



# Bioprospecting deep-sea marine animal lipids from Azores for therapeutic applications



Bio prospecção em lípidos de animais marinhos de profundidade dos Açores  
para utilizações terapêuticas

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**Supervision:**

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**Co- Supervision:**

Maria Ana Almeida Colaço, Ph.D.

Susana P. Gaudêncio, Ph.D.

Dissertation presented to Universidade dos Açores for obtaining the doctoral  
degree in Marine Sciences, specialty in Marine Resources

Horta, May, 2015

UNIVERSIDADE DOS AÇORES

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## Cooperation Institutions:



Department of Oceanography and Fisheries at University of the Azores/ Institute of Marine Research/ LARSyS - [Associated] Laboratory of Robotics and Systems in Engineering and Science.



Centro de Malária e Doenças Tropicais (IHMT) – Universidade Nova de Lisboa). Professor Doutor Virgílio do Rosário and Doutora Dinora Ferreira.



Organic Geochemistry Department at University of Bremen/ Hinrichs Lab at MARUM. With supervision by Professor Marcus Elvert.



Center for Marine Biotechnology and Biomedicine - Scripps Institution of Oceanography at University of California. With supervision by Distinguished Professor Bill Fenical.



Faculdade de Ciências e Tecnologia at Universidade Nova de Lisboa:

UCIBIO-REQUIMTE, Life sciences Department- Group lidered by Doutora Alexandra R. Fernandes.

LAQV –REQUIMTE, Chemistry Department, with supervision by Doutora Susana P. Gaudêncio.



Faculdade de Farmácia at Universidade de Lisboa/ Instituto de Investigação do Medicamento. Group lidered by Professora Doutora Cecília Rodrigues.

This research was only possible through support of FCT that financed it with Ph.D. scholar ref. SFRH/BD/72154/2010.

## **FCT** Fundação para a Ciência e a Tecnologia

MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

Several projects financed part of laboratory consumables and chemicals products used during this research, namely PTDC/QUI-QUI/119116/2010-"Ocean Treasures", PTDC/MAR/111749/2009-DeepFun; PEst-C/EQB/LA0006/2013, Pest-OE/BIA/UI0457/2011-CREM, FP7/2007-2013 n° PCOFUND-GA-2009-246542 and LA 9 – 2013-2014 – PEST OE/EEI/LA0009/2013, financed by FCT, Ministério da Educação e Ciência, Portugal and FEDER.



Dedicado à minha família  
Pena, Milagre e Monteiro que  
me ensinou a importância da  
diversidade.

Advertency: This thesis was written at the sound of *Chamarritas...*

## Acknowledgments

So many people contributed in some way for this thesis to become a reality. I hope I did not forget anyone...my apologies in that case.

Abroad and foreigner...

My great appreciation to Professor Bill Fenical, whom I have to thank for all that I have learned during these four years about marine natural products. Your patience planning next to me how to proceed with the research when the funding from FCT was cut unexpectedly was vital to continue and allowed me to finish my research. Your immense experience with marine natural products and your availability to continue teaching about what you know are of true inspiration. To his team, in particular to Lauren Paul for performing the cytotoxicity tests with MRSA and HCT-116 bioassays: your experience and professionalism is possible to see in the very low standard deviations values- thank you for your patience and kindness, and to Ellen Zefrir (Ph.D.), for introduce me to the NMRs machines and helping me with the preliminary NMR analysis.

To Andreas Eich for his great help processing the biological samples, especially by lyophilizing a big amount of coral samples for this study

To the chief scientists from missions MenezMar (Doctor Nicole Dubilier), and MomarSat 2011 (Doctor Pierre-Marrie Sarradin and Doctor Mathilde Cannat), for welcoming me onboard and allowing sample collection for my research. All scientific and technical crews from R/V "Meteor "and R/V "Pour quoi Pas?" and ROVs "Quest" and "Victor-6000".

