# CARIBBEAN MARINE BIODIVERSITY AS A SOURCE OF NEW COMPOUNDS OF BIOMEDICAL INTEREST AND OTHERS INDUSTRIAL APPLICATIONS

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**Summary**

The Cuban marine flora and fauna is characterized by its richness in species that represents an extraordinary natural source of unexplored biomolecular diversity. Many of those biomolecules could represent important tools for industrial applications. For this reason, in our Centre the main aim was focused on the isolation, purification and characterization of bioactive compounds present in marine invertebrates with particular emphasis on sponges, cnidarians, seaweeds and marine plants. Micro organisms represent also an important source of biologically active compounds that were included in our studies. In our Institution, research is performed by a multidisciplinary team. The purpose of this article is to review the most important results of the Centre of Marine Bioproducts in the last few years.
Key words. Marine organisms, sustainable use, marine bioproducts.

It is a real fact that the importance of marine organisms as a source of new substances is growing. Marine organisms represent approximately a half of the total global biodiversity, it covers more than half of the earth surface and this source has been poorly exploited. The sea offers an enormous resource for novel compounds and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. These compounds could be used for basic research, in industry and biomedicine. A very different kind of substances has been obtained from marine organisms. There are several reasons to support this fact: great biodiversity (chemical diversity) poorly studied, organisms submitted to unique environmental conditions and non specific relationships (predation, defence). This environment is very different in many aspects from the terrestrial, a situation that demands the production of quite specific and potent active molecules. Cuban marine ecosystem represents a valuable source for this kind of studies.

A multidisciplinary strategy that combines the internal capacities and the collaboration with national and foreign institutions is followed in our studies. Our main objectives are: to obtain new structures with novel mechanism of action and to search for new products for medical application and/or others purposes. The general approach comprises a first step to obtain an extract for the screening of some pharmacological activity. Once selected the most promised extract, bio-guide purification steps are followed. Finally, for structural determination and pharmacological characterization are usually included in collaboration with foreign institutions and specialized laboratories. The main aim of this article is to summarize the most important results of the Centre of Marine Bioproducts in the last few years.

## Anti-inflammatory, analgesic and antioxidants

Since 1980, when Manoalide was isolated by de Silva and Scheuer[1], the pharmacologists have been studying the marine bioactive substance with anti-inflammatory, analgesic and antioxidant activity. There are a number of marine compounds that are able to interact with enzymes involved in the inflammatory process, especially

phospholiphase $A_2$ ($PLA_2$) [2-5]. Thus, marine natural products can be new experimental probes to establish the mechanisms of inflammation, in addition to their potential role as novel anti-inflammatory agents.

On the other hand, the production of reactive oxygen species is also prevalent in the world's oceans, and oxidative stress is an important component of the stress response in marine organisms exposed to a variety of insults as a result of changes in environmental conditions such as thermal stress, exposure to ultraviolet radiation, or pollution[6]. Therefore, as an adaptative response, many marine organisms are protected against oxidative insult by producing strong natural antioxidants. These molecules that can safely interact with free radicals to terminate the chain reaction are thought to replace the synthetic antioxidants that are widely used at present time. Since, active oxygen and free radicals are related to various pathophysiological events, such as inflammation, aging, mutagenicity and carcinogenicity [7,8], marine organisms are an invaluable source of bioactive compounds with potential therapeutic effect.

In order to find anti-inflammatory and/or antioxidants effects in marine organism present in Cuban's sea shore, in the last few years our group has carried out a screening of 80 extracts prepared from 60 species of algae, sponges, cnidarians and marine plant presents in Cuban's sea shores [9-13]. Our general approach has comprised an initial "in vivo" test of the hidroalcoholic extract of these species as anti-inflammatory agents after intraperitoneal (ip) administration in mice by using classical models for the evaluation of anti-inflammatory drugs such as, carrageenan paw oedema, croton oil ear oedema and arachidonic acid (AA) ear oedema. Furthermore, according to the previous results in such a model, we tested the "in vitro" effects of the selected extracts on enzymes involve in AA pathway and other pro-inflammatory mediators. Together with this, we also evaluated the effect of the extract as analgesic agent by the use of the classical writhing test in mice. We also tested the possible antioxidant potential of the extract, evaluating their "in vitro" radical trapping properties and the "in vitro" inhibition of Fe-mediated lipid peroxidation in rat brain homogenates.

