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Winter School on

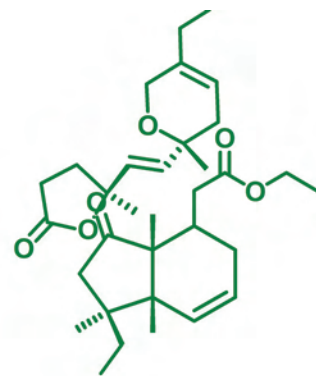
Recent advances in bioactive compounds from marine organisms and development of high value products for health management

23 January to 12 February 2018



Marine Biotechnology Division
ICAR-Central Marine Fisheries Research Institute

Post Box No. 1603, Ernakulam North P.O., Kochi-682 018, Kerala, India



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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

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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
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P R E F A C E

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.


January, 2018

Kajal Chakraborty
Course Director



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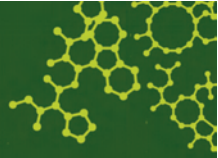
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CORALS AND SPONGES: IMPORTANT RESOURCE BASE OF BIOACTIVE COMPOUNDS

CHAPTER

30

K. Vinod

Calicut Research Centre of Central Marine Fisheries Research Institute

Ocean is a reservoir of millions of flora and fauna; many of which are still unknown to man. Although exploratory surveys are being carried out by many maritime nations on a continuous basis to unravel the species diversity and richness of world oceans; our knowledge of marine biota is very meager. The species unknown to man would be many times more than the species that are known. With technological advancements and improved deep-sea exploratory capabilities, our mission to explore the marine environment has been a regular process and many species are being discovered and described every day; some of them even from the abyssal depths.

The marine resources are integral part of human life and man has been dependent on these resources from time immemorial. The ocean not only provides us protein food, but also medicines and products which are useful in other industrial applications. The marine natural products have attracted the attention of biologists and chemists all over the world since the last five decades and approximately 16,000 marine natural products have been isolated from marine organisms and reported in approximately 6,800 publications (Datta et al., 2015). The marine invertebrates in particular have been attracting the attention of researchers due to the plethora of bioactive substances hidden in it. Most of the marine invertebrates are known for their special adaptations to thrive in different environments, to escape from predators, for prey capture etc. Many of the invertebrates produce bioactive substances ranging from peptides to alkaloids to terpenoids to steroids for defense and preservation of their natural integrity. Ocean is considered as important source of potential drugs, as some of these bioactive compounds or secondary metabolites have biomedical potential.

The bioactive substances formed by marine organisms such as protozoans and invertebrates viz., poriferans, cnidarians, annelids, arthropods, molluscs and echinoderms have attracted the interest of researchers due to their antiviral, antifungal, antiprotozoal, antihelminthic and anticancer activities (Zapata and Amemiya, 2000). A vast number of bioactive compounds are produced by phylogenetically diverse organisms that have different chemical activity and structure. Until the end of the first decennium of this century, more than 15,000 natural compounds that belong to various families of chemicals have been isolated from marine invertebrates. During the past few years, a large number of novel compounds have also been reported (Pomponi, 1999; Jimeno, 2002), and some of these compounds are expected to be used as therapeutics in the near future.



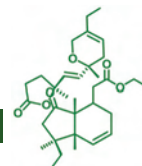
BIOACTIVE COMPOUNDS FROM CORALS

Cnidarians are well known producers of complex mixtures of proteinaceous toxins used for defense and for prey capture. In spite of their toxicity, cnidarians are believed to be potential source of bioactive compounds of pharmaceutical value. The corals, particularly the soft corals (Alcyonaceans) have been found to possess many secondary metabolites useful in the pharmaceutical industries. A large number of highly active anti-tumor compounds have been isolated from many invertebrates including corals. Eleutherobin from Eleutherobia family of corals, sarcodictyin from Mediterranean Stolonigeran corals are some good examples. The soft corals have large variety of sesquiterpenoids and Diterpenoids and many of these compounds were reported as toxic. The soft coral *Clavulariaviridis* which belongs to the family Clavulariidae is reported to yield cytotoxic steroids, stoloniferones a-d (Kobayashi et al., 1984). *Plexaurahomomall* which belongs to the family Plexauridae and found in the Caribbean Sea is one of the richest natural source of prostaglandins, so far discovered in soft corals. The glycosides, cervicosides and prostanoids claviridenones, from the soft corals *Sinulariacervicornis* and *Clavulariaviridis* were shown to have antitumor activity against human cancer cell lines (Zhang et al., 2006). A number of diterpenoids of the xenicane groups from the soft coral, *Xenia* sp. exhibited mild to-potent cytotoxic activities against human lung carcinoma (H460) and liver carcinoma (HepG2) cell lines (Su and Wen, 2011).

Secondary bioactive metabolites were also discovered in gorgonians. They are mainly steroids and terpenoids and several lipid compounds. Pseudopterolide, a diterpene which exhibited unusual cytotoxic properties was extracted from the gorgonian *Pseudopterogorgiaacerosa* (Banduraga et al., 1982). Guaiazulene from the gorgonian *Euplexauraerecta* exhibited mild activity against *Pseudomonas aeruginosa* (Fusetani et al., 1981). Subergorgic acid, a cardiotoxin was obtained from the gorgonid *Subergorgiasuberosa* (Groweiss et al., 1985).

