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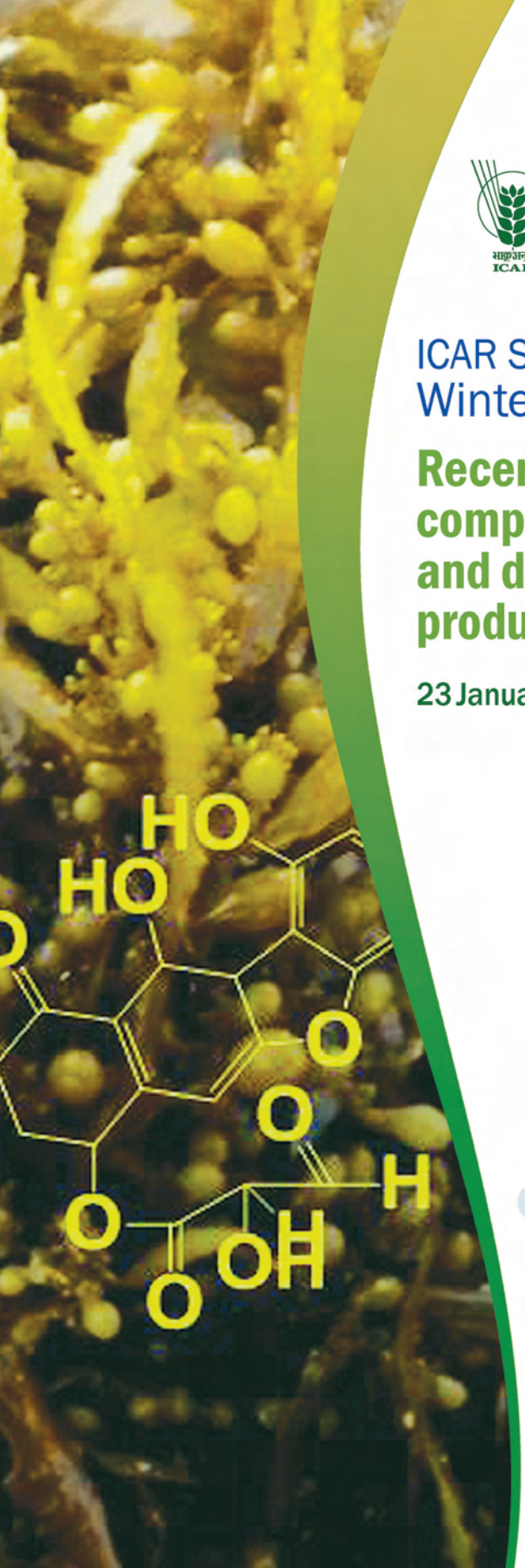
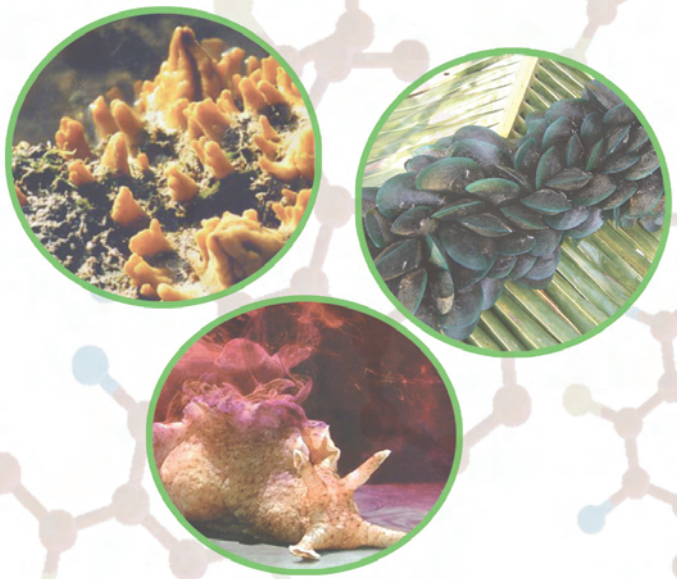


ICAR Sponsored Winter School on

Recent advances in bioactive compounds from marine organisms and development of high value products for health management

23 January to 12 February 2018



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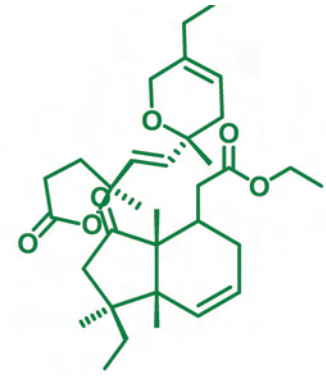

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Course Manual

ICAR-Winter School on

Recent advances in bioactive compounds from marine organisms and development of high-value products for health management

CMFRI Training Manual Series No. 13/2018
ICAR-Central Marine Fisheries Research Institute,
23 January - 12 February, 2018

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Edited by Foxit Reader

FOREWORD

There has been a growing interest in the marine derived bioactive compounds in the recent years, and the functional foods, enriched with natural ingredients have been proved to provide beneficial action for human health. Marine derived bioactive components and the functional food ingredients demonstrated to possess potential health benefits. High value secondary bioactive metabolites from the marine organisms are attracting attention because of the growing demand for new compounds of 'marine natural' origin, having potential applications in pharmaceutical fields, and concerns about the adverse effects by synthetic drugs and their derivatives. The pioneering R & D works at ICAR-Central Marine Fisheries Research Institute on marine bioprospecting envisaged a systematic approach involving chemical profiling of major species of marine organisms for bioactive pharmacophore leads for activity against various diseases, and a library of molecules with bioactive potential. The research work in this institute developed protocols to prepare various pharmaceutical leads, nutraceuticals/ functional food supplements enriched with lead molecules with different properties against various drug targets for use against various life-threatening diseases.

ICAR-Central Marine Fisheries Research Institute is the pioneering marine research institute in India to work in the frontier area of bioactive molecule discovery from marine organisms as promising therapeutic agents against various diseases, aquatic food product technology, and development of high value products for health management. This prestigious research institute of Indian Council of Agricultural Research is working in the broad national interest of producing high value bioactive leads from the marine organisms, which would provide promising therapeutic agents against various diseases. This institute has developed and commercialized the nutraceutical products Cadalmin™ Green Algal extract (Cadalmin™ GAe) and Antidiabetic extract (Cadalmin™ ADe) as green alternatives to synthetic drugs to combat rheumatic arthritic pains and type-2 diabetes, respectively to a leading biopharmaceutical company in India. The anti-inflammatory nutraceutical Cadalmin™ Green Mussel extract (Cadalmin™ GMe) from Asian green mussel Perna viridis has been commercialized with Amalgam Group of Companies. Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) has been developed from seaweeds to combat dyslipidemia leading to obesity, and the product was out-licensed to a leading Indian MNC in wellness and obesity management. Antimicrobial therapeutic product from marine bacteria as oral applicant has been developed and the product is in pipeline for commercialization. Seaweed-derived natural template inspired synthetic derivatives as potential pharmacophores were designed and developed. Several nutraceutical and cosmeceutical products from marine organisms are in pipeline, and are being commercialized.

The objective of the National level ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is to provide up-to-date information and acquaint the participants with the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance from marine organisms, general and advanced methods of isolation procedures by chromatography, classification of organic compounds and their characterization by advanced spectroscopic experiments. This program further aims to give exposure to the chemical perspectives of marine organisms, primary and secondary bioactive metabolites from fish and marine organisms to develop bioactive compounds and high-value functional food products. Theory and practical classes will be conducted in these areas to provide the participants a hands-on experience.

This ICAR Winter School is organized with the full funding support from ICAR, New Delhi, and the twenty-five participants from various parts of India who are attending this programme were selected after scrutiny of their applications based on their bio-data. They are serving as academicians, such as Professors/Scientists, and in similar posts. The faculties include the knowledgeable scientists and professors from various parts of India and abroad. This training will enable the participants to efficiently carry out their academic programmes, and to plan research on bioactive molecule discovery in their respective laboratories and institutes so that they can formulate the strategies for research.

The Winter School on "Recent advances in bioactive compounds from marine organisms and development of high value products for health management" is very ideal for the current scenario of increasing lifestyle diseases and human health. Understanding the importance of natural products in the health care system of India, ICAR-Central Marine Fisheries Research Institute has reasonably contributed in the various aspects. The Manual released on this occasion covers all aspects of marine natural products prepared by the experts in their respective fields. I congratulate the Course Director of this programme, Dr. Kajal Chakraborty and Head of the Marine Biotechnology Division, Dr. P. Vijayagopal, along with other staff members of Marine Biotechnology Division and Central Marine Fisheries Research Institute for their sincere efforts in bringing out the manual in time, and to arrange the programme in a befitting manner.

A. Gopalakrishnan
Director, ICAR-Central Marine Fisheries Research Institute
Kochi, Kerala

PREFACE

Marine-derived bioactive components and the functional food ingredients with potential health benefits are an emerging area of research. The rich diversity of flora and fauna in the marine and coastal habitats of the Indian subcontinent represent an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use. Considering the underutilization of these groups of marine organisms, exploring bioactive compounds and development of any biologically useful products have benefits as health products. Comprehensive analyses demonstrated that during the last decade the average proportion of bioactive compounds among the new compounds is declining, though there are a large number of marine natural products yet to be explored. This may indicate that the research level of bioactivity is not keeping up with the discovery of new compounds. Thus, the research tools and methods for finding bioactivity need to be improved. The first improvement is about methods of spectral and bioactivity-guided separation and purification of marine-derived secondary metabolites, which combine the discovery of new compounds. These improvements in technology are dependent upon the automation in spectroscopy, which also allows the study of the functions of new compounds extracted from the target marine organisms. Second, for the discovery of new lead compounds and artificial intelligence for drug development evolved to a more mechanistic approach that targets specific molecular lesions. Combined with high-throughput screening through a large number of drug targets, bioactivity research against various life-threatening diseases will be effective in revealing the potentially useful biological properties of marine natural products. Furthermore, the discovery of new bioactive compounds from marine metabolites will form the basis for new drug leads. Thus, the new compounds will absolutely compose an abundant resource for future bioactivity research and drug development. Various medicinal and biomedical products from marine flora and fauna provide a myriad of benefits for human health and multiple life-threatening diseases, and therefore, are the attractive options for the food and pharmaceutical industry. The increasing interest in marine-based functional food ingredients and nutraceutical formulations in the last decade along with increased number of patents filed/granted have appropriately demonstrated the possibilities of bioactive from marine organisms to maintain and improve human health and well-being.