One of the studied extract, the hidroalcoholic extract of the seaweed of the genus *Stypopodium* [14] , showed after single i.p administration (5, 10, 20 $mk.kg^{-1}$) prior to the application of the pro-inflammatory agent, that did not inhibit the inflammation-induced by croton oil. On the contrary, at the same concentration range a significant inhibition on the AA-induced ear edema was observed that reached a plateau of about 70% at dose between 10 and 20 $mg/kg^{-1}$. Accordingly, the extract ($10\mu g.mL^{-1}$) inhibited "in vitro" almost completely human recombinant and bee poison $PLA_2$ activities. After administration within the same dosage range the extract also inhibited acetic acid-induced writhing. Besides, potent "in vitro" radical trapping properties were observed against ABTS ($5\ \mu g.mL^{-1}$) and superoxide anion ($670\ \mu g.mL^{-1}$), which agrees with the potent inhibitory effect ($250\ \mu g.mL^{-1}$) on the generation of NFκβ induced by TNF. Taking together, the anti-inflammatory activity of the hidroalcoholic extract of *Stypopodium sp.* seems to be mainly related with the inhibition of lipoxygenase-derived proinflammatory mediators. However, a contribution of its antioxidant effect leading to an inmuno-regulation of pro-inflammatory cytokines can not be ruled out.

The extract of the sponge *Ircinia sp.* and the Coelenterate Anthipates *sp.*, also exhibited anti-inflammatory actions [15,16] (10, 20 and 50 $mg.kg^{-1}$), that seems to be related with different mechanism of action. Thus, both extracts, administered in the same manner that the previous one, inhibited carrageenan paw edema, reaching a maximum in inhibitory effect of about 80% and 90%, respectively, when administered at 50 $mg.kg^{-1}$. Additionally, the sponge's extract inhibited croton oil and AA-induced ear edema (maximum inhibitory effects 80 and 45%, respectively). However, the Coelenterate's extract exhibited lower potency, since only a partial inhibition of about 50 and 15% was observed on both inflammatory processes when administered at the maximum dose assayed (50 $mg.kg^{-1}$). This finding agrees with the slight "in vitro" inhibitory effects on $PLA_2$ activity found for *Anthipates sp.* extract. Further, both extracts potently scavenged ABTS cation and showed strong superoxide anion scavenger capacity (80%) when added ($670\ \mu g.mL^{-1}$) at the incubation medium. In addition for both, analgesic effects were found (maximum inhibitory effect of 80%) when administered within the same dosage range.

As it is observed, all the above mentioned extracts showed anti-inflammatory effects, however results from "in vivo" and "in vitro" models suggest different mechanism of action. This fact seems to be quite reasonable according to different chemical composition of the extract. Thus, the extract from *Ircinia sp.* is mainly composed by a mixture of alkaloids, aminoacids and saponins, whereas in the extract of *Stypopodium sp.* no alkaloid were detected but phenolic and terpenic structures were found among its main components.

## Antitumorals

The cancer is the second cause of death in Cuba and in many developed countries of the world, then as result of some researcher institutions of international prestige, as the National Institute of the Cancer of United States and Pharmamar of Spain, have been involved in the systematic search of substances of chemotherapy value, so much of terrestrial origin and as marine. One of the studied compounds in marine origin that it is in clinical phase III is the Ecteinoascidine 743 commercially called Yondelis [17,18] referred to a family of extracted products from a tunicate *Ecteinascidia turbinate*. Also, in the scientific literature there are different reports concerning to the anti-tumoral actions of seaweeds extracts [19,20,21].

In Cuba these studies were initiated in the period 1980-1990 in the National Institute of Oncology [22,23,24,25,26] and they were continued more recently in collaboration with our Centre [27,28]. Results demonstrated that of the 69 seaweed extracts evaluated, 60 were very cytotoxic in front of a brine shrimp *Artemia salina* a alternative toxicity test ($CL_{50}$ <10 $\mu g.mL^{-1}$), 40% belongs to extracts of green algae, 28% to extracts of red and 32% to extracts of brown algae, while the rest of the extracts (6) were moderately toxic for the referenced test [29]. Also, of the extracts evaluated as toxic, 30 of them, were tested by the interchange of DNA test in from of the bioluminesce of the bacteria *Photobacterium leiognathi* according to the developed method by Steinberg[30] modified by Miravet [31]. The experiments showed that 18.6 were positive. Six of these extracts, classified as moderately toxic, were evaluated in the National Oncology Institute, in front of two signal murine tumours: leukaemia P388 and adenocarcinome 755. The results showed that two extracts significantly increased the survival of the mice implanted with the referred tumours and in comparison to 5-fluorouracil (PPF) like positive control.