All technical and scientific team at HinrichsLab, for their great help in the laboratory, especially Bernhard Viehweger, Jenny Wendt and Xavier Prieto-Mollar for their help with the GC-MS machines, and Jessica Arndt for her immense patience with all the tuning and change of columns to accomplish the perfect separations with LC-MS machines. Doctor Julius Lipp for his wise technical suggestions. Very especially both to Doctor Lars Wörmer who gave me a great help with the *Leiodermatium* project, and Professor Marcus Elvert,

for kindly reviewing parts of the thesis. Professor Elvert, also for his immense patient teaching me how to analyze MS spectra, as well as other chemical techniques that gave new insights in my Ph.D. research and for always being available to answer my endless doubts. A word of appreciation to Professor Kai-Uwe Hinrichs for accepting to host me at his lab in MARUM, although my work was about a complete different subject, and to my colleges at the HinrichsLab for their enthusiastic discussions about my work, always available to help with my technical hesitations and for the healthy scientific environment.

In Portugal...

To Luís Pires for his essential support and especially to Dr. Valentina Costa and Dr. Humberto for providing all conditions to work at the chemistry laboratories in DOP. Ricardo Medeiros, for providing ArcGIS maps. M.Sc. Valentina de Matos for her support with biological samplings, taxonomic identifications and for providing photos from biological samples. M.Sc. Andreia Braga-Henriques, for coral taxonomic identifications and discussions about valid hypothesis for those animals. Doctor Joana Xavier and M.Sc. Raquel Pereira, for sponge's taxonomic identifications (or the samples would still be referred along this work as *Porífera 1* and *Porífera 3!*). To Daniel Pereira and all not identified biological observers onboard fishery vessels, who had the kindness of bring me the biological samples well preserved enough to be used in this work, even in rough sea conditions...

To my college and friend M.Sc. Berta Solé, for all the help provided with samplings and for contributing with a good working environment at the laboratory. Doctor Gui Menezes for his wise and expert suggestions with statistic methods. Colleague Ph.D. student José Nuno Pereira, for providing photos from his own doctoral work, from sponges *in situ* taken by ROVs. Doctor Fernando Tempera for bring several samples collected during his own research at channel Pico-Faial. João Santos, for developing an informatics program for facilitating fatty acids data exploring (GC-FID). Carlos and Luís Monteiro for designing the cover symbol. Sr. Santos, the most available do-it-all technician at DOP in Azores University. Professor Virgílio do Rosário and

Doctor Dinora Ferreira for accepting me at IHMT to experiment crudes as antimalarials and especially to Marta Machado, with whom I have done most part of the *in vitro* bioassays. Doctor Florbela Pereira, for doing the NMR analysis, all support provided at FCT-UNL (Lisboa) laboratories and help interpreting NMR data. To both teams from Professor Alexandra Fernandes (Joana Silva e Pedro Martins) and Professor Cecilia Rodrigues (Sofia e Pedro), for continuing with anticancer bio-essays with HCT-116 for *Petrosia* and *Leiodermatium* extracts. To the head chiefs from both groups for accepting to perform the experiments...

My appreciation especially to my supervisors: Doctor Ricardo S. Santos, Doctor Susana P. Gaudêncio and Doctor Ana Colaço. Without this unique blend of expertise it would not be possible to do this research. The freedom they gave me to explore wrong paths were fundamental to learn and I deeply thank them for their confidence, funding with their own projects all materials I have needed in laboratory. A word of gratitude to Doctor Ana Colaço, for being the first who courageously accepted to embark in this completely new research, so differently from what we done until that moment. I recognize now how productive our discussions were and at the end, they were all worth it.

Last, but not least, a profound gratitude to all my friends and family, for their unconditional support. They have always believed in me, even when myself was starting to doubt...without your final push at the right moment, none of this would be possible.

My sincere appreciation to You All.

