14th International Congress of Ethnopharmacology

VIII Simposio Internacional de Química de Productos Naturales y sus Aplicaciones




23rd - 26th September 2014

Hotel Patagónico, Puerto Varas, Chile

Book of Abstracts

http://14ise-slf.utalca.cl/

WELCOME

Welcome to Puerto Varas and the 14th International Congress of Ethnopharmacology and VIII Simposio Internacional de Química de Productos Naturales y sus Aplicaciones

The 14th International Congress of the Society for Ethnopharmacology is held for the first time in a Spanish-speaking country from South America.

We are holding the meeting in Puerto Varas, a city located in the Provincia de Llanquihue, Region de los Lagos, at the shore of the Llanquihue lake, one of the largest natural lakes in South America. Puerto Varas is a touristic city, well known for its German traditions, food, fish and seafood, natural environment which includes the perfect cone of the Osorno Volcano and the snowcapped peaks of the Volcanos Calbuco and Tronador, clearly visible from the lakefront. Puerto Varas is one of Chilean's most beautiful cities: a charming convention city full with all the facilities you would expect from a large city, while maintaining the individuality and friendliness of the south of Chile.

We have put together an exciting and broad program covering all of the major themes of Ethnopharmacology and Natural Products research, including analytical methods in phytochemical analysis, bioactivity and mechanisms of action of natural and synthetic compounds, bioprospecting, ethnobotany, metabolomics and food plants, nature-based design of bioactive compounds, phytochemistry and chemosystematics, synthesis of bioactive compounds, traditional medicines, among many others.

The plenary and keynote lectures constitute a wonderful opportunity for us to hear and interact with world famous speakers. You are encouraged to take advantage of the relaxed and informal tone of this meeting and to engage scientific discussions. The oral program will be complemented by poster sessions given by researchers and students from all over the world. The meeting will also provide a series of workshops designed to provide attendees with an overview of a particular field and also to give insight into focus areas for current development and future directions.

Many of the activities at the meeting will be centered on the exhibition area (poster sessions, lunches and coffee breaks) so that you will have several opportunities to view and interact with the exhibitors.

The meeting is jointly supported by the International Society of Ethnopharmacology, IRD (France), Sociedad Latinoamericana de Fitoquímica and the División de Química de Productos Naturales, Sociedad Chilena de Química. On behalf of the entire team of 14th ISE Puerto Varas, we welcome you to this exciting event.

Guillermo Schmeda-Hirschmann
Chairman ISE 2014-SLF

COMMITTEES

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Hermine Vogel
Felipe Jiménez-Aspee

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Guillermo Schmeda Hirschmann

Contact

Guillermo Schmeda Hirschmann, Dr.rer.nat.
Chairman ISE 2014 - SLF
Instituto de Química de Recursos Naturales
Universidad de Talca, Talca, Chile
Phone: +56 71 2200 288
E-mail: schmeda@utalca.cl

Dr. Maria del Pilar Caramantín Soriano
Organizing Secretariat
Instituto de Química de Recursos Naturales
Universidad de Talca, Talca, Chile
Phone: +56 71 2200 290
E-mail: mcaramantin@utalca.cl

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14TH INTERNATIONAL CONGRESS OF ETHNOPHARMACOLOGY

HISTORY AND FUTURE MEETINGS

The International Society of Ethnopharmacology (ISE) is a small, collaborative, interdisciplinary group of scientists – anthropologists, pharmacists, pharmacologists, ethnobotanists, phytochemists, and others – all fascinated by the study of the global use of medicines. The ISE is particularly concerned about understanding the medicinal uses of plants in traditional societies. It seeks to understand the cultural and the pharmacological dimensions of human medicinal plant use everywhere. Since the first congress in 1990, the ISE has the ambition to arrange biannual international congresses for its members and other interested participants. Over the last years the intention has been to hold these congresses alternately on various continents, preferably in collaboration with local societies.

We extend a kind invitation to you to participate in the "15th International Congress of the International Society for Ethnopharmacology" which will be held on May 5-8, 2015, at the BeitZaman Hotel & Resort, Petra, Jordan.

http://15icse.bau.edu.jo/inv.html

PLENARY SPEAKERS

Dr. Ana H. Ladio
INIBIOMA-Laboratorio Ecotono; Universidad Nacional del Comahue, Río Negro, Bariloche, Argentina
Email: ahladio@gmail.com

Dr. Anthony Booker
Centre for Pharmacognosy and Phytotherapy, UCL School of Pharmacy, UCL, London, WC1N 1AX, UK
Email: anthony.booker.11@ucl.ac.uk

Dr. Anna Jäger
University of Copenhagen, Copenhagen, Denmark
Email: ankj@sund.ku.dk

Dr. Bruce K. Cassels
Facultad de Ciencias, Universidad de Chile, Santiago, Chile
Email: bcassels@u.uchile.cl

Dr. David Pessoa-Mahana
Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile
Email: cpessoa@uc.cl

Dr. Claudia Mardones
Facultad de Farmacia, Universidad de Concepción, Concepción, Chile.
Email: cmardone@udec.cl

Dr. De-an Guo
Shanghai Institute for Biological Sciences, Shanghai , People's Republic of China
Email: daguo@mail.shcnc.ac.cn

Dr. Edward J. Kennelly
Lehman College, The City University of New York, NY, USA
Email: kennelly@lehman.cuny.edu

Dr. Erdem Yesilada
Faculty of Pharmacy, Yeditepe University, Atasehir, Istanbul, Turkey
Email: yesilada@yeditepe.edu.tr

Mr. Eric Brand
School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China
Email: ericbrand@gmail.com

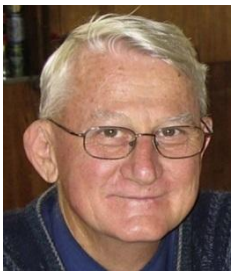
Dr. Fernando Javier Durán
UMYMFOR-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
Email: fduran@qo.fcen.uba.ar

Dr. Geneviève Bourdy
UMR 152 Pharmadev IRD-Universidad de Toulouse, Toulouse, Francia
Email: genevieve.bourdy@ird.fr

Dr. Guillermo Schmeda Hirschmann
Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile
Email: schmeda@utalca.cl

Dr. Jacobus N. Eloff
University of Pretoria, Pretoria, South Africa
Email: kobus.eloff@up.ac.za

Dr. Jacqueline Takahashi
Instituto de Ciências Exatas, Departamento de Química, Universidad Federal de Minas Gerais, Belo Horizonte, Brazil
Email: jat@qui.ufmg.br



Dr. Johannes Van Staden
University of KwaZulu-Natal, Pietermaritzburg, South Africa
Email: vanstadenj@ukzn.ac.za

Dr. John Pezzuto
College of Pharmacy, University of Hawaii, Hilo, Hawaii, USA.
Email: pezzuto@hawaii.edu

Dr. Luis Manuel Peña
Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México
Email: lmanuel@cicy.mx

Dr. Marcia Regina Piuvezam
Universidade Federal da Paraíba, João Pessoa, Brazil
Email: mrpiuvezam@ltf.ufpb.br

Dr. Marco Leonti
University of Cagliari, Cagliari, Italy
Email: marcoleonti@netscape.net

Dr. Maria das Graças Lins Brandao
Faculdade de Farmacia, Universidade Federal Minas Gerais, Belo Horizonte, Brazil
Email: mglinsbrandao@gmail.com

Dr. Mario Pino Quivira
Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
Email: mariopino@uach.cl

Dr. Mario Simirgiotis
Universidad de Antofagasta, Antofagasta, Chile
Email: mario.simirgiotis@uantof.cl

Dr. Massuo J. Kato
Instituto de Quimica, Universidade de São Paulo, Butantã, Brazil
Email: massuojorge@gmail.com

Dr. Michael Heinrich
School of Pharmacy, University College London, London, UK
Email: m.heinrich@ucl.ac.uk

Dr. Michel Sauvain
UMR 152 Pharmadev IRD-Universidad de Toulouse, Francia y Universidad Peruana Cayetano Heredia, Lima, Perú
Email: michel.sauvain@ird.fr

Dr. Mirtha Parada
Instituto de Salud Pública de Chile, Chile.
Email: mparada@ispch.cl

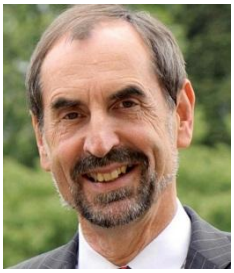
Dr. Mohamed Haddad
UMR 152 Pharmadev IRD-Universidad de Toulouse, Toulouse, France
Email: mohamed.haddad@ird.fr

Dr. Nibaldo Inestrosa
Pontificia Universidad Católica de Chile, Santiago, Chile
Email: ninestrosa@bio.puc.cl

Dr. Pastor Arenas
Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET), Buenos Aires, Argentina
Email: pastorarenas@yahoo.com.ar

Dr. Peter Caligari
Instituto de Biologia Vegetal y Biotecnologia, Universidad de Talca, Talca, Chile
pcaligari@utalca.cl

Dr. Peter Winterhalter
Technische Universität Braunschweig, Braunschweig, Germany
Email: p.winterhalter@tu-braunschweig.de



Dr. Reinhold Carle
Universität Hohenheim, Stuttgart, Germany
Email: reinhold.carle@uni-hohenheim.de

Dr. Robert Verpoorte
Institute Biology Leiden, Leiden University, Leiden, The Netherlands
Email: verpoort@chem.leidenuniv.nl

Dr. Rosario Rojas
Universidad Peruana Cayetano Heredia, Lima, Perú
Email: rosario.rojas@upch.pe

Dr. Rudolf Bauer
Karl-Franzens-Universität Graz, Graz, Austria
Email: rudolf.bauer@uni-graz.at

Dr. Russell Barrow
Research School of Chemistry, The Australian National University, Canberra, Australia; University of Goroka, Goroka, Eastern Highlands, Papua New Guinea.
Email: rab@anu.edu.au

Dr. Valérie Jullian
UMR 152 Pharmadev IRD-Universidad de Toulouse, Toulouse, Francia
Email: jullian@cict.fr

Dr. Vanderlan da Silva Bolzani
Universidade Estadual Paulista, Instituto de Química, Araraquara, Sao Paulo, Brazil
Email: bolzaniv@iq.unesp.br

Dr. Wan-sheng Chen
Department of Pharmacy, Changzheng Hospital Second Military Medical University, Shanghai, People's Republic of China
Email: chenwanshengsmmu@aliyun.com

Dr. Yusuf Öztürk
Department of Pharmacology, Faculty of Pharmacy Anadolu University, Tepebasi TR-26470 Eskisehir Turkey
E-mail: yozturk@anadolu.edu.tr

ISE STUDENT TRAVEL GRANT AWARDS

The International Society of Ethnopharmacology (ISE) has provided a limited number of travel grants (free registration) for PhD candidates presenting posters or talks at the 14th ISE conference. The eligibility criteria for this award were:

1. Applicant was a student and has not got his PhD title yet
2. Applicant intended to present a poster or lecture
3. Applicant was a member of the International Society of Ethnopharmacology

Congratulations to the Award Winners:

WINNERS:

1. Eric Brand: ericbrand@gmail.com (USA/China)
2. Cristina Elizabeth Ramírez-Serrano: valeria_vida_soy@hotmail.com (Mexico)
3. Verónica Sánchez Sánchez: vera1o@outlook.es (Mexico)
4. Gabriela Belén Martínez Hernández: gabmh89@gmail.com (Mexico)
5. Gabriela Vargas-Villa: vagy@live.com.mx (Mexico)
6. Marta Gruca: gruca@biology.au.dk (Denmark)
7. Ivana Vrhovac ivrhovac@imi.hr (Croatia)
8. Josip Madunic jmadunic@biol.pmf.hr (Croatia)
9. Santanu Bhadra sanbhadra@gmail.com (India)
10. Edna Ooko: ednaooko@gmail.com (Germany)

BEST POSTER WINNERS

- **Screening and identification of neuroprotective compounds relevant to Alzheimer's disease from medicinal plants of S. Tomé e Príncipe**

  Currais A[1], Chiruta C[1], Goujon-Svrzic M[1], Costa G[2], Santos T[2], Batista MT[2], Paiva J[3], Madureira MC[3], Maher P[1]

  [1]The Salk Institute for Biological Studies, Cellular Neurobiology Laboratory, 10010 N. Torrey Pines Rd., La Jolla, 92037 CA, USA; [2]Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, and Center for Neurosciences and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal; [3]Centre for Functional Ecology, Faculty of Sciences and Technology, University of Coimbra, 3001-455 Coimbra, Portugal

- **Plants smoked by pre-Hispanic populations of central Chile: chemical and pharmacological aspects**

  Echeverría J, Espinoza S, Niemeyer HM

  Facultad de Ciencias, Universidad de Chile, Casilla 653, Las Palmeras 3425, Santiago, Chile

- **Chilean *Prosopis* mesocarp flour: polyphenolic content, antioxidant activity and fingerprint analysis**

  Schmeda-Hirschmann G[1], Quispe C[1], Soriano MDPC[1], Theoduloz C[2], Jiménez-Aspee F[1], Pérez MJ[3], Cuello AS[3], Isla MI[3]

  [1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [2]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Chile; [3]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA-CONICET). Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina

- **Comparative chemical profiling of fungal endophytes associated to *Bursera simaruba* from Colombia**

  Fierro J, Vargas-Medina L, Jiménez P, Coy-Barrera E

  School of Science, Universidad Militar Nueva Granada, AA49300, Cajicá, Colombia

PROGRAM

TUESDAY 23RD, SEPTEMBER 2014			
10:00 - 17:00	**REGISTRATION** (Entrance Centro de Convenciones)		
		Room Osorno B	Room Osorno A
09:00- 13:00		**Minicourse 1** **Ana Ladio:** Ethnoecology, medicinal and food plants. A theoretical, epistemological and methodological approach (in Spanish)	
14:00 -18:00		**Minicourse 2** **Pastor Arenas:** Ethnobotany: an interdisciplinary field with multiple applications (in Spanish)	14:00-15:30 **ISE Board Meeting** 15:30-17:30 **Workshop** **Robert Verpoorte, Michael Heinrich**: How to write a first class scientific paper. Editors and Associated Editors from international journals
18:30 - 19:00	**OPENING CEREMONY** (Rooms Calbuco + Tronador)		
19:00 - 20:00	**Mario Pino:** First human settlement in the Americas: the Monte Verde site, Puerto Montt, South of Chile		
20:00 -21:00	**COCKTAIL** (Hotel Restaurant)		

WEDNESDAY 24th, SEPTEMBER 2014			
	Room Calbuco	Room Tronador	Room Osorno B
Session chairs		*Pastor Arenas*	*Esteban Ferro* *Marcia Regina Piuvezam*
09:00 - 09:30		09:00-09:15 **José Bravo:** Chemical valorization of Bolivian native pharmacopoeia 09:15-09:30 **Patricia Mollinedo:** Antioxidant properties of Bolivian plants	**Guillermo Schmeda-Hirschmann:** Native berries from southern Chile: from prehispanic food sources to potential nutraceuticals
09:30 - 10:00		**Valerie Jullian:** From malaria to cancer: *Quassia amara* and simalikalactone E (SkE)	09:30 - 09:45 **Goran Gajski:** Combined antitumor effect of bee venom and cisplatin on human glioblastoma A1235 cells 09:45 - 10:00 **Barbara Oliveira Henriques:** In vitro inhibition of TNF-α release induced by Brazilian medicinal plants
10:00 - 10:30		**Rosario Rojas:** Peruvian-Amazonic plants for cosmetic use	**Marta Gruca:** New categories for traditional medicine in the Economic Botany Data Collection Standard
10:30 - 11:00	**COFFEE BREAK**		
Session chairs		*Rosario Rojas* *José Bravo*	*David Pessoa-Mahana* *Jacqueline Takahashi*
11:00 - 11:30		**Michel Sauvain:** Stomach cancer and the fight against *Helicobacter pylori.*	**Marcia Regina Piuvezam:** *Cissampelos sympodialis* (Menispermeaceae): A novel phytotherapic weapon against allergic diseases
11:30 - 12:00		**Mohamed Haddad:** Combine Ethnopharmacology and Chemotaxonomy: the case of saponins with antileishmanial properties	**Andrés Mauricio Rojas Sepúlveda** Biologically active secondary metabolites present in species of the genus *Bursera*
12:00 - 12:30		**Genevieve Bourdy:** Knowledge, attitudes and practices, a new focus on the validation of traditional use of medicinal plants (in Spanish)	**Francisco Silva Zamora:** Synthesis of triterpene prodrugs as potential inhibitors of cancer cells
12:30 - 13:00		12:30 - 12:45 **Billy Cabanillas:** Leishmaniasis and Peruvian medicinal plants. 12:45 - 13:00	**Luis Manuel Peña:** The added value of biodiversity. Recent examples of bioactive metabolites from the Yucatán medicinal flora

		Veronica Olate: Variability in the resin composition of the Cupressaceae *Austrocedrus chilensis*	
13:00 - 14:30	**LUNCH** (Hotel Restaurant)		
Session chairs	*Claudia Mardones* *Fernando Javier Durán*	*Massuo J. Kato* *Genevieve Bourdy*	*Carla Delporte* *Maria das Graças Lins Brandão*
14:30 - 15:00	**Patricia Manzano:** Estudio Farmacognóstico de *Vernonanthura patens* (Kunht) H. Rob	**Verónica Paeile** (Merck)**:** Plataformas Merck Millipore para la determinación de analitos de Importancia biológica en fluidos y células	**Marco Leonti:** Bioprospecting and Ethnopharmacology: evolutionary considerations
15:00 - 15:30	**Eric Brand:** Applied macroscopic identification: an overview of common adulterants found in TCM school clinics in the USA	**Guillaume Odonne:** A call for a new approach against neglected parasitic diseases: the *AmazInG project*, a holistic view onto traditional Amazonian anti-leishmanial remedies.	**Anna Jäger:** Plant-based treatment of snake bites
15:30 - 16:00	**Jürg Leuenberger** (CAMAG)**:** HPTLC for identification of botanical materials - a practical approach	**Gabriel Vargas Arana:** Argininyl esters and alkaloids from the venom of the Amazonian toad *Rhinella* (*Bufo*) *marinus*	**Jacobus N. Eloff:** Using plants to protect humans and animals
16:00 - 16:30	**Mirtha Parada:** Chilean regulation of medicines made from plants	**Russell Barrow:** Discovery of metabolites from fungi used traditionally in the Highlands of Papua New Guinea	**Johannes Van Staden:** African Traditional Medicine in South Africa
16:30 - 17:00	**COFFEE BREAK**		
Session chair	*Russell Barrow*	*Mariano Pertino*	
17:00 - 17:30	**Nibaldo Inestrosa:** Mechanism of action of natural products for neurological diseases: APP processing, Wnt signaling and neurogenesis		
17:30 - 18:00	**Vanderlan da Silva Bolzani:** Center for Innovation in Biodiversity and Drug discovery: CIBFar: an innovative approach to identify secondary metabolites from tropical biodiversity as hits and leads	**Nancy Torres** (Genesys) Soluciones tecnológicas para estudios metabolicos de nuevos fármacos	
18:00 - 18:30	**Nina Etkin Award** (Room Calbuco)		
18:30 - 19:30	**POSTER SESSION A (posters 1-90)** (Room Osorno A)		