Two of the above mentioned extracts were submitted to a bio-guide purification procedure. The results show a semi-purified fraction that increased the survival in 51.5 % of inoculated mice with leukaemia P388 getting better results in comparison with the positive control (5-fluorouracil (PPF), a compound traditionally used in the cancer chemotherapy). Studies of the chemical composition of the fraction showed the presence of flavonoides and flavones. While effective treatments exist for acute lymphocytic leukaemia, particularly in the case of children, and for chronic mylogenous leukaemia, more efficacious treatments for other forms of acute and chronic forms of the disease. So, seaweeds could represent a promising source in the search of this kind of compounds[32].

## Neuropharmacologically active compounds

Our work in this field since its very first days has mainly been focused on the isolation, purification and characterization of novel compounds present in seaweeds and cnidarians. These studies have been developed by the aim of finding new tools to investigate on neurobiology at a molecular level and to obtain promising molecules or products potentially used for medical and others applications.

Several sea anemone toxins have become useful pharmacological tools for studying the structure and function of voltage-gated ionic channels, to the design of new drugs and to analyse functional process. These organisms are relatively abundant along Cuban seacoasts and have proved to be very interesting sources of bioactive compounds being the majority of them of peptidic nature. Marine peptides have opened a new perspective for

pharmaceutical developments and they are considered also as promising lead drug candidates. The most studied sea anemones for our group have been: *Bunodosoma granulifera, Condylactis gigantean, Stichodactyla helianthus and Phyllactis flosculifera.* From these animals we have studied two pore-forming toxins, phospholipases, $K^+$ channel inhibitors, $Na^+$ channel toxins and others compounds. The toxins acting upon $Na^+$ and $K^+$ channels are basic peptides of molecular masses 4-5 kDa and three disulphide bridges crosslinked.

BgK[33] is a potassium channel blocker from the sea anemones *B. granulifera* that competes with dendrotoxin-I for binding to rat brain synaptosomal membranes with a Ki of 0.7 mM, and suppress $K^+$ currents in rat dorsal root ganglion (DRG) neurons in culture. BgK partially and reversibly blocks $K^+$ currents in snail neurons with maximum blocking effect at 100 nM [34].

BgII and BgIII from *B. granulífera* cause toxicity in mice when injected intracerebroventricularly and exhibit a markedly different binding ability to rat brain synaptosomes; being both effects higher for BgII[35]. In dorsal root ganglia neurons both toxins produce a concentration-dependent slowing of the TTX-S sodium current inactivation ($IC_{50}$=4.1±1.2 and 11.9±1.4 μM, respectively) with no significant action on activation time course or current peak amplitude[36]. In rat cardiomyocytes, BgII and BgIII slow the rapid inactivation process and increased the current density with $EC_{50}$ of 58 and 78 nM respectively[37] Moreover, on the cloned hH1 cardiac sodium channel expressed in *Xenopus laevis* oocytes, both toxins slowed down the inactivation process of $Na^+$ currents ($EC_{50}$= 0.38 and 7.8 μM respectively)[37] . The evaluation of these toxins on five different cloned sodium channels expressed in *Xenopus laevis* oocytes showed that they possess the highest efficacy for the insect sodium channel. BgII is specially potent on the insect sodium channel with an $EC_{50}$ value of 5.5 ± 0.5 nM[38]. In addition, there were evaluated other peptides from sea anemones: CgNa (*Condylactis gigantea),* ApC (*Anthopleura elegantissima*) and BcIII (*Bunodosoma caissarum*). They were studied in DRG neurons using whole cell patch clamp technique[39,40,41]. Under current clamp condition these peptides increase the action potential duration. This effect is due to slowing down the inactivation process of the sodium current without modifying the activation process. They show different affinities to the sodium channel and exhibit some peculiarities in their action. The $IC_{50}$ values were: ApC 1.25 ±1.06 μM; CgNa 1.46±1.2 μM and BcIII 2.7±2 μM.

In a very recent investigation by using a purification strategy comprising gel filtration, ion exchange and reversed-phase chromatography, new toxins acting upon glutamate receptors and acid-sensing ion channels (ASICs) have been isolated. Further characterization experiments are in progress.

