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CBiOS Science Sessions - 2016 -

Unravelling new ethnopharmacological roles of *Plectranthus* species: biological activity screening

*Revelando novas acções etnofarmacológicas das espécies de *Plectranthus*: screening da actividade biológica*

Joana Andrade

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Abstract

This work focused on the screening of biological activities of several *Plectranthus* spp. (*P. madagascariensis*, *P. neochilus*, *P. grandidentatus*, *P. ecklonii*, *P. porcatus*, *P. prostratus*, *P. ornatus* and *P. saccatus*). The biological activity screening aimed at unraveling novel ethnopharmacological roles regarding antioxidant, anti-skin ageing, anti-inflammatory and anti-mycobacterial activities, of several extracts (aqueous, methanol, acetone and ethyl acetate) and isolated compounds (rosmarinic acid; chlorogenic acid; a mixture of β -sitosterol : stigmasterol (1:1); a mixture of α -amyrin : β -amyrin (3:1); a mixture of oleanolic acid : ursolic acid (1:4); a mixture of 1,6-di-O-acetylforskolin : 1,6-di-O-acetyl-9-deoxyforskolin (1:1); (11R*,13E)-11-acetoxylalima-5,13-dien-15-oic acid; (11R*,13E)-15-butyryloxyhalima-5,13-dien-11-ol; (11R*,13E)-halima-5,13-diene-11,15-diol; (11R*,13E)-11-acetoxylalima-5,13-dien-15-oic methyl ester; (13S,15S)-6 β ,7 α ,12 α ,19-tetrahydroxy-13 β ,16-cyclo-8-abietene-11,14-dione; 1 α ,6 β -diacetoxo-8 α ,13R*-epoxy-14-labden-11-one; Parvifloron D; 6,7-dehydroroyleanone; 7 α -acetoxo-6 β -hydroxyroyleanone; and 6 β ,7 α -dihydroxyroyleanone).

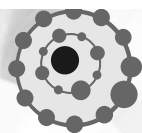
Antioxidant activity (DPPH method) revealed an increased activity of the organic extracts (20-76%), possibly due to polyphenols and abietane diterpenes. Acetylcholinesterase was studied in vitro and organic extracts showed no significant inhibition. Concerning the tyrosinase inhibition assay, it was observed high inhibition for the organic and aqueous extracts (56-68%), justified with a synergistic effect from mainly present compounds (46-75%). Also, in the collagenase assay, all tested samples showed high enzymatic inhibition (around 70%). In contrast to the results of the previous enzymatic assays, the anti-elastase assay revealed that the organic extracts of *P. grandidentatus* (ethyl acetate 42.84 \pm 4.2%) and *P. neochilus* (acetone 38.23 \pm 1.8%) were the most active.

In the anti-inflammatory assay, the non-cytotoxic compounds revealed unable to reduce NO production, after LPS stimulated inflammation. Finally, for the anti-tubercular activity screening, one halimane compound showed *Mycobacterium tuberculosis* H37Rv growth decrease with 2.1 \times 10⁵ CFU/mL, similar to the positive controls isoniazid (1.2 \times 10⁵ CFU/mL) and ethambutol (2.0 \times 10⁵ CFU/mL), suggesting its potential for further research development.

Overall, this was the first screening on *Plectranthus* spp. medicinal plants (concerning its tyrosinase, elastase, and collagenase inhibition, and also antioxidant, anti-inflammatory and anti-tubercular activities) of extracts and compounds for their corresponding preliminary scientific validation upon ethnopharmacological uses.