Note: In respect to the Portuguese who do not speak English and should have the possibility to understand how grateful I am to them, I add this note translating the acknowledgments part that concerns them:

Em Portugal...

Ao Luís Pires pelo fundamental apoio no laboratório de química e especialmente à Dr<sup>a</sup> Valentina Costa e Dr. Humberto por garantirem as condições necessárias à execução dos trabalhos no laboratório do DOP. Ao Ricardo Medeiros que pacientemente fez vários mapas em ArcGIS, até termos o "certo". À Mestre Valentina de Matos pelo apoio na recolha de amostras da colecta e identificação taxonomia de grande parte dos espécimens e cedência de fotos do material biológico. À Mestre Andreia Braga-Henriques, pela identificação taxonómica dos corais e discussão acerca de hipóteses válidas para o trabalho com estes animais. À Doutora Joana Xavier e à Mestre Raquel Pereira, pela identificação taxionómica das esponjas (se não fosse o trabalho da Raquel, ainda estaria a considerar *Porífera 1* e *Porífera 3!*). Ao Daniel Pereira e outros observadores não identificados a bordo de barcos de pesca que tiveram a gentileza de me trazerem amostras em muito boas condições de preservação, mesmo quando o estado do mar não era o melhor para preocupações com amostras de esponjas e corais...

À minha colega, Mestre Berta Solé, pelo apoio na recolha, processamento dos espécimes biológicos e camaradagem em laboratório. Ao Doutor Gui Menezes pelos seus valiosos conselhos acerca da metodologia estatística. Ao Mestre José Nuno Pereira, por ter cedido as fotos das esponjas dentro de água, capturadas por vários ROVs, as quais constituem parte do seu próprio trabalho de doutoramento. Ao Doutor Fernando Tempera por ter cedido material biológico recolhido no âmbito dos seus trabalhos de investigação no canal Pico-Faial. Ao João Santos, por ter desenvolvido um programa informático que permitiu agilizar todo o processo de análise de dados dos ácidos gordos (GC-FID). Aos criativos irmãos Carlos e Luís Monteiro por terem feito o design do símbolo para a capa. Ao Sr. Santos, a pessoa que sempre me "desenrascou" com alternativas criativas para os inúmeros

problemas em laboratório e pela sua preocupação em ter um ambiente de laboratório no DOP que compromettesse o mínimo possível minha saúde. Ao Professor Doutor Virgílio do Rosário e Doutora Dinora Ferreira por me aceitarem no IHMT para fazer a parte experimental relativa aos testes anti-maláricos e em especial à Marta Machado, por ter realizado grande parte dos ensaios *in vitro*. À Doutora Florbela Pereira, pelas análises de química estrutural por NMR, apoio em laboratório na FCT-UNL (Lisboa) e toda ajuda a interpretar os dados de NMR. À equipa da Professora Doutora Alexandra, (Joana Silva e Pedro Martins), bem como à equipa da Professora Doutora Cecilia Rodrigues (Sofia e Pedro), por terem realizado os testes em HCT-116 com os extratos de *Petrosia* e *Leiodermatium*. Aos chefes dos respectivos grupos por aceitarem proceder às análises...

Por fim, muito especialmente aos meus orientadores: Doutor Ricardo S. Santos, Doutora Susana P. Gaudêncio e Doutora Ana Colaço. Sem esta combinação invulgar de orientadores, de áreas tão distintas, não teria sido possível realizar este trabalho. A liberdade que me deram para poder explorar caminhos errados foram fundamentais para aprender, e agradeço a confiança que depositaram em mim para realizar este trabalho, costeando através dos seu projetos próprios os materiais e reagentes que necessitei em laboratório. Agradeço especialmente à minha orientadora Doutora Maria Ana Colaço, por ter sido a primeira a ter a coragem de embarcar nesta investigação bastante diferente do que ambas tínhamos feito até à altura. Reconheço que as discussões foram produtivas e no fim, valeram a pena.