The present ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is designed to acquaint the participants with the advances in marine bioactive compounds with emphasis on the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance. The course is planned in such a way that it covers both theoretical and practical aspect of recent advances in bioactive compounds from marine organisms. This programme will strengthen the knowledge of participants with regard to

the general and advanced methods of isolation procedures by chromatography, and their characterization by advanced spectroscopic experiments aspects.

I wish to thank the Education Division of Indian Council of Agricultural Research for giving us an opportunity to organize this ICAR Winter School. We are grateful to Dr. A. Gopalakrishnan, Director, ICAR-Central Marine Fisheries Research Institute, for his guidance, continuous interest in the course and providing all necessary facilities. I am highly obliged to Dr. P. Vijayagopal, Head, Marine Biotechnology Division for his guidance and support for the programme. All the scientists of Marine Biotechnology Division, technical staff, supporting staff and research scholars supported us in organizing the ICAR Winter School. I recall with gratitude the marvellous effort and help in preparing this manual by Minju Joy, Research Scholar of Marine Biotechnology Division. I take this opportunity to thank all the faculty members who have devoted their valuable time and contributed material for the preparation of the manual. I am confident that the Course Manual would aid the participants to enhance their knowledge and competence in the area of marine bioactive compounds and their applications for the development of high-value products for health management.

Kajal Chakraborty
Course Director

January, 2018

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CHAPTER

03

MARINE ORGANISMS-TREASURE HOUSE OF VALUABLE PRODUCTS AND THEIR CHEMICAL PERSPECTIVES

Kajal Chakraborty, Minju Joy, Soumya Salas, Soumya Krishnan

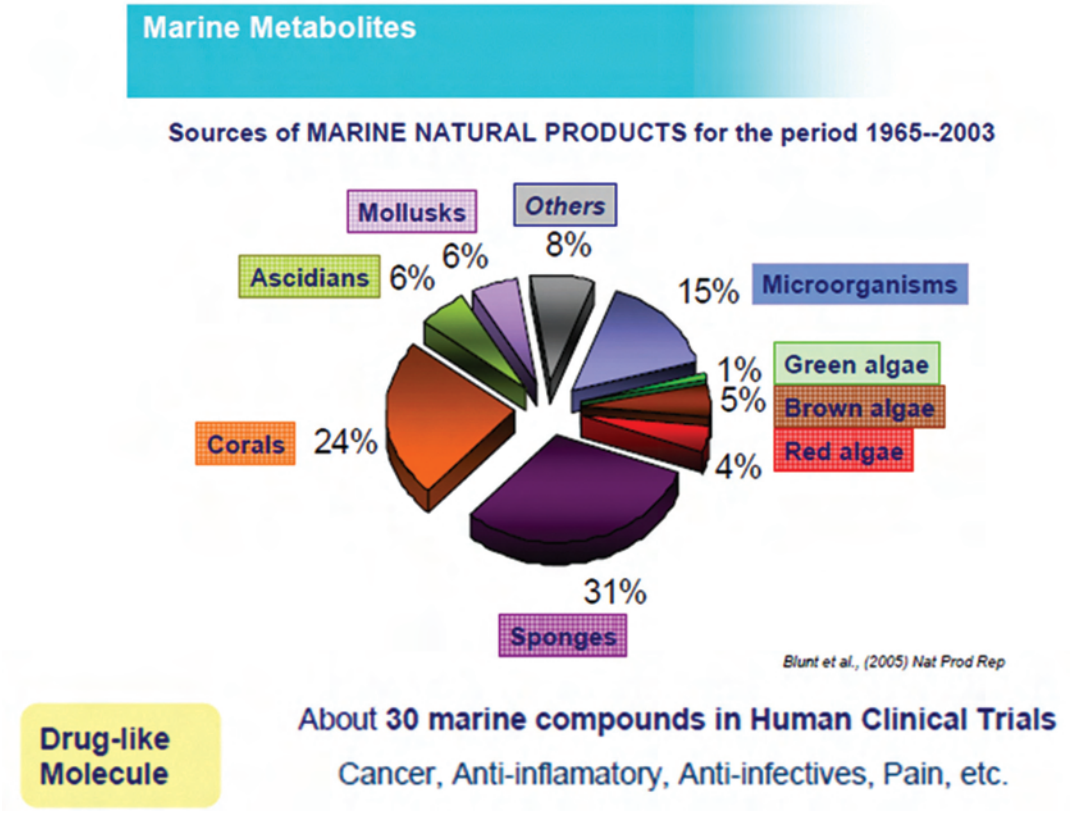
Bioprospecting Section of Marine Biotechnology Division,
ICAR-Central Marine Fisheries Research Institute, Kochi

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 speciation at all phylogenetic levels, from unicellular microorganisms to mammals. New metabolites from marine organisms have resulted in the isolation of more or less 10,000 metabolites, many of which are endowed with pharmacodynamic properties. Considering its great taxonomic diversity, investigations related to the search of new bioactive compounds from the marine environment can be seen as an almost unlimited field. Of note is that the chemical compounds with pluralities of bioactive properties are present in the marine organisms as an adaptive mechanism to survive against the extreme stress factors in the oceanic ecosystems, which cannot be found in terrestrial organisms.

The rich diversity of marine organisms represents an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use, and there is considerable potential for exploitation of these compounds as functional food ingredients. Marine derived bioactive components and the functional food ingredients with potential health benefits demonstrated to possess advantageous as functional food with added health benefits.

The marine fishes are rich natural resources of biologically active compounds such as long chain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and proteins with high biological value, whilst marine bacteria, sponges, and macroalgae contributed as potential reservoirs of bioactive secondary metabolites such as terpenoids, steroids/sterol glycosides, phenolics, amino acids, fatty alcohol esters, glycolipids etc as natural antioxidants, anti-inflammatory, and antimicrobial compounds. 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 functional food ingredients and naturally occurring bioactive substances with defined health benefits can potentially be derived from the marine micro/macroalgae, microorganisms, sponges, tunicates, and molluscs etc. all of which contain their own unique set of biologically active molecules. Macroalgae are a source of biologically active phytochemicals with beneficial use in healthcare. The marine algae can be considered as natural bioreactors, and were demonstrated to produce pluralities of

bioactive molecules, which is an appealing attribute to the functional food industry. The balanced n-3 to n-6 polyunsaturated fatty acid ratio of macroalgae adds to their efficacy as dietary supplements. Resolvins are novel families of anti-inflammatory mediators formed from n-3 polyunsaturated fatty acids EPA and DHA, termed E- and D-series resolvins. These mediators may explain many of the anti-inflammatory actions of omega-3 fatty acids. As photosynthetic organisms, marine algae play a key role in the productivity of marine ecosphere and constitute the basis of the marine food web. The majority of bioactive marine molecules have been isolated from benthic species such as sponges, echinoderms, polychaetes, bryozoans, ascidians, mollusks and cnidarians including the microbial diversity in the marine environment. Several studies have demonstrated that the marine living surfaces represent an environment rich in epibiotic microorganisms that produce bioactives. Many of these marine microorganisms can be easily cultured and manipulated in bioreactors and, therefore, represent an excellent renewable source of biologically active compounds. The marine-derived bioactive molecules therefore have recognized applications against myriad of human ailments.

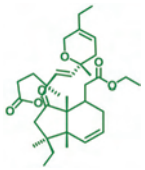


(Source: Marine Literature Database, Vpc 14.3, June, 2007)

Various nutraceutical or functional food formulations, pharmaceutical and biomedical products from marine flora and fauna including the fishery by-products provide a myriad of benefits for human health and multiple life threatening diseases, and therefore, are the attractive options for the food and pharmaceutical industry. It is of note that the effect of the bioactive compounds in the functional food or nutraceutical products may be invisible towards the health parameters over relatively short periods. However, these ingredients contribute significantly to health when they are consumed throughout life as part of the daily diet. The health promoting effects of marine-derived naturally occurring bioactive ingredients are preventive, fundamentally differentiating them from curative drugs with multiple adverse effects on human well-being. The increasing interest in marine-based functional food ingredients and nutraceutical formulations as evidenced by the scientific papers published in the last decade correlating diet and human health have shown the possibilities of bioactive compounds and nutritional elements from marine organisms to maintain and improve human health and well-being.

NATURAL BIOLOGICALLY ACTIVE COMPOUNDS 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 alga *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.

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 et al., 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 triterpene sulphate 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 et al., 2004). Monocyclic diterpenes have been purified from the Tasmanian green alga *Caulerpa trifaria* (Handley and Blackman, 2000). The green alga, *Caulerpa racemosa*, was reported to yield a bioactive sesquiterpene acid (Anjaneyulu et al., 1991). Halitunal, a novel antiviral diterpene aldehyde has been isolated from the marine alga, Halimeda tuna (Koehn et al., 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 et al., 1990). Kahalalide F, a cytotoxic, antiviral and antifungal cyclic depsipeptide, was isolated from a Hawaiian species of *Bryopsis* sp. (Hamann and 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*

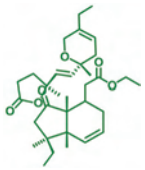
parahaemolyticus and *Vibrio alginolyticus* with minimum inhibitory concentrations of 30 ìg/ml by ULV2, and 40 ìg/ml by ULV4, respectively against the former and 30 ìg/ml by ULV2, and 80 ìg/ml by ULV4, respectively, against the latter (Chakraborty et al., 2010). Two new guaiane sesquiterpene derivatives, 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 and 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 et al., 1993). A product containing anti-inflammatory and antioxidant principles from seaweeds (Cadalmin™ GAe) for use against inflammatory disorders with an ecofriendly "green" technology has been developed by CMFRI. The product know-how has been patented (Chakraborty et al., IP 2064/CHE/2010), and is under commercialisation. The active ingredients in Cadalmin™ GAe also suppress the build-up of uric acid in hyperuricemic patients.