Lecturer's resumé

BSc in Biochemistry by the Faculty of Sciences (University of Lisbon), has recently accomplished the MSc in Biopharmaceutical Sciences from the Faculty of Pharmacy with First Class classification. Her thesis project entitled "Unravelling new ethnopharmacological roles for *Plectranthus* species: Biological activity screening" developed at the Research Center for Biosciences & Health Technologies (CBiOS), founding body of the Universidade Lusófona de Humanidades e Tecnologias (ULHT), and iMed.Ulisboa, Research Institute for Medicines and Pharmaceutical. Under supervision of Prof. Dr. Patrícia Dias Mendonça Rijo (PhD) and Prof. Dr. Célia Maria Cardona Faustino (PhD). To accomplish some thesis research milestones has also developed investigation at the Center for Marine Sciences CCMAR, University of Algarve for evaluation of anti-inflammatory properties of *Plectranthus* isolated compounds on RAW 264.7 macrophage cells stimulated with LPS (Biosafety Laboratory Level 2). Also, this year was awarded for a Short Term Scientific Mission: "Natural diterpenoids as potential anti-tubercular drugs" at the National Institute for the Infectious Disease Lazzaro Spallanzani - Istituto di Ricovero e Cura a Carattere Scientifico, Rome, Italy.



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Morphometric studies in experimental pathology: when the researchers aim to turn a histologic image into a statistical datum

Estudos Morfométricos em Patologia Experimental: Quando os investigadores pretendem transformar uma imagem histológica num dado estatístico

Pedro Faisca, José Catarino

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IGC - Instituto Gulbenkian da Ciência

Abstract

Nos dias que correm, com o incansável avanço da ciência através da experimentação animal, o patologista experimental surge com um papel cada vez mais importante na comunidade científica. Contudo são lhe apresentados desafios de cariz um pouco diferente do patologista clínico que, o obrigam a utilizar ferramentas algo diferentes. A morfometria permite transformar uma observação em variáveis constantes, algo que é muito prezado pelos investigadores pois permite efectuar tratamento estatístico das mesmas e assim dar ainda mais força aos dados da sua investigação. Nesta pequena apresentação abordaremos isso mesmo – a importância da morfometria no dia a dia de um patologista experimental bem como algumas ferramentas e metodologias que poderão tornar o seu trabalho mais proveitoso e menos trabalhoso.

Lecturer's resumé

Obteve o título de Médico Veterinário em 2000 na Universidade de Trás-os-Montes e Alto Douro e obteve o grau de doutorado (PhD) na área de patologia e experimentação em animais de laboratório na Universidade de Liège em 2007. Está creditado pela DGV desde 2007 como apto para efectuar experimentação animal. É desde 2005 professor convidado na Faculdade de Medicina Veterinária da Universidade Lusófona de Humanidades e Tecnologias no qual é regente das Unidades Curriculares de Citologia e Histologia I e II e Anatomia Patológica I e II. Fora da universidade exerce funções de histopatologista veterinário numa empresa privada (DNatech) e é também o patologista experimental da Unidade de Histopatologia do Instituto Gulbenkian da Ciência (IGC). É o presidente em funções da Sociedade Portuguesa de Patologia Animal.

Fez parte da organização de vários eventos na universidade (ex: Organização do Curso de Experimentação Animal segundo os requisitos da FELASA da Universidade Lusófona) e fora dela (ex: Organização do XX Encontro da SPPA-Ictiopatologia na FMV-ULHT em 2015), é autor e co-autor de artigos científicos e posters em variadas áreas como histopatologia veterinária, oncologia veterinária, cultura de células, biologia molecular, patologia de animais de laboratório entre outras publicados em revistas com impacto científico. Realizou comunicações livres em encontros de relevo bem como participou em várias arguções de teses de mestrado e doutoramento.

Analysis of the effects of massage on the peripheral circulation

Análise dos efeitos da massagem sobre a circulação periférica

Clemente Rocha

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Abstract

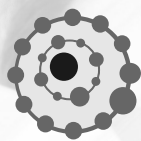
The peripheral circulatory disease is the third most important cause of cardiovascular disease in developed countries, representing a huge burden for health systems. Disease control and therapeutic effectivity is far from satisfactory, motivating new approaches. Massage is an ancient technique used in conventional and non conventional medicine, and has been used to relief symptoms and improve vascular functions. Nevertheless, its physiological implications and mechanisms are still poorly understood. In this study, we explore the impact of two massage maneuvers (upward and downward) in the peripheral microvascular response in the human lower limb, using non-invasive measurement techniques such as laser Doppler flowmetry (LDF) and photoplethysmography (PPG).