In addition to the previously described polypeptide toxins, a purine derivative has also been obtained from B*unodosoma granulifera*: Bainh. acting on different ionic currents[42]. We have also evaluated some pharmacological properties of two cytolytic toxins from the sea anemone *Stichodactyla helianthus*. In molluscan central neurons both cytolisins irreversible decreased the cholinergic responses ($EC_{50}$ 0.6 μM for StI whereas for StII the maximum blocking effect (50%) was reached at 6.6 μM). Moreover, it was observed an increase in the pacemaker action potential duration in the concentration range assayed. In electrically stimulated guinea pig isolated left atria by contractility assays, St I and St II elicited a concentration dependent increase (partially reversible after washing), of the contraction force amplitude (EC50 0.3 and 0.03 μM for StI and StII respectively. Studies with pH-inactivated cytolysins suggested the existence of an additional pharmacological mechanism to pore formation in guinea pig that could contribute to the described pharmacological action [43,44] .

Moreover, there are several polypeptide toxic fractions from sea anemones and a jelly fish that seem to exhibit unique and potential useful properties: a fraction with phospholipase A activity that acts on ionic channels, others semi-purified fractions that interact with cholinergic receptors obtained all from *Condylactis gigantea*[45,46,47,48], a fraction from the sea anemone *Phyllactis flosculifera* that acts as on glutamate receptors[49] as well as two fractions from *Physalia physalis* showing anticholinergic and antiglutamatergic effects, respectively[50,51,52].

The seaweeds constitute another natural resource of great variety and abundance in the Cuban coralline reefs. In the scientific literature actions of extracts and isolated compounds of brown algae have been described on the Nervous System[53-56]. We have studied the neuropharmacological properties of several seaweeds from the genus *Turbinaria, Sargassum*, *Dictyota, Ulva* and *Padina* using behavioural and electrophysiological techniques. Extracts were intraperitoneally and/or orally administered to mice (OF-1 line, 18-22 g body weight) as single doses (40-1000 $mg.kg^{-1}$ weight). The extracts were also evaluated as anticholinergic and antiglutamatergic agents using snail receptors (0.2 – 1 $mg.mL^{-1}$). Our results showed that the most relevant effects were; sedative effect as well as anxiolytic action for the single oral administration of *Dictyota´*s extracts and anticolvulsivant effects for *Sargasum* extract. Some of the extracts act at the level of cholinergic and glutamatergic snail receptors at the concentration range tested. Besides, single intraperitoneal administration of the extracts showed that all, except that from *Ulva sp.*, significantly increased the onset of convulsions induced by ammonium. The fact that the pharmacological actions in mice were observed after single administration suggests that the active components of the extracts can rapidly cross the blood barrier allowing them to act on the Central Nervous System. The neuropharmacological actions presented here open new therapeutical possibilities for these species[57,58] .

## Compounds for biotechnological purposes.

Marine biodiversity is extremely high, as a direct consequence of the extraordinary variability of the marine biosphere. The potential of marine organisms for commercial development and exploitation impinges on virtually every area of biotechnology. Marine microorganisms are widely recognized not only because of the role in the marine ecosystem and because they represent a valuable source of novel natural substances of biotechnological interest[59]. They posses the capacity to degrading complex structures such as xenobiotics, hydrocarbons. In addition, from microorganisms have been isolated novel compounds to develop of pharmaceutical industry.

Since the end of 1989, the Institute of Oceanology and at the present the Centre of Marine Bioproducts was able to establish a Marine Micro organism Collection (MMC) isolated from Cuban coastal zone. Actually the Collection is composed by more than 400 bacterial strains isolated from marine ecosystems, waters and sediments. The more representative *genus* in the MMC are *Bacillus, Micrococcus* and *Pseudomonas.* On the other hand, it was possible to isolate some new genera of bacteria from Cuba, such as *Burkholderia*, *Chryseomonas, Pasteurella* and *Stenotrophomones*. Up to the present we have identify the following activities from these bacterial strains: 26% are capable to produce antitumoral compounds, 7% produce antifungal metabolites, 20% produce antibacterial metabolites, 15% produce antifungal and antibacterial metabolites. Also, from them, 42% shows proteolytics activity, 50 % hemolytics and 25% are capable to degrade hydrocarbons using different conditions. As many as 57 % of all the bacteria present in the collection showed wide-spectrum activities.