GREEN ALGAE

The green seaweed *Ulva fasiata* is known to produce a novel Sphingosine derivative which has been found to have antiviral activity (Lahaye, 1996; , Leiro et al., 2007). A polysaccharide isolated from the green marine algae Ulva lactuca exhibited dose-dependent, strainspecific and selective inhibition of the viral reproduction (Ivanova et al., 1994). Work on Caulerpa racemosa (Zhanjiang coastline, China), previously the source of caulerpin and two related caulerpin derivatives (Liu et al., 2012) led to the discovery of two prenylated para-xylenes caulerprenylol A (44) and B (45) that were each weakly antifungal (Liu et al., 2013). Interesting results were uncovered from the screening and careful bioassay-guided analysis of a collection of Floridian marine eukaryotic algae using an ARE-luciferase reporter gene assay that led to the detection and isolation of three monounsaturated fatty acids 46-48 from Ulva lactuca as activators of the ARE response. Each contained the identical D7,9-keto motif (wang et al., 2013)

BROWN ALGAE

The number of new compounds characterised in 2013 from the Ochrophyta was dominated by terpenoid chemistry. Based on the in vitro cytotoxicity of a crude *Dictyota*

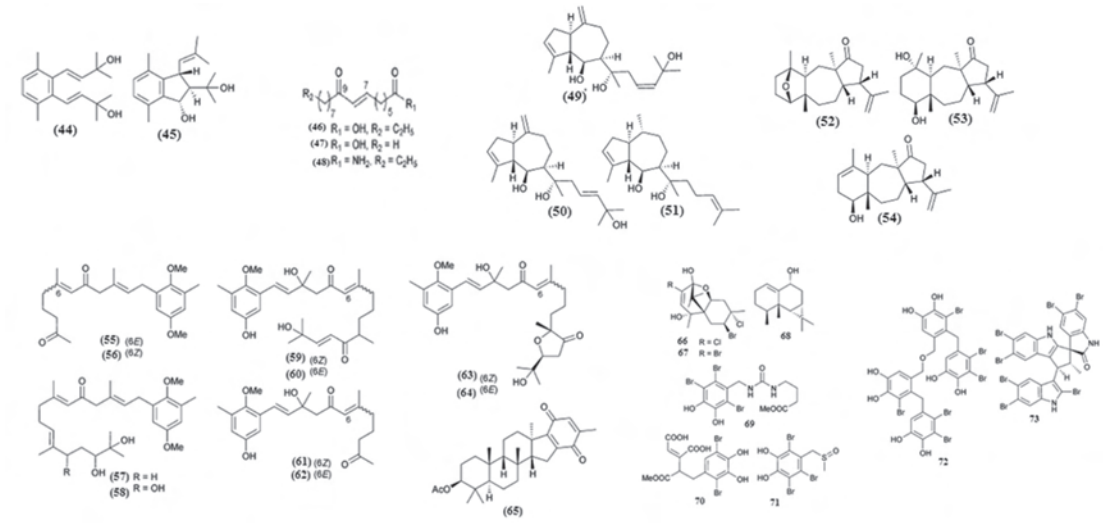


dichotoma (Abu-Bakr, Red Sea, Egypt) extract an investigation was mounted and three new diterpenoids (Z)-pachydictyol B 49 , (E)-pachydictyol B 50 and pachydictyol C 51 were characterised along with the known pachydictyol A (Hirschfeld et al., 1973) and several other well-known brown algal metabolites (Abou-El-Wafa et al., 2013). Re-investigation of *Dilophus spiralis* (Elafonissos Is., Greece) resulted in the isolation of three new dolastanes 52-54 and five previously reported perhydroazulenes. The cytotoxic meronorse squiter penoids cystoazorone A 55 and B 56 and meroditer penoids cystoazorol A 57 and B 58 were isolated from *Cystoseira abies* - marina (Mosteiros, Sao Miguel Is., Azores) (Gouveia et al., 2013) while a series of meroditerpenoids cystodione A-F 59-64, all with strong antioxidant properties in the ABTS assay, were isolated from *Cystoseira usneoides* (Gibraltar Strait) (de los et al., 2013). The mildly antiproliferative meroditerpenoid zonaquinone acetate 65 was obtained from a Jamaican *Stypopodium Zonale*. Other known brown algal metabolites were co-isolated and these included flabellinone (Sabry et al., 2005) not previously identified in *S. zonale*, stypoldione (Gerwick et al., 1979) and sargaol (Numata et al., 1992)

RED ALGAE

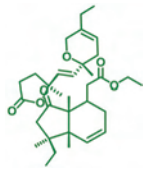
The nine new compounds reported from red algae in 2013. The chamigrane sesquiterpenes yicterpene A 66 and B 67 were isolated from *L. composita* (Pingtan Is., China) (Li et al., 2013). Of the 7 compounds isolated from *L. similis* (Sepanggar Is., Kota Kinabalu, Sabah), ent-1(10)-aristolen-9b-ol 68 was claimed as an enantiomer of a known compound (Kamada et al., 2013, Shide et al., 1987). Two bromophenols 69 and 70 with radical scavenging activity were obtained from *Symphyocladia latiuscula* (Qingdao, Shandong Province, China) (Xu et al. 2013). This same collection of *S. latiuscula* also provided the weakly antifungal bromophenol sulfoxide 71.

One new 72 and three known bromophenols isolated from *Vertebrata lanosa* (Oldervik, Ullsfjorden, Norway) had cellular antioxidant activities, the first time this activity has been reported for this class of compounds (Olsen et al., 2013). The unprecedented polybrominated spiro-trisindole similisine A 73 and its enantiomer similisine B were obtained from *Laurencia similis* (S. China Sea) (Sun et al., 2013). Five known bromophenols from a variety of red algae had inhibitory activity against glucose 6-phosphate dehydrogenase, this being the first report of such inhibitors from red algae (Mikami et al., 2013). Analysis of the metabolite compositions of seasonal collections of *Graciliaria gracilis* (Lesina Lagoon, S. Adriatic Sea, Italy) led to the proposition for using *G. gracilis* as a multi products source for biotechnological, nutraceutical and pharmaceutical applications. Bioactive metabolites isolated from Asparagopsis taxiformis were found to have little potential for therapy services to fish infected with Streptococcus iniae (Mata et al., 2013). The aqueous extracts of the red seaweed *Hypnea musciformis* were capable of inhibiting the replication of HSV-1 suggesting that the main effective components in these extracts could be polysaccharides (Rhimou et al., 2010).



BIOACTIVE METABOLITES FROM MOLLUSKS

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 produce deadly nerve toxins. 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 bioprospecting, but only recently they have been recognized as potential sources for bioactive compounds. In the marine environment, where the animals are constantly exposed to the threat of biofouling, mollusks remain relatively free of biofouling, due to the ability of these sedentary organisms control fouling epibionts by effective antimicrobial mechanisms (Tincu and Taylor, 2004; Bansemir et al., 2006; Mayer et al., 2007). Preliminary studies indicated marine bivalves and cephalopods as rich sources of structurally diverse compounds with antibacterial potential (Chandran et al., 2009). There is evidence that bivalve mollusks are useful in the treatment of inflammatory joint diseases (Couch et al., 1982; Miller et al., 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 and 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 al., 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 of researchers 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).

The extract from Indian green mussel (*Perna viridis*) has earlier been found to be active against influenza, herpes and hepatitis viral strains (MitraDebasis, 2004). Compounds isolated from molluscs were reported to be used in the treatment of rheumatoid arthritis and osteoarthritis (Chellaram and Edward 2009a). A study conducted by Przeslawski et al., (2005) has shown that intertidal gastropod eggmasses contain (MAAs) mycosporine-like aminoacids that absorb potentially damaging ultraviolet radiation. The study conducted by Benkendorff (2013) confirmed the presence of brominated indoles with anticancer activity in extracts from the egg masses of the Australian mollusc *Dicathais orbita*. Dye secretion (Baker and Duke, 1976) and extracts from the hypobranchial (Cooksey, 2001) and reproductive glands

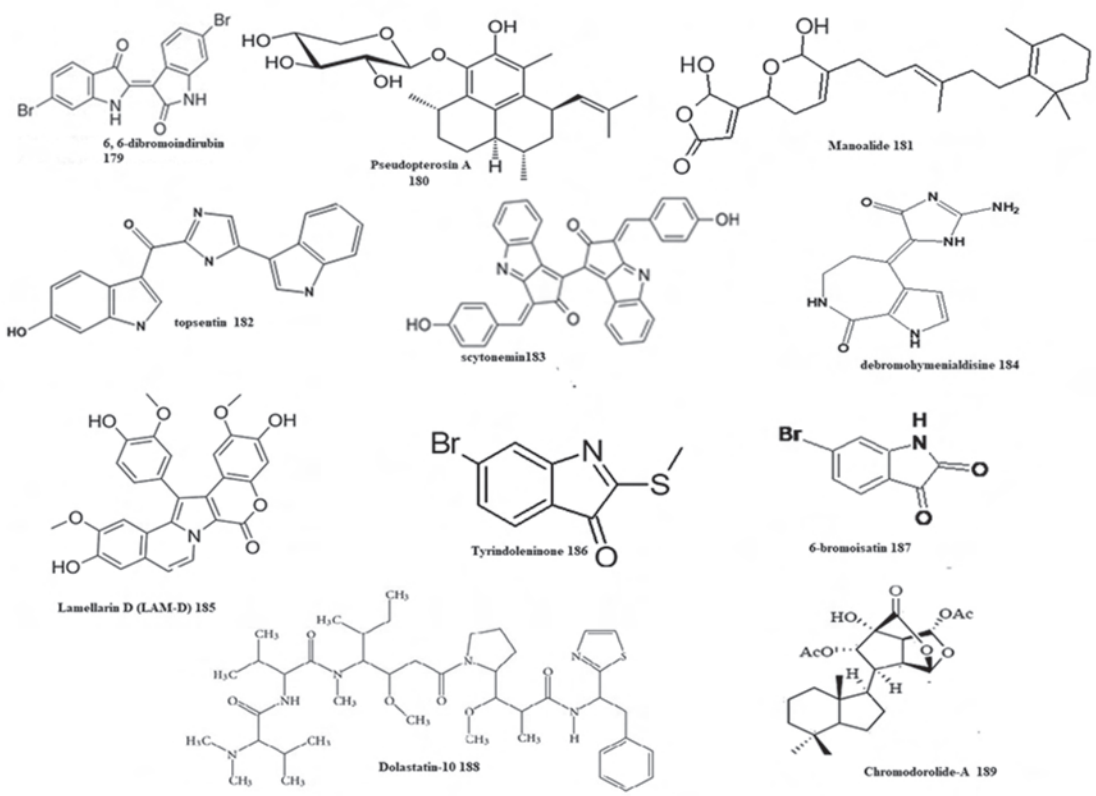
of male muricids (Westley and Benkendorff, 2008) contain the structural isomer of tyrian purple, 6, 6-dibromoindirubin 179 which is identified as a potent protein kinase inhibitor. A study on the antiinflammatory activity of the gorgonian (sea whip) *Pseudopterogorgia elisabethae* resulted in the identification of Pseudopterin 180 which possessed potent anti-inflammatory activities 300 mg/kg (Look et al., 1986). Manoalide 181 pseudopterosins, topsentins, 182 scytonemin183 and debromohymenialdisine 184 are some of the compounds isolated from molluscs that exhibited anti-inflammatory activity (Chellaram and Edward, 2009a). Pseudopterosins have anti-inflammatory and analgesic activity, with a mechanism of action different from the common non-steroidal anti-inflammatory drugs, commercially, pseudopterosins are found in skin creams as topical anti-inflammatory agents. An alkaloid Lamellarin D (LAM-D) 185, initially isolated from a prosobranch mollusk of the genus Lamellaria, exhibits cytotoxicity against many different tumors.

