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Bioactive compounds and nutraceuticals from marine organisms

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Marine flora and fauna and their potential use

Ocean harbors a large biodiversity of marine fauna and flora with about 5,000 species of sponges, 9,000 species of annelids, and 66,535 species of molluscs. Among the mollusks, 15,000 species of bivalves and 600 species of cephalopods have been reported to occur. Marine biotechnology is the science in which marine organisms are used in full or partially to make or modify products, to improve plants or animals or to develop microorganisms for specific uses. With the help of different molecular and biotechnological techniques, humans have been able to elucidate many biological methods applicable to both aquatic and terrestrial organisms. Only 10% of over 25,000 plants have been investigated for biological activity. The marine environment may contain over 80% of world's plant and animal species. In recent years, many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and marine organisms. The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites, many of which are endowed with pharmacodynamic properties. The deep knowledge about nerve transmission has been learnt using squid and its giant nerve axons and the mesenteries of vision have been unraveled using the eyes of horseshoe crabs, sharks and skates. The surf clam is proving an excellent model for the cell cycle and its regulation while the sea urchin is a model for understanding the molecular basis of cellular reproduction and development. The objective of this review is to highlight some of the recent developments and findings in the area of marine biotechnology with special reference to the biomedical potential of marine natural products. Therefore, this rich diversity of marine organisms represents an enormous resource for the discovery of potential compounds with valuable pharmaceutical and biomedical potential (Kamboj, 1999). These bioactive compounds belong to different chemical classes' viz., terpenoids, steroids/sterol glycosides, phenolics, amino acids, fatty alcohol esters, glycolipids etc. Of the few bio-evaluated, some showed interesting biological activity with respect to antibacterial, antioxidant, and anti-inflammatory properties. It is well known that during the past 20 years, pharmaceutical industry has been relatively successful in containing problems due to single resistant determinants.

Introduction to Marine Natural Products (MNPs)

Natural products have long been used as foods, fragrances, pigments, insecticides, medicines, etc. Due to their easy accessibility, terrestrial plants have served as the major source of medicinally

useful products, especially for traditional or folk medicine. About 25% of all pharmaceutical sales are drugs derived from plant natural products and an additional 12% are based on microbially produced natural products. The marine environment covers a wide thermal range (from the below freezing temperatures in Antarctic waters to about 350°C in deep hydrothermal vents), pressure range (1-1000 atm), nutrient range (oligotrophic to eutrophic) and it has extensive photic and non-photic zones. This extensive variability has facilitated extensive speciation at all phylogenetic levels, from microorganisms to mammals. Despite the fact that the biodiversity in the marine environment far exceeds that of the terrestrial environment, research into the use of marine natural products as pharmaceutical agents is still in its infancy. This may be due to the lack of ethno-medical history and the difficulties involved in the collection of marine organisms. But with the development of new diving techniques, remote operated machines, etc., it is possible to collect marine samples and during the past decade, over 5000 novel compounds have been isolated from shallow waters to 900-m depths of the sea.

Marine Bacteria as a Source of MNP

Microorganisms continue to be a productive and successful focus for much marine natural products research. Symbiotic marine bacteria from corals may be responsible to produce secondary metabolites displaing antibacterial properties. Zhang et al (2005) highlighted the importance of marine microbes as potential source of novel bioactive compounds. The putative probionts belonging to *Bacillus, Pseudomonas,* and *Micrococcus* sp. are the major candidate species from marine ecosystem (Gatesoupe, 1999). In marine *Pseudomonas aeruginosa* culture supernatants, two compounds of the chemical group diketopiperazines (DKPs), *viz.,* cyclo (ÄAla-L-Val) and cyclo (L-Pro-L-Tyr), respectively were identified capable of activating an N-acylhomoserine lactone (AHL) biosensor. Thallusin, isolated from marine bacterium (Japan), is a potent differentiation inducer. An analogue of the tambjamine alkaloids has been isolated from the marine bacterium *Pseudoalteromonas tunicate*. Cell-free culture supernatant of the psychrophilic aerobic bacterium *P. haloplanktis* obtained from seawater contained a diketopiperazine. The diastereoisomeric quinolinones were isolated from *P. janczewskii* derived from surface water (German Bight, Helgoland Island). Both compounds were cytotoxic to a range of human tumour cell lines, with strongly cytotoxic to SKOV-3 cells (human ovarian carcinoma). Use of fluorescent Pseudomonads as biocontrol agent was reported. Torrento and Torres (1996) reported the in vitro inhibition of *V. harveyi* by a *Pseudomonas* species isolated from the the aquatic environment. Gram *et al.* (1999) demonstrated the protection of rainbow trout administered with *P. fluorescens* AH2 when challenged with *V. anguillarum.* Another study by Smith and Davey (1993) demonstrated that bathing Atlantic salmon in a strain of *P. fluorescens* reduced subsequent mortality from stress-induced furunculosis. Specific inhibition of *V. harveyi* by *Pseudomonas aeruginosa* has been reported earlier by Torrento and Torres (1996). Pridmore et al. (1996) reported that variacin, a bacteriocin produced by *Micrococcus varians*, inhibited other Gram-positive bacteria. It was reported to be a new lanthionine-containing bacteriocin, variacin, displaying a broad host range of inhibition against Gram-positive food spoilage bacteria, has been identified from two strains of *Micrococcus varians* isolated from meat fermentations. Variacin, like lacticin 481, contains lanthionine and beta- methyllanthionine residues. El-Shafei (1997) observed that the introduction of *Micrococcus* to fungal cultures resulted in lysis and inhibition of fungal growth, and attributed this to the production of mycolytic enzymes. A *Bacillus* sp, which enables them to digest