A group of young healthy volunteers, both sexes was included in the study after informed written consent. Following acclimatization, each volunteer was submitted to two massage protocols in one randomly chosen limb (test), lying supine, one in the ascending direction (upward) from the root of toes until mid-leg, and another in the descending direction (downward) from the mid-leg until the toes root. Each protocol consisted of three phases - I rest, II the massage and III the recovery. The contralateral leg was used as control and both foot were kept in the same position during protocols. Both protocols were executed in a random order, separated by a 30 min washout period. Circulatory changes were quantified by LDF and PPG.

Results shows that massage improves the lower limb perfusion in both protocols. These results also suggest that the simultaneous use of LDF and PPG, enables us to detect discrete changes in blood flow that could not be detected with a single technique approach.

Lecturer's resumé

Degree in Computer Engineering from ISPGAYA and MSc in Computer Engineering (ISCTE-IU Lisboa). Has dedicated an important part of his activity to Naturopathy (General Course of Naturopathy and Holistic Traditional Sciences - Institute of Traditional Medicine). Specialist in Antiaging, Ozone Therapy and Tallasotherapy by Universidad Europea del Atlántico / FUNIBER, and current PhD student in the doctoral program in Health Sciences at U Lusófona where he concluded an advanced training in Clinical Trials. He is particularly interested in peripheral vascular disease and non-conventional therapeutic approaches for its management and treatment.



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Encapsulation of biomolecules in Liquid and Solid Nano- & Micro-particles

Encapsulação de biomoléculas em nano e micro-partículas líquidas e sólidas

Luís Fonseca

Instituto Superior Técnico

Isolation, synthesis and nanoencapsulation of cytotoxic compounds from *Plectranthus* spp

*Isolamento, síntese e nanoencapsulação de compostos citotóxicos de *Plectranthus* spp*

Catarina Garcia

CBiOS - Research center for Health Sciences & Technologies,
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Abstract

Today, the encapsulation of active biomolecules in liquid and solid nano- & micro-particles is a great challenge for applications on industrial biotechnology, and development of delivery carriers of cosmeceuticals, nutraceuticals and pharmaceuticals.

The liquid heterophase systems have shown to be suitable methods for producing those materials matching the previous requirements of functionality and encapsulation of active and sensible biomolecules.

For the active these goals NanoLipCar Technology is the most recent development on Emulsion technologies that continues to be an exciting method and procedure for high-value applications in those industrial fields.

Lecturer's resumé

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<http://scholar.google.pt/citations?user=2IHjgNoAAAAJ>

http://www.researchgate.net/profile/Luis_Fonseca2/

Research Areas and expertise

Luís P. Fonseca is Associate Professor at the Department of Bioengineering of Instituto Superior Técnico (I.S.T.), Lisboa at University of Lisbon (UL).

Current research of Luis P. Fonseca focus on the use of oil-in-water emulsions in particular nanoemulsions for production of fruit flavors by enzyme esterification that led later to the design and development of encapsulation method based on hydrogels and lipid nanoparticles of a range of high-value products from flavors and fragrances, emollients, specialty and fine chemicals, nutraceutical and pharmaceutical that led to the development of actual NanoLipCar Technology at I.S.T.

Abstract

Cancer is one of the major causes of death worldwide. New attempts to find new anticancer compounds have been investigated, and plants are a valuable source for the discovery of bioactive compounds.

Plectranthus is a large and widespread genus with a diversity of ethnobotanical uses. In this work, 31 acetonetic, ethanolic and methanolic extracts were prepared from 16 different *Plectranthus* species, and a biological activity screening was carried out. A preliminary assessment of the toxicity of some *Plectranthus* spp. extracts was performed through a lethality test against *Artemia salina* L. brine shrimp. Their anticancer properties were assessed in different cell lines. Two acetonetic extracts were selected as the most active in both models (brine shrimp and cancer cell lines).

One of the cytotoxic spp. previously evaluated - *P. madagascariensis* - was phytochemically studied and a cytotoxic diterpene was isolated from its essential oil in high amounts. The anticancer properties of this compound, 6,7-dehydroroyleanone, were assessed in different cell lines, and the mechanism responsible for cell death was enlighten for the first time.