THURSDAY 25th, SEPTEMBER 2014			
	Room Calbuco	Room Tronador	Room Osorno B
Session chair	*Guillermo Schmeda-Hirschmann*	*Marco Leonti*	*Anna Jäger*
09:00 - 09:30	**Edwin Castillo Martinez:** Ethnomycology: characterization of the bioactive constituents from the mushroom, *Fulaga dive* (Amanitaceae) used traditionally in Papua New Guinea	**Pastor Arenas:** Los flujos de sangre en la mujer: una enfermedad entre las indígenas y criollas del Gran Chaco. Etiología, terapéutica, temores y prevenciones (in Spanish)	**Jacobus N. Eloff**: The correlation between taxonomy and antimicrobial activity of southern African tree leaf extracts
09:30 - 10:00	**De-an Guo:** Traditional Chinese medicine is marching towards the evidence-based medicine	**Ana Ladio:** Cultural landscapes and traditional medicinal knowledge among Mapuches: adaptation, maintenance and replacement	09:00 - 09:15 **Debora Scariot:** 4-nitrobenzaldehyde thiosemicarbazone derived from S-limonene causes ultrastructural changes on *Leishmania amazonensis* cells 09:15 - 09:30 **Nathalia Camaforte Henriques:** Hypoglycemic action of *Bauhinia holophylla* (Steud.) through glycogenesis stimulation and gluconeogenesis inhibition in streptozotocin-diabetic mice
10:00 - 10:30	**Felipe Jimenez-Aspee:** Antioxidant activity and characterization of consitutents in Copao fruits (*Eulychnia acida* Phil., Cactaceae) by HPLC-DAD-MS/MS^n	**Maria das Graças Lins Brandão:** Natural History of Pharmacy in South America: rediscovering the medicinal plants of Brazil	**Iris Catiana Zampini:** Nutritional and functional properties of native fruits of Argentina: Revalorization use in the design of functional foods
10:30 - 11:00	**COFFEE BREAK**		
Session chair	*Bruce Cassels*	*Michael Sauvain*	*Jorge Retamales*
11:00 - 11:30	**Robert Verpoorte:** Do negative results exist?	**Gastone Zanette:** Ethnopharmacology: a past Italian pioneer	**Michael Heinrich:** Metabolomics in medicinal and food plant research along the value chain from producer to consumer
11:30 - 12:00	11:30 - 11:45 **Olubunmi Abosede Wintola:** Toxicological evaluation of aqueous extract of *Aloe ferox* Mill. in loperamide-induced constipated rats	**Jacqueline Takahashi:** The remarkable structural diversity achieved by fungal biotransformations in biologically active compounds	**Rudolf Bauer:** Natural products and crude drugs in traditional medicine research: how to deal with complexity?

	11:45 - 12:00 **Esameldin Elgorashi:** Genotoxicity of *Acacia seyal* (Mimosaceae) wood smoke		
12:00 - 12:30	**Massuo J. Kato:** Chemical variability in Piperaceae species: cause or consequence?	**Ashwell Ndhlala:** Effects of different cultivation practices on the pharmacological properties of *Artemisia annua* L. (Asteraceae)	**Peter Caligari:** The domestication of native berries: *Fragaria chiloensis* as a Chilean example
12:30 - 13:00		**Pouya Faridi:** *Lapis judaicus:* a traditional drug for kidney stone- Randomized double blind clinical trial	**Claudia Mardones:** Calafate: the promising berrie from Patagonia
13:00 - 14:30	**LUNCH** (Hotel Restaurant)		
Session chair	*Anna Jäger*		
14:30 - 16:00	**James Oluwagbamigbe Fajemiroye** Interactive session and networking opportunity: student member of ISE to meet Senior colleagues in a friendly and relaxed atmosphere		
16:00 - 16:30	**COFFEE BREAK**		
Session chair	*Peter Caligari*	*Cristina Quispe*	
16:30 - 17:00	**John Pezzuto** Resveratrol: A highly promiscuous molecule leads the way to monogamous interactions	**Leonardo del Carpio** (Del Carpio): Latest development in LC/MS/MS High resolution with Orbitrap Technology	
17:00 - 17:30	**Anthony Booker:** Value chains of *Rhodiola rosea* (*Sedum roseum* (L.) Scop., Crassulaceae): an investigation of wild collection, cultivation and adulteration		
17:30 - 18:30	**ISE MEMBERS MEETING** (Room Osorno B)		
18:30 - 19:30	**POSTER SESSION B (posters 91-180)** (Room Osorno A)		
20:00 - 22:00	**DINNER** (Hotel Restaurant)		

FRIDAY 26TH, SEPTEMBER 2014		
	Rooms Calbuco + Tronador	Room Osorno B
Session chair	*Michael Heinrich*	*Vanderlan da Silva Bolzani*
09:00 - 09:30	**Mario Simirgiotis:** Native fruits from southern South America: Antioxidant capacity and HPLC-PDA-ESI/ToF-MS profiling of phenolic compounds	**Fernando Javier Durán:** Semisynthetic derivatives with potential cytostatic, anti-inflammatory and antifeedant activity derived from cucurbitacins isolated from south American medicinal plants
09:30 - 10:00	**Edward Kennelly:** Jaboticaba fruits for chronic obstructive pulmonary disease	**David Pessoa-Mahana:** Endocannabinoid system: an attractive target for new drug developing
10:00 - 10:30	**Reinhold Carle:** *Bactris gasipaes* and *Carica papaya* - potent fruits for the prevention of hidden hunger	**Bruce Cassels:** Nature-based design of bioactive compounds: the aporphinoids
10:30 - 11:00	**COFFEE BREAK**	
Session chair	*Edward Kennelly*	
11:00 - 11:30	**Peter Winterhalter:** Bioactives from side streams of fruit processing	
11:30 - 12:00	**Robert Verpoorte:** Synergy?	
12:00 - 12:30	**CLOSING CEREMONY** (Rooms Calbuco + Tronador)	
12:30 - 14:00	**LUNCH** (Hotel Restaurant)	
14:30 → (about 4 h)	**EXCURSIONS** (Saltos del Petrohué or Volcán Osorno)	

LECTURES

L-1

First human settlement in the Americas: the Monteverde site, Puerto Montt, South of Chile

Pino M

Science Faculty, Universidad Austral de Chile, Independencia 631, Valdivia

The archeological site Monte Verde (41˚ 30' 14.7'' S, 73˚ 12' 22.2''W), was discovered by local farmers in 1976. From 1978 until today the site has been excavated, analyzed and interpretate [1-5]. In 1932 the discovery of the Clovis site in New Mexico laid the basis for proposing that all Americans descended from these hunters. Tens of Clovis sites have been dated between 11,050 to 10,800 ^{14}C yr B.P. Using plant materials and bones of megafauna Monte Verde was dated in 12,500 ^{14}C yr B.P. Since Monte Verde is located in southern Chile, the diference of 1500 ^{14}C yr B.P. between Clovis and Monte Verde is not real. Must be added the time that takes the travel of human groups from northern North America to southern Chile, which means that the ancestors of the people of Monte Verde entered North America about 20,000 years ago. But Monte Verde is not only, until today, the oldest archaeological site in the Americas. It is an incredibly well-preserved site, consisting of a "toldo" and a special area. The quality of preservation is related to a flooding over the site, probably by a rise of ground water, and was transformed into an anoxic wetland. Thus, a layer of peat seals the site. In Monte Verde a human footprint, and remains of bones and mat of gomphoteres, ropes, knots and a wide range of edible and medicinal continental plants and algae are recorded, including the oldest wild potato of America.

References: [1] Dillehay, T.; Pino, M.; Davis, M.E.; Valastro, S.; Varel, A.; Casamiquela, R. 1982. Monte Verde: Radiocarbon dates from an early-man site in South-Central Chile. Journal of Field Archaeology. 9: 547-550. [2] Pino, M. & Dillehay, T. 1988. Monte Verde, South-central Chile: Stratigraphy, Climate Change and Human Settlement. Geoarchaeology: Vol.3, N3, 177-191. [3] Dillehay, T. 1989. Monte Verde. Vol. I. Palaeoenvironment and site context, Smithsonian Press. [4] Dillehay, T. 1997. Monte Verde. Vol. II. Archaeological Context and Interpretation. Smithsonian Press. [5] Dillehay, T.; Ramírez, C.; Pino, M.; Collins, M.; Rossen, J.; & Pino-Navarro, J.D. 2008. Monte Verde: Seaweed, Food, Medicine, and the Peopling of South America. Science 320 (5877): 784 -786.

L-2

Biosynthesis and metabolic regulation of valuable secondary metabolites in *Salvia miltiorrhiza*

Chen W-S

Second Military Medical University, Shanghai, China

Salvia miltiorrhiza Bunge (Dan-shen in Chinese), is a commonly used traditional Chinese medicine for improving body function and cardiac symptoms treatment. The phenolic acids aroused scientists' interest in the last twenty years because of their significant bioactivities that may contribute to the therapeutic effects of *S. miltiorrhiza*. Here, the in vivo phenolic biosynthetic pathway was firstly mapped in *S. miltiorrhiza* using ^{13}C Tracer, and then the involved catalytic enzymes were successively isolated. By establishing "gene-to-metabolite" network, the key enzymes for target phenolic acids production were successfully discovered. The above knowledge about phenolic biosynthetic pathway in *S. miltiorrhiza* promoted the possibility of metabolic

regulation for the enhanced synthesis of active pharmaceutical compounds. For example, abiotic-elicitation systems, as well as signal molecule (MeJA) biosynthetic key genes-mediated intensive regulation strategies were established, enhancing Lithospermic acid B and Tanshinone IIA accumulations 6.5 and 16.6-fold, respectively. In conclusion, the well-developed research system including pathway discovery and biosynthesis regulation in *S. miltiorrhiza* will provide an excellent model to investigate metabolic regulation of valuable secondary metabolites in TCM.

L-3

Phytochemicals with nuclear factor-kappa B inhibitory activity from Turkish folk medicines

Yesilada E

Yeditepe University, Faculty of Pharmacy, Atasehir, Istanbul, Turkey

Nuclear Factor kappa B (NF-κB) plays a pivotal role in the expression and activation of pro-inflammatory transcription factors. Eventually it has been employed as a biological target to assess activities on inflammatory and immune disorders as well as cancers. In Turkish folk medicine the leaves of *Sambucus ebulus* L. [SE], *Sambucus nigra* L. [SN] (Adoxaceae) and *Cistus laurifolius* L. (Cistaceae) [CL] were frequently used against several inflammatory conditions. Aim of this study was to find evidences for their traditional use by in vivo/in vitro techniques, to isolate the active constituents by bioassay-guided processing and to betray the activity mechanisms. Two flavonoids (isorhamnetin-3-*O*-β-D-glucopyranoside [**1**], isorhamnetin-3-*O*-β-rutinoside [**2**]), two iridoids (Sambulin A [**3**] and B [**4**]) were isolated from SE as the NF-κB inhibitory components. Furthermore [**1**] inhibited NO, iNOS, TNFα. and [**2**] inhibited PGE_2/COX_2. They exerted their effects through p38/IκBα. Iridoids suppressed iNOS/COX_2 levels, NO/PGE_2, TNFα, JNK and IκBα phosphorylations. Additionally [**4**] inhibited IL-6 and [**3**] inhibited p38 phosphorylation. Three flavonoids were isolated from the chloroform subextract of CL as the anti-inflammatory principles; 3-*O*-methylquercetin; 3,7-*O*-dimethylquercetin; 3,7-*O*-dimethylkaempferol by in vivo techniques. While only the remaining H_2O subextract was found to be active on NF-κB. However the activity was lost on further fractionation and thus a synergistic activity might be discussed. This subextract also decreased the levels of NO, PGE_2 and the expression levels of iNOS/COX_2 proteins as well as JNK and IκBα phosphorylations. A sterol mixture was determined to be responsible for the NF-κB activity of SN. This fraction also decreased the levels of NO, PGE_2 and the expression levels of iNOS/COX_2 proteins and IκBα phosphorylation. Consequently, in vitro/in vivo experimental data have provided molecular and mechanistic evidences supporting the traditional use of these plants.

L-4

New categories for traditional medicine in the Economic Botany Data Collection Standard

Gruca M[1], Cámara-Leret R[2], Macía MJ[2], Balslev H[1]

[1]Department of Bioscience - Research Group Ecoinformatics and Biodiversity, Aarhus University, Build. 1540, Ny Munkegade 114, DK-8000, Aarhus C, Denmark; [2]Departamento de Biología, Área de Botánica, Universidad Autónoma de Madrid, Calle Darwin 2, ES - 28049 Madrid, Spain

The *Economic Botany Data Collection Standard* (EBDCS) proposed by Cook [1] was a major step forward in comparative ethnobotany and it has been successfully followed by ethnobotanists investigating plant uses in many parts of the world. However, we have encountered some cases in our study of traditional medicine where the standard seems incomplete and inaccurate when it is applied to plant uses of rural or indigenous societies in developing countries. Not all plant uses in

traditional medicine can be associated with commonly known diseases, and therefore be analysed based on conventional medical system categories offered by the EBDCS. Cultural and spiritual aspects are inextricable part of traditional medicine and excluding them from the medical context only exposes a part of traditional medicinal knowledge. To fill this gap we propose two categories: Cultural Diseases and Disorders, and Ritual/Magical Uses – to be added to the EBDCS. We believe that the proposed changes will give a more accurate insight into traditional medicine, as well as contribute to developing an integrative medicinal data collection protocol, which will make the studies more comparable.

Reference: [1] Cook F. (1995) Economic Botany Data Collection Standard, KEW, Royal Botanical Gardens.

L-5

From malaria to cancer: *Quassia amara* and Simalikalactone E (SkE)

Jullian V [1,2], Bourdy G [1,3], Deharo E[1,3], Fabre N[1,3], Valentin A[1,3], Le HL[1,3], Robert G[4,5], Auberger P [4,5]

[1]Université de Toulouse ; UPS ; UMR 152 Pharma-DEV ; Université Toulouse 3, Faculté des Sciences Pharmaceutiques, F-31062 Toulouse cedex 09, France; [2]Institut de Recherche pour le Développement (IRD), UMR 152, Mission IRD Casilla 18-1209, Lima, Peru; [3]Institut de Recherche pour le Développement (IRD) ; UMR 152 Pharma-DEV F-31062 Toulouse cedex 09, France; [4]INSERM/U1065, C3M, Equipe 2: Morts Cellulaires, Différenciation, inflammation et Cancer, Nice, France; [5] Université de Nice, France

In our constant effort to find new therapeutic solutions against malaria from traditional pharmacopeias, our team has confirmed at the end of an extensive ten years research program, the medicinal potential of a broadly used and cultivated plant all over Amazonia: *Quassia amara* L. (Simaroubaceae). Bioguided fractionations of this plant lead to the isolation of the Simalikalactone E (SkE). SkE has been first isolated from the traditional decoction made with *Q. amara* leaves. In order to investigate further its promising biological activities, we have then perfected the extraction protocol to achieve a reproductible yield of 40 mg/kg of plant.
In our recognized laboratory models, this product displayed very interesting antimalarial and anticancer activity *in vitro* and *in vivo*. SkE inhibited murine malaria growth of *Plasmodium vinckei petteri* by 50% at 1 mg/kg of body weight/day, by oral route [1]. In a model of chronic myeloid leukemia (CML) cells implanted in athymic mice, a significant reduction of tumor size was obtained after treatment with SkE at 1 mg/kg of body weight/day, by ip route [2]. In these experiments, SkE showed pharmacological properties equivalent and sometimes more effective than reference compounds used in classical therapy (such as chloroquine for malaria or Imatinib for cancer). Investigation of its mechanism of action on CML cells showed that SkE acts on the ERK pathway, inhibiting ERK1/1, MEK ½ et B-Raf phosphorylation [2]. We developed a quantification procedure of SkE in blood samples and analyzed mice blood after oral and ip administration of SkE. We found out that SkE is barely detectable 4h after administration. We suggested that SkE could be rapidly metabolized, and therefore, the *in vivo* antimalarial and anticancer pharmacological properties may be due to its metabolites.

References: [1] Cachet N. et al Antimicrobial Agents and Chemotherapy, 2009, 53, 10, 4393-4398. [2] Robert G. et al, Oncotarget, 2012, 3(12), 1688-1699.

L-6

Peruvian-Amazonic plants for cosmetic use

Rojas R

Unidad de Investigación en Productos Naturales, Universidad Peruana Cayetano Heredia

Con miras a desarrollar 2 cremas cosméticas a base de extractos hidroalcohólicos de hojas de *Bixa orellana* (BO) y *Oenothera rosea* (OR), ambas muestras fueron sometidas a diversos tests químicos, enzimáticos, celulares e *in vivo*. Los extractos de BO y OR mostraron buena actividad antioxidante en los tests de DPPH (EC_{50}= 10.65 y 10.78 µg/mL, respectivamente) y TEAC (0.52 y 0.2, respectivamente). BO y OR inhiben moderadamente la enzima elastasa (EC_{50}= 72.68 y 112.04 µg/mL, respectivamente) y altamente la enzima colagenasa (EC_{50}= 166.23 y 194.89 µg/mL, respectivamente). La toxicidad de BO y OR contra melanocitos B16 es baja (GI_{50}= 240.24 y 270.80 µg/mL, respectivamente). Solamente el extracto de BO promueve la síntesis de colágeno *in vitro* (11.2% a c= 25 µg/mL); mientras que solo el extracto de OR fue capaz de proteger a los fibroblastos contra los daños de la irradiación UV-B (24.5% a c= 25 µg/mL). El contenido de compuestos fenólicos en los extractos de BO y OR fue 14.9 y 15.4 mg de ácido gálico/g extracto, respectivamente. El contenido de ácido ascórbico del extracto de OR (210.64 mg/100 g extracto) fue mayor que el de BO (136.63 mg/100 g extracto). En cuanto a los marcadores químicos, BO contiene 8.66 mg de ácido elágico/g extracto; mientras que el de OR contiene 2.39 mg de quercetina/g extracto. Se prepararon 2 cremas con cada uno de los extractos y se realizó el "Patch test" en 20 voluntarios sanos. Ninguno de los sujetos mostró algún tipo de efecto adverso cuando las cremas fueron aplicadas en forma tópica. Ambas cremas tuvieron resultados positivos en la evaluación subjetiva de eficacia reportada por los voluntarios.