plant cellulose since fish do not produce the enzyme was reported by Saha et al. (2006). An improvement in survival of trout was observed by means of enhanced adhesion in the intestines by *B. animalis* (Ibrahim et al. 2004).

MNPs from Marine Algae

The fact that microalgae/cyanobacteria in general and marine forms in particular are one of the richest sources of known and novel bioactive compounds including toxins with wide pharmaceutical applications is unquestionable. Among the five divisions of microalgae, studies of biomedical natural products have been concentrated on only two divisions, i.e., Cyanophyta (blue-green algae) and Pyrrophyta (dinoflagellates). Although several metabolites have been isolated from cyanophytes, most of them are isolated from fresh water species, which are cultured easily in comparison to marine organisms. Lyngbyatoxin-A and debromoaplysiatoxin are two highly inflammatory but structurally different metabolites isolated from toxic strains of *Lyngbya mausculata* collected in Hawaii, and anatoxin-a from *Anabaena ciecinalis*. Some of the marine cyanobacteria appear to be potential sources for large-scale production of vitamins of commercial interest such as vitamins of the B complex group and vitamin-E. The carotenoids and phycobiliprotein pigments of cyanobacteria have commercial value as natural food colouring agents, as feed additives, as enhancers of the color of egg yolks, to improve the health and fertility of cattle, as drugs and in the cosmetic industries. Some anti-HIV activity has been observed with the compounds extracted from *Lyngbya lagerhaimanii* and *Phormidium tenue*. More than 50% of the 100 isolates from marine sources are potentially exploitable bioactive substances. The substances tested for were either the ones that killed cancer cells by inducing apoptotic death.

MNPs from seaweeds

Seaweeds are abundant in the intertidal zones and in clear tropical waters. However, they have received comparatively less bioassay attention. Seaweeds, popularly known as green algae, are widely distributed in both inter-tidal and deep-water regions of the seas. These seaweeds are of immense pharmaceutical and agricultural value. A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids, have been reported from various seaweeds (Blunt, Copp, Munro, Northcote, & Prinsep, 2006), amongst which terpenoid compounds represent a major share. For example, Caulerpa brownii from Australia was reported to yield a number of bioactive novel diterpenoids and terpenoid esters (Handley & Blackman, 2005). Capisterones A and B are triterpenesulphate esters that were isolated from the tropical green alga, Panicillus capitatus, and were found to exhibit potent antifungal activity against the marine algal pathogen Lindra thallasiae (Puglisi, Tan, Jensen, & Fenical, 2004). Monocyclic diterpenes have been purified from the Tasmanian green alga Caulerpa trifaria (Handley & Blackman, 2000). The green alga, Caulerpa racemosa, was reported to yield a bioactive sesquiterpene acid (Anjaneyulu, Prakash, & Mallavadhani, 1991). Halitunal, a novel antiviral diterpene aldehyde has been isolated from the marine alga, Halimeda tuna (Koehn, Gunasekera, Neil, & Cross, 1991). 2-Hydroxy-10-methylzeatin has been purified from seaweeds, NIO-143, and the absolute configuration of the said cytokinin has been determined by spectroscopic procedures (Farooqi, Shukla, Shukla, & Bhakuni, 1990). Kahalalide F, a cytotoxic, antiviral and antifungal cyclic depsipeptide, was isolated from a Hawaiian species of *Bryopsis* sp. (Hamann & Scheuer, 1993). A method to purify labdane diterpenois as major constituents of