Along with 6,7-dehydroroyleanone, another diterpene previously isolated (7 α -acetoxy- β -hydroxyroyleanone) were used to perform Mitsunobu hemi-synthetic reactions, with the propose of substituting the C-12 hydroxyl group and improve the original properties. The structure elucidation of the synthesized compounds was carried out by 1D and 2D NMR spectroscopy. The biological activity of the new derivatives was evaluated and some structure-activity relationships were taken for future synthesis of new bioactive agents.

Lecturer's resumé

Catarina Garcia is a Health Sciences PhD student in Universidade Lusófona de Humanidades e Tecnologias (Lisbon, Portugal) in partnership with Universidad de Alcalá (Madrid, Spain).

She attended both Universidade Lusófona de Humanidades e Tecnologias (Lisboa, Portugal), where she was given a Scholarship attributed by Diploma of Merit, in 2010; and Faculdade de Farmácia da Universidade de Lisboa (Lisboa, Portugal), where she concluded her MSc in Pharmaceutical Sciences in December 2015.

Her MSc thesis focused on new approaches for the stimulation of dendritic cells regarding antitumoral applications, and she's visited the immunotherapy center and clinic Praxisgemeinschaft für Zelltherapie, Duderstadt GmbH & Co. KG, in Duderstadt, Germany.

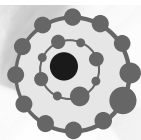
She has developed work in microbiology, micology, pharmacognosy, phytotherapy, phytochemistry and nanotechnology. She has attended to international training schools as well as practical courses on in vitro evaluation of anticancer compounds, NMR assignment and hematopoietic neoplasms hematologic evaluation. She's has participated in several workshops and symposiums, where she was able to present her work through 16 panel communications, 9 being international, and 1 nationally recognized as the 'Best Poster Award for Medicinal Plants and its Products'. She has also presented her investigational work through 2 oral communications (1 of them international). Currently, she has 2 published articles.

January | Janeiro 2017

Universidade Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal

February | Fevereiro 2017

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Development of polymeric nanoparticles for the treatment of yeast infections

Desenvolvimento de nanopartículas poliméricas para o tratamento de infecções fúngicas

Luís Roque

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Abstract

A administração oral é uma das vias mais atractivas e utilizadas para administrar agentes terapêuticos convencionais, mas também é muito utilizada para administrar novos fármacos que são obtidos a partir de fontes naturais (como extractos ou compostos isolados), devido à sua facilidade de administração e elevada adesão por parte do doente. Apesar destes factos, muitos destes novos fármacos, quando administrados oralmente apresentam baixa biodisponibilidade. Uma das soluções para superar esta limitação é a incorporação destes em novos sistemas de distribuição como as nanopartículas (NPs). A nanotecnologia tem demonstrado a sua capacidade de manipular material a nível molecular para atingir áreas específicas do corpo e controlar melhor a libertação de fármacos. Neste trabalho, as NPs foram produzidas utilizando diferentes polímeros mucoadesivos, tais como o poli (ácido láctico-co-glicólico) (PLGA), ácido poli-l-láctico (PLA) e alginato (Alg) e totalmente caracterizados.

Lecturer's resumé

Mestre em Ciências Farmacêuticas e Doutorando em Ciências da Saúde. Tem desenvolvido a sua carreira em investigação académica, colaborando em diversos projectos de investigação do CBiOS. Os seus interesses de investigação focam-se na área da nanotecnologia e na sua aplicação no tratamento de infecções fúngicas, associando para esse efeito, produtos naturais. Realizou algumas comunicações em congressos nacionais e internacionais e publicou artigos nestes âmbitos investigacionais.