## Development of New products.

In our Centre has been obtained, from marine plants and seaweeds, some products with anti aging properties (or skin protection) and stimulates hair growth. From a marine plant, that is highly abundant in Cuban coasts, a product; named BM21, was obtained. Experimental studies showed that BM21 (at 10 %) helps in the recovery of the normal properties of the epidermis and the irradiated damaged dermis, and particularly in the disposition and organization of the collagen and elastic fibres[60,61]. Also, BM21 promotes hair growth in an “in vivo” assay in mice the same range of effectiveness than a commercial product from human placenta with the same specificity, used as positive control in our experiments (results certified by LIORAD Laboratories, Cuba). Also, BM-21 shows anti-inflammatory and antioxidant properties and fulfil the requirements for micro-organism content. This product was considered potentially non toxic for human in toxicology studies of dermal irritability

in rabbits; ophthalmic irritability (test of HET-CAM) and dermal sensitization (results certified by LIORAD Laboratories, Cuba). Its stability studies demonstrated that BM21 maintains its chemical characteristics and biological properties at least for a period of twelve month after being elaborated. The product BM-21 has its Approval Certificate granted by the National Registration Office, Institute of Nutrition and Food Hygiene that belongs to Ministry of Public Health of Cuba as "A raw material to be used in cosmetic industry" with number **041/01-XII** and was patented in the Republic of Cuba with number **CU 22931.**

Other product named UL-2, obtained from a seaweed that is highly abundant in Cuban coasts, also improved the recovery of the normal properties of the epidermis and the damaged dermis of irradiated mice skin, and particularly in the disposition and organization of the collagen and elastic fibres[62]. Different than BM-21, this product does not stimulate hair growth.This product has its Approval Certificate granted by the National Registration Office of the Institute of Nutrition and Food Hygiene that belongs to Ministry of Public Health of Cuba as "A raw material to be used in cosmetic industry" and its is patent pending. Searching for new products, studies with another seaweeds extracts are in progress. These studies have revealed promising results for the cosmetic industry.

It is known that organisms are capable to catch different things from the environment and use it to enhance their growth and metabolism. On this unique characteristic is based the fundamental principle of bioremediation; "to use microorganism to catch contaminated substances from the environment and/or convert it to a nontoxic form."

In this regard, the Centre of Marine Bioproductos has developed different products based on the capacity of marine bacteria to degrade hydrocarbons and to produce different metabolites. We have obtained four products against oil pollution in natural ecosystems, they were patented and called: BIOIL, k-BIOIL, CBM-225 y BIOIL-FC. BIOIL, the first of these products, is based in the immobilization of cell in a biodegradable source whereas BIOIL-FC is used in form of free cells. BIOIL-FC has been successfully used since 1992, in different oil spill in Cuban coastal zone, such as in Matanzas Bay, Cienfuegos Bay, Levisa Bay, Varadero Beach and Jibacoa Beach. There were obtained good results that were supported by High-Resolution Gas Chromatography analyses. On another hand, we demonstrated their capacity for degrading different fractions of oil, including the asfaltenes. In all cases, the removal of oil was higher than 75 % in only 30 days.
Also, from a marine bacterium, we have obtained a biosurfactant called IDO-503. It was used in the enhancement of oil recovery from some different oil spills in the north of Havana. The capacity of oil wells production was triplicate, when the product was added to the field.

## Conclusions

The derivative consequences of the natural and human activity represent a danger for the protection and the sustainable use of the marine diversity. As a response to environmental insult, marine organisms are capable to synthesize numerous compounds that can act on a variety of molecular target that could potentially contribute to various pharmacological classes. These factors, together to the richness of the Cuban marine ecosystems, still scarcely studied, reinforces the interest to accelerate the studies about the potentialities of non conventional marine organisms as source of interest for the biomedical and others industrial applications. With this aim, a multidisciplinary approach has been followed in our centre where different laboratories and disciplines (ecologists, taxonomists, chemists, and pharmacologists) have been integrated under a common purpose. Under these criteria, important results have been obtained in terms of new molecules/activities or products of potential biomedical interest, as well as other of successfully introduced in our country. However, a future strategy will promote the integration of different groups of investigation in this field to improve the exchange of technologies and knowledge. This will contribute to strength the scientific and technological facilities in this field. Therefore, it would be necessary to elaborate strategies for the future development and the application of scientific results mainly those related to public health.

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