Um último agradecimento pessoal aos meus amigos e família, pelo apoio incondicional pois sempre acreditaram em mim, mesmo quando eu própria começava a duvidar...sem o vosso empurrão na altura certa, nada disto seria possível!

A todos vocês, o meu sentido agradecimento."

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## Abbreviations

Br-FAs	Branched fatty acid(s)
CDCl <sub>3</sub>	Deuterated chloroform
COSY	Homonuclear correlation spectroscopy
CWCs	Cold-water coral(s)
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DOP	Departamento de Oceanografia e Pescas
DUFAs	Di-unsaturated fatty acid(s)
EEZ	Economic exclusive zone
ESI	Electrospray ionization
FAMEs	Fatty acid methyl ester(s)
FAs	Fatty acid(s)
FID	Flame ionization detection
GC	Gas chromatography
GL	Glycolipids
HSQC	Heteronuclear single-quantum correlation spectroscopy
HVF	Hydrothermal vent font
LAQV	Laboratório Associado para a Química Verde
MAR	Mid Atlantic ridge
MDS	Multi dimensional scaling
MIC	Minimum inhibitory concentration
MNPs	Marine Natural Products
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MUFAs	Monounsaturated fatty acid(s)
NCI	National Cancer Institute
NIST	National Institute of Standards and Technology
NL	Neutral lipids
NMIs	Non-methylene-interrupted (fatty acid)(s)
NMR	Nuclear Magnetic Resonance (spectroscopy)
NPs	Natural Products

NSA	Not significantly active
PL	Polar lipids
PUFA	Poly unsaturated fatty acid(s)
PUFAs	Polyunsaturated fatty acid(s)
QToF-MS	Quadrupole time-of-flight mass spectrometer
R/V	Research vessel
REQUIMTE	Rede de Química e Tecnologia
ROV	Remote operated vehicle
SD	Standard deviation (statistic)
SFAs	Saturated fatty acid(s)
Smt	Seamount
TIC	Total ion current (chromatogram)
UCIBIO	Research Unit on Applied Molecular Biosciences
Uw Vol	Underwater volcano
WHO	World Health organization

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## Resumo

A região dos Açores apresenta uma grande diversidade de comunidades faunísticas em ecossistemas marinhos de profundidade, com numa grande variedade de "hotspots" no fundo do mar, tais como montes submarinos, encostas das ilhas e fontes hidrotermais. No entanto, apesar desta enorme biodiversidade, os seus invertebrados marinhos de profundidade nunca foram bio prospectados enquanto produtos naturais marinhos funcionais. Esta tese teve como objectivo fazer-lo.

Foram extraídos lípidos de dezanove espécies animais, num total de trinta e seis extractos brutos, subsequentemente testados *in vitro* como antibacterianos, antimaláricos e anticancerígenos. Da diversidade de animais de profundidade que incluiu corais, esponjas, ouriços e invertebrados das fontes hidrotermais, os resultados revelaram que os extractos apresentam actividade para todos os ensaios biológicos testados, com as esponjas a apresentarem-se como os animais mais úteis como fonte de compostos naturais marinhos anticancerígenos e os corais de água fria, como antimaláricos.

Foi usada uma estratégia de fracionamento guiado pela bioactividade, nos extractos dos animais mais activos: as mega-esponjas *Petrosia* sp. e *Leiodermatium* sp.. Os esteróis (Petrosterol, Sitosterol e 23, 24-Dihydrocalysterol) foram identificados nas fracções de *Petrosia* sp. mais anticancerígenas mas devido a perda de actividade durante a separação dos compostos, não foi possível confirmá-los como principais anticancerígenos presentes. O estudo com a esponja *Leiodermatium* sp. resultou no isolamento de uma mistura complexa de compostos, demonstrando um potente efeito anticancerígeno ( $IC_{75}=0,08 \mu\text{g/ml}$ ). Os compostos foram identificados como pertencentes a uma mesma família de macrólidos e foram denominados "azorelides". Foi possível isolar os compostos azorelide A e azorelide B mas a esteroquímica absoluta de ambas as moléculas não foi determinada devido às suas escassas quantidades (1,1 mg de azorelide A/kg e 0,47 mg de azorelide B/kg de esponja *Leiodermatium* seca).