L-7

Possible antineoplastic actions of three *Hypericum* species, extracts of aerial parts of *H. perforatum*, leaf and flowers of *H. montbretii* and *H. origanifolium:* A comparative study with paclitaxel

Öztürk Y[1], Güzey G[1]*, Öztürk N[2], Potoglu-Erkara I[3]

*Anadolu University, Faculty of Pharmacy, Departments of [1]Pharmacology and [2]Pharmacognosy, [3]Eskisehir Osmangazi University, Faculty of Science and Art, Department of Biology, Eskisehir, Turkey (*Present address: Hospital of Eskisehir Osmangazi University, Department of Pharmacy, Turkey) E-mail: yozturk@anadolu.edu.tr*

Hypericum species have been reported to be used in traditional medicine for various curative purposes including wound healing and neoplastic diseases. Previous reports have indicated that *H. perforatum* (St.-John's Worth) possesses wound healing[(1,2)] and antiproliferative activities[(1,3)] in experimental studies. The primary purpose of the present study was to compare the antiproliferative effects of methanolic extracts of leaf and flowers of *H. perforatum*, *H. montbretii* and *H. origanifolium* on A549, HeLa cells (cancer cell lines) and NIH3T3 cells (normal cells) with paclitaxel in culture media. Possible antineoplastic effects were studied using MTT, neutral red, acridine orange experiments and soft agar gel colony forming assay. Extracts were added in media in a concentration range of 1-250 µg/ml and incubated with cells up to 4 days while their preparation procedures and phytochemical compositions have been previously reported[(3)]. All extracts applied exhibited inhibitory effects in colony formation assay, although they exhibited antiproliferative/cytotoxic activities in different profiles of action and at different concentrations in other experiments performed for antiproliferative activities. Results obtained here suggested that these three *Hypericum* species have potentials for antineoplastic activity, which may deserve further future investigations.

References: [1] Öztürk N, Korkmaz-Çetin S, Öztürk Y. *J Ethnophar*macol 111, 33-39, 2007. [2] Dikmen M, Öztürk Y, Sagratini G, et al. *Phytother Res* 25, 208-211, 2011. [3] Güzey G, Ibadova S, Öztürk Y, et al. *Med Arom Plant Sci* Biotechnol 5(Sp Issue 1), 91-99, 2011.

L-9

Stomach cancer and the fight against *Helicobacter pylori*

Sauvain M

Director de Investigación en el IRD, Francia; Director del Laboratorio Mixto Internacional LAVI; "Laboratorio Andino Amazónico de Química de la Vida"; IRD - Universidad Peruana Cayetano Heredia (UPCH); Avenida Honorio Delgado 430 San Martín de Porres 34, Lima, Perú

El cáncer del estómago es la tercera causa de cáncer y la secunda causa de mortalidad por cáncer en el mundo. En América Latina, es la quinta causa de cáncer y la secunda causa de mortalidad (http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=962). La infección a *Helicobacter pylori* se considera como una de las causas principales de la aparición del cáncer del estómago.En el Perú, en las capas sociales más desfavorecidas, la superpoblación y las malas condiciones sanitarias facilitan la transmisión de *H. pylori*. Se infecta a los niños a partir de su más joven edad - más del 70% son seropositivos a partir de la edad de 5 años - y un 90% de los adultos que tienen una endoscopia gastrointestinal para buscar síntomas de dispepsia también son positivos. El tratamiento para erradicar las bacterias falla en muchos casos, debido principalmente a la resistencia a los antibióticos y malas condiciones sanitarias facilitando su transmisión, por lo tanto, surge la necesidad de desarrollar mejores regímenes terapéuticos. Trabajos efectuados en América Latina muestran el interés de los activos presentes en las plantas medicinales y alimentarias para luchar contra la infección. En el Perú, existe un vasto conocimiento tradicional sobre la utilidad de plantas medicinales y alimenticias para el tratamiento y la prevención de los males de estómago, principalmente las úlceras, por ello, nuestro grupo busca nuevas sustancias naturales que controlen la proliferación bacteriana. Se colectó 124 especies seleccionadas por métodos etnofarmacológicos a través de encuestas realizadas en las comunidades Yanesha (15), de Huaraz (73) y de Chiclayo (36). La selección de los extractos más activos se realizó utilizando el cultivo de *H. pylori* proveniente de un aislado clínico. A partir de los extractos crudos se realizaron pruebas de microdilución en caldo para determinar la concentración inhibitoria media (CI50), la concentración mínima inhibitoria (CMI) y la concentración mínima bactericida (CMB). Se utilizó Claritromicina y Amoxicilina como drogas de referencia. El IC_{50} y la CMI fueron determinados a las 24 horas y 72 horas respectivamente, la CMB fue determinado después de 2 días de cultivo. Se evaluó la actividad anti-*Helicobacter* de los extractos provenientes de las 124 plantas medicinales o alimentarias. Cinco especies presentaron buena actividad, obteniéndose un CI_{50} entre 33.7 y 1000 µg/mL, una CIM y CMB entre 125 y 1000 µg/mL. La amoxicilina tuvo un CI_{50} de 1 µg/mL, una CIM de 2 µg/mL y una CMB de 2.5 µg/mL, mientras que la claritromicina tuvo un IC_{50} de 0.002 µg/mL, una CIM de 0.5 µg/mL y una CMB de 1 µg/mL (Gadea et al., 2014). Se estudio dos de las cinco plantas a fin de aislar y caracterizar los compuestos activos. La actividad se concentró en ciertas fracciones que permitieron aislar compuestos bioactivos. Los datos espectroscópicos de los compuestos aislados y sus actividades farmacológicas serán presentados y discutidos en relación con los usos reportados en medicina ancestral y el interés de complementar este trabajo con modelos capaces de medir otros parámetros farmacológicos del tratamiento tradicional de la ulcera gástrica.

L-10

*Cissampelos sympodialis (*Menispermaceae): a novel phytotherapic weapon against allergic diseases

Piuvezam MR, Bezerra-Santos CR, Vieira C

Federal University of Paraiba, PB, Brazil

Thirty percent of the world-wide population suffers from allergies and the prevalence of these diseases comes increasing gradually in the whole world. *Cissampelos sympodialis* Eichl (Menispermaceae) is a plant popularly used in northeastern Brazil to treat allergic conditions like asthma. We have previously demonstrated that *Cissampelos sympodialis* extract induces the production of regulatory (IL-10) and Th1-type cytokines. The goal of the study was to evaluate the effect of the leave hydroalcoholic extract and its alkaloids warifteine and methylwarifteine on the pulmonary allergic inflammation by analyzing the eosinophils recruitment, deposition of mucus and collagen fiber, IgE production and allergic mediators. Intranasal or oral treated animals with *Cissampelos sympodialis* or warifteine showed inhibition of the OVA-specific IgE serum titer as compared with the control group. In the lung histological stain analysis both treatments showed reduction of mucus and collagen fibers deposition. Also the plant extract and its alkaloid were demonstrated to attenuate airway inflammation and cysleukotrienes generation in asthma model. We also focused on the potential effect of *C. sympodialis* and warifteine to modulate airway hyperreactivity (AHR) and IL-13 production as a key mediator implied in AHR. Mice were sensitized twice with OVA plus Alum and challenge for a long-term protocol. These animals were orally treated with *C. sympodialis* or warifteine once before (prophylactic) or after the last OVA challenge. As control groups were used saline-treated or dexamethasone-treated animals. Then mice were submitted to concentrations of methacholine aerosol and the respiratory pause (Penh) was measured. Mice were euthanized and the bronchoalveolar lavage collected with buffer solution to subsequent IL-13 quantification. AHR response and IL-13 production were attenuated by prophylactic treatment with *C. sympodialis* as compared with non-treated mice. Similarly, both pre- and post-oral treatments with warifteine inhibited AHR response as compared with non-treated mice. These data shows that *C. sympodialis* ameliorates AHR by IL-13 dependent mechanisms. In addition, we also tested other isolated alkaloid from the plant extract and by the flow cytometric analysis we showed that inhaled methylwarifteine (MW) and, mainly, the inhaled extract reduced the number of CD3+T cells and eosinophil-like cells. Therefore, the alkaloids and the extract of *Cissampelos sympodialis* lead to down regulate the inflammatory cell infiltration with remarkable decrease in the number of T cells in an experimental model of respiratory allergy, suggesting that the plant can be delivered by oral or intranasal routes to treat allergic asthma.

Acknowledgements: CNPq/INCT Cancer.

L-11

Value chains of *Rhodiola rosea* (*Sedum roseum* (L.) Scop., Crassulaceae): an investigation of wild collection, cultivation and adulteration

Booker AJ, Jalil B, Heinrich M

Centre for Pharmacognosy and Phytotherapy, UCL School of Pharmacy, UCL, London, WC1N 1AX, UK

Rhodiola rosea (Golden Root) is a high-value herbal medicinal product, licensed in the UK for the treatment of stress-induced fatigue and anxiety. The aim of the project is to assess the diverse value chains that lead to the production of *Rhodiola rosea* as a herbal medicinal product and

assess phytochemical variation between a product registered under the Traditional Herbal Medicine Products Directive[1] and un-registered products. Different *Rhodiola* products are available (Fig. 1) and the principal aim is to establish how these different products are treated and whether there is evidence of adulteration. Several Chinese species are used in traditional Chinese medicine and we investigate their phytochemistry and assess their potential as adulterants.
The sustainability of medicinal plants is an important issue and we will establish what strategies exist for the sustainable cultivation of the plant material and if there are instances of depletion in the wild due to over-collection. Fieldwork examines *Rhodiola* wild-collection and cultivation and investigates which products are traceable through being integrated into a value chain. Approximately 50 products have been sourced from different suppliers. We analyse these samples using HPTLC and NMR spectroscopy coupled with multi-variate analysis software following a method previously developed by our group for the analysis of turmeric products.[2] A phytochemical analysis is currently underway and we will present the results as they become available. The variation in phytochemical constituents present in *Rhodiola* products are determined and important phytochemical differences between cultivated plant material and wild sourced material are assessed.

Acknowledgements: We are grateful to Dr. Willmar Schwabe GmbH & Co. KG, Germany for the funding of this project.
References: [1] European Parliament C. 2004. Directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004 amending, as regards traditional herbal medicinal products, Directive 2001/83/EC on the Community code relating to medicinal products for human use. ed.: EUR-Lex. [2] Booker A, Frommenwiler D, Johnston D, Umealajekwu C, Reich E, Heinrich M 2014. Chemical variability along the value chains of turmeric (*Curcuma longa*): A comparison of Nuclear Magnetic Resonance Spectroscopy and High Performance Thin Layer Chromatography. Journal of Ethnopharmacology doi: 10.1016/j.jep.2013.12.042.

L-12

Combine Ethnopharmacology and Chemotaxonomy: the case of saponins with antileishmanial properties

Haddad M[1,2], Vasquez P[3], Vaisberg A[3], Castillo D[3], Sauvain M[1,2], Rojas R[3]

[1]Université de Toulouse, UPS, UMR152 PHARMA-DEV, Toulouse, France; [2]IRD UMR 152 PHARMA-DEV; Mission IRD, casilla 18-1209, Lima, Peru; [3]Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru

Recent studies on a peruvian plant (Theophrastaceae) led to the isolation of triterpenes molecules with significant leishmanicidal properties and absence of toxicity. These compounds are structurally similar but simpler than maesabalides, which are 13,28-epoxy-oleanane triterpene saponins with strong antileishmaniasis properties, isolated from the vietnamese medicinal plant *Maesa balansae* (Myrsinaceae). Since previous works have shown the importance of the 13,28-epoxy bridge in the skeleton for the antileishmania activity, we decided to investigate further the peruvian biodiversity for structural analogues based on this structural feature. Two new triterpenoid saponins, named magnosides A (**1**) and B (**2**) were isolated from the ethanolic extract of roots of *Cybianthus magnus*. Both compounds were found to possess the 13,28-epoxy bridge. The bioactivity of compounds **1** and **2** was evaluated *in vitro* against different cellular models including *Mycobacterium tuberculosis*, *Leishmania amazonensis* axenic amastigotes, mouse peritoneal macrophages and eight cancer cell lines. While neither of the tested compounds displayed any activity against *M. tuberculosis*, both exhibited anti-leishmanial activity against axenic amastigotes as well as *in vitro* growth inhibitory activity.

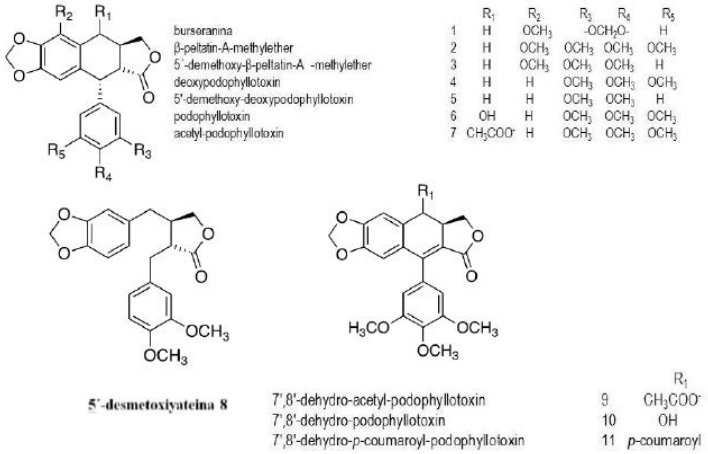
L-13

Biologically active secondary metabolites present in species of the genus *Bursera*

Rojas-Sepúlveda A[1], Antúnez, M[2], Durango C[1], Marquína S[2], Villarreal M[3], Álvarez L[2]

[1]Docente-investigador, Universidad Antonio Nariño Cra 3 Este # 47 A-15, Facultad de Ciencias, Sede Circunvalar, Bogotá, Colombia; [2]Docente-Investigador, Universidad Autónoma del Estado de Morelos, Centro de Investigaciones Químicas (CIQ), Cuernavaca, Morelos, México; [3]Docente-Investigador, Universidad Autónoma del Estado de Morelos, Centro de Investigación en Biotecnología (IBT), Cuernavaca, Morelos, México

Cancer chemotherapy can often reduce symptoms, prolong life and promote healing of this disease. In this sense, plants are a source of new and approved drugs for the treatment of diseases in humans and play an important role in the process of discovering and developing new drugs, where more than 60% of drugs have proven source natural. [1, 2] In this study different species of Bursera were analyzed. The isolation of the active compounds present in the extracts obtained from the bark, leaves, stems and seeds of *B. fagaroides*, var. fagaroides, *B. morelensis*, *B. pinnata, B. copallifera,* and *B. grandilfolia*, allowed the isolation and identification of such podophyllotoxin lignan eleven identified as burseranina **1**, β-peltatin-A-methylether **2**, 5´-demethoxy-β-peltatin-A -methylether **3**, deoxypodophyllotoxin **4**, 5'-demethoxy-deoxypodophyllotoxin **5**, desmethoxy-yatein **8**, podophyllotoxin **6**, acetyl-podophyllotoxin 7, 7',8'-dehydro-acetyl-podophyllotoxin **9**, 7',8'-dehydro-podophyllotoxin **10** and 7',8'- dehydro-p-coumaroyl-podophyllotoxin **11**, from extracts of *B. fagaroides* var. fagaroides, through the analysis of data from 1D and 2D NMR and compared with reported values. Compounds 8, 2-5, and 6-8 above were isolated from different sources, while 9-11 are novel natural products, first described in a kind of Bursera. Using as a biological model in vitro cytotoxic against human cancer cell lines KB (nasopharyngeal), PC-3 (prostate), MCF-7 and H (breast) and F-6 (colon) activity. Lignans isolated showed potent activity against these cell lines, which exhibited higher activity than camptothecin against KB (IC_{50} = 0.189, $1.91x10^{-6}$ µg/mL). In general, the best effect was observed against PC-3 with IC_{50} values of $1x10^{-5}$ µg/mL. (IC_{50} = $7.1x10^{-6}$ mg/mL) was the most potent against HF-6, followed by (IC_{50} = 0.00018 mg/mL) [3].



		R_1	R_2	R_3	R_4	R_5
burseranina	1	H	OCH_3	-OCH_2O-		H
β-peltatin-A-methylether	2	H	OCH_3	OCH_3	OCH_3	OCH_3
5´-demethoxy-β-peltatin-A -methylether	3	H	OCH_3	OCH_3	OCH_3	H
deoxypodophyllotoxin	4	H	H	OCH_3	OCH_3	OCH_3
5'-demethoxy-deoxypodophyllotoxin	5	H	H	OCH_3	OCH_3	H
podophyllotoxin	6	OH	H	OCH_3	OCH_3	OCH_3
acetyl-podophyllotoxin	7	CH_3COO^-	H	OCH_3	OCH_3	OCH_3

		R_1
7',8'-dehydro-acetyl-podophyllotoxin	9	CH_3COO^-
7',8'-dehydro-podophyllotoxin	10	OH
7',8'-dehydro-*p*-coumaroyl-podophyllotoxin	11	*p*-coumaroyl

Acknowledgements: Universidad Antonio Nariño - Vicerrectoria de Investigaciones VCTI.
References: [1] Mann, J. (2002). Cancer (2):143-148. [2] Newman, D. et al. (2003). J. of Natural. Products. (66):1022-1037. [3] Geran, R., et al (1972) Cancer Chemotherapeutic Reports: Part 3 (2).

L-14

Pharmacognostic study of *Vernonanthura patens* (Kunht) H. Rob

Manzano Santana P[1, 2], Miranda M[1]

[1]Escuela Superior Politécnica del Litoral. Centro de Investigaciones Biotecnológicas del Ecuador, ESPOL-CIBE. Campus Gustavo Galindo, Km. 30.5 vía Perimetral, Apartado 09-01-5863. Guayaquil, Ecuador; [2]Facultad de Ciencias Naturales y Matemáticas (FCNM), Departamento de Ciencias Químicas y Ambientales (DCQA), Escuela Superior Politécnica del Litoral; Campus Gustavo Galindo, Km. 30.5 vía Perimetral, Guayaquil, Ecuador

En este trabajo se presenta los resultados del estudio químico-biológico de las partes aéreas de *Vernonanthura patens (Kunth) H. Rob*., que crece en la costa ecuatoriana. La identificación estructural química de las fracciones y compuestos aislados de la especie se realizaron por cromatografía Gaseosa-Espectrometría de Masas y RMN. Se evaluó la actividad antileishmanial y citotóxica de los extractos frente a *Leishmania amazonensis* cepa MHOM/77BR/LTB0016; y, contra la línea celular neuroblastoma de ratón Neuro 2 A respectivamente. Del análisis por CG-EM se asignaron estructuras a 110 compuestos, todos informados por primera vez para la especie. Del total de compuestos, cuatro fueron aislados, e identificados como lupeol, acetato de lupeol, palmitato de lupeol y epilupeol por técnicas de RMN. Los principales grupos químicos identificados fueron: hidrocarburos, ácidos grasos libres y sus ésteres metílicos y etílicos, terpenoides (mono, sesqui, di y triterpenoides), fitosteroles, azúcares, vitamina E, alcaloides y amidas de ácidos grasos. El extracto etanólico de las hojas presentó una actividad y selectividad antileishmanial, con una CI_{50} de 24, 3 µg/mL y un IS de 12, significativamente mayor que el control positivo Pentamidine (CI_{50} 1,3; IS 9). Todas las muestras ensayadas frente a la línea celular Neuroblastoma de ratón Neuro 2 A, presentaron una CI_{50} < 165 µg/mL. Los resultados de la presente investigación se informan por primera vez para la especie. Este trabajo forma parte de las investigaciones encaminadas a validar y proteger el uso ancestral de laritaco en el sur de Ecuador.

