti-inflammatory, anti-cancer, anti-tumour properties offering tremendous opportunity to harness this resource for production of new drugs. The chemical adaptations of some species also provide best cues for synthesis of new compounds to battle many diseases. Thus marine molluscs can stand as a competent source of secondary metabolites and that could be of great therapeutic use in the new millennium.

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**References**


82. Santhi, V., Sivakumar, V., Thilaga, R. D and Boriga, J.F., Bioactive potential of Tonna galea (Linn, 1758) from Gulf of Mannar, Glob J of Pharmacol. 5:3(2011) 130-135
84. Li H., Parisi, M. G., Parrinello, N., Cammarata, M and Roch, P., Molluscan antimicrobial peptides, a review from activity-based evidences to computer-assisted sequences, ISIJ, (2011). 8: 85-97