dichloromethane-soluble fraction green alga *Ulva fasciata* has been illustrated. Antimicrobial assay showed that the compounds labda-14-ene-3a,8a-diol (ULV2) and labda-14-ene-8a-hydroxy-3-one (ULV4) were inhibitory to the growth of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* with minimum inhibitory concentrations of 30 ìg/ml by ULV2, and 40 ìg/ml by ULV4, respectively against the former and 30 ìg/ml by ULV2, and 80 ìg/ml by ULV4, respectively, against the latter (Chakraborty et al., 2010). Two new guaiane sesquiterpene derivatives, guai-2-en-10a-ol (G1) and guai-2-en-10a-methanol (G2), were chromatographically purified as major constituents of the $CHCl_3/CH_3OH$ (1:1, v/v) soluble fraction of *Ulva fasciata*. Acetylation of G2 furnished guai-2-en-10a-methyl methanoate (G3) with acetyl group at C_{11} position. Compounds G2 and G3 exhibited significant inhibition to the growth of *Vibrio parahaemolyticus* with minimum inhibitory concentrations of 25 and 35 mg/mL, respectively (Chakraborty et al., 2010). The antiinflammatory agent produced by *Ulva lactuca* was identified as 3-O-b-glucopyranosylstigmasta-5,25-diene (Awad, 2000). A survey of the metabolites of U. lactuca led to the proposal that 4-hydroxybenzoic acid is the most likely biosynthetic precursor of 2,4,6-tribromophenol, an antibacterial compound (Flodin & Whitfield, 1999). Two new antimicrobial terpenes, taxifolione and 7,7-didehydro-6-hydroxy-6,7-dihydrocaulerpenyne, were purified from Caulerpa taxifolia, a tropical green alga from Cap Martin, France (Guerriero et al., 1993). Neomeris annulata, from Kwajalein Atoll, was reported to possess three brominated sesquiterpenes, shown to deter fish feeding (Paul, Cronan Jr., & Cardellina II, 1993).

Metabolites from molluscs

More than 2600 scientific studies over the last 20 years testify to the important contribution of toxins extracted from marine mollusks to medicine and cellular biology. To date, only 100 out of a potential 50,000 toxins have been extracted and analyzed. The *Conus* species have evolved deadly nerve toxins and small, conformationally constrained peptides of 10-30 amino acids. Some of the conotoxins block channels regulating the flow of potassium or sodium across the membranes of nerve or muscle cells; others bind to N-methyl-D-aspartate receptors to allow calcium ions into nerve cells; and some are specific antagonists of acetylcholine receptors responsible for muscle contraction. Thus, conotoxin are valuable probes in physiological and pharmacological studies. Bivalve mollusks and cephalopods are widely used in different parts of the world for various studies, but only recently they have been recognized as potential sources for bioactive compounds. Preliminary studies indicated marine bivalves and cephalopods as rich sources of structurally diverse compounds with antibacterial potential (Chandran *et al.,* 2009). In the marine environment, where all surfaces are constantly exposed to the threat of surface colonisation, invertibrates like bivalve mollusks and cephalopods remain relatively free of biofouling. It is apparent that these sedentary organisms control fouling epibionts by effective antimicrobial mechanisms (Tincu & Taylor, 2004). Therefore these marine invertebrates appeared to offer a source of potential antimicrobial compounds (Bansemir *et al*., 2006; Mayer *et al.,* 2007). There is evidence that bivalve mollusks are useful in the treatment of inflammatory joint diseases (Couch *et al.,* 1982; Miller *et a*l., 1993). Nonsteriodal anti-inflammatory drugs (NSAIDs), viz., aspirin and ibuprofen, are often used for inflammatory conditions. However, most of these medications can produce the unfortunate side effects, which may lead to stomach ulcer if taken frequently. Therefore exploring the bivalve mollusks for their anti-inflammatory and antioxidant activities and development of product therefrom may significantly reduce adverse side effects resulting from taking NSAIDs. There are reports of dried flesh of the New Zealand mussel