Tyrindoleninone 186 and 6-bromoisatin 187 are indole derivatives from marine mollusk *Dicathais orbita* that induces apoptosis in female reproductive cancer cell lines ovary, granulosa, and choriocarcinoma (OVCAR-3, KGN, Jar), respectively (Edward et al., 2012). The indole derivatives tyrindolinone 186, and 6,62 -dibromoindirubin 179, from the Muricidae family of marine gastropods, also have reported anti-cancer properties (Benkendorff et al., 2011, Vine et al., 2007, Westley et al., 2010) Dolastatin-10 188, and 15, derived from the shell-less mollusc *Dolabella auricularia* (Pettit et al., 1987) were reported to have anti-tumor activity against breast and liver cancer in phase I clinical trials (Tran et al., 1997). Chromodorolide-A 189 isolated from *Chromocloris cavae* exhibits *in vitro* antimicrobial and cytotoxic activities (Morris et al., 1990). A team from the University of Melbourne has extracted the conotoxin from a cone-shell snail which not only inhibits pain as being 10,000 times more powerful than morphine, but also accelerates the recovery of injured nerves. (Holmes et al., 2002).

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. 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

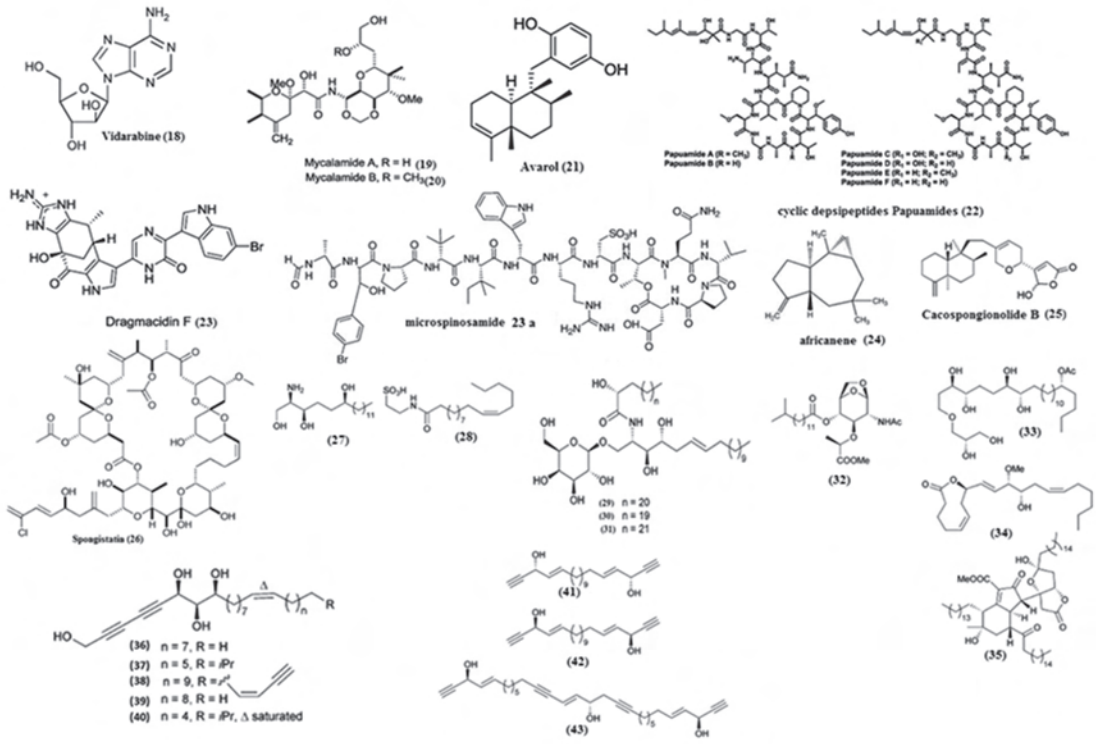
small cell Lewis lung carcinoma. Halichondrin-B, a polyether macrolide from Japanese sponge *Theonella* spp., has generated much interest as a potential anticancer agent. The theopederins are structurally related to mycalamide-A from marine sponge, *Mycale* spp. collected in New Zealand and onnamide-A 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 tumor 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.



Perry et al., (1988) first reported the isolation and in vitro antiviral activity of mycalamide A (19) and mycalamide B (20) from a New Zealand sponge of the genus Mycale in 1988 and 1990, respectively. Avarol (21) a sesquiterpenoid hydroquinone with a rearranged drimane skeleton, was first isolated from the marine sponge *Disidea avara* in 1974. The anti-HIV and cytotoxic cyclic depsipeptides Papuamides, (22), were isolated from the sponges *Theonella mirabilis* and *Theonella swinhoei*. Cutignano et al., reported the isolation of a new bromoindole alkaloid, dragmacidin F (23) from a marine sponge of the genus Halicortex which was responsible for the *in vitro* antiviral activity against HSV-1 and HIV-1 exhibited by Halicortex extracts (Cutignano, 2000, Garg et al., 2004). Isolation of microspinosamide (23a), a cyclic depsipeptide from an Indonesian collection of the sponge *Sidonops microspinosa* was reported in 2001. The Anti-HIV activity of crude extract of *S. microspinosa* was first discovered during the National Cancer Institute's primary anti-HIV screening (Boyd, 1988). Sesquiterpene africanene (24) isolated from the soft coral *Sinularia leptoclados* resulted in a more potent reduction of paw volume than that produced by 100 mg/kg body weight of ibuprofen, in carrageenan-induced rat edema assay. A novel sesterterpene Cacospongionolide B isolated from the sponge *Fasciospongia cavernosa* was found to be an inhibitor of human synovial phospholipase A2. It irreversibly inhibited both secretory PLA2 in vitro and group II secretory PLA2 in vivo. A marine macrolide, Spongistatin (26) isolated from the sponge *Hyrtios erecta* demonstrated potent microtubule-severing activity. Mechanism of action of was significantly different from all other antimicrotubule agents. The modified sphingoid base halisphingosine B 27 was isolated from *Haliclona tubifera* (Santa Catarina, Brazil) (Molinski et al. 2013) while taurinated fatty acid 28 was isolated from Axinella sp. (Hainan Is., S. China Sea) (Huang et al., 2013.) An Axinyssa djiferi found attached to mangrove tree roots (Djifer, Senegal) yielded axidjiferosides A-C 29-31, a mixture of which inhibited chloroquine-resistant *P. falciparum* (Farokhi et al., 2013). An acetylated nitrogenous glycolipid 32 was isolated from *Plakinastrella clathrata* (Gneerings Reef, Queensland, Australia), with the absolute configuration confirmed by synthesis of lipidchain analogues. The compound was claimed to be a moderate anti-inflammatory by inhibition of PGE2 (Katavic et al., 2013).

Mycalol (33) is glycerol ether isolated from *Mycale acerata* (Terra Nova Bay, Antarctica). A combination of chiroptical and Mosher's methods were used to assign the absolute configuration of this speciesc inhibitor of human anaplastic thyroid carcinomas, the most aggressive and currently untreatable thyroid gland malignancies, but inactive against other solid tumors (Cutignano et al., 2013). The absolute configuration of topsentolide C2 (34) (Topsentia sp.) (Luo et al., 2006) was established by total synthesis of four possible diastereomers (Towada et al., 2013). The moderately antimicrobial fatty acid trimer manzamenone O (35) was isolated from Plakortis sp. (Manzamo, Okinawa) (Tanaka et al.,

2013). Sponges from the genus Petrosia are a rich source of new polyacetylenes. The report of petrosiols A-E (36-40) from *Petrosia strongylata* (Ishigakijima Is., Okinawa) as inducers of nerve growth factor-like neuronal differentiation in PC12 cells was followed rapidly by reports that 36 inhibits proliferation and migration of platelet derived growth factor-induced vascular smooth muscle cells and hence could be used as a lead for vascular disorders(Choi et al., 2013). A racemic mixture of C20 bisacetylenic alcohols (41) and (42) has been isolated from Callyspongia sp. (Iriomote Is., Okinawa), and separated by chiral HPLC. Total synthesis of both enantiomers and detailed biological evaluation showed 41 was more active than its enantiomer against HeLa and temperature sensitive rat lymphatic endothelial cells, thus defining the 1-yne-3-ol moiety as an essential pharmacophore. Petrosiacetylene E (43) (Petrosia sp. Dokdo Is., S. Korea) was a low mM inhibitor of multiple HTCLs.