L-15

Knowledge attitude practice (KAP) study: a methodology stemming from epidemiology and applied to the validation of antimalarial remedies in French Guiana (South America)

Bourdy G, Deharo E, Vigneron M, Deparis X

Université de Toulouse; Université Paul Sabatier; Pharma-Dev UMR 152; Faculté de Pharmacie; 35 Chemin desmaraîchers, F-31062, Toulouse cedex 9, France.b Institut de Recherche pour le Développement (IRD); Pharma-Dev UMR 152; Faculté de Pharmacie, 35 Chemin des maraîchers, F-31062, Toulouse cedex 9, France

Regarding ethnopharmacology driven studies applied to the validation of medicinal plants uses and drug discovery, 3 different types of methodologies can be used, stemming from ethnobotany, epidemiology and anthropology. In order to have a broad panorama of medicinal plants used against malaria in French Guiana and to select promising antimalarial remedies for further studies, an epidemiological KAP study was undertaken [1], applied to 117 people from 5 different ethnic groups or nationalities. This study highlight that preferred treatments for people suffering from malaria are a mix of plants and drugs, and that strong importance is given to prophylactic treatments, always based on medicinal plants. Composition of curative remedies is based on the use of wild plants selected amongst 34 different species, arranged in order to maximise synergy between compounds, hence reduce doses and potential toxicity. Preventive drinks called "*amers*" (bitter) are always complex remedies, made out of cultivated or introduced species and the plant

association pattern is linked with the people cultural group. The parasitological evaluation *in vitro* and *in vivo* of most used remedies confirms their antimalarial potential, and highlight the importance of *Quassia amara* as a key ingredient in all antimalarial preparations thus leading to select a traditional remedy made out of leaves of this plant for further evaluation. Bio-guided fractionation of this remedy ultimately led to the discovery of a new original promising molecule, the simalikalactone E (SkE) [2].

References: [1] Vigneron M, et al. (2005) Antimalarial remedies in French Guiana: A knowledge attitudes and practices study- Journal of Ethnopharmacology 98, 351–360. [2] Cachet N., et al. 2009. Antimalarial activity of Simalikalactone E, a new quassinoid from *Quassia amara* L. (Simaroubaceae). Antimicrobial Agents and Chemotherapy 53, 10, 4393-4398.

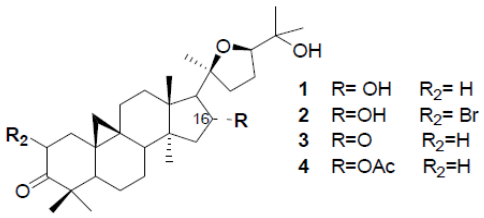
L-16

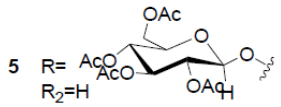
Synthesis of triterpene prodrugs as potential inhibitors of cancer cells

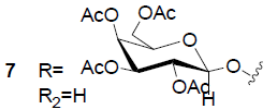
Silva-Zamora F[1], Parra-Delgado H[1,3], Tiburcio MS[2]

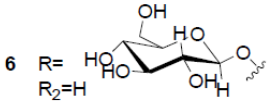
[1]Actual address: Facultad de Ciencias Químicas, Universidad de Colima, Campus Coquimatlán, Km 9, Carr. Colima-Coquimatlán, CP 28400, Colima, México; [2]Facultad de Química and [3]Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, Distrito Federal, México

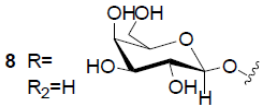
Argentatina A (**1**) is a cycloartane-triterpene which is isolated from “guayule” resin (*Parthenium argentatum* Gray). Our studies demonstrate that **1** and some derivatives are cytotoxic against cancer cells [1]. Taking into consideration the poor water solubility of triterpenes as well as the ability to direct specifically deactived prodrugs through an ADEPT model [2], we synthesized derivatives **2**-**8** in 60-80%yield. Preparation of **2** was made from **1** by treatment with bromine under acid conditions. **1** was selectively oxidized using Dess-Martin reagent to give **3**. Also, monoacetyl derivative **4** was prepared by treatment of **1** with Ac2O in pyridine. Finally, glucosides (**5**-**6**) and galactosides (**7**-**8**) were obtained by reaction of **1** with the adequate glucosyl o galactosyl donor. Products were purified using conventional chromatography and their characterization was carried out by 1H and 13CNMR, IR and MS. Our biological studies on K562 cancer cells at 50μM demonstrated that **3**-**5** were less active than **1** and **2**. That result suggests these compounds can be propose as prodrugs.











References: [1] Parra-Delgado H. et al. (2005). Bioorg. Med. Chem. Lett. 15:1005-1008. [2] Bagshawe K.D. (2006). Rev. Anticancer Ther. 6:1421-1431.

L-17

Novel anticancer compounds from *Garcinia* plants

Xu HX

School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200 Cai Lun Road, Shanghai, China

Garcinia is the most important genus of the Guttiferae family with about 450 species widely distributed in tropical Africa, Southeast Asia and Southern China. Novel phytochemicals embracing xanthones, biflavonoids, and benzophenones from *Garcinia* species have diverse pharmacological properties including cytotoxic, anti-microbial, anti-fungal, anti-plasmodial, anti-viral and anti-inflammatory effects. In tradition Chinese Medicine, the processed gum-resin of *G. hanburyi* is used internally as a drastic purgative, emetic and vermifuge to treat tapeworm and externally for the treatment of chronic dermatitis, hemorrhoids and bedsore. However, the active ingredients of related species are not well characterised or studied. Therefore, in the past several years, we have made great efforts to acquire 15 members of the *Garcinia* family plants from the Xishuanbanna (西雙版納), Yunnan (雲南), and Guangxi (廣西) areas of China. A new approach was used to identify novel anti-cancer compounds from *Garcinia* plants. This approach combines the strengths of advanced chemical separation techniques with the power of a newly developed fluorescent biosensor-based high throughput drug screening platform. Through our research, more than 200 bioactive xanthones and benzophenones were isolated and identified. Many novel xanthones, phenols and flavonoids were isolated from *Garcinia* species, most of which exhibited potent cytotoxicity against different tumor cell lines. Our results demonstrated that xanthones from *Garcinia* species have strong cytotoxic effects and some of them have great potential to be developed into new medicines. We continue to isolate more active compounds from *Garcinia* species and perform further studies on the phytochemistry, bioactivity, and structure-activity relationship of xanthones and related compounds.

L-18

The added value of biodiversity. Recent examples of bioactive metabolites from the Yucatán medicinal flora

Peña LM

Laboratorio de Química Orgánica, Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Calle 43 No. 130, Col. Chuburná de Hidalgo, Mérida, Yucatán, México CP 97200

The historical importance of natural products as new and better pharmaceuticals is evident when we mention products such as taxol, vincristine, or artemisinin. Plants, and particularly medicinal plants, still represent the most important source of bioactive natural products today. Mexico is considered one of the richest countries in terms of biodiversity and in terms of cultural diversity. More than fifty ethnic groups recognize and use over 3,000 plants in their practice of traditional medicine. However, it is believed that less than 5% of these species have been studied in terms of their production of biologically active secondary metabolites. The general objective of the research carried out in our laboratory, is to promote the conservation of the biodiversity of the Yucatan Peninsula by carrying out research in the area of bioactive natural products. It has been suggested that discovering natural products with biological activity in little known and less studied plant species could contribute to the protection of biodiversity, since recognizing the economic value of a particular natural source could ensure its survival. This presentation will mention some of the

different types of biological activities (e.g. antiprotozoan, antifungal, antituberculosis, antioxidant, analgesic, anti-inflammatory, anti-AGEs, etc.) that are currently used by our group to guide the purification of bioactive metabolites produced by native medicinal plants of the Yucatan Peninsula. Additionally, the structures of a number of recently identified bioactive metabolites will be presented.

L-19

Bioprospecting and Ethnopharmacology: evolutionary considerations

Leonti M

Department of Biomedical Sciences, University of Cagliari, Via Ospedale 72, 09124 Cagliari (CA), Italy

Ethnopharmacology and the pharmacologic investigation of indigenous pharmacopoeias have considerably contributed to the discovery of new drugs and molecular targets. In the last few decades ethnopharmacology has become more important with respect to the evaluation of herbal medicines, while bioprospecting for single compound drugs or drug leads from traditional medicines has become less effective. Natural product chemists often follow the concepts of chemotaxonomy and pharma-phylogeny for bioprospecting biodiversity [1,2]. Recently phylogenetic, taxonomic and cross-cultural approaches have been discussed in the scientific literature as tools for bioprospecting ethnopharmacopoeias [3]. Meanwhile, the European Union has sponsored a multi-partner project dedicated to the phylogenetic exploration of medicinal plant diversity [4]. The aim of this presentation is to highlight the challenges and limitations of phylogenetic and taxonomic approaches with respect to the search for new bioactive compounds from traditional pharmacopoeias. The interpretation of the emic and ethnographic field-data is not straightforward since the selection of traditional or local pharmacopoeias is driven by co-evolution between ecological and cultural synergies. We have shown that indigenous pharmacopoeias as well as plant-derived drugs in general are obtained from widespread and species-rich families [5]. We hypothesize that as a function of evolution, widespread taxa contain a broader range of ecologically-relevant information encoded in their genes with respect to locally occurring taxa. This information is expressed through the synthesis of allelochemicals having a wide ecological radius with broad-spectrum biota-specific interactions, including the targeting of proteins in mammals and primates [5].

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L-20

Applied macroscopic identification: An overview of common adulterants found in TCM school clinics in the USA

Brand E., Zhao Z

School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

Authentic medicinal materials are essential for the safe and effective practice of Chinese herbal medicine. While significant research has been conducted to assess the prevalence of misidentified medicinal materials in regions such as Hong Kong, little systematic research has been conducted to determine the prevalence of adulterants in TCM clinics in the USA. Based on previous research, 25 commonly misidentified Chinese herbs that can be effectively differentiated based on morphological characteristics were selected for organoleptic assessment in our preliminary field investigation [1,

2]. 159 samples were collected from the teaching clinics of 8 ACAOM-accredited schools of Chinese medicine in the USA, and the samples were authenticated based on macroscopic identification by Prof. Zhao Zhongzhen of Hong Kong Baptist University. The participating schools were selected for maximum geographic diversity and retention samples were deposited in the herbarium of the Bank of China Chinese Medicine Center of HKBU. Although issues of misidentification only affect a small minority of the Chinese herbs found in U.S. trade, our preliminary investigation suggests that misidentification of certain herbs remains a significant issue. For example, mislabelling is common for the Chinese herbal material *mutong*; although no specimens of the banned adulterant *Aristolochia manshuriensis* Kom. were observed, many schools labelled *mutong* material with the name *Akebia trifoliata* (Thunb.) Koidz. despite the fact that the actual product stocked was derived from *Clematis armandii* Franch. Altogether, nearly 33% of the samples had errors in botanical nomenclature or identity, though significant safety concerns such as potential contamination with aristolochic acid-containing species were uncommon.

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L-21

A call for a new approach against neglected parasitic diseases: the *AmazInG project*, a holistic view onto traditional Amazonian anti-leishmanial remedies

Odonne G[1], Bourreau E[2], Vonthron C[3], Nardella F[3], Eparvier V[4], Makerri C[4], Jahn-Oyac A[5], Deharo E[6], Bourdy G[6], Stien D[4,7], Houël E[5]

[1]USR 3456 CNRS, 2 av. Gustave Charlery, 97300 Cayenne, French Guiana; [2]Institut Pasteur de la Guyane, 26 av. Pasteur, 97300 Cayenne, French Guiana; [3]UMR 7200 CNRS-Unistra, 74 rte du Rhin, 67401 Illkirch, France; [4]UPR2301 ICSN-CNRS, 1 av. de la Terrasse, 91198 Gif-sur-Yvette, France; [5]UMR CNRS-EcoFoG, Campus Agronomique, 97379 Kourou, French Guiana; [6]UMR 152 IRD-UPS PharmaDEV, 35 Ch. des Maraîchers, 31062 Toulouse, France; [7]CNRS-UPMC, USR 3579-LBBM, Observatoire Océanologique, 66650 Banyuls/Mer, France

The AMAZING project* aims at improving the understanding of traditional remedies vivid use against leishmaniasis, by linking plants, people and diseases in a single perspective. Leishmaniasis is a neglected parasitic disease widespread in tropical South America, and its symptoms are well described by the Amazonian populations, making the study of the medicinal uses of the local flora relevant. However, the classical approach for the study of these remedies – *in vitro* bioassay performed on organic solvent extracts – in most cases fails to explain their good reputation. We finalized a data-base including more than 500 antileishmanial use-reports (one plant/one culture), from our field studies and bibliography. Based on an original way to estimate a *distribution index*, we were able to define a ranking of the most promising plant that worth further studies. This approach is also original by linking for the first time pharmacology, cultural parameters, and ecology in a single holistic project. We hope that this will generate synergies between South American and European teams to find new effective and affordable medicines against *Leishmania*.

Prevailing parasitic diseases in **Amazonia: an **in**tegrated approach for the rationalization of medicinal plant uses in French **G**uiana. Funded by a CEBA grant* (CEBA, ref. ANR-10-LABX-25-01).

L-22

Plant-based treatment of snake bites

Jäger A

Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, 2 Universitetsparken, 2100 Copenhagen, Denmark

Snakes have been feared by humans of all cultures from before mythological times, and with good reason as many snakes are deadly venomous. The majority of the Worlds dangerous snakes occur in developing countries, where access to treatment is limited. Snake venoms contain compounds with specific effects on the nervous system, coagulation of the blood or on the cardiac muscle, but also enzymes causing necrosis of the tissue. 6 million people are bitten annually, resulting in 125.000 deaths, and 500.000 permanent disabilities due to tissue necrosis. We have created a database on plants to treat snakebites worldwide, based on available literature from PubMed, Web of Sciences and Science Direct. Currently, the database holds 1500 species. We have collected plants in Mali, Congo, South Africa, China, Pakistan and Peru. Extracts of the plants have been tested for inhibition of various classes of necrotic enzymes. The assays include proteases, phospholipases and hyaluronidase, all performed in microtitre plates. The most active extracts were evaluated by HPLC for tannin content, tannins are well-known for inhibiting enzymes, and might be a useful strategy for topical treatment of snakebites, but it is an unspecific activity. Tannins were removed from the extracts, and extracts still exhibiting activity were further investigated by HPLC-NMR-BIOASSAY for direct structure elucidation of the active compounds. Further, it was investigated if active extracts could pass over the skin, and whether they promoted cell-growth to aid wound healing. We hope this will lead to an accessible plant-based treatment of the necrotic tissue damage many survivors of snakebite suffer from.

L-23

HPTLC for identification of botanical materials - a practical approach

Leuenberger J

CAMAG, Sonnenmattstrasse 11, Muttenz, CH-4132, Switzerland

Not only in current Good Manufacturing Practice (cGMP) for dietary supplements and herbal medicines but also in ethnobotanical and phytochemical research proper identification of botanical materials is of fundamental importance. High Performance Thin Layer Chromatography (HPTLC) is ideally suited for an identification based on chemical fingerprints. The key elements of HPTLC are a standardized methodology, validated methods, and suitable instruments including HPTLC plates. The United States Pharmacopoeia has just recently published guidance documents for HPTLC methodology which can serve as the basis for global standardization in the Pharmacopeial Forum PF 40(3)[1]. The International Association for the Advancement of HPTLC is developing a method collection currently including identification of more than 160 plants[2]. Through its laboratory CAMAG, the leading manufacturer of instrumentation for HPTLC, has been collaborating for more than a decade with industry, academia, and regulatory bodies in the development of methods and pragmatic concepts for identification of botanicals as part of quality control in a cGMP environment. Powerful instruments and state of the art software can help the analyst to perform reliable identification with ease and confidence. This presentation illustrates the individual steps of the process.

References: [1] http://www.usp.org/usp-nf/pharmacopeial-forum; [2] http://www.hptlc-association.org/methods.cfm.

L-24

Argininyl esters and alkaloids from the venom of the Amazonian toad *Rhinella* (*Bufo*) *marinus*

Vargas-Arana G[1], Quispe C[2], Theoduloz C[3], Schmeda-Hirschmann G[2]

[1]Universidad Científica del Perú, Av. Abelardo Quiñones Km 2.5, Iquitos, Perú; [2]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, 3460000 Talca, Chile; [3]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, 3460000 Talca, Chile

Little is known on the chemistry and bioactivity of the amphibian defense substances in Peruvian Amazon frogs. The large toad *Rhinella marina* (Linnaeus, 1758), formerly known as *Bufo marinus* is common in the Amazon forest and swamp areas nearby Iquitos, in the Peruvian Amazon Province of Loreto. Compounds occurring in the toad skin secretions and venom of *Rhinella* (*Bufo*) species have been shown to display a wide array of biological activities and are used as a crude drug in Chinese traditional medicine. *Rhinella marina* is reported to be used to treat erysipelas and scorpion stings in Brazil (1). A study was undertaken to assess the chemistry and cytotoxicity of the parotid glands venom of *R. marina* from two different locations in Loreto. HPLC-ESI-MS/MS and NMR analysis showed a complex pattern of constituents, including argininyl diacid esters, argininyl esters of marinobufagin, telocinobufagin and bufalin. Several compounds are reported for the first time for South American *Rhinella marina*. Clear differences were found in the composition of both venom samples. The cytotoxicity of the crude venom and main compounds was assessed on human lung fibroblasts and a panel of human cancer cells. Very high activity was found for the crude venom and compounds, with IC_{50} values in the range of 0.154-0.296 µg/mL for SK-MES-1 lung cancer cells (HTB-58) and 0.169-0.212 µg/mL for J82 bladder carcinoma cells (HTB-1), respectively. The chemical diversity and bioactivity found in the samples encourages further work on Peruvian amphibians.

Acknowledgements: G V-A thank the Universidad Científica del Perú for financial support.
References: [1]. Alves RRN, Alves HN (2011). The faunal drugstore: animal-based remedies used in traditional medicines in Latin America. Journal of Ethnobiology and Ethnomedicine 7:9 doi:10.1186/1746-4269-7-9.