Perna canaliculus possessig polyunsaturated fatty acids (PUFAs) with possible anti-inflammatory effects (Croft, 1979; Zwar, 1994; Gibson & Gibson, 1981). The anti-inflammatory, antioxidant, and anti-prostaglandin activities were reported in green lipped mussels of New Zealand (Couch *et al.,* 1982; Miller *et a*l., 1993). Neosurugatoxin isolated from *Babylonia japonica* is useful in characterizing two classes of acetylcholine receptors. Dolastatin, a cytotoxic peptide from *Dolabella auricularia* is an antineoplastic substance. Ulapualide-A, a sponge-derived macrolide isolated from the nudibranch *Hexabranchus sanguineus* exhibits cytotoxic activity against L 1210 murine leukemia cells and antifungal activity, which exceeds that of clinically useful amphotericin-B. Chromodorolide-A isolated from *Chromocloris cavae* exhibits *in vitro* antimicrobial and cytotoxic activities. Onchidal from *Onchidella bieyi* is a useful probe for identifying the active site residues that contribute to binding and hydrolysis of acetyl cholinesterase. A team from the University of Melbourne extracted the conotoxin from a cone-shell snail. It not only inhibits pain as being 10,000 times more powerful than morphine, but also accelerates the recovery of injured nerves. The absolute stereochemistries of membrenones A–C, -dihydropyrone-containing polypropionates isolated from the skin of the Mediterranean mollusc *Pleurobranchus membranaceus*, have been determined by stereocontrolled syntheses of the enantiomers. The first synthesis of siphonarin-B has confirmed the absolute stereochemistry of the metabolite isolated from the molluscs *Siphonaria zelandica* and *S. atra*. Bursatellanin-P, a 60-kDa protein was purified from the purple ink of the sea hare *Bursatella leachii*. The protein exhibited anti-HIV activity. The first total syntheses of aplyolides B–E, ichthyotoxic macrolides isolated from the skin of sea hare *Aplysia depilans*, have been reported confirming the absolute stereochemistry reported for the metabolites. Cephalopods, gastropods, and bivalve mollusks constitute a major share of marine fauna, and were reported to possess structurally diverse anti-stress metabolites with respect to antibacterial, antioxidant, and anti-inflammatory properties (Chandran *et al.* 2009). A product (Cadalmin™ GMe) developed by CMFRI containing 100% natural anti-inflammatory ingredients was prepared from green mussel *Perna viridis* to combat joint pain and inflammatory diseases (Chakraborty *et al.,* 2010a; Chakraborty *et al*. 2010b). Polysaccharides, lysolecithin, and phenolic components in *P. viridis* were found to competitively inhibit inflammatory COX and LOX in an inflammation and oxidative stress reaction (Chakraborty et al., 2010b).

Metabolites from Sponges

Approximately 10,000 sponges have been described in the world and most of them live in marine waters. A range of bioactive metabolites has been found in about 11 sponge genera. Three of these genera (*Haliclona*, *Petrosia* and *Discodemia*) produce powerful anti-cancer, anti-inflammatory agents, but their cultivation has not been studied [61]. The discovery of spongouridine, a potent tumor-inhibiting arabinosyl nucleoside in Caribbean sponge *Cryptotethia crypta*, focused attention on sponges as a source of biomedically important metabolites. The compound manoalide from a Pacific sponge has spawned more than 300 chemical analogs, with a significant number of these going on to clinical trials as anti-inflammatory agents. An aminoacridine alkaloid, dercitin, has been isolated from the deep-water sponge, *Dercitus* spp. that possesses cytotoxic activities in the low nanomolar concentration range and in animal studies, prolongs the life of mice-bearing ascitic P388 tumours, and is also active against B16 melanoma cells and small cell Lewis lung carcinoma. Halichondrin-B, a polyether macrolide from Japanese sponge *Theonella* spp., has generated much interest as a potential anticancer agent [14,63]. The theopederins are structurally related to

mycalamide-A from marine sponge, *Mycale* spp. collected in New Zealand and onnamide-A from marine sponge, *Theonella* spp. collected in Okinawa, which show *in vitro* cytotoxity and *in vivo* antitumour activity in many leukemia and solid tumour model systems. Isoquinolinequinone metabolite cribostatin from the Indian Ocean sponge *Cribrochalina* spp. shows selective activity against all nine human melanoma cells in National Critical Technologies (NCT) panel. Spongstatin, a macrocytic lactone from the Indian Ocean collection of *Spongia* spp., is the most potent substance known against a subset of highly chemoresistant tumour types in the NCT tumour panel. Two new -pyrones (herbarin) along with a new phthalide, herbaric acid, were isolated from two cultured strains of the fungus *Cladosporium herbarum* isolated from the sponges *Aplysina aerophoba* and *Callyspongia aerizusa* collected in the French Mediterranean and in Indonesian waters, respectively.

Conclusions

"Poison kills the poison," the famous proverb is the basis for researchers in finding the biomedical metabolites from living organisms. Sea has got plenty of metabolites and other resources in living or dead form. Sponges (37%), coelenterates (21%) and microorganisms (18%) are the major sources of biomedical compounds followed by algae (9%), echinoderms (6%), tunicates (6%), molluscs (2%) bryozoans (1%), etc. The main emphasis is given in the search of drugs for deadly human diseases as cancer and AIDS. The scientists at different parts of the world have extracted various drugs for such diseases in recent years.

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