## Abstract

Azores region presents a great diversity of deep-sea ecosystems with faunal communities in a variety of benthic hotspots, as seamounts, islands slopes and hydrothermal vents. Despite its high biodiversity, these deep-sea invertebrates were never bio prospected in regard to their natural products. This thesis aims to do so.

Lipids were extracted from nineteen animal species and a total of thirty-six crude extracts were tested *in vitro* as potential antibacterial, antimalarial and anticancer activities. From a deep-sea diversity of corals, sponges, sea-urchins and hydrothermal vents invertebrates, results showed that extracts were active for all tested bioassays, with sponges presenting the best outcomes as anticancer and corals as antimalarial natural product sources.

A bioactivity-guided fractionation strategy was used in the most active animal extracts: the mega sponges *Petrosia* sp. and *Leiodermatium*. Sterols (Petrosterol, Sitosterol and 23, 24- Dihydrocalysterol) were identified in the most active anticancer fraction from *Petrosia* sp. but, due to activity lost during compounds isolation, it was not possible to confirm these as the main anticancer compounds present. Study with *Leiodermatium* sponge extracts lead to isolation of a complex mixture of compounds, showing potent anticancer activities ( $IC_{75}=0.08 \mu\text{g/ml}$ ). Compounds were identified as belonging to the same macrolide family and were named "azolelides". It was possible to isolate azorelide A and azorelide B, but their absolute stereochemistry was not achieved due to amount scarcity (1.1 mg of azorelide A/kg and 0.47 mg of azorelide B/kg of dry *Leiodermatium* sponge).

## 1 Introduction

There is a growing recognition of Ocean's biotechnological potential. If European Commission describes it as "one of the most exciting technological sectors", Institutes as Marine Engineering, Science, and Technology (IMarEST) recognize the sea as a "biotechnological frontier waiting to be explored". The marine biotechnological products have enormous potential and their most known usages go from anticancer agents, bulk chemicals such as adhesives, feed additives for aquaculture, to remediation of environmental damage. Current applications for high-value marine-derived products exists in several markets including antifoulants, biofilm inhibitors, bioremediation, high- and low-temperature tolerant enzymes with unique activities, human and animal tissue repair, nutraceuticals, and personal care products (Allen and Jaspers, 2009). Probably related to their roles in chemical defense, the marine environment is a rich source of complex, biologically active compounds as starting points for drug discovery, with marine invertebrates identified as one of the richest sources of bioactive compounds (Rocha *et al.*, 2011). Their biotechnological potential attracts scientific and economic interests Worldwide and although the number of research publications about new marine natural products from these animals increases every year (Leal *et al.*, 2012), it is still barely investigated compared to the vast range they live in. As most research on marine invertebrate's chemical ecology has been focused on tropical and temperate environments, a shift towards untapped regions and habitats increasing bioprospecting efforts targeting these regions is of great importance. Deep-sea areas may be more difficult to approach but their high biodiversity may provide unprecedented bioactive compounds due to environmental specificities those animals live in. Nevertheless, to fully realize the potential that these areas offer in terms of new products and processes it requires a concerted effort of many parties, bringing together skills and equipment's from a wide array of disciplines, including taxonomy, marine invertebrate biology, marine microbiology, molecular biology, chemistry, biochemistry and biomedicine (Allen and Jaspers, 2009). Effective collaboration from all these areas of knowledge is essential to attain any novel

marine biotechnological products, requiring interdisciplinary, multifaceted approaches and training in relevant disciplines in order to solve unique problems posed by the deep-sea marine environment.