L-25

Using plants to protect humans and animals against microorganisms and parasites

Eloff JN

Phytomedicine Programme, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa 0110

Compounds originating from plants form an important part in human and animal medicine. Microorganisms and parasites have developed a worrying resistance to many compounds [1]. Complex plant extracts may resolve some of the problems. Plants may be selected based on traditional use or on a random screening or taxonomical base. Random screening of leaf extracts of more than 700 trees have led to products with promising activity in handling diarrhoea [2] and fungal infections [3]. Factors useful in identifying promising leads include biological activity, cytotoxicity, selectivity index, mechanism of activity and activity in preclinical trials. Analysis of the data also led to identification of tree plant taxa with promising activities and selectivity against different pathogens. Promising products based on traditional use have been developed to treat infestations by helminths [4], ticks and blowflies [5]. There are however many difficulties between

discovering an effective plant extract and developing a commercially viable product. This aspect requires diverse expertize usually outside the sphere of academics. Protecting the intellectual property may also stifle collaboration and advances in using plants to increase the quality of life of poor rural inhabitants.

References: [1] Eloff JN, McGaw LJ 2014 pp 163-173 in Gurib-Fakim A (Ed) Novel Plant Bioresources: Applications in Food Medicine and Cosmetics, John Wiley. [2] Ahmed, A.S (2012) J. Ethnopharm. 143: 826-839. [3] Suleiman MM et al. (2012) BMC Vet. Res. 2012, 8:210 [4] Adamu M et al. (2013) BMC Vet. Res.2013 9:38. [5] Mukandiwa L., et al. (2012) J. Ethnopharm.143: 812-818.

L-26

Chilean regulation of medicines made from plants

Parada M

Instituto de Salud Pública. Chile, Casilla 48, Santiago, Chile

At present, the developed countries such as those in developing show accelerated drug use made with medicinal plants growth. These plants are used as raw material for a wide range of businesses that use less or greater degree of industrialization compounds derived from them. Thus, infusions, oils, extracts, powders, fragrances, personal care products, dietary supplements, functional foods, herbal medicines and products for industrial use and application in agriculture are developed. The regulations governing them have been modified and supplemented according to current requirements. Today is the force S.D. N°. 3/10, (which approves the Regulation of the National Control of Pharmaceuticals for Human Use), in which the definitions of herbal medicines and the requirements for its regulation are included, with the Public Health Institute of Chile (ISP) who shall be applied, the ISP is the government body responsible for implementing regulatory changes, as functionally decentralized public service, which has management autonomy with legal personality and its own assets. The Ministry of Health for approval of its policies, rules and plans of activities and in monitoring their implementation. The aim of this paper is to describe the scope that has legislation in Chile for medicines made from medicinal plants.

Acknowledgements: Instituto de Salud Pública de Chile.
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L-27

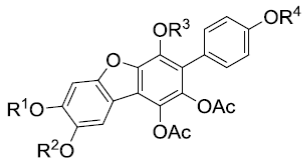
Discovery of metabolites from fungi used traditionally in the Highlands of Papua New Guinea

Barrow RA[1,2], Beekman AM[1], Wossa SW[1,2], Kevo O[3]

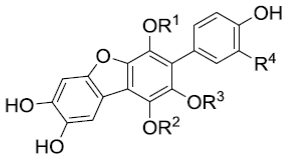
[1]Research School of Chemistry, The Australian National University, Canberra, 0200, Australia; [2]University of Goroka, Goroka, Eastern Highlands, 441, Papua New Guinea; [3]Village Chief, Kiovi Tribe, Eastern Highlands, Papua New Guinea

The boletopsins are highly oxygenated *p*-terphenyl fungal metabolites, first isolated in 1987 by Steglich and co-workers [1]. While examining the biodiversity of mushrooms traditionally used in Papua New Guinea several new boletopsins were discovered. The origin of these metabolites was a *Boletopsis* sp. of mushroom, which is traditionally used by the people of the Kiovi village, in the

Eastern Highlands Province, as a food source as well as a treatment for gastrointestinal complaints. Bioassay guided isolation of the metabolites highlighted potential cytotoxic and antibacterial activity. This rare class compounds are found exclusively in fungi, with only ten previously reported members. The present work explores the elucidation and total synthesis of these newly discovered fungal metabolites.



	R^1	R^2	R^3	R^4	boletopsin
1	Ac	Ac	Ac	Ac	1
2	Ac	H	Ac	H	2
3	H	Ac	Ac	H	3
4	H	H	Ac	H	4
5	Ac	Ac	Ac	H	5
6	H	H	Ac	Ac	6
7	H	H	H	H	7



	R^1	R^2	R^3	R^4	boletopsin
8	Ac	H	H	H	8
9	H	Ac	Ac	OH	9
10	Ac	H	H	OH	10

Acknowledgements: RAB acknowledges funding from the NHMRC (1028092).
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L-28

African Traditional Medicine in South Africa

Van Staden J, Moyo M, Nair JJ

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, *Pietermaritzburg, Scottsville 3209, South Africa*

South Africa is characterized by a population known for excessive reliance on its plant resources for medicinal, cultural and spiritual needs. Trading of medicinal plants in urban and semi-urban streets and shops is one of the peculiar features that signify the importance of the practice of African Traditional Medicine in South Africa. This paper highlights the role South Africa has played in recent years, in contributing to the worldwide increase in research in the field of ethnopharmacology. Medicinal plant research in South Africa is primarily focused on investigating the efficacy of these plants in the search for new therapeutic compounds. Examples will be presented from our research on the screening of traditionally used plants. In particular, emerging research areas including anticancer, antiplasmodial, neurodegenerative as well as infectious and neglected diseases such as tuberculosis and venereal diseases will be emphasised. In addition, other important aspects of ethnopharmacology research related to the safety of indigenous medicinal plants and the conservation of this unique floral heritage using plant biotechnology will be discussed.

L-29

Mechanism of action of natural products for neurological diseases: APP processing, Wnt signaling and neurogenesis

Inestrosa N

Center for Aging and Regeneration (CARE), Faculty of Biological Sciences, P. Catholic University of Chile, Santiago +5626862724, Chile

The use of natural products is an emergent conceptual strategy in the search of drugs with therapeutic potentials for the treatment of neurodegenerative disorders. Here we will describe the

neuroprotective activity of two compounds which have been studied in our laboratory for the last 5 years. The first one hyperforin, is the active molecule for the anti-depressant activity of the St. John´s Wort (*Hypericum perforatum*), the second one is Andrographolide a labdane diterpene, an active product of the plant *Andrographis paniculata*, used in the Ayurvedic Medicine. Both compounds, prevents cognitive deficit, evaluated by behavioral analysis, synaptic electrophysiology including long-term potentiation and depression, tau phosphorylation and amyloid-b deposition in an Alzheimer´s disease (AD) model, a double transgenic APPswe/PS-1 mouse. We will present here, some of the putative mechanisms of action of these natural products for AD, and may be for others neurological diseases: (a) *"APP processing":* where the amyloid precursor protein (APP) became subject to a proteolytic process that leads to the generation and accumulation of the Aβ peptide in the brain of AD patients. (1). (b) "*The Wnt signaling*" is an essential pathway involved in the differentiation of synapses and of neuronal proliferation. The activation of Wnt signaling leads to the inactivation of glycogen synthase kinase-3β (GSK-3β), which determines the expression of Wnt target genes (2). (c) "*Adult neurogenesis*" consists in the generation of new neurons in the adult brain from neural stem/progenitor cells. This process occurs in the subgranular zone of the hippocampal dentate gyrus, and the adult-born neurons play a role in the formation of new memories (3).

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L-30

Center for Innovation in Biodiversity and Drug discovery: CIBFar: an innovative approach to identify secondary metabolites from tropical biodiversity as hits and leads

Bolzani V

Sao Paulo State University - UNESP, Institute of Chemistry, NuBBE, Araraquara, CEP 14.800-900, SP, Brazil

In Brazil, the establishment of the Centers for Research, Innovation and Dissemination (CEPID) by the State of São Paulo Research Foundation (FAPESP) was an extraordinary initiative to biodiscovery research. One of the most important challenges in drug discovery is the identification of promising hits and the generation of high quality leads in the early stages of this complex process. Brazilian biodiversity still have been a huge source of new secondary metabolites and thus, the most successful source of interesting compounds for drug discovery. One of the challenges in drug discovery using natural products as models is the lack of how to access natural products containing all botanical, chemical, pharmacological and toxicological information together. This fact motivated us to create a database of natural products and derivatives from Brazilian biodiversity containing all molecular information on all natural products identified by our group[1]. Taking in account all information on taxon, compounds isolated, semi-synthetic derivatives, chemical properties (molar mass, *c*Log*P*, number of hydrogen bond donor and acceptor), Lipinski's 'rule of 5' violation, chemical structure as SMILES and Mol2, and biological, pharmacological and toxicological properties some hits have been identified for design of new lead molecules. Examples of new proteases inhibitors and antimalarial compounds design will be presented.

Reference: [1] M. Valli, R. N. dos Santos, L. D. Figueira, C. H. Nakajima, I. Castro-Gamboa, A. D. Andricopulo, and V. da S. Bolzani. *Journal of Natural Products*, *76*, 2012, 445-449.

L-31

Bleeding in women: a disease among indigenous and other local women in the Gran Chaco. Etiology, therapeutics, fears and prevention

Arenas P

Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 piso 16, CP 1121,Buenos Aires, Argentina

Bleeding in women (menstrual, puerperal or hemorrhagic, and especially at menarche) is thought of as a disease, both by the indigenous peoples and by the Criollos (non-indigenous locally born people) of the Gran Chaco in South America. The subject is addressed from the perspective of the ethnosciences (particularly ethnomedicine and ethnobotany), considering in the first instance the vision and discourse of the actors. Secondly, an analysis and an interpretation are made from the viewpoint of academic science. Besides the physical discomfort that bleeding may provoke in the person, this situation involves a degree of danger, which is at times extreme, for the patient, their family and the community in which she lives. This presentation contains a description of the corpus of beliefs, the etiological explanations of the disease, the associated fears and taboos, the consequences and punishments for violating any prohibitions, and the naturalist therapeutics applied, especially those in connection with phytodrugs. It includes a special reference to the ceremonies linked with the menarche in various ethnic groups of the Chaco. Finally, mention is made of how these concepts of folk medicine influence official health plans, the daily life of the people, and even the local economic activities.

L-32

The correlation between taxonomy and antimicrobial activity of southern African tree leaf extracts

Pauw E, Eloff JN

Phytomedicine Programme, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa 0110

Traditional leads are not useful to identify plants with high antimicrobial activity because traditional healers mainly use water as extractant and water does not extract the high-activity intermediate polarity compounds. We determined the antibacterial and antifungal activity of 714 acetone leaf extracts of 537 different tree species against *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Cryptococcus neoformans*. Several extracts had MICs as low as 0.02 mg/ml. We analysed 14 out of the 38 tree orders where more than 8 different species were investigated, representing 89% of all species examined. There were statistically significant differences in some cases. Celastrales, Rosales and Myrtales species had the highest activity against Gram-positive bacteria, Myrtales and Fabales against Gram-negative bacteria and Malvales and Proteales against fungi. Fabales species had the highest activity against all the microorganisms tested. There was substantial selectivity in some orders. Proteales species had very high activities against fungi and very low activities against bacteria. Celastrales and Rosales species had very low antifungal activities and very high Gram-positive bacterial activities. In all cases orders with high activity species also included low activity species and vice versa. Nevertheless, there was a twofold increase in probability of finding extracts with interesting antifungal activity from orders with high mean activity than from orders with low mean activity. The probability increased to threefold and fivefold for Gram-positive and Gram-negative bacteria respectively. The taxonomic relationship with antimicrobial activity may guide the selection of families, genera and species to deliver useful compounds or extracts.

L-33

Ethnomycology: characterization of the bioactive constituents from the mushroom, *Fulaga dive* (Amanitaceae) used traditionally in Papua New Guinea

Castillo Martinez E[1], Wossa SW[1,2], Kevo O[3], Barrow RA[1,2]

[1]Research School of Chemistry, The Australian National University, Canberra, 0200, Australia; [2]University of Goroka, Goroka, Eastern Highlands, 441, Papua New Guinea; [3]Village Chief, Kiovi Tribe, Eastern Highlands, Papua New Guinea

As a part of an ongoing study examining the traditional use of fungi by communities' in the Highlands of Papua New Guinea our attention was focussed on the extract of the mushroom, Fulaga Dive, used as a food source by the Kiovi tribe, in the Eastern Highlands Province. The mushroom demonstrated antibacterial activity against the Gram (+) *S. epidermidis* (IC_{50} 305 µg/mL) and represented a new species within the genus and subgenus *Amanita* as determined by DNA analysis. Bioassay guided isolation and chemical structure elucidation revealed the presence of two novel furan fatty acids (FFAs) in abundant quantities. While FFAs are rarely detected in nature they have recently gained special attention due to their usefulness as active components of functional foods and their potential as anti-inflammatories and radical scavengers [1, 2]. The aim of the present work was to explore the pathway from ethnomycology through isolation, structural elucidation and synthesis of these compounds.

Acknowledgements: ECM acknowledges the ANU for an Australian Postgraduate Award. RAB acknowledges funding from NHMRC (1028092).
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L-34

Cultural landscapes and traditional medicinal knowledge among Mapuches: adaptation, maintenance and replacement

Ladio A

INIBIOMA (Instituto de Investigaciones en Biodiversidad y Medio Ambiente- CONICET-UNCo), Quintral 1250, 8400, Barioche,Argentina.

Mapuche rural populations living NW Patagonia (Argentina) have developed several strategies for the maintenance of their rich traditional medicine [1, 2]. More than ten years of ethnoecological studies have shown that they utilize different cultural landscapes as source of therapeutic plants (composed in total by more than 120 species), which are delineated by diverse learning and adaptation processes to deal with the present socio-cultural and environmental circumstances. The aim of the work will be to show, by means of quali-quantitative approach, that the differential use of the natural surroundings, the ecological transformation of the land through cultivation, and the in situ protection given to certain wild medicinal plants, are behaviours that demonstrate the strong bio-cultural roots underlying their traditional medical system. We also found that the cultural importance of the different units of the landscape has an organoleptic pattern; it depends on the sensorial characteristics, relative importance and symbolic value of the medicinal species they are composed of. At present, this pharmacopeia is also enriched with exotic plants that come from distant cultural scenarios promoted by massive media, which are obtained through barter with neighbouring populations. Hybridization processes are occurring where the adaptation,

maintenance and replacement are part of this interplay. This re-conversion implies revitalization and re-articulation, and makes it possible the survival of the Mapuche traditional medical wisdom.

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L-35

Natural history of pharmacy in South-America: rediscovering the medicinal plants of Brazil

Brandão MGL

DATAPLAMT, Museu de História Natural e Jardim Botânico & Faculdade de Farmácia, Universidade Federal de Minas Gerais, 30180-010, Belo Horizonte, Brazil.

Plants have been used as food and medicinal sources by thousands of years by the American Indians. The Portuguese and Spanish brought several of these species to Europe at the beginning of the 16th century and the use of Brazilian plant remedies, such as copaiba (Copaifera spp.), ipecacuanha (Carapichea ipecacuanha (Brot.) Stokes) and curare (Chondrodendron spp.) expanded to several parts of the world. Historical research can be very helpful for recovering valuable ethnopharmacological data regarding the use of plants. In Brazil, this is important and necessary due to the intense and continuous destruction of native vegetation, since the discovery of the country in 1500. Much of the available information about the Brazilian biodiversity was compiled by European naturalists who traveled throughout the country in the 19th century. The data and specimens recorded by them, and deposited in European Institutions, are an important source of information about the uses of the plants. At that time, the native flora was more conserved, and Brazilian native species were predominantly used in traditional medicine. Since 2003, our research group has worked in recovering data and images of useful plants native to Brazil in historical bibliography, and has providing this information at a website (www.dataplamt.org.br) and in historical books that have been translated into Portuguese. A range of material for popularization of science have been produced (books, toys, documentaries, mini-laboratory, web-serie) and fellowships (from CNPq) are distributed for students of schools from inner, in order to help the teachers to introduce the importance of Brazilian medicinal plants and the necessity to improve the scientific studies with them. The final objective of all the work is to contribute to the preservation and better use of the Brazilian biodiversity and Native American traditional heritage.

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L-36

Nutritional and functional properties of native fruits of Argentina: Revalorization use in the design of functional foods

Isla MI[1,2], Zampini IC[1,2], Alberto MR[1,2], Ordoñez RM[1,2], Cuello AS[1], Torres S[1,2], Sayago J[1,2]

[1]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA.CONICET); [2]Facultad de Ciencias Naturales e IML. Universidad Nacional de Tucumán; [3]Fundación Miguel Lillo. (4000) S.M. de Tucumán, Tucumán, Argentina

The aim of this project is the study of nutritional and functional benefits of fruits from Argentinean Northwestern as well as its relation with the chemical composition. The fruits of some native

Leguminosae such as *Prosopis* species were used as food by native people and the flour of such fruits was introduced into the Argentinian Food Code). Other attractive fruits of our region due to their potential applications in the food industry are "mistol " (*Ziziphus mistol*) that recently joined the list of edible fruits of the Argentinian Food Code, " tree tomato" (*Solanum betaceum*), "mato" (*Myrcianthes pungens*), "chañar" (*Geoffroea decorticans*), fruits of some cactus (*Opuntia, Rhipsalis*), among others. All of them are used in the local cuisine in fruit salads, juices, as candy, jellies, preserves and other regional products by different aboriginal and rural communities and are now being rediscovered as potential new crops. Recently, we demonstrated that somefruits from plants native toNOA have nutritional and functional properties and can be exploited for designing functional food as such or minimally processed.

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L-37

R & D of depsides salts from *Salvia miltiorrhiza*

Xuan L

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 haike Road, Shanghai 201203, China

Modernization of traditional Chinese medicine (TCM) is strategically significant for healthcare of China's population and for the sustainable development of society and economy. *Salvia miltiorrhiza* Bunge, a traditional Chinese herbal medicine known as “Danshen,” has been widely used in clinics to treat cardiovascular disease. Based on the identification of depsides salts (mainly magnesium lithospermate B) as its primary active compound, a modernized TCM, named as Depsides Salts from *Salvia miltiorrhiza*, was developed with clear ingredients and mechanism, controllable quality, and definite efficacy and safety. After a decade of study, Depsides Salts has been confirmed to be safe and effective for the treatment of patients with coronary artery disease and chronic angina pectoris in multi-centre clinical trials. Since it was licensed by the China Food and Drug Administration (CFDA) in 2005 and launched in 2006, its pharmaceutical revenue has been grown quite rapidly. The sales exceeded RMB 3.5 billion in 2013 and it was applied to more than 5 million patients.