The influence of lyophilization on polymeric nanoparticles for delivery of therapeutic proteins

Influência da liofilização de nanopartículas poliméricas para a veiculação de proteínas terapêuticas

Pedro Fonte

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UCiBIO - REQUIMTE, Department of Chemical Sciences - Applied
Chemistry Lab of Faculty of Pharmacy, University of Porto, Portugal

Abstract

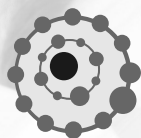
Therapeutic proteins are used to treat specific pathological conditions and severe diseases. The encapsulation into polymeric nanoparticles is a powerful strategy to deliver them in a sustained manner and preserve their stability. However, polymeric nanoparticles may get unstable in aqueous suspension, leading to their aggregation or fusion. The lyophilization comes to the front line as an useful approach to avoid the instability of nanoparticles. Nevertheless, this dehydration process may induce stresses to nanoparticles and encapsulated proteins. The use of excipients such as cryo- and lyoprotectants and the optimization of lyophilization cycle, may be useful strategies to avoid such stresses. Thus, the main aim of this work was to undergo an optimization of lyophilization parameters of polymeric nanoparticles for delivery of therapeutic proteins, to preserve the stability of nanoparticles, and with utmost importance, better preserve the structural stability and bioactivity of proteins. For this purpose, poly(lactic-co-glycolic acid) (PLGA) as polymer and insulin as therapeutic protein were used as models.

Lecturer's resumé

Pedro Fonte is Assistant Professor at Escola de Ciências e Tecnologias da Saúde of Universidade Lusófona and member of the Development of Delivery Systems Group of Universidade Lusófona Research Center for Biosciences & Health Technologies (CBiOS), Lisbon, Portugal. He is also Research Associate in UCiBIO, REQUIMTE, Department of Chemical Sciences - Applied Chemistry Lab of Faculty of Pharmacy, University of Porto, Portugal.

He obtained his PhD in Pharmaceutical Sciences – Nanotechnologies specialty from the Faculty of Pharmacy, University of Porto, Portugal in collaboration with the University of Copenhagen, Denmark, in 2016. Currently, his area of scientific activity is on development of new drug delivery systems by means of colloidal carriers, structural characterization of proteins entrapped into micro- and nanocarriers, lyophilization of biopharmaceuticals, targeted and controlled delivery of drugs across biological barriers, solid state characterization of pharmaceuticals and drying of pharmaceuticals and biopharmaceuticals. He is also interested in the up-scale of pharmaceutical and food products and is involved in entrepreneurship activities.

In the development of his work acted as partner of 5 competitively funded projects (FCT and CESPU), received several awards and distinctions, and published 8 book chapters and 23 peer-reviewed papers. He is also member of the editorial board of international peer-reviewed journals (3) and referee of top-ranked journals in his area of expertise.



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Abietane Cationic Amphiphiles (ACA)_loaded polymeric microspheres to tackle resistance bacteria

Microsfersas poliméricas de abietanos catiónicos anfifílicos (ACA) aplicadas a bactérias resistentes

Íris Neto

CBiOS - Research center for Health Sciences & Technologies,
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Use of the jellyfish *Catostylus tagi* in human food

*Utilização da medusa *Catostylus tagi* na alimentação humana*

António Raposo

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Abstract

This work focused on the development of novel abietane cationic amphiphiles (ACAs) by chemical modification of dehydroabietic acid (DHA), leading to cationic amide derivatives by condensation of the DHA carboxyl function with the amine group of biogenic polyamines (spermine) and basic proteinogenic amino acids (arginine).

The antimicrobial activity screening of DHA and novel ACA derivative showed that the compounds had inhibited the planktonic and biofilm growth of a collection of Gram-positive and Gram-negative bacteria and a yeast.

The microencapsulation of the compounds and the stability studies demonstrated that DHA remains stable within alginate microspheres.

Regarding cytotoxic evaluation, further studies are needed since no cytotoxicity was shown on the HaCat cell culture assay.

Future perspective is to contribute further to the studies of DHA and its derivatives, to scientifically validate their uses, understand their safety, and unravel new bioactive compounds with therapeutic potential and specific targets.

Lecturer's resumé

Íris Neto is a Master degree student in Biopharmaceutical Sciences at Faculty of Pharmacy, University of Lisbon. She is also a pharmacy technician that works at Cordeiro and Praxis pharmacy group.