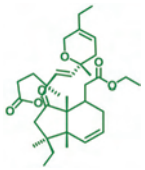
ECHINODERMS

A commercially available specimen of the starfish Asterias rollestoni (Xiamen food market, China) afforded the tetraosides 190 and 191 (Zhang et al., 2013). The species *Astropecten polyacanthus* (Cat Ba, Haiphong, Vietnam) contained the inactive or mildly cytotoxic sterols astropectenol A-D 192-195 (Cuong et al., 2013) which reported to inhibit

the expression of pro-inflammatory cytokines in bone marrowderived dendritic cells (Thao et al., 2013). Aphelasteroside E 196, which contains the rare sulfation at C-26, was isolated from *Aphelasterias japonica* (Poset Bay, Sea of Japan) (Popov et al., 2013) and C-24-arabinosides pectinioside H-J 197-199 were identified in extracts of *Asterina pectinifera* (Dalian coast, Yellow Sea, China) (Li et al., 2013). Tetraosides typicoside A1 200 (the 24E isomer of previously reported intercedenside A (*Mensamaria intercedens*) (Zou et al., 2013), A2, B1, C1 and C2 201-204 are minor metabolites isolated from the sea cucumber Actinocucumis typica (Vizhinjam coast, Arabian Sea, India) (Silchenko et al., 2013). Antifungal, haemolytic and cytotoxic evaluations of the five NPs identified widespread activity, with typicoside C1 being markedly less active in all assays. Of the disulfated pentaosides cucumarioside I1, I3 and I4 205-207 (*Eupentacta fraudatrix*, Peter the Great Gulf, Sea of Japan), only 205 exhibited biological activity including cytotoxicity (weak) and (strong) haemolytic activity (Silchenko et al., 2013). Extracts of the starfish *Astropecten monacanthus* (Cat Ba, Haiphong, Vietnam) afforded the hexaosides astrosterioside A–C 208- 210 and pentaoside astrosterioside D 211 (Thao et al., 2013). While 208 and 210 exhibited mild inhibition of IL-6 production by stimulated bone marrow-derived dendritic cells, diketo-containing 211 exhibited potent inhibition of production of IL-6,IL-12 p40 and TNF-a. The pyrrole and furan oligoglycosides astebatherioside A–D 212-215were reported from the starfish Asterina bather (Catba, Haiphong, Vietnam) (Thao et al., 2013), while 212 was either inactive or weakly active, 213-215 demonstrated inhibition of IL-12 p40 production, and to a lesser extent of IL-6 production, in LPS-stimulated bone marrow-derived dendritic cells.

CNIDARIANS

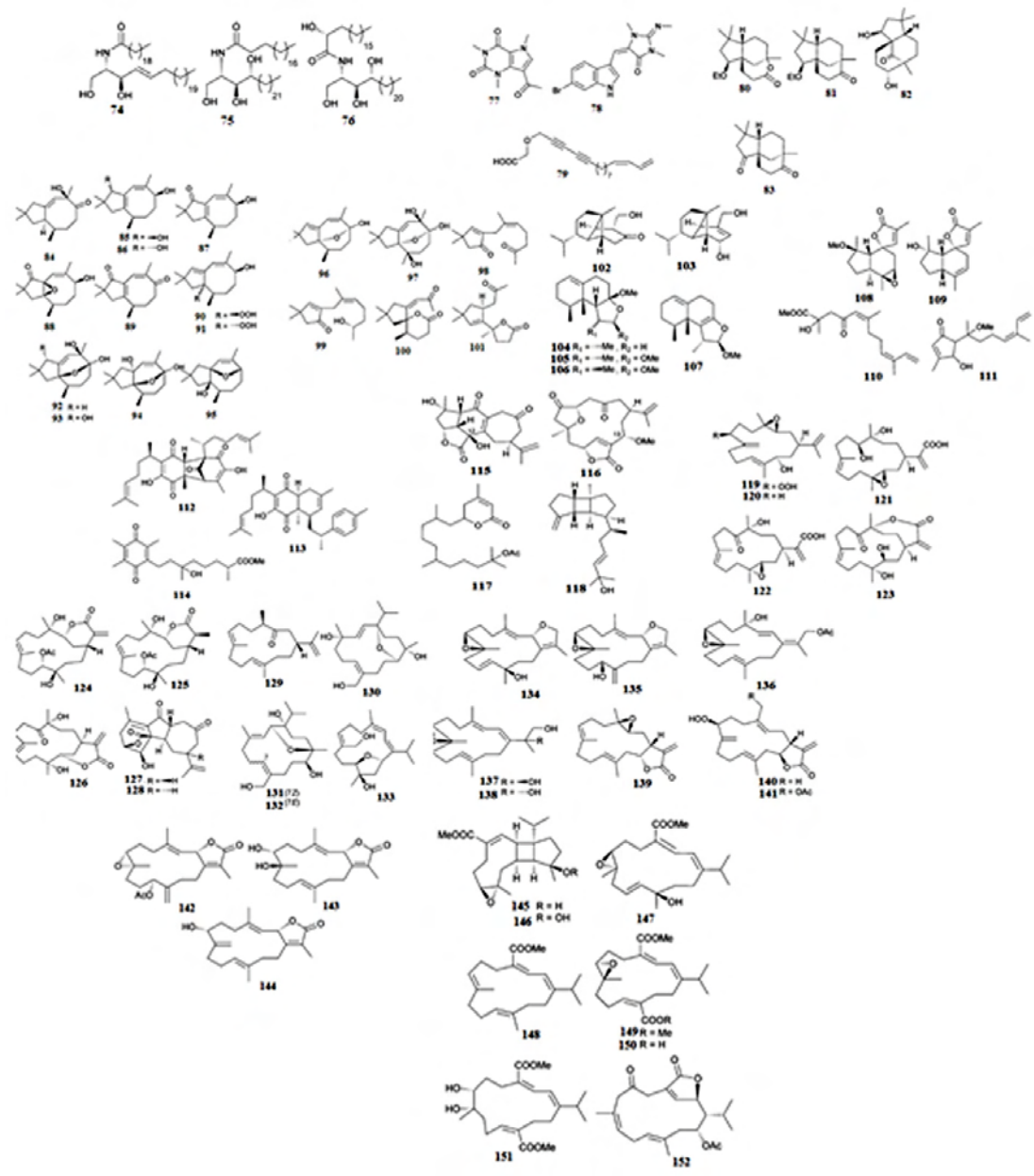
The number of new compounds reported from cnidarians in 2013 (Imran et al., 2010) has increased by 38% over the average for each of the previous 10 years. In addition to an epidioxysterol, three ceramides 74-76 were isolated from *Sinularia candidula* (Safaga, Egyptian Red Sea) (Ahmed et al., 2013). Of the three ceramides, 74 was the most potent anti-H5N1 virus agent. Pyrimidinedione 77 was reported from *Verrucella umbraculum* (Hainan Is., S. China Sea) (Huang et al., 2013) while Mediterranean specimens of the scleractinian coral *Astroides calycularis* afforded the new aplysinopsin analogue 78. (Cachet et al., 2013). Polyacetylenic montiporic acid D 79 (*Montipora digitata*, Sesoko Is., Okinawa, Japan) exhibited mild antibacterial and antioxidant properties (Kodani et al., 2013). New clovane-type sesquiterpenes rumphellclovane C-E 80-82 and four unnamed variants 83-86 were reported from the same collection of *Rumphella antipathies* (Southern Taiwan) (Chung et al., 2013). The latter four compounds are reported as NPs for the first time. Clovane 83 inhibited superoxide generation and elastase release by stimulated human neutrophils. Sesquiterpenes capillosanane A-N 84-97 and seco-variants capillosanane O-R 98-101 were isolated from *Sinularia capillosa* (Sanya Bay, Hainan Province, China) (Cheng et al., 2013). Capillosanane



A exhibited antifouling activity against *B. amphitrite*. Further examples of tricyclic sesquiterpenes were reported from *Lemnalia philippinensis* (philippinlins A 102 and B 103 collected at Lanyu, Taiwan (Xio et al., 2013) and *Paralemnalia thyrsoides* (parathyrsoidins A-D 104-107 collected at Sansiantai, Taitong County also in Taiwan (Tseng et al., 2013).

Spiro-butenolides sinularianins C108 and D 109 and potential biosynthetically-related precursors sinularianins E 110 and F 111 were isolated as mild inhibitors of NF-kB activation from *Sinularia sp*. (Dongluo Is., Hainan Province, China) (Yang et al., 2013). Perezoperezone 840 and curcuperezone 841 (Pseudopterogorgia rigida, Caribbean Sea) are envisaged to arise, in the case of 840, from non-symmetrical dimerisation of known cometabolite perezone (Wagner et al., 1965)and in the case of 841, through coupling of perezone and á-curcumene (Georgantea et al., 2013). Flexibilisquinone 842 (cultured specimen of *Sinularia flexibilis*) (Lin et al., 2013.) was claimed to be the enantiomer of sarcophytonone (*Sarcophyton crassocaule*). Of two new C19-norditerpenes 12-hydroxy-scabrolide A 115 843 and 13-epi-scabrolide C 116 (*Sinularia maxima*, Nha Trang Bay, Vietnam) the latter was identified as an inhibitor of the production of IL-6 and IL-12 by LPS-stimulated bone marrow derived dendritic cells (Thao et al., 2013). ä lactone 117 845 was isolated from *Scleronephthya gracillimum* (Green Is., Taiwan) as a modest inhibitor of expression of iNOS and COX-2 in stimulated macrophages (Fang et al., 2013). The weakly cytotoxic spatane diterpene leptoclalin A 118 was reported from cultured specimens of *Sinularia leptoclados* (Tsai et al., 2013). A diverse array of cembranoid diterpenes have been reported from soft corals till date. Arbolides A 119 and B 120, epoxy-alcohols with the former also containing a hydroperoxide functional group, were obtained from *Sinularia arborea* (southern Taiwan) (Chen et al., 2013). Similarly functionalised cembranes flexibilins A121 and B 122 in addition to 3-lactone-containing flexibilin C 123 were reported from *S. flexibilis* also collected from southern Taiwan (Hu et al., 2013). Of the ä-lactones 11-acetylsinuflexolide 125 and dihydro analogue 126 (S. flexibilis, Pingtung county, Taiwan), only the former exhibited cytotoxicity. *Sinularia flexibilis* (southern Taiwan) was also the source of flexibilin D 127 and of known congener 5-dehydrosinulariolide (Lin et al., 2009). The same publication also described sinulanorcembranolide A128 and the 1-epi-diastereomer 129 from the same collection of *S. gaweli* (Sansiantai, Taitung County, Taiwan). One of sinulariols T-Z5 130-141 (*S. rigida*, Sanya Bay, Hainan Is., S. China Sea), specifically 136, exhibited effects against the model fouling organisms *B. amphitrite* and *B.neritina* (Lai et al., 2013)