L-38

Ethnopharmacology: a past Italian pioneer

Zanette G, Facco E

University of Padova, Department of Neurosciences, via Giustiniani 5, 35125 Padova, Italy

We suppose that most of you know the meaning of the words “*set and setting*”. So here, the “***setting”*** is represented by Milan, on the edge of the 18th and 19th century, whereas the “***set”*** is represented by an eclectic Italian natural philosopher, Paolo Mantegazza (PM). Physician, anthropologist, writer and politician, PM founded the first Italian University Chair of Anthropology, the National Museum and the Italian Society of Anthropology and Ethnology, the Journal “Archives for Anthropology and Ethnology”. Back from South America, this ethnobotanist “on the field”, introduced in the European pharmacopeia *Erythroxylum coca*, source of the first local anaesthetic, cocaine hydrochloride, introduced by Freud in psychotherapy. In “*Tales Of Human Nature. Festivals And Inebrations*” [1] PM proposed a classification of mind altering substances 60 years before

Lewis Lewin's "*Phantastika*". In this remarkable effort to collect the knowledge about Ethnopharmacology, PM, following a common medical practice of that time, shared know-how, performed self-experimentation and reported his personal experiences. In Mantegazza's vision of the natural world, the mind altering plants, called "*nervous feeding*", are integral part of nature and, therefore, of *H. sapiens*: «…*Under their influence the consciousness of our existence becomes deeper, moral pains are mitigated and forgotten, happiness is resuscitated with the possibility to reach the inner enlightenment*…».

Reference: [1] Mantegazza P. Quadri Della Natura Umana. Feste Ed Ebbrezze. Bernardoni Editore, Milano, 1871.

L-39

Metabolomics in medicinal and food plant research along the value chain from producer to consumer

Booker AJ[1], Johnston D[2], Heinrich M[1]

[1]*Centre for Pharmacognosy and Phytotherapy, UCL School of Pharmacy, UCL, London UK;*
[2]*School of Oriental and African Studies, London UK*

Ethnopharmacology has for many decades focused on what generally has been termed 'traditional' knowledge and practice, often focusing on remote places and exotic people. However, in an increasingly interconnected world alternative themes have increasingly come into the center of scientific attention, including, for example, the importance of knowledge transmission, the use of herbal substances in urban contexts or by migrant groups and the commodification of such knowledge. Value chain analysis has been applied to a variety of food commodities (e.g. tea, coffee, cocoa) in order to better understand the sometimes complex interventions involved in bringing a finished product onto the market. This includes the inputs necessary, the actors involved in each of the steps, who has power and governance in the chain and which interventions constitute value addition. Little attention however has been paid to the value chains of medicinal plants, an area that is prone to supply chain difficulties including exploitation through middlemen, over-harvesting of wild medicinal plants, adulteration and contamination of products at different stages along the chain and a general lack of traceability through the different stages of production. The quality of the finished product is one variable that can be directly linked to value but in order to produce a high quality herbal medicinal product it is necessary for each stage of the value chain to have effective quality controls in place. Moreover it is important that the quality of herbal material can be measured objectively at each stage. This is especially important if finished products are destined to be exported to countries where higher entry standards and regulatory requirements exist. Analytical analysis provides a method to achieve this measurement and through investigating and documenting the quality of a product as it moves along the value chain, it is possible to provide better and more defensible justification for value addition and consequently these quality driven interventions result in a higher standard of product for the end consumer.

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L-40

The remarkable structural diversity achieved by fungal biotransformations in biologically active compounds

Takahashi JA

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais (UFMG). Av. Presidente Antonio Carlos, 6627, CEP 31.270-901 - Belo Horizonte, MG, Brazil

Medicinal plants are a major source of biologically active molecules. A number of derivatives from these naturally occurring molecules have been prepared for structure-activity screenings. Studies on structure versus activity have showed that, in most cases, in terpenes, biological activity increases with the increase of functionalization. Since naturally occurring terpenes usually have a limited number of functional groups to be used as targets for semi-synthetic modifications, production of terpene derivatives has been mostly achieved through the use of fungal biotransformations. In this review, selected examples the wonderful chemical diversity produced by fungi in terpene skeletons will be presented. General biotransformation protocols as well as fungal with consistent regio- and stereoselectivity will also be discussed.

L-41

Natural products and crude drugs in traditional medicine research: how to deal with complexity?

Bauer R

Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitätsplatz 4/I, A-8010 Graz, Austria

The use of plant extracts in traditional medicine is regularly following a holistic concept. A single compound – single target strategy is not adequate for elucidation of the active principle, but multiple active compounds and multiple targets have to be considered. For that reason, an alternative strategy is emerging: the application of metabolomics and systems biology [1,2]. Omics technologies are promising tools which allow us to deal with multiple active constituents and multiple pharmacological activities. Gene expression profiles demonstrate the activity spectrum of an extract, and may be also used for extract standardization [3]. However, the functional aspect should also be considered. The pharmacological targets must be verified on protein level in-vitro, and finally with the desired effects in-vivo. The constituents relevant for pharmacological effects can be traced by LC-MS and NMR based metabolic profiling and correlation with activity using bioinformatics tools [4,5]. Principle component analysis and orthogonal partial least square discriminant analysis (OPLS-DA) are frequently used [6]. By using these techniques, we could distinguish the chemical profiles of active and non-active samples of *Lonicera* and *Clematis* species, and were able to identify the constituents which are most relevant for activity [7,8].

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L-42

Creation of the national Cambodian Pharmacopoeia: a new tool for the quality control of the medicinal plants, the plant extracts and the plant-based medicines

Bessioud P[1], Fabre B[2], Kim S[1], Leti M[2], Mandeau A[2]

[1]University of Health Sciences, 73 Bd Monivong, Phnom Penh, CAMBODIA. [2]Institut de Recherche Pierre Fabre, 3 avenue Hubert Curien – BP13562, 31035 Toulouse, France

The medicinal plant is a drug: it has biologic activities, it can show toxicity, and it is intended for a fragile population. In the light of this, the medicinal plant needs scientific expertise (both botanical, chemical, pharmacological, clinical and toxicological) and its distribution should be supervised. Traditional plant-based medicine takes up an important place in Cambodian primary health care system. Several hundreds of plants are indeed known for their prophylactic and healing properties [1-3]. Yet, as for today, Cambodia has a poor legislation toward traditional medicine. The authorities have then recently decided the creation of a national Pharmacopoeia as a tool to ensure the quality, the efficiency and the safety of plant-based medicines. To cope with this, the appointed plants have been selected through rigorous criteria, their monographs set up according to international standards and a regulatory framework is shaped. Today, the first monographs of raw plants have been established at the Laboratory of Phytochemistry of the University of Health Sciences (*Herba cum radice Andrographidis* and *Curcumae longae rhizome*) and new monographs are currently being developed.

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L-43

Effects of different cultivation practices on the pharmacological properties of *Artemisia annua* L. (Asteraceae)

Ndhlala AR[1], Ncube B[2], Mulaudzi R[1], Abdelgadir HA[1], van Staden, J[2], du Plooy CP[1]

[1]Agricultural Research Council, Vegetable and Ornamental Plants Institute (VOPI), Private Bag X293, Pretoria 0001, South Africa; [2]Research Centre for Plant Growth and Development, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3201, South Africa

The use of traditional herbal medicines is increasing in developing countries due to lack of adequate health care systems. The demand for medicinal plants has meant that sustainable harvesting from the wild is no longer feasible. The solution is to develop medicinal plants as crops through farming. It is therefore important to establish good agricultural practice to ensure high yield, product quality and safety. In this study, factors known to affect the pharmacological effectiveness of *Artemisia annua* were investigated. The parameters investigated include: plant spacing and pruning. Oven-dried plant material was extracted with 50% methanol, acetone, 70% ethanol and water. The methanolic extracts were subjected to phytochemical and antioxidant tests while the rest were tested for antimicrobial activity against two bacterial strains and *Candida albicans*. The extracts exhibited a broard spectrum of activities. The antimicrobial activity ranged from 0.195 to

12.5 mg/mL. Differences were observed in the bioactivities of the extracts from different treatments. Highest bioactivities were observed for the extracts from pruning methods that involved trimming off the tips of all branches in combination with spacing of 30x70 cm. More phytochemical compounds were detected in the same pruning and spacing treatment. There was a positive correlation between the antioxidant activity and the phytochemical levels as determined by the DPPH and β-carotene-linoleic acid assays. This could be due to wounding effects on the plant that triggered the production of more phytochemical compounds as defense mechanism against microbial infections. Different cultivation methods affect the expression of secondary metabolites and consequently bioactivities.

L-44

The domestication of native berries: *Fragaria chiloensis* as a Chilean example

Caligari PDS[1], Retamales JB[2]

[1]Instituto de Ciencias Biológicas y Centro de Mejoramiento Genético y Fenómica Vegetal, Universidad de Talca, Casilla 747, Talca, Chile; [2]Facultad de Ciencias Agrarias y Centro de Mejoramiento Genético y Fenómica Vegetal, Universidad de Talca, Casilla 747, Talca, Chile

The native Chilean species *F. chiloensis* is the "mother" of the commercial strawberry (*F. x ananassa)* to which it contributed important horticultural characteristics such as large fruit size, and high photosynthetic rate. It has two botanical forms, one of which is white-colored (*F. chiloensis* f. *chiloensis*) and was domesticated by the native Chilean people. At the present, its culture is restricted to small plantings in areas with coastal influence in South-Central Chile. A long-term program to re-establish the white-colored *F. chiloensis* as a commercial species has been carried out for the last 15 years at the Universidad de Talca. The program has included: breeding, physiology, chemistry, biochemistry, market studies and management practices. For this, ecotypes were collected in various sites in southern Chile (Lat. 35 to 43º S); they were characterized both morphologically and through molecular means, and propagated both by stolons and *in vitro* techniques. Plants were established in various trials and in the greenhouse to optimize horticultural management (substrate, mulch, irrigation, hydroponics, planting distances, germplasm, etc.). The breeding program is currently evaluating plant material generated from various crosses between *F. chiloensis* accessions as well as interspecific crosses with the commercial strawberry. Two possibly interesting aspects of the white strawberry, as opposed to the commercial red strawberry, are that it has ellagic acid as the main phenolic component and that would be less allergenic. There is a need for more work in order to transform the Chilean native strawberry into a commercially-viable sustainable crop.

L-45

Chemical variability in Piperaceae species: cause or consequence?

Kato MJ[1], Yamaguchi LF[1], Yoshida NC[1], Gaia AM[1], Martins M[1], Costa VB[1], Valero-Gutierrez LY[1], Oliveira-Jr CRO[1], Correia MV[1], Fokoue HH[1], Cebrian-Torreyon G[1], Massad TJ[1], Egydio APM[2], Salatino ML[2], Salatino A[2]

[1]Research Support Center in Molecular Diversity of Natural Products, Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, 05508-000, São Paulo, SP, Brazil; [2]Department of Botany, Institute of Biosciences, University of São Paulo, Rua do Matão, 277 - 05429-970 - São Paulo, SP - Brazil

The diversity of Piperaceae with some 4000 species is impressive among basal Angiosperms [1]. The biotic interactions with dispersers such as bats, the small size and large number of seeds, and

the relatively short life-cycle could in part account for such variability of species [2]. Their chemistry are diversified and characterized by amides, chromenes, meroterpenes, piperolides, pyrones, and polyketides but the distribution of compounds according to the evolutionary prospects is not clear yet [3]. Thus, species belonging to the two major genera, *Piper* and *Peperomia*, were subjected to the molecular phylogenetic studies using ITS and *mat*K in order to determine the relationship among the species. On the other hand, chemical profiling of crude extracts using ^{1}H NMR and ESI data as well as detailed phytochemical characterization have been made in order to evaluate their chemotaxomical importance. Further studies involving ontogenetic studies [4] as well as plant-insect interactions [5,6] have contributed to a better understanding of cause-effect relationship behind such chemical variability.

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L-46

Lapis judaicus: a traditional drug for kidney stone-Randomized double blind clinical trial

Faridi P[1], Seradj H[2], Mohammadi-Samani S[3], Mohagheghzadeh A[1], Roozbeh J[4]

[1]Department of Traditional Pharmacy; [2]Department of Pharmacognsy; [3]Department of Pharmaceutics, School of Pharmacy; [4] Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Aim of study: Kidney stones are a common disorder of the urinary tract and cause a great deal of morbidity and economic loss. Due to side effects and costs of current treatment, procedures researchers are interested to find medicinal therapies. Iranian traditional medicine recommended Lapis judaicus for preventing and treatment of kidney stones [1, 2]. We have studied efficacy and safety of Lapis judaicus on the size of calcium kidney stones and related blood and urine factors.
Materials and Methods: Sixty kidney stone disease patients were included in a double blind randomized clinical study. Patients received 2 g of *Lapis judaicus* or placebo capsule per day for 10 weeks. Ultrasonography, blood and urine samples were collected before and after the study period to evaluate the efficacy and safety of Lapis judaicus in calcium kidney stone patients (NIH registration number: NCT01443702).
Results: The size of kidney stones reduced significantly ($p<0.001$) in drug group. In 9 patients stone completely dissolved. Urine calcium concentration and specific gravity were reduced and urine magnesium increased ($p<0.05$). Lapis judaicus didn't affect BUN, creatinine, ALT, AST.
Conclusion: Countercurrent to placebo group, the size of kidney stones were reduced significantly in drug group after orally use of Lapis judaicus. A further study involving larger population of patients will be necessary to confirm the evidence seen in the present clinical study.

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L-47

Calafate (*Berberis microphylla*): a super fruit from Patagonia

Mardones C[1], Ruiz A[1], von Baer D[1], Hermosín-Gutierrez I[2], Vergara C[1], Bustamante L[1], Hinrichsen P[3]

[1]Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Concepción, Chile; [2]Instituto regional de Investigación Científica y Aplicada, Universidad Castilla-La Mancha, Ronda de Calatrava 7, 13071, Ciudad Real, Spain; [3]Inia La Platina, Santiago

Calafate (*Berberis microphylla* G. Forst) is a plant from Patagonia which produces a dark blue berry, with a diameter between 7 and 11 mm. Our research has been focused in promoting calafate nutraceutical qualities and also to establish the knowledge basis for its domestication, considering metabolomic profiles, manily of phenolic compounds and genetic diversity of the fruits. By HPLC-DAD-ESI-MS/MS, profiles and concentrations of anthocyanins, flavonols and hydroxycinnamic acids (HCADs) in a large number of samples obtained in two years in different areas of Patagonia were studied. Using HPLC-FL-ESI-MS/MS, the presence of alkaloids in these samples was also evaluated. Besides the antioxidant capacity ($TEAC_{CUPRAC}$ and $TEAC_{ABTS}$) and ascorbic acid contents were studied. Using molecular techniques (AFLP, SSR), genetic diversity of the samples was also studied and all results, including genetic and metabolic profiles, were analyzed using chemometric tools. Differences depending on the origin of the samples were evaluated. The main results show that the calafate fruit was noted for its diversity and HCADs content (1.18-6.28 μmol/g), this being greater than levels described in other fruits. Calafate contain also relevant anthocyanin concentrations (4.7-31.2 μmol/g). The most abundant anthocyanin was delphinidin-3-glucoside, while 5-caffeoylquinic acid was the main HCADs, along with a group of caffeoyl-glucaric derivatives, whose structures were confirmed by NMR. The fruits of calafate contained very low concentrations of berberine (nd-3.26 mg/100g) and interesting levels of flavonols (0.85-1.83 μmol/g), especially certain derivatives of quercetin and isorhamnetin. All these results let qualify calafate as a "super fruit". Chemometric analysis revealed that although differences between samples from Aysén and Magallanes are observed, it is not possible to classify them as different sets. The year of collection does not change the topography of the observed clusters. There is a correlation between the genetic structure of the population and its metabolic profiles, since both agree that this is the same set of samples, except for a subset of samples from Aysén, differentiated both by their phenolic profiles as their genetic data. However, the phenolic profiles support the conclusion that although it is the same set of samples, there differentiators, attributable more to environmental than to genetic factors.

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L-48

Resveratrol: A highly promiscuous molecule leads the way to monogamous interactions

Pezzuto J

Professor and Dean, College of Pharmacy, University of Hawaii at Hilo, Hilo, Hawaii 96720

Cancer chemoprevention entails the ingestion of dietary or pharmaceutical agents that can prevent, delay or reverse the process of carcinogenesis. We have been actively engaged in the systematic discovery and characterization of natural product cancer chemopreventive agents. As part of this project, an extract obtained from a nonedible Peruvian legume, *Cassia quinquangulata* Rich. (Luguminosae), was evaluated and found to be an inhibitor of cyclooxygenase. The active

component was identified as resveratrol (*trans*-3,4',5-trihydroxystilbene). Further tests with resveratrol revealed surprisingly broad spectrum activity indicative of potential to inhibit carcinogenesis at the stages of initiation, promotion and progression. This discovery has led to many additional research efforts; there are now around 5,000 papers concerning some aspect of resveratrol action, and several clinical trials have been or are being conducted. This is rather astonishing since the resveratrol is extremely promiscuous and rapidly metabolized. Following oral administration, serum concentrations are very low. Conversely, using resveratrol as a chemical scaffold, we have produced structural derivatives that demonstrate much greater potency and specificity, as well as more favorable pharmacokinetic properties. Some perspectives on future development will be presented.

L-49

Native berries from southern Chile: from prehispanic food sources to potential nutraceuticals

Schmeda-Hirschmann G

Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile

The western Andean slopes of southern South America are rich in native berries that were gathered by the Native Americans as food source. Some of the most remarkable species include the Chilean strawberries *Fragaria chiloensis*, several currants (*Ribes* spp.) and the Chilean raspberry *Rubus geoides*. The aim of our study was to assess the phenolic content and composition of the Chilean red and white strawberries as well as that of native currants and raspberry fruits. *In vitro* antioxidant activity was measured in methanol and enriched-in-phenolic-extracts (XAD) of the ripe fruits by the ability to scavenge free radicals (DPPH and ABTS) and ferric reducing power (FRAP). Total phenols, total flavonoids and total anthocyanin content were determined. The fruits presented high antioxidant activity, total phenol and total anthocyanin. The polyphenol content in fruits was assessed by HPLC-DAD-MS/MS^n. Flavonoids, tannins and phenyl propanoids were identified or tentatively identified from the Amberlite-retained ripe fruits extracts. The chemical profiles allow a clear differentiation of the wild berries and pointed out to differential nutraceutical properties for the South American species.

Further studies are underway to get a better picture of the chemical composition of South American berries and to disclose its nutraceutical potential.

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L-50

Native fruits from southern South America: Antioxidant capacity and HPLC-PDA-ESI/ToF-MS profiling of phenolic compounds

Simirgiotis M

Laboratorio de Productos Naturales, Departamento de Química, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile

Phenolic compounds are important components of several fruits, vegetables and beverages, contributing to their flavor, color and biological properties, including antioxidant activity. Besides, they are associated with the prevention of diseases thought to be induced by oxidative stress, such as inflammation, cancer and cardiovascular diseases. A number of important technological

advances in high-performance liquid chromatography (HPLC) have achieved significant improvements in both the speed and the separation of polyphenols in the past decade. Recent advances in mass spectrometry, such as high-resolution and sequential collision ion trap-mass spectrometry (HR-MS and MS^n) as well as the use of improved HPLC-MS interfaces including thermospray, plasmaspray, electrospray, etc. allow rapid tentative identification of phenolic compounds in plants, food products and beverages. Furthermore HPLC coupled to mass spectrometry detectors are the most sensitive, selective and reproducible methods for quantifying phenolic compounds. This presentation deals with our applications of liquid chromatography hyphenated with diode array and mass detection (HPLC-PDA- ESI- and ToF-MS) to the study of Chilean food plants with antioxidant properties in the recent years.