BIOACTIVE COMPOUNDS FROM MARINE SPONGES

Sponges occur in marine as well as in freshwater ecosystems; however, a majority of them are marine. The marine forms are found in inshore as well as in deeper waters. The 'World Porifera Database' enlists 8877 valid species of sponges (Van Soest et al., 2018). Sponges have proved to be the best source among marine organisms of biologically active metabolites for use as drug leads or biomedical tools (Ireland et al., 1993). Sponges are known for their capacity to produce bioactive secondary metabolites (Koopmans et al., 2011). More than 5300 different products are recorded from sponges and their associated microorganisms and every year around 200 new metabolites are reported (Lipton and Shine, 2009). Although concerted efforts resulted in the development of many new bioactive compounds from marine sponges, only very few compounds have reached the clinical trial stage. One of the



reasons for this, as cited by many workers is that many of the sponge bioactive metabolites are very toxic, thus leading to a low therapeutic index.

In India, a total of 486 species of sponges were reported (Thomas, 1998), the Gulf of Mannar and Palk Bay has the highest (319 species), followed by Andaman and Nicobar Islands (95 species), Lakshadweep (82 species) and Gulf of Kachchh (25 species) (Venkataraman, 2005). According to (Sivaleela 2014), the Gulf of Mannar and Palk Bay accounts for over 67% of sponge diversity of India. The sponge fauna of India is dominated by species of Demospongia followed by Hyalospongiae and Calciospongiae (Venkataraman, 2005). A recent report on the sponge diversity in India by Chandra *et al.* (2016) listed 476 species; the Palk Bay and the Gulf of Mannar having the highest species diversity (329 species), followed by Andaman and Nicobar Islands (126 species).

The pioneering discovery of bioactive compounds from marine sources was the serendipitous isolation of C-Nucleosides, spongouridine and spongothymidine from the Caribbean sponge *Cryptotheca crypt* in early 1950s (Bergmann and Feeney, 1950). These compounds have potential antiviral activity and their synthetic analog was an anticancer drug (McConnell *et al.*, 1994). The compound Manzamine A has potent antimalarial activity against rodent malarial parasite *Plasmodium berghei* *in vivo* (Anget *et al.*, 2000). There are several bioactive compounds *viz.* sterols, terpenoids, phenolic or quinoid, carotenoids etc. from marine sponges reported for medicinal values.

Marine sponges are a good source of unusual sterols and the sulphated and alkaloid sterols have exhibited antimicrobial activity. A bioactive compound, halistanol was reported from the sponge *Halichondriamooriei* (Fusetani *et al.*, 1981). Halistanol trisulfate, a sulfated steroid derivative was isolated from the extracts of *Topsentia* spp. (Slate *et al.*, 1995). Townsend *et al.* (1992) reported that the pigmented human melanoma cell line, MM418, became demelanized when treated continuously with halistanol trisulphate (HTS), a C29 steroidal detergent isolated from a marine sponge. The steroidal alkaloids plakinamine A and plakinamine B which are antimicrobial metabolites were obtained from *Plakinasp.* and the compound was found to inhibit the growth of *Staphylococcus aureus* and *Candida albicans* (Rosser and Faulkner, 1984). The sterols from the sponge *Toxadociazum* inhibited the growth of two species of bacteria namely *Staphylococcus aureus* and *Bacillus subtilis* (Nakasue *et al.*, 1983).

The extracts of *Zygomycetes* sp. showed potent antibacterial activity against bacteria such as *Bacillus megaterium*, *Klebsiella pneumoniae* and *Sterptococcus pyogenes* and exhibited antifungal activity against all pathogenic test strains (Johnson *et al.*, 2012). *Spongia officinalis*, the common bath sponge is a rich source of terpenoids. The compounds sesquiterpenoid and avarol, which exhibited antimicrobial activity and also found active against "AIDS" virus



was first isolated from a Mediterranean sponge *Disidea avara* (Minale et al., 1974). The compound triprenylphenols have been isolated from the red sponge *Halichondria panacea* (Cimino et al., 1973; Casapullo et al., 1993; Jaspars et al., 1995). Phyllofenone A and 20, 24-diethyl-25-norscalarane sesterterpene with antifungal activity from sponge *Phyllospongia foliascens* have been isolated by Kimuchi et al., (1981; 1983).