Two separate collections of Lobophytum sp. yielded epoxycembranes 134-138 Ximao Is., Sanya Bay, Hainan, China) and á-methylene-í-lactones 139-141 (Sanya Bay, Hainan, China). This is the first report 138 as an NP. Epoxycembrane 136 was a modest inhibitor of NO production by stimulated macrophages, while 139-141 were each found to be moderately cytotoxic towards a range of human and murine tumour cell lines. A Red Sea (Hurghada)

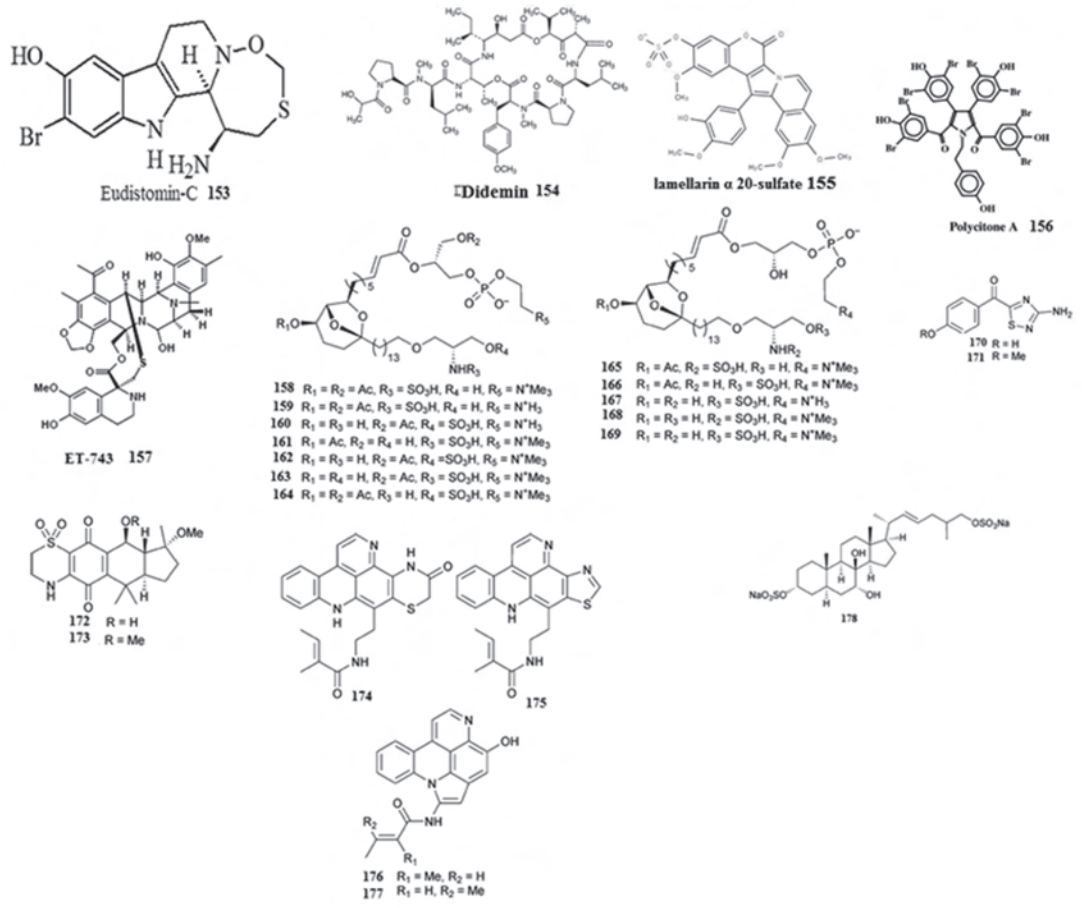


collection of *S. trocheliophorum* provided trochelioids A 142 and B143 and 16-oxosarcophytonin E 144, later reported for first time as NP (Hegazy et al., 2013). Sixteen new cembranoids were reported from *S. trocheliophorum* (Yalong Bay, Hainan, China). Of methyl sarcotroates A 145 and B 146, and sarcophytonolides M-R 147-152, ,only

hydroperoxide-containing 146 and sarcophytonolide N 148 were found to inhibit human PTP1B (Liang et al., 2013).

TUNICATE

Significant antiviral activity was observed for a series of indole alkaloids, Eudistomin-C 153 from Tunicate *Eudistoma olivaceum* sbeing most effective against Human Simplex Virus (Munro et al., 1939).



The tetrahydro-â carbolines generally exhibited higher levels of biological activity than their fully aromatic relatives; for example, the oxathiazepino-eudistomins, exhibit the highest level of antiviral activity and were also endowed with antimicrobial activity. Eudistomin K is significantly active against Herpes simplex Type I (HSV-1) and Polio virus (Marialuisa et al., 2011). The cyclic depsipeptide Didemin-B 154 isolated from Tunicate, *Tridemnum solidium*

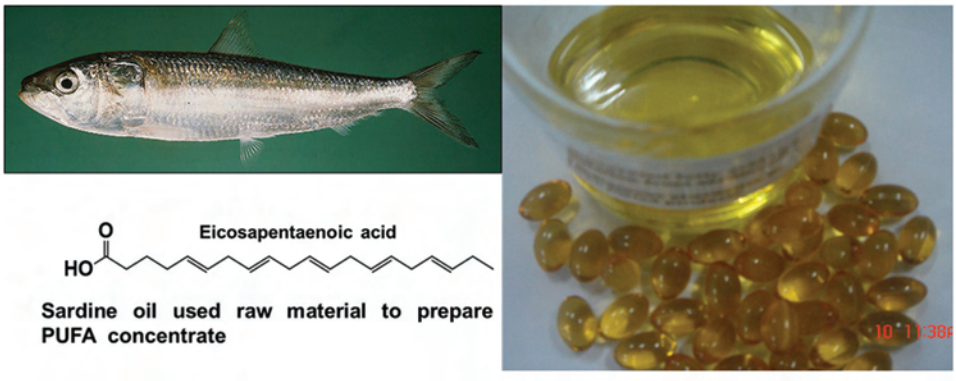
shows *in vivo* antitumor activity, besides its strong antiviral activity (Rinehart 1998, Rinehart, 1999). Alkaloid lamellarin á 20-sulfate 155 in an unidentified ascidian showed selective in vitro inhibition of HIV-1 integrase. Lamellarins form a group of more than 30 polyaromatic pyrrole alkaloids isolated from diverse marine organisms, mainly ascidians and sponges. Polycitone A 156 isolated from the ascidian *Polyctor* sp.is a potent inhibitor of the reverse transcriptase of HIV & both C and B retroviruses, as well as a general inhibitor of cellular DNA polymerases. As polycitone A is a general inhibitor of DNA polymerases it cannot serve as an anti-HIV drug but structural modifications of polycitone A could lead towards the rational design of new derivatives with anti-HIV reverse transcriptase activity. Ecteinascidin 743 (157), a complex alkaloid derived from the ascidian *Ecteinascidia turbinata* (Rinehart et al., 1990; Wright et al., 1990) and licensed by the University of Illinois to PharmaMar S.A. is in Phase I clinical trials for ovarian cancer and other solid tumors in the United States and Europe. The sulfonated serinol lipids siladenoserinol A-L 158-169 isolated from the colonial Tunicates Didemnidae from North Sulawesi, Indonesia) inhibited the interaction of tumor suppressor p53 with Hdm2, potentially leading to reactivation of p53 and induction of apoptosis in cancer cells. (Nakamura et al., 2013). Two new examples of the rare 1,2,4-thiadiazole ring system, polycarpathiamine A 170 and B 171 were isolated from *Polycarpa aurata* (Ambon, Indonesia). While 170 exhibited submicromolar cytotoxicity (L5178Y), 171 was inactive (Pham et al., 2013). The structures of the modestly cytotoxic dioxothiazinomeroterpenes conthiaquinone A 172 and B 173 from *Aplidium conicum* collected from Porto Cesareo, Lecce, Italy were established by interpretation of NMR data (Menna et al., 2013). Four new examples of pyridoacridine alkaloids, shermilamine F 174, dehydrokuanoniamine F 175 arnoamine C 176 and D 177 (*Cystodytes violatinctus*, Solomon Is.) exhibited modest cytotoxicity towards a panel of HTCLs.(Bontemps et al., 2013). A sperm activation and attractant 178 was isolated from egg seawater of *Ascidia sydneiensis* (Fong et al., 2013).