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L-51

Semisynthetic derivatives with potential cytostatic, anti-inflamatory and antifeedant activity derived from cucurbitacins isolated from South American medicinal plants

Duran FJ[1], Lang KL[2], Schenkel EP[3], Silva IT[3], Deagosto E[4], Oliveira Simoes CM[3], Rossini C[4], Campos Bernardes LS[3], Palermo JA[1]

[1]UMYMFOR-CONICET, Department of Organic Chemistry, Faculty of Science, University of Buenos Aires, Ciudad Universitaria, Pabellon 2, 1428, Buenos Aires, Argentina; [2]Department of Pharmacy, Federal University of Juiz de Fora (UFJF), Campus Governador Valadares, MG, Brazil; [3]Department of Pharmaceutical Sciences, Federal University of Santa Catarina (UFSC), 88040-900 Florianópolis, SC, Brazil; [4] Chemical Ecology Laboratory, Department of Organic Chemistry, Faculty of Chemistry, University of the Republic, General, Flores 2124,11800 Montevideo, Uruguay

The cucurbitacins are highly oxygenated triterpene derivatives, occurring mostly, but not exclusively in plants of the family Cucurbitaceae.[1] Since cucurbitacins are intensely bitter and some of them are also highly toxic to many organisms including insects and vertebrates,[1] they are generally supposed to act as plant defence substances.[2] Recent works have reported that cucurbitacins and related compounds are active against different tumour cell lines, via suppression of STAT3 (Signal Transducer and Activator of Transcription-3) phosphorylation.[3] STAT3 affects apoptotic processes, and is considered a valid target for novel anticancer drug design.[4] Based on these promising results, a research project was started, aimed at the preparation and biological evaluation of semisynthetic analogues of cucurbitacins, in order to enhance their biological activity. This project combines the efforts of three groups, in a multidisciplinary approach to study how different structural modifications on these highly functionalized scaffolds, may affect the bioactivity. The starting compounds for this study were extracted from two species of Cucurbitaceae, *Wilbrandia ebracteata* Cogn. and *Luffa operculata* Cogn. which are sustainable sources of these substances. In this presentation, the latest results on the semisynthesis of cucurbitacin derivatives will be discussed, especially modifications of cucurbitacin B and dihydrocucurbitacin B. Further results on the evaluation of the biological activities of the natural and semisynthetic compounds will also be presented, such as the in vitro cytotoxic activity against lung cancer cells (A549)[5] and the behaviour against the insect, *Epilachna paenulata* Germar (oligophagous on Cucurbitaceae).[6]

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L-52

Jaboticaba fruits for chronic obstructive pulmonary disease

Wu S-B[1,2], Long C[2], Kennelly EJ[1,2]

[1]Department of Biological Sciences, Lehman College and The Graduate Center, The City University of New York, 250 Bedford Park Boulevard West, Bronx, New York, USA; [2]College of Life and Environmental Sciences, Minzu University of China, 27 Zhong-guan-cun South Avenue, Haidian District, Beijing 100081, China

The fruits of *Myrciaria cauliflora* (Mart.) O. Berg, known commonly as jaboticaba or jabuticaba, are edible, and consumed *in natura*, or in products such as juices or jams. As part of a program to examine polyphenols from edible fruits for antioxidant and anti-inflammatory activity for chronic obstructive pulmonary disease (COPD) treatment, we have identified a number of bioactive compounds from jaboticaba, including a novel depside jaboticabin. Jaboticabin displayed antioxidant activity in a number of assays, as well as anti-inflammatory activity using interleukin-8 and matrix metalloproteinase-1 as markers in small airway epithelial cells exposed to cigarette smoke extract *in vitro*. For *in vivo* assays, we have conducted a complete synthesis of jaboticabin in eight steps, and the synthesized jaboticabin was fed to animals in a smoke-exposed mouse model for COPD. We were able to detect jaboticabin in the murine lungs by LC-MS-ToF, thus demonstrating its bioavailability. We have looked for other sources of jaboticabin including *Myrciaria vexator* McVaugh, known commonly as false jaboticaba, and the wood of *Myrciaria cauliflora*. Employing chemometric tools, we identified jaboticabin from *M. vexator*. However, jaboticabin was not detected in the wood of *M. cauliflora*. We are now reporting the isolation and identification of ellagic acid derivatives from the methanolic extract of jaboticaba wood as well as their antioxidant and anti-inflammatory activity. The fruits of jaboticaba and false jaboticaba are both good sources of polyphenols, including the novel depside jaboticabin. Our laboratory continues to conduct research on *M. cauliflora* and related species to explore polyphenols for the treatment of COPD.

L-53

Endocannabinoid system: an attractive target for new drug development

Pessoa-Mahana D

Facultad De Química, Pontificia Universidad Católica de Chile, Santiago, Chile

Cannabis sativa has been widely used for hundreds of years as a medicine. Δ9-Tetrahydrocannabinol (Δ9-THC), the predominant active ingredient of cannabis, and other cannabinoids are known to have therapeutic benefits for treating pain, seizures, emesis, anxiety, glaucoma, sleep disorders, appetite disorders, cancer, Alzheimer's disease, and epilepsy. Cannabinoids act on G protein-coupled receptors, CB1 and CB2. During the last years, our research group has been interested in the synthesis of benzimidazole derivatives as ligands of the type 1 cannabinoid receptor. In past screening tests using mice brain synaptosomes, compound JM-6 was identified as a high affinity CB1 agonist (Ki = 98.2 nM) [1]. Considering the structural resemblance of JM-6 to the reported ligands, we defined a strategy to develop new and more active CB1 ligand derivatives. Docking simulation studies of JM-6 with our reported CB1 model [2] allowed us to propose the existence of three major interaction regions: (a) a hydrogen bond between the pyridine ring and the quaternary amino group of the side chain of residue K3.28(192); (b) the

naphthalene moiety establishing hydrophobic and aromatic interactions with the side-chain residues F2.57(170), F2.61(174), L3.32(196), I5.31(267), F7.35(379), A7.36(380); and (c) the benzene ring of the benzimidazole core which displayed hydrophobic and aromatic interactions with the side chain residues F2.57(170), F3.36(200), W6.48(356) and L6.51(359) (Figure 1).

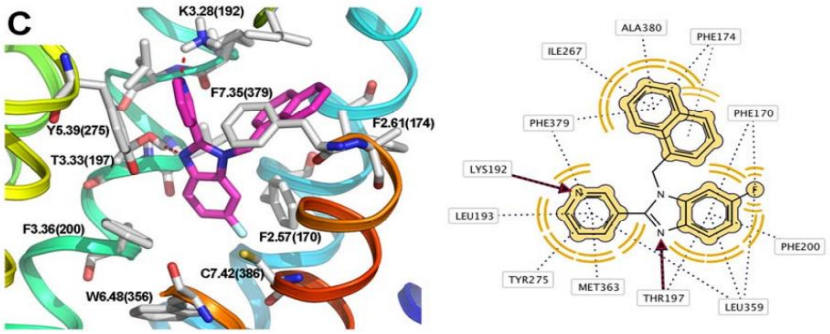

Figure 1. Docking simulation studies of JM-6 with our CB1 model.

Significant affinity (nanomolar range) was observed for 21 of the 47 synthesized compounds, while another eight compounds had affinities in the micromolar range. Three benzimidazoles exhibited IC_{50} values around 20 nM and four molecules exhibited an exceptional affinity with IC_{50} values under 10 nM [3]. A 3D-QSAR model was obtained from CoMFA analysis [3]. This model has high r2 (0.998), F (944.13), and q2 (0.71), as well as a small SEE (0.057), suggesting that it is reliable and predictive. The steric and electrostatic contour maps of CoMFA, which sustained the rational design for new series are displayed in Figure 2. In addition, a new 3D-QSAR model was recently obtained for a series of indole cannabinoid ligands [4].

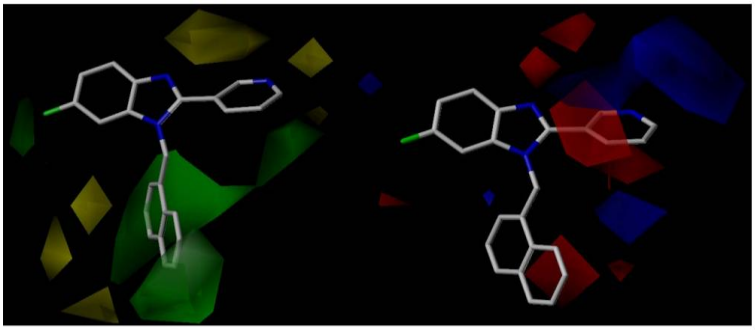

Figure 2. 3D-QSAR model obtained from CoMFA analysis

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L-54

Bactris gasipaes and *Carica papaya* - potent fruits for the prevention of hidden hunger

Hempel J[1], Schweiggert RM[1], Carle R[1,2]

[1]*Institute of Food Science and Biotechnology, Hohenheim University, D-70599 Stuttgart, Germany;*
[2]*Biological Science Department, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

High dietary consumption of carotenoids has been associated with diverse health benefits, and metabolic conversion of specific carotenoids to vitamin A is important for the growth and soundness of cells. The so-called hidden hunger, i.e. a diet chronically low in vitamin A and its precursor carotenoids, rapidly leads to night blindness, increased morbidity, and mortality.
Bactris gasipaes Kunth ("peach palm") fruits are abundant in the Latin America. They have been consumed by the native populations; but nowadays, they are an underutilized crop. Due to their high levels of β-carotene, lycopene, and the rare γ-carotene, predominantly found in the chromoplasts of orange and yellow-colored fruits, peach palm is an excellent source of readily bioavailable carotenoids exerting high provitamin A activity. First proof of their *in vivo* absorption is provided. The lipid-dissolved storage of carotenoids was documented using microscopy. Furthermore, evaluation of micro- and macronutrients revealed the nutritional value of the fruit, which may help to alleviate vitamin A deficiency.
Increased papaya (*Carica papaya* L.) consumption may also contribute to diminish vitamin A deficiency. High levels of provitamin A carotenoids went along with further vitamins and antioxidants. Lycopene only occurred in red-fleshed genotypes. Due to a heterosis effect, all carotenoid contents in hybrid genotypes exceeded those of their inbred lines. In a human study, carotenoids were shown to be easily bioavailable. β-carotene bioavailability was 3 times higher than from carrot and tomatoes, while lycopene was 2.6-fold more bioavailable from papaya than from tomato. In addition, an efficient conversion of provitamin A carotenoids was observed after papaya consumption. This is due to their unusual deposition. While β-carotene was lipid-dissolved forming liquid-crystalline aggregates, solid crystals were found in carrot and tomato, and also lycopene aggregates were smaller in papaya than in tomato.

L-55

Nature-based design of bioactive compounds: the aporphinoids

Cassels BK

Department of Chemistry, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile

The aporphinoids form one of the largest and most diverse families of isoquinoline alkaloids, including the aporphines, nor-, dehydro-, oxo-, pro- and secoaporphines, aristolactams and aristolochic acids, azafluoranthenes, benzoquinolinediones, indenopyridinones, and even the apparently anomalous oxoisoaporphines. Many papers are published every year reporting the isolation and structure elucidation or identification of new or previously known natural aporphinoids, often including the results of in-house bioassays. The biological activities are most varied, from antioxidant through antibacterial, antiparasitic or antiproliferative to cytoprotective, isolated organ studies, cardiovascular, enzyme inhibitory, receptor-dependent and behavioral. Nevertheless, research on the structural modification of natural products or the *de novo* synthesis of analogs pursuing medicinal chemical goals such as improved potency or selectivity, or a deeper understanding of structure-activity relationships and pharmacological mechanisms, remains rare.

The latter aspects have focused almost exclusively on:

a) the aporphine skeleton itself, usually isolated from nature or obtained by rearrangement of

natural or modified opioids, with the aim to develop structure-activity relationships primarily in the monoaminergic field;

b) the secoaporphine (1-aminoethylphenanthrene) scaffold, obtained from natural or modified aporphines or by total synthesis, often examining the compounds' antiproliferative activities;
c) the naturally rare oxoisoaporphine (1-azabenzanthrone) system, synthesized from simple starting materials and studied in the context of its cytotoxicity but also of its monoamine oxidase inhibitory properties.

This presentation will review classical work in the field, discussing its contribution to medicinal chemistry and pharmacology, and address more recent advances in these areas, particularly from the author's laboratory, and their future prospects.

L-56

Bioactives from side streams of fruit processing

Winterhalter P

Institute of Food Chemistry, Technische Universitaet Braunschweig, Schleinitzstrasse 20, 38106 Braunschweig, Germany

Fruit processing and winemaking generate a number of side streams which can be used for the recovery of bioactive compounds, such as anthocyanins, procyanidins and stilbenes. Due to reported beneficial physiological effects of these compounds, they are highly esteemed for the production of neutraceuticals/food supplements – a market within the consumer health industry that is steadily growing. A combination of membrane technology (membrane adsorber Sartobind S) with countercurrent chromatography (CCC) has been applied to the fractionation of crude natural extracts obtained from fruit pomace (bilberry, blackberry, aronia). In a first step, membrane technology yields a pure anthocyanin fraction free of other copigments and polymeric phenols [1]. In the second step, the bioactives in the remaining copigment fraction are separated by preparative countercurrent chromatography [2]. CCC is a support-free liquid-liquid chromatographic technique which allows gentle separations in the g to kg scale. Techniques that will be presented include *inter alia* high-speed countercurrent chromatography (HSCCC) and spiral-coil low speed rotary countercurrent chromatography (spiral-coil LSRCCC). With regards to polymeric constituents present in fruit pomace - especially the procyanidines - a depolymerization process converts high-molecular compounds into bioavailable ones (dimers and trimers) [3]. Finally bioactivity-guided isolation of bioactive oligomeric stilbenes from grape vine shoots for will be presented [4].

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L-57

Synergy?

Verpoorte R, Kim HK, Choi YH

Editor-in-chief Journal of Ethnopharmacology, Natural Products Laboratory, Institute of Biology Leiden, Leiden University, PO Box 9505, 2300RA Leiden, The Netherlands

In the discussion on medicinal plants often synergy is used as an argument to explain activity, and even to argue that they superior to single pure compounds. But how much real evidence is there for synergy? Synergy in simple words means that 1+1>2. To proof synergy between two compounds for a certain biological activity isobolograms are used in which one can see that the activity of the mixtures of two compounds is higher than the sum of the two. Most papers on synergy describe this method to proof synergy of two compounds, however, this requires that one knows the active compound(s) of a medicinal plant. If these are not known it becomes difficult, particularly when in bioassay guided fractionation the activity is lost, e.g. when activity is fully dependent on the presence of two or more compounds. The only solution is a systems biology approach. By measuring the metabolic profile of different extracts of a medicinal plant or fractions thereof and combining that information with the results of the bioassays of these samples one may identify the signals that correlate with activity. These signals may be due to one or more compounds. After identification of these compounds, e.g. after isolation via metabolomics guided fractionation, one can test these compounds for synergy.
If synergy would play an important role in medicinal plants, the synergistic effect is on the system as a whole, and thus may have many forms. That means for studies on synergism one should use preferably in-vivo bioassays, and if possible even apply this approach in clinical trials, as besides synergy also prodrugs may be present in medicinal plants.

L-58

Discussion on the Origin, Development, Function and Practical Significance of Tea Culture

Peng Y, Xu L, He C, Xiao W, Xiao P

Institute of Medicinal Plant Development, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing 100193; China

Chinese tea culture was developed rapidly in Tang Dynasty, the population is closely linked with the economic, political and culture. Moreover, the tea contains the caffeine which can adjust the spirit, while those non- Camellia teas do not contain caffeine. Thus, the popularization of the tea has become a thriving situation. This article further described the formation process of the tea culture. Since ancient times, the plants used as a 'tea' present the diversity. And those teas for disease prevention are not single one. However non-Camellia tea has been still used by different folks in China. It is significant to study and develop non-camellia tea, for promoting Chinese traditional culture, developing agrarian economy and preventing chronic disease. Besides the elaboration of tea drink definition and classification, the paper has proposed the future perspective, and also suggested that considering the study and development of tea drink as an important issue in future human health plans.

L-59

Design, synthesis and anti-tumor evaluation of new bufalin analogs

Hu L, Guo D, Liu X

Shanghai Institute of Materia Medica, Chinese Academy of Sciences,Shanghai 201203, China

Epidemiological data as well as in vitro and in vivo studies indicated that cardiac glycosides had anti-cancer activities and also emphasized the possibility of developing this class of compound as anti-cancer agents. Promising compounds such as Anvirzel and UNBS1450 are now in clinical trials in USA and Belgium, respectively. In China, CHANSU, a traditional medicine containing bufadienolides, has long been used for treatment of cancer. Presently, preparations such as Huachansu Injection are still popularly used in clinic. The active components of Huachansu Injection were bufadienolides including bufalin, cinobufagin, resibufogenin and etc. Among the bufadienolides, bufalin was showed as the most active one. However, its narrow therapeutic window of limits its therapeutic application. The LD_{50} of bufalin in mice (i.p.) is only 2.2 mg/kg.
Based on the recent understanding about the structure-activity relationship of cardiac glucosides and their anti-cancer activity as well as cardiotonic activity, we designed and synthesized series of bufalin derivatives. By evaluating their anti-cancer activities and toxicity, we have successfully found a bufalin derivative, BF211·HCl, as a promising candidate for anti-cancer therapy. Compared with bufalin, BF211·HCl exhibited better water-solubility, stronger in vitro and in vivo anti-tumor activity and lower toxicity (ZL201210017717.8, CN201210285301.4, PCT201210017717.8, US13508827, Steroids, 2013, 78: 508-512.).

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KEYNOTE LECTURES

K-1

In vitro inhibition of TNF-α release induced by Brazilian medicinal plants

Henriques BO, Correa O, Castilho RO, Braga FC

Faculdade de Farmácia, Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil

TNF-α plays a pivotal role in chronic inflammatory diseases like rheumatoid arthritis and ulcerative colitis. Several TNF-α inhibitors have been proven to be clinically effective to treat such diseases, but their use has some restrictions. Therefore, there is demand for new TNF-α inhibitors that can be further developed as anti-inflammatory drugs. The aim of this study was to evaluate the anti-TNF-α effect of eight Brazilian plants, selected by ethnopharmacological or chemosystematic approach. The extracts were prepared by percolation of the dry plant materials with EtOH 96%, following solvent elimination under reduced pressure. The effect of the extracts (62.5, 125 and 250 µg/mL) on TNF-α release by LPS-stimulated THP-1 cells was determined by ELISA, whereas cell viability was evaluated by the MTT assay [1]. All extracts showed cell viability higher than 90% at the concentration of 250 µg/mL. The extracts of *Stryphnodendron obovatum* (stem bark) and *Vernonia phosphorea* (leaves) elicited a significant concentration-dependent inhibition of TNF-α release (54.2 ± 3.0% and 96.2 ± 1.1% at the highest concentration, respectively), in comparison to non-treated LPS-stimulated cells. The extracts of *Mikania glomerata* (leaves), *Bowdichia virgilioides* (leaves) and *Terminalia glabrescens* (stem) fail to inhibit TNF-α release, whereas *Vitex polygala* (leaves), *Campomanesia lineatifolia* (stem) and *Licania tomentosa* (leaves) increased the cytokine production. Chromatographic analysis of the active extracts by TLC on silica gel and RP-HPLC indicated the predominance of polar compounds, mainly polyphenols, which may contribute to the observed activity.