She graduated in Pharmacy in 2013 at Escola Superior de Saúde Ribeiro Sanches and will obtain her MSc in Biopharmaceutical Sciences from the Faculty of Pharmacy, University of Lisbon, Portugal in collaboration with the Universidade Lusófona das Humanidades e Tecnologias, Lisbon, Portugal in 2017.

In the development of her work she published 1 book chapters, 5 conference posters, 1 conference virtual presentation and 4 peer-reviewed papers.

Abstract

The studies for a sustainable future of mankind by international organizations such as FAO are unanimous in alerting for the need to change many of today's consumption habits. Innovative food sources, as well as readily available, are widely sought nowadays and jellyfish are considered one of them.

We aimed to study *Catostylus tagi*, an edible jellyfish native of the estuaries of Tagus and Sado rivers, Portugal. An alternative storage and cooking process was developed to replace the traditional Asian salting procedure. After chemical characterization, the obtained food product was submitted to microbiological, heavy metals, and allergenic control as well as to sensory evaluation.

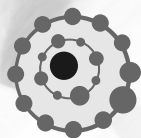
The results of our investigations encourage the intake of boiled umbrellas by Europeans since no allergic reaction was found even in seafood allergic patients and panelists scored it at the level of good acceptance.

The continuity of the studies points to a gastronomic elaboration of the product.

Lecturer's resumé

António Raposo is Assistant Professor at Escola de Ciências e Tecnologias da Saúde of Universidade Lusófona and Member of the Food Sciences and Phytochemistry Group of Universidade Lusófona Research Center for Biosciences & Health Technologies (CBiOS), Lisbon, Portugal. He is also Research Associate in CiiEM, Centro de Investigação Interdisciplinar Egas Moniz, in the Department of Animal Pathology, Animal Production, Bromatology and Food Technology, Faculty of Veterinary, University of Las Palmas de Gran Canaria, Spain and in the Ton Duc Thang University, Vietnam.

He graduated in Nutritional Sciences in Instituto Superior de Ciências da Saúde Egas Moniz, 2009 and obtained his PhD in Food Safety from the Faculty of Veterinary, University of Las Palmas de Gran Canaria, 2013



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Applicability of microalgae in the formulation of cosmetic products

Aplicabilidade das microalgas na formulação de produtos cosméticos

Ana Morocho

Universidade de São Paulo- Departamento de Farmácia.

Abstract

Microalgas são organismos eucariontes, unicelulares e fotossintetizantes com grande potencial para produção de ácidos graxos, pigmentos como os carotenóides e clorofila, bem como outros biocompostos de interesse na indústria de alimentos, farmacêutica e de cosméticos. Este projeto avaliou o potencial biotecnológico de duas espécies de microalgas, a saber, *Neochloris oleoabundans* UTEX 1185 e *Ankistrodesmus braunii* UTEX 245. Ambas as espécies foram cultivadas em fotobiorreatores fechados de 3,5 L tipo "air-lift", sob diferentes condições visando à otimização do crescimento e produtividade celular, o que, conseqüentemente, leva ao aumento da produtividade em biocompostos de valor agregado como os metabolitos com atividade fotoprotetora e antioxidante. Foram testadas as atividades antimicrobiana, antioxidante e citotoxicidade de diferentes extratos (metanólico, acetônico e aquoso) de biomassa liofilizada de ambas as espécies de microalgas. A segurança e eficácia clínica de formulações dermocosméticas contendo os extratos ativos dessas microalgas está a ser avaliada.

Lecturer's resumé

Possui graduação em Bioquímica Farmacêutica – Universidad Central del Ecuador (2004), mestrado (2010) e doutoramento (2014) em Tecnologia Bioquímico-Farmacêutica – Universidade de São Paulo. Foi assistente nas disciplinas: Química Orgânica, Toxicologia, Fitoquímica e Farmacologia na Escola de Bioquímica e Farmacia da Universidad Central del Ecuador (2005-2008). Tem experiência nas áreas de Farmácia e Biotecnologia, com ênfase em bioprocessos industriais, tecnologia de fermentações, cultivo de micro-organismos fotossintetizantes e tratamentos desses meios de cultivo.