POLYUNSATURATED FATTY ACIDS FROM MARINE FISH

Long-chain polyunsaturated fatty acids (LC-PUFAs), viz., eicosapentaenoic acid (EPA, 20:5 n13), docosahexaenoic acid (DHA, 22:6 n23) and linolenic acid (LA, 18:3 n33) are widely available in a large variety of marine organisms, like microalgae, polychaetes, fin fish and shellfish. These LC-PUFAs are recognised to have special pharmacological and physiological effects on human/animal health (Harris, 1989). The n3 and n6 long-chain polyunsaturated fatty acids (LCPUFAs), viz., eicosapentaenoic acid (EPA, 20:5n3), docosahexaenoic acid (DHA, 22:6n3), and arachidonic acid (AA, 20: 4n6), are essential fatty acids in the diet of animals and human beings, because they cannot synthesize it *de novo* from precursor molecules (Chakraborty et al., 2010). Therefore they require greater concentrations of PUFAs for their growth, reproduction and survival (Cahu et al., 1994). Diets deficient in these PUFAs

particularly EPA have been found to have a negative effect on various vital biochemical and physiological pathways (Chakraborty et al., 2009). The important natural sources of n3 LC-PUFAs are marine fish oils such as sardine, mackerel, cod, shark, and menhaden, which contain PUFA levels of about 30%. For this reason, marine fish oils are preferentially used as raw material to prepare n3 PUFA concentrates. Additionally, it was reported that n3 PUFAs were moderately absorbed by the intestine as triglycerides and most promptly absorbed when free fatty acids (FFA) were given orally. Therefore, it is convenient to prepare n3 concentrates as FFA after chemical hydrolysis of marine oils. Argentation silica gel chromatography of urea inclusion adducts from cod liver oil yielded highly pure DHA in the process, while for EPA, the recovery in the combined process was 29.6%. EPA and DHA have been concentrated from shark liver (*Isurus oxyrinchus*) in one single step, in which fish liver oil was simultaneously extracted, saponified, and concentrated. Additionally, the PUFA concentrate was winterized to crystallize the remaining saturated fatty acids, resulting in a further increase in the concentration of DHA and EPA. The polyunsaturated fatty acids EPA and AA have been purified from the red microalga *Porphyridium cruentum* by the urea inclusion method followed by silica gel column chromatography of the urea concentrate. The unique substrate specificity of microbial lipases has been utilised for the enhancement of PUFA content in fish oils by several groups (Harris, 1989; Matori et al., 1991). Isolates of *Pseudomonas fluorescens* have been found to produce enzymes active on lipolytic substrates under alkaline conditions (Kojima & Shimizu, 2003). Lipase genes from *Pseudomonas* have been cloned and expressed in Escherichia coli, due to their potential industrial applications (Kojima et al., 2003). *P. fluorescens* SIK W1 was found to produce extremely heat-stable lipase that has high lipolytic activity for short- to medium-chain triacylglycerols (Chung et al., 1991). An extracellular alkaline metallolipase with molecular weight of 74.8 kDa derived from cultures of *Bacillus licheniformis* MTCC 6824 was found to enrich D5 olefinic double bond fatty acids, viz., EPA and AA (Chakraborty et al., 2008b).

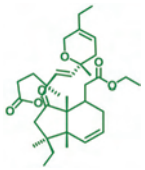
POLYUNSATURATED FATTY ACID (PUFA) ENRICHED FORMULATION



A thermophilic and alkalophilic lipase from *Bacillus coagulans* BTS-3 was purified and biochemically characterised (Kumar et al., 2005). A lipase produced by recombinant B. licheniformis was found to be stable at alkaline pH of 12.0 (Nthangeni et al., 2001). These results are in contrast to thermotolerant lipases from *Bacillus thermoacetenulatus* and *Bacillus thermoleovorans*, which display maximum activity at pH 8.0 (Lee et al., 1999; Rua et al., 1997). *B. coagulans* NCIMB 9365 has been reported to possess an intracellular carboxylesterase (Molinari et al., 1996). The substrate specificity of lipase has been utilised for the recovery of EPA (D5) and DHA (D4) from marine oils and c-linolenic acid (D6) from borage seed oil (Morioka et al., 1987). DHA-rich triglycerides were prepared from fish oil with lipases obtained from *Candida cylindracea* and *Chromobacterium viscosum* (Tanaka et al., 1992, 1994). Substrates containing D2–D7 isomers of 18:1 were resistant to pancreatic lipase-catalysed hydrolysis, resulting in higher concentrations of oleic acid, and the discrimination was the greatest for the D5 isomer (Heimermann et al., 1973). An extracellular lipase purified from *Pseudomonas fluorescens* MTCC 2421 was used to enrich sardine oil triglycerides with eicosapentaenoic acid and linolenic acid to 35.28% and 8.25%, respectively (Chakraborty et al., 2010). An extracellular lipase derived from *Bacillus circulans*, isolated from marine macroalga, *Turbinaraia conoides*, was used to prepare n-3 polyunsaturated fatty acid (PUFA) concentrates from sardine oil triglycerides. The enzyme was purified 132-fold with specific activity of 386 LU/mg. The purified lipase was able to enrich sardine oil with 37.7 ± 1.98% 20:5n-3 and 5.11 ± 0.14% 18:3n-3 in the triglyceride fraction (Chakraborty et al., 2010).

MARINE BACTERIA AS A SOURCE OF MARINE NATURAL PRODUCTS

It has been argued that because of the high dilution effect of seawater, marine-derived bioactive compounds may have evolved great potency. This theory was supported in 2004 with the report of a first-in-class antimicrobial compound from a marine isolate *Verrucosispora*. Renewed interest in marine microorganisms and their ability to produce antimicrobials has resulted in numerous reports of novel antimicrobial compounds. The period of antimicrobial drug discovery from the early 1940s to the 1960s is referred to as the Golden Age. During this time, the industrialization of penicillin production created the expertise and facilities to make significant quantities of antimicrobial compounds by fermentation. The clinical use of antibiotics heralded a health care miracle; deaths due to bacterial infections were significantly reduced, resulting in increases in life expectancy. The majority of compounds that were discovered during this period were isolated from soil bacteria, most notably the filamentous *Actinobacteria*. Microorganisms are a prolific source of structurally diverse bioactive metabolites and have yielded some of the most important



products of the pharmaceutical industry. Microbial secondary metabolites are now being used for applications other than antibacterial, antifungal and antiviral infections. It was during 1928s when Alexander Fleming (Fleming, 1929) began the microbial drug era when he discovered in a Petri dish seeded with *Staphylococcus aureus* that a compound (penicillin) produced by a fungus/mold killed the bacteria. Later, penicillin was isolated as a yellow powder and used as a potent antibacterial compound during the Second World War. Following this extraordinary discovery by Flemming, the antibiotics chloramphenicol and streptomycin, were isolated. Naturally occurring antibiotics are produced by fermentation, an old technique that can be traced back almost 8000 years. Owing to technical improvements in screening programs, and separation and isolation techniques, the number of natural compounds discovered exceeds 1 million (Ecker et al., 2005). Among them, 50-60% are produced by plants (alkaloids, flavonoids, terpenoids, steroids, carbohydrates, etc.) and 5% have a microbial origin. Of all the reported natural products, approximately 20–25% show biological activity, and of these approximately 10% have been obtained from microbes. Furthermore, from the 22 500 biologically active compounds that have been obtained so far from microbes, 45% are produced by bacteria or bacteria-like microbes, 38% by fungi and 17% by others (Berdy, 2005). The increasing role of microorganisms in the production of antibiotics and other drugs for treatment of serious diseases has been dramatic. However, the development of resistance in microbes to various life-threatening diseases and in aquaculture has become a major problem and requires renewed research effort to combat it. Antimicrobial development after the Golden Age was characterized by semi-synthetic modifications of compounds that were already clinically proven. The poor antimicrobial discovery rate from microbes, coupled with the availability of chemically synthesized small molecule libraries, led to the abandonment of microbial screening programmes in the majority of pharmaceutical companies. To date, small chemical libraries have failed to deliver a new antimicrobial compound to the clinic, prompting many to speculate that the withdrawal of microbial screening was premature, exacerbating the threat of antibiotic resistant bacteria.

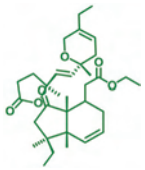
MICROBIAL NATURAL PRODUCTS

Microbial natural products that have reached the market without any chemical modifications are a testimony to the remarkable ability of microorganisms to produce drug-like small molecules. Although still in clinical trials, a feature example of this is salinosporamide A (NPI-0052), a novel anticancer agent found in the exploration of new marine environments (Fenical et al., 2009). In 2008, over 1000 marine natural products were reported (Blunt et al., 2010). However, out of the 19 microbial-derived drugs reported in 2008, no natural products from marine microbes were present, signifying the novelty of their systematic exploration

(Ganesan, 2008). Currently, >30 compounds of marine microbial origin are in clinical or preclinical studies for the treatment of different types of cancer (Simmons et al., 2005), clearly demonstrating that potential of marine microorganisms as an essential resource in the discovery of new antibiotic leads. The evolution of marine microbial natural product collections and development of high-throughput screening methods have attracted researchers to the use of natural product libraries in drug discovery. These libraries include subsections of crude extracts, pre-fractionated extracts (automated HPLC-MS fractionation) and purified natural products. A research group in Ireland has developed a two-dimensional chromatographic strategy that includes a protocol to generate purified marine natural product libraries that are accurately characterized by mass spectroscopy during production to expedite dereplication of known compounds and identification of novel chemotypes. Although the biosynthetic and regulative crosstalk of secondary metabolite biosynthesis is complex within and between microorganisms, all levels can be influenced by imitating natural environmental changes. Development and testing of new culture media for the maximum expression of secondary metabolites is important as chemical diversity in the construction process of a marine natural products library. An optimization of 'one strain, many active compounds' can be used together with 'fingerprint' methods (HPLC and nuclear magnetic resonance) including tandem analytical techniques such as MS/MS, GC-EI/MS, HPLC-SPE-NMR, LC-MS-MS and LC-NMR for the optimization/selection of culture media for high-throughput fermentation of novel strains. Tormo et al. (2003) developed a method for the selection of production media for bacterial strains based on their metabolite HPLC profiles, that yielded the highest metabolite diversity and least overlapping HPLC profiles were selected for large-scale fermentation. Targeted high-throughput screening methods are important for the speed and accuracy of identification of novel antimicrobials. From these evaluation models, many crude extracts or purified compounds were obtained as positive hits. In addition for evaluation purposes, it is worthy to note that these screening assays also provide mode of action hypothesis from the crude extracts.