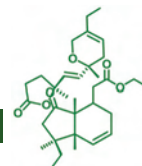
CONCLUSION

Sponges are often known to produce metabolites which are beneficial to mankind, however the main issue is the production of requisite biomass as sponges produce metabolites in trace quantities (Osinga et al., 1999). Hence, in order to having potential applications in various fields, and production of metabolites in trace quantities creates an issue with respect to mass production. Large quantities of sponges are to be exploited in order to produce considerable quantity of required metabolites. The available natural population of sponges will not be sufficient enough for utilization as it could threaten the existing natural stock. Culturing sponges is a way to address supply problem (Laportet et al., 2009), as aquaculture is one of the best possible methods to produce large quantity of requisite sponge biomass. With the advancement of aquaculture technologies in recent days, it is easy to produce large biomass of sponges without harnessing its natural populations. Sponges have been experimentally farmed for over 100 years with early attempts done in the sea to supply bath sponges (Duckworth, 2009). For commercial sponge aquaculture, increased productions of sponge biomass coupled with retaining high biosynthesis of target bioactive metabolites are the major pre-requisites (Duckworth and Battershill, 2003). The similar is the case with soft corals too. There is a need to develop suitable culture techniques for some of the potential soft coral species for mass production which can be utilized for extraction of compounds.



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Inauguration of winter school 2018 by Padma Bhushan Dr. Manju Sharma



Photo with Dr. K. Gopakumar, Formerly DDG ICAR (Fy)



Field visit to India Sea Foods



Field visit to BOS Naturals



Field visit to Accelerated Freeze Drying Co. Ltd

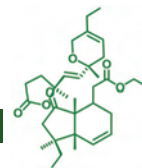


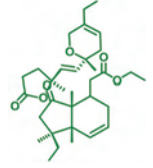
Photo with Dr. Meledath Govindan



Lectures and Interactive Sessions



Practical Sessions





ICAR-CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Ernakulam North P.O., Kochi-682018, Kerala

ICAR Sponsored Winter School on Recent Advances in Bioactive Compounds from Marine Organisms and Development of High Value Products for Health Management
January 23 to February 12, 2018

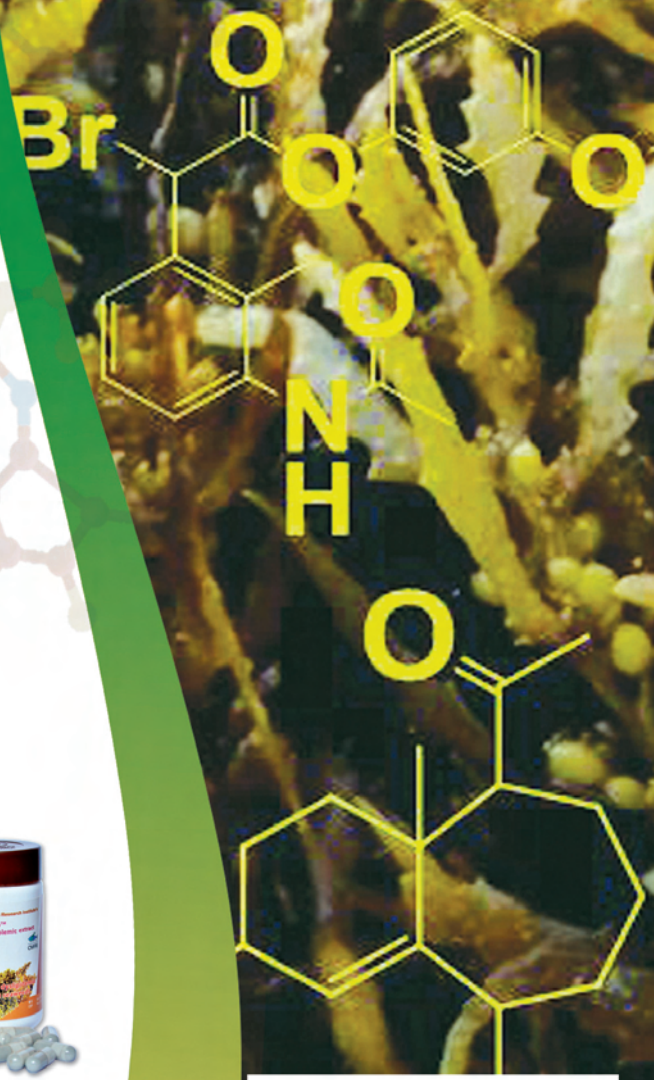




കേന്ദ്രീയ സമുദ്ര മാത്സ്യകൃി അസൂസുധാല സംസ്ഥാല
Central Marine Fisheries Research Institute
കേരള മാത്സ്യകൃി അസൂസുധാല സംസ്ഥാല

Sitting (L to R) Minimol K.C., Grace Thomas, Kajal Chakraborty (Course Director), P. Vijayagopal (Head, Marine Biotechnology Division), A. Gopalakrishnan (Director), Paulson Mathew, Sathu T., Radhakrishnan E.K.

Standing (L to R) Aswathy Elizabeth Mani, Sreemol C.K., Prima Francis, Soumya Krishnan, Minju Joy, V. Rani, Seeja Thomachan Panjikkaran, Sheneya Festus, Drishya K., Anie Y., Suja Rani S., Sindhu Issac, Teena P. Varghese, Magna Thomas, Santwana Palai, Norma Xavier Chelat, Naheef K., Satya Narayan Sahoo, Jaimin Hareeshbhai Bhatt, Ajoy Saha, Senthil Kuppusamy, Kedar Shashikant Damle, Shubhajit Dhara, Midhun Dominic C.D., Manukuttan K.S., Suji Chandru, Tima Antony, Soumya Salas



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