ANTIBIOTICS FROM MARINE MICROBES

During recent decades, we have seen an increasing number of reports on the progressive development of bacterial resistance to almost all available antimicrobial agents. In the 1970s, the major problem was the multidrug resistance of Gram-negative bacteria, but later in the 1980s the Gram-positive bacteria became important, including methicillin-resistant staphylococci, penicillin-resistant pneumococci and vancomycin-resistant enterococci (Moellering, 1998). In the past, the solution to the problem has depended primarily on the development of novel antimicrobial agents. However, the number of new classes of

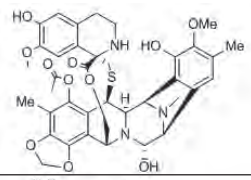
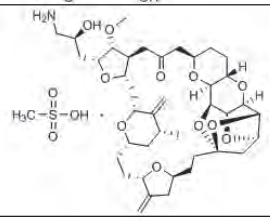
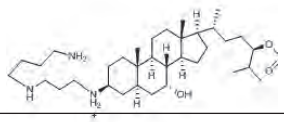
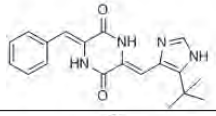
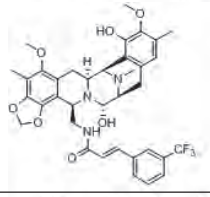
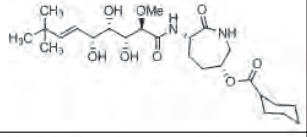
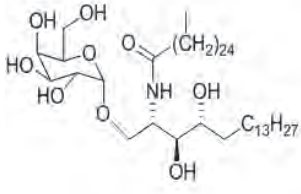


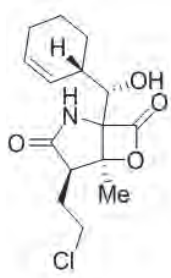
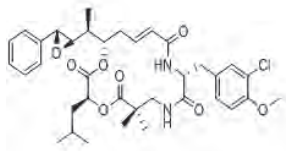
antimicrobial agents being developed has decreased dramatically in recent years. The conventionally used antibiotics/drugs become resistant to most of the natural antimicrobial agents that have been developed over the past 50 years (Hancock, 2007) thereby limiting the effectiveness of current antimicrobial drugs. In 2004, more than 70% of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics (Katz et al., 2006). The so-called 'superbugs' (organisms that are resistant to most of the clinically used antibiotics) are emerging at a rapid rate. *S. aureus*, which is resistant to methicillin, is responsible for many cases of infections each year (Balaban et al., 2005). The incidence of multidrug-resistant pathogenic bacteria is increasing. The Infectious Disease Society of America (IDSA) reported in 2004 that in US hospitals alone, around 2 million people acquire bacterial infections per year (dedicated website: http://www.idsociety.org/Content.aspx). There are also other examples of Gram-positive (*Enterococcus* and *Streptococcus*) and Gram-negative pathogens (*Klebsiella, Escherichia, Enterobacter, Serratia, Citrobacter, Salmonella* and *Pseudomonas*) (Cragg and Newman, 2001). Among them, *Pseudomonas aeruginosa* accounts for almost 80% of these opportunistic infections. They represent a serious problem in patients hospitalized with cancer, cystic fibrosis and burns, causing death in 50% of cases. Other infections caused by *Pseudomonas* spp include endocarditis, pneumonia and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin and musculoskeletal system (Levin and Bonten, 2004). New families of anti-infective compounds are needed to enter the marketplace at regular intervals to tackle the new diseases caused by evolving pathogens. At least 30 new diseases emerged in the 1980-2000s and they are growing. Emerging infectious organisms often encounter hosts with no prior exposure to them and thus represent a novel challenge to the host's immune system. Several viruses responsible for human epidemics have made a transition from animal host to humans and are now transmitted from human to human. HIV, responsible for the acquired immunodeficiency syndrome (AIDS) epidemic, is one example. Although it has not been proven, it is suspected that severe acute respiratory syndrome (SARS), caused by the SARS coronavirus, also evolved from a different species (Kremer et al., 2000). One additional reason for developing new antibiotics is related to their own toxicity. As with other therapeutic agents, the use of antibiotics may also cause side effects in patients. Some side effects are more severe and, depending on the antibiotic, may disrupt the hearing function (aminoglycosides), kidneys (aminoglycosides and polypeptides) or liver (rifampin). In recent times, several research groups are making concerted efforts to find novel antimicrobial agents as a solution towards multiresistant antibiotic and drug molecules.

AQUACULTURE GRADE ANTIMICROBIAL CHEMICALS FROM MARINE MICROBES

Disease caused by bacterial pathogens has been widely recognized as a major cause of economic loss in many commercially cultured fish and shellfish species in India, with mortality of larval stages in hatcheries and the growing stages in different mariculture systems. Pathogenic vibrios are involved in significant mortalities in the larviculture and growout phases of famed finfish and shellfishes. In an attempt to control the proliferation of pathogenic vibrios, the prophylactic and therapeutic use of antibiotics has been practiced in commercial hatcheries, creating more serious problem of antibiotic resistance among the microflora in the environment. With safety concerns about synthetic antibiotics, and the antibiotic resistance problems, considerable interest has arisen in finding alternative natural sources (Gomez-Gil et al., 2000). Screening and development of aquaculture-grade chemicals from bacterial flora could be a highly promising approach to produce these bioactive moleules. Members of the genus *Pseudomonas* and *Bacillus* either free living or associated with marine flora are common beneficial bacterial candidates, and are known to produce a wide range of secondary metabolites (Raaijmakers et al., 1997) inhibiting a wide range of pathogenic bacteria (Rengpipat et al., 1998). The metabolites 6-oxo-de-O-methyllasiodiplodin, (E)-9-etheno-lasiodiplodin, lasiodiplodin, de-O-methyllasiodiplodin, and 5-hydroxy-de-O-methyllasiodiplodin were isolated from the mycelium extracts of a microbe obtained from South China Sea (Yang et al., 2006). Marine bacterial strain, *Pseudomonas*, producing inhibitory compounds against shrimp pathogenic vibrios including *Vibrio harveyi*, *V. fluvialis*, *V. parahaemolyticus*, *V. damsela* and *V. vulnificus* was reported by Chaitanya et al., (2002). Bioactive compounds were isolated from a marine bacterium *Bacillus circulans* (Chakraborty et al., 2010). Labda-14-ene-3a,8a-diol and labda-14-ene-8a-hydroxy-3-one were found to be inhibitory to the growth of *Vibrio parahaemolyticus* with minimum inhibitory concentrations of 30-40 µg/mL (Chakraborty *et al.*, 2010), and their structures have been elucidated by ^{1}H NMR and ^{13}C NMR spectra, including 2D NMR. Several bacterial flora were isolated from marine ecosystem (*Bacillus subtilis*, *B. amyloliquifaciens*, *Pseudomonas putida,* and *P. aeroginosa*) with potential activities (> 20 mm inhibition zone) against pathogenic Vibrios (Chakraborty et al., 2010). The antibacterial component in the $CHCl_3$ fraction of *P. aerogenosa* was found to be N-substituted methyl octahydro-1-phenazinecarboxylate. The other important antibacterial molecules were found to be propyl 2-oxoacetate and phenethyl 2-oxoacetate. About 4530 bacterial isolates were purified from seaweeds and sediments, and 23 isolates (*B. subtilis* MTCC 10402, 10403 & 10407, *B. amyloliquifaciens* 10456, *P. putida* MTCC 10458, *P. aeroginosa* MTCC 10610) were found to be potential against pathogenic *Vibrios*, Pyocyanins, *N*-substituted phenazinecarboxylate, propyl/phenethyl-2-oxoacetates were the major antibacterial molecules in bacteria (Preetha *et al.* 2010).

Clinical status of marine derived antitumor agents, their chemical class and mode of action

Compound Name	Structure	Chemical Class	Organism	Company	Status
Trabectedin (ET-743)		Alkaloid	Tunicate	PharmaMar	Approved
Eribulin Mesylate (E7389)		Macrolide	Sponge	Eisai Inc.	Phase III
Squalamine lactate		Amino-steroid	Shark	Genaera	Phase II
Plinabulin (NPI-2358)		Diketopipe-razine	Fungus	Nereus Pharma-ceuticals	Phase II
Zalypsis		Alkaloid	Nudi-branch	Pharma Mar	Phase II
LAF389		Amino acid	Sponge	Novartis	Phase I
KRN7000		α -galacto sylceramide	Sponge	Kirin	Phase I

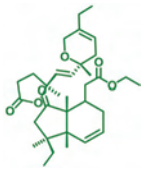
Marizomib, Salinosporamide A;NPI-0052)		Beta-lactone-gammalactam	Bacterium	Nereus Pharma-ceuticals	Phase I
LY355703, CRYPTO 52		Cryptophycin	Cyano-bacterium	-	Preclinical

NUTRACEUTICALS FROM MARINE ORGANISMS: PIONEERING WORKS OF ICAR-CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

The rich diversity of marine organisms represents an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use. The pioneering research work at ICAR-Central Marine Fisheries Research Institute envisages a systematic approach involving chemical profiling of major species of marine organisms for bioactive compounds with potential biological activities against different disease.

Nutraceuticals developed by ICAR-Central Marine Fisheries Research Institute for use against type-2 diabetes, obesity/dyslipidemia and rheumatoid arthritis

The research works at ICAR-Central Marine Fisheries Research Institute developed a hitherto unraveled database of marine organisms with small molecular weight bioactive molecules responsible to combat various life-threatening diseases. This prestigious marine fisheries research institute of Indian Council of Agricultural Research (ICAR) has developed and commercialized the nutraceutical products Cadalmin™ Green Algal extract (Cadalmin™ GAe) and Antidiabetic extract (Cadalmin™ ADe) as green alternatives to synthetic drugs to



combat rheumatic arthritic pains and type-2 diabetes, respectively. The anti-inflammatory nutraceutical Cadalmin™ Green Mussel extract (Cadalmin™ GMe) from Asian green mussel *Perna viridis* has been commercialized. The active principles in Cadalmin™ GMe isolated from *P. viridis* exhibited potential capacities to inhibit experimentally induced inflammation, and can act as dual inhibitors of membrane arachidonate oxygenation by cyclooxygenase-2 and lipoxygenase pathways, thus decreasing pro-inflammatory prostaglandin/leukotriene synthesis and down-regulating the inflammatory sequence. Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) has been developed from seaweeds to combat dyslipidemia leading to obesity, and the product was out-licensed for commercial production and marketing. Several products are in pipeline, and are being commercialized.

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. The main emphasis is given in the search of drugs for deadly human diseases as cancer and AIDS. Efforts of the researchers around the world are continuing their efforts in finding new molecules and drug candidates in the context of deadly diseases such as AIDS, cancer, cardiovascular disease, arthritis, diabetes, etc.

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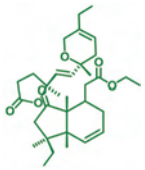

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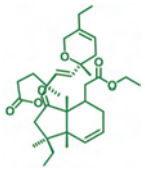

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