Acknowledgements: This work received financial support from CNPq and FAPEMIG. CAPES is also acknowledged for a PhD fellowship (B.O.H.)
Reference: [1] Weiss T. et al. (2004) Antimicrob. Agents Ch. 48:1974-1982.

K-3

Chemical valorization of indigenous pharmacopoeia in Bolivia

Bravo J

Institute of Natural Products Research IIPN, Department of Chemical Sciences, Pure and Natural Sciences School, Universidad Mayor de San Andrés UMSA, Calle Andrés Bello y Calle 27, Cota Cota, P.O. Box 303, La Paz, Bolivia

Bolivian indigenous populations possess ancient medicinal traditions, derived from a profound knowledge of the use of plants. Chemical valorisation of these traditions started in the middle od 1980's. Bolivian and French researchers used an ethno-pharmacological approach to study the traditional medicine of Bolivian eastern and western indigenous ethnic groups. The results obtained found important applications of traditional medicine on parasitic sicknesses including malaria, leishmaniasis, and Chagas' disease. A review of the most important discoveries in this research implies a description of the biological effectiveness of the active plant extracts, characterisation of the isolated molecules, as well as their activity indexes. The ethnic groups studied in these research efforts include the Chacobo, Raqaypampeno, Tacana, Chiman, and Aymaro-Quechua. [1-4]

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K-4

Antioxidant activities of Bolivian plants

Mollinedo P

Institute of Natural Products Research IIPN, Department of Chemical Sciences, Pure and Natural Sciences School, Universidad Mayor de San Andrés UMSA, Calle Andrés Bello y Calle 27, Cota Cota, P.O. Box 303, La Paz, Bolivia

Certain climatologically-adverse conditions in the basin of the Poopo lake, located on the high plateau of the Bolivian Altiplano, have prevented the existence of a reliable agricultural system and the impact on the population of the area has been severe. These problems have been the main motivation in choosing this region for this particular study. However, many species of plants have somehow managed to survive and adapt to this environment and they have developed the capacity to synthesize secondary metabolites that have not only helped them to survive, but also to counteract the oxidative stress. Some 35 plant species and 5 lichen species were collected in the region, in order to evaluate their antioxidant activity. 28 compounds with anti-oxidant capacity have been identified from extracts of these species. All of the crude extracts, chromatographic fractions and pure compounds were studied using 4 assays (ABTS, DPPH, TBARS and β-carotene). Two plant species that have presented the greatest activity have been selected for certain in-depth studies. From *Rheedia acumminata* four compounds were isolated as potent antioxidant compounds, three of them (named acuminoxanthone, acuminophenone A and acuminophenone B) have not been reported previously. From *Adesmia spinosisima*, taxifolin, fustin and a phenyl derivate were isolated from the extract with high antioxidant activity, and it is possible that the compounds give a synergistic effect. Depsides and benzofurane compounds isolated from five lichen species were evaluated with the same antioxidant assays. All of the compounds showed high antioxidant capacity, compared with Trolox. Perlatolic acid is the best antioxidant compound among the lichen molecules. The TEAC antioxidant activity was correlated with various parameters in order to study QSARs. The analysis pointed out that the most important parameter involved in the antioxidant activity, as a free radical scavenger, is the energy of the highest occupied molecular orbital. A mathematic function was determined to predict the antioxidant activity for similar compounds. The great applicability of antioxidant products make these plants a potentially useful resource that could help to support the sustainable development of the region. The harvesting of these plants does not require the change of the current climatologic environment, mostly because these species have adapted so well to the region [1-4].

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K-5

Combined antitumor effect of bee venom and cisplatin on human glioblastoma A1235 cells

Gajski G[1], Čimbora-Zovko T[2], Rak S[2], Rožman M[3], Osmak M[2], Garaj-Vrhovac V[1]

[1]Mutageneis Unit, Institute for Medical Research and Occupational Health, 10000 Zagreb, Croatia; [2]Laboratory for Genotoxic Agents, Division of Molecular Biology, Ruđer Bošković Institute, 10000 Zagreb, Croatia; [3]Laboratory for Chemical Kinetics and Atmospheric Chemistry, Division of Physical Chemistry, Ruđer Bošković Institute, 10000 Zagreb, Croatia

Cisplatin is one of the most widely used anticancer-drugs. However, cisplatin-resistance is the major obstacle for successful treatment of cancer patients. We investigated possible combined anticancer ability of bee venom (BV) that tops the list of the many natural compounds which have been newly introduced as anticancer agent, and cisplatin towards human glioblastoma A1235 cells. Further, we identified several peptides in the BV sample used in the course of our study and determined the exact concentration of the melittin (MEL) by mass spectrometry. Peptides MEL, apamin, MCD peptide and tertiapin were identified as components of BV and are in agreement with previous findings regarding peptide components of BV. Additionally, the concentration of MEL in BV was determined and its mass fraction was estimated at 0.19. BV applied alone in concentrations of 2.5 to 30 μg/mL displayed dose-dependent cytotoxicity, as evaluated with MTT assay, against A1235 cells with IC_{50} value of 22.57 μg/mL. Treatment with BV alone induced a necrotic type of cell death, as shown by characteristic morphological features and fast staining with ethidium-bromide. Combined treatment of BV and cisplatin induced cell sensitization, suggesting that BV could enhance the killing effect of selected cells when combined with cisplatin. The isobologram method used to determine the extent of synergism on combining two agents for their possible therapeutic effect showed that combined treatment induced an additive and/or synergistic effect towards cells depending on the concentration of both. Therefore, our results suggest that a greater anticancer effect could be triggered if BV was used in the course of chemotherapy. Results also suggest that combined treatment with BV could be useful from the point of minimizing the cisplatin concentration during chemotherapy, consequently reducing and/or postponing the development of cisplatin resistance, making BV a perfect candidate as a future anticancer modality.

K-6

Leishmaniasis and Peruvian medicinal plants

Cabanillas B[1], Le lamer A-C[2], Fabre N[2], Sauvain M[2], Bourdy G[2]

[1]Instituto de Investigaciones de la Amazonia Peruana (IIAP), Av. Abelardo Quiñones Km 2.5, apartado postal 784, Iquitos, Perú; [2]Université de Toulouse III, PHARMA-DEV, UMR 152, 118 Route de Narbonne, 31062 Toulouse Cedex 9, France

La Leishmaniasis, enfermedad causada por el parasito *Leishmania*, ha sido catalogada por la Organización Mundial de la Salud (OMS) como un problema de salud pública mayor [1]. La enfermedad bajos sus tres formas clínicas está presente en Europa, América, África y la India. Los tratamientos actuales basados en sales antimoniales, la amphotericina B y la miltefosina han mostrado ser en muchos casos tóxicos, costosos y con una creciente resistencia en pacientes. La medicina tradicional se presenta como una herramienta importante en el descubrimiento de nuevas drogas. La información recogida acerca de las plantas medicinales empleadas por dos comunidades amazónicas, en el tratamiento de leishmaniasis y de otras enfermedades de naturaleza infecciosa o inflamatoria, permitió identificar plantas con buena actividad sobre amastigotes axénicos de *L. amazonensis* (CI_{50} < 20 μg/mL) [2,3]. Como parte de este trabajo se realizaron el aislamiento bioguiado de los compuestos con actividad leishmanicida de las especies

Piper dennisii, *Piper sanguinesipicum* y *Renealmia thyrsoidea*, lográndose identificar 37 compuestos entre: derivados del ácido cafeico, derivados del ácido benzoico, lignanos, flavonoides, sesquiterpenos y diarilhepatanoidos. Los compuestos más activos sobre los amastigotes fueron probados en el modelo de macrófago infectado para medir la efectividad de los compuestos en un ambiente más similar al de un organismo vivo.

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K-8

4-nitrobenzaldehyde thiosemicarbazone derived from S-limonene causes ultrastructural changes on *Leishmania amazonensis* cells

Scariot DB[1], Britta EA[1], Silva CC[2], Falzirolli, H[2], Ueda-Nakamura T[1], Nakamura CV[1]

[1]Departamento de Ciências Básicas da Saúde, Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil; [2]Departamento de Química, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil

4-nitro benzaldehyde thiosemicarbazone (BZTS) is a synthetic compound whose antileishmanial activity was previously determined for promastigote and axenic amastigote forms of the parasite. The aim of this study was to evaluate the activity of BZTS on intracellular amastigote forms of *Leishmania amazonensis* and the main ultrastructural cell changes elicited by the compound. Intraperitoneal macrophages were collected from BALB/c mice, cultivated on glass coverslips, infected with promastigote forms, treated with different concentrations of BZTS, incubated at 34 °C, 5% CO2 and stained with Giemsa. IC_{50} obtained for intracellular amastigote forms was 8.8 µM. Axenic amastigote forms were cultivated and treated with BZTS (IC_{50} and IC_{90}, respectively, 7.0 and 27.7 µM), fixed on glutaraldehyde, post-fixed on OsO4, dehydrated in acetone and embedded in Epon for analysis in TEM. The images showed extensive swelling and disorganization in inner mitochondrial membrane beyond strongly presence of lipid droplets in the cytoplasm. Confirming that, promastigotes were exposure to different concentrations of BZTS, for 24 h and incubated with Rhodamine 123 to evaluate mitochondria membrane potential through flow cytometer. The results showed Rh 123 fluorescence intensity decrease, evidencing mitochondria membrane depolarization. Furthermore, parasites were treated with IC_{50} and IC_{90}, washed in PBS and directly stained with Nile Red, and observed on epifluorescence microscope. Several lipid bodies have been observed, which could indicate alteration of phospholipid and sterol content [1]. Finally, the results permit to conclude that BZTS compound is responsible to cause strong mitochondrial damage, which is an important and peculiar target on *L. amazonensis* [2].

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K-9

Hypoglycemic action of *Bauhinia holophylla* (Steud.) through glycogenesis stimulation and gluconeogenesis inhibition in streptozotocin-diabetic mice

Camaforte NAP[1], Vareda PMP[1], Saldanha LL[2], Dokkedal AL[2], Bosqueiro JR[3]

[1]Institute of Biosciences, Botucatu-SP; [2]Department of Biological Sciences; [3]Department of Physical Education, Bauru-SP, São Paulo State University, UNESP, Brazil

Diabetes mellitus is a chronic disease characterized by hyperglycaemia resulting from defects on insulin action, secretion or both [1]. The genus *Bauhinia* has many species used in the treatment of diseases like inflammation, infection and diabetes [2]. Previous studies in our laboratory showed that the treatment with *Bauhinia holophylla* crude extract has hypoglycaemic activity, decreasing fasting glycaemia and increasing hepatic glycogen content. The aim of this study was to verify the mechanism of action of *B. holophylla* in streptozotocin-diabetic mice. Male Swiss mice (90-days old, 40g) were divided in 4 groups: CTLSAL (normal mice treated with saline), CTLEXT (normal mice treated with *B. holophylla*, 14 days, 400 mg/kg.day), STZSAL (diabetic mice treated with saline) and STZEXT (diabetic mice treated with *B. holophylla*, 14 days, 400 mg/kg.day). The treatment with crude extract of *B. holophylla* showed that hypoglycaemic activity occurs through increase of GSk3b expression, involved in glycogenesis, and inhibition of G6Pase and PEPCK, important enzymes of gluconeogenesis.

Acknowledgements: FAPESP.
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K-10

Toxicological evaluation of aqueous extract of *Aloe ferox* Mill. in loperamide-induced constipated rats

Wintola OA, Sunmonu TO, Afolayan AJ

Medicinal Plant and Economic Development Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa

Aloe ferox Mill. is a widely used medicinal plant in South Africa for the treatment of many ailments including constipation [1]. Herbal remedies are commonly employed in developing countries for the treatment of various diseases including constipation. The rationale for utilization of medicinal plants rested largely on the belief that they are safe and free of side effects [2].The present study evaluated the toxicological effect of aqueous leaf extract of the herb at 50, 100 and 200 mg/kg body weight for 7 days on the haematological parameters as well as liver and kidney function indices in loperamide–induced constipated rats. The extract did not cause any significant ($p > 0.05$) effect on the kidney and liver–body weight ratio as well as the kidney function indices including serum levels of creatinine, uric acid, urea, calcium and potassium ions at all the dosages investigated. Whereas the serum levels of total protein, albumin, bilirubin and gamma glutamyl transferase (GGT) were not affected, the elevated activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in the untreated constipated animals were normalized following treatment with extract. The data obtained with respect to the haematological analysis indicated that the extracts had no significant ($p > 0.05$) effect on the haematological parameters with the exception of lymphocyte count which was increased in the untreated constipated rats. This was however attenuated after administering the herb. The available evidence in this study suggests that *A. ferox* may be safe as an oral remedy for constipation. Generally, the effect of the extract

compared favourably well with senokot, a recommended drug for the treatment of constipation.
Acknowledgments: This research was supported by grants from National Research Funds (NRF) and Govan Mbeki Research and Development Centre (GMRDC), University of Fort Hare, South Africa.

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K-11

Genotoxicity of *Acacia seyal* (Mimosaceae) wood smoke

Elgorashi E[1], Eldeen I[2], Makhafola T[3], Eloff J[3], Verschaeve L[4]

[1]Toxicology Section,ARC- Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 110, South Africa; [2]Faculty of Forestry,University of Khartoum, Shambat 13314, Sudan; [3]Phytomedicine Programme, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 110, South Africa; [4]Laboratory of Toxicology, Scientific Institute of Public Health, B-1050, Brussels, Belgium

Acacia seyal Del. is a thorny tree that grows in many parts of Africa especially north of the equator. It also occurs in east and southern Africa [1]. The tree is used in African traditional medicine to treat various ailments. Exposure to the smoke of the wood of the tree is believed to relieve rheumatic pain, smooth skin, heal wounds and achieve general body relaxation [2]. The aim of this study was to investigate the potential genotoxic harmful effects that may arise from the long term exposure to the smoke of the tree using the *in vitro* Ames, micronucleus/cytome and comet assays. All condensed smoke concentrations tested in Ames test induced mutation in both TA98 and TA100. The condensed smoke also significantly increased the frequency of micronuclei, nucleoplasmic bridges and buds formation in a dose dependent manner when evaluated using the micronucleus/cytome assay. Moreover, significantDNA damage, expressed as % DNA and tail length, was observed in the comet assay. In conclusion, the smoke bath from wood of *A. seyal* should be used with caution as it may induce gene mutation, chromosomal aberration and DNA damage.

Acknowledgements: The study was supported financially by the National Research Foundation (NRF), South Africa.
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K-12

Antioxidant activity and characterization of constituents in copao fruits (*Eulychnia acida* Phil., Cactaceae) by HPLC–DAD–MS/MSn

Jimenez-Aspee F[1], Quispe C[1], Soriano MDPC[1], Fuentes Gonzalez J[1], Hüneke E[1], Theoduloz C[2], Schmeda-Hirschmann G[1]
[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, 3460000 Talca, Chile; [2]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, 3460000 Talca, Chile

Copao (Eulychnia acida Phil., Cactaceae) is an endemic species occurring in arid areas of northern Chile. The fruits are commercialized by peasants within the Elqui and Limari valleys and areappreciated for its acidic and refreshing taste. We now report the total phenolic (TP) and total flavonoid (TF) content, antioxidant activity, phenolic composition and main phenolic distribution in

pulp and epicarp of copao fruits from different harvesting places from both valleys. The ascorbic acid content was determined in fresh fruit pulp, epicarp and juice. The phenolic-enriched extract was analyzed for antioxidant effect and composition. Ferulic acid, 9,10-dihydroxy-4,7-megastigmadien-3-one hexoside, isorhamnetin and quercetin glycosides were identified by HPLC-DAD-MS/MS analysis.The main compounds were isolated and fully characterized by NMR techniques. The main phenolic in the samples was isorhamnetin-3-O-[α-rhamnopyranosyl-(1→6)-β-glucopyranoside]. The HPLC pattern of the phenolic-enriched extracts of the fruits allows a differentiation of samples from the Elqui and Limari valleys. All fruit extracts and Amberlite-retained fraction from the methanolic extract were devoid of toxicity against human gastric AGS cells and human lung fibroblasts, with IC50 values >400 µg/mL for AGS and 344 to >400 µg/mL for fibroblasts, respectively. The compounds identification, associated with the antioxidant activity and insignificant cell toxicity, add relevant information for the possible development of this native fruit into a new crop.

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K-13

Variability in the resin composition of the Cupressaceae *Austrocedrus chilensis*

Olate VR[1], Soto A[2], Schmeda-Hirschmann G[1]

[1]Instituto de Química de Recursos Naturales, Laboratorio de Química de Productos Naturales, Universidad de Talca, Casilla 747, Talca, postal code 3460000, Chile; [2]Instituto de Matemática y Física, Universidad de Talca, Casilla 747, Talca, postal code 3460000, Chile

The chemical composition of resins from South American native gymnosperms is an interesting but poorly explored field. The investigations in this area include members of the families Cupressaceae, Araucariaceae and Podocarpaceae, among others [1, 2]. One of the most important native species in the southern part of our continent is the Cupressaceae *Austrocedrus chilensis.* It is a dioecious species covering a wide area in both sides of the Cordillera de los Andes, in Chile and Argentina, and it is locally known as “ciprés de cordillera”. The chemical composition of the resin of this species has been described as a mixture of labdane, abietane and isopimarane diterpenes [3]. When resins of female and male trees were separately analyzed, some differences in its profiles were detected. The aim of the study was to evaluate a possible variability in resin profiles of *A. chilensis*. Single resin drops from different trees were collected in different seasons of the year (spring, summer and winter), including samples from male and female individuals. Resins were analyzed by GC-MS and ^{1}H-NMR techniques. The results were compared by multivariate statistical analysis [4]. The resin profiles showed differences in its composition according to the season of the year. This variability was verified by statistical means. The results also suggested a variation in the relative composition of the resin diterpenes according to the sex of the trees.

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POSTERS

P-1

Molecular mechanisms that underlie the sexual stimulant actions of *Avicennia marina* and *Crocus sativus*

Alharbi AJ[1], Alhowiriny TA[1], El Tahir KEH[2], Al-Taweel AM[1], Perveen S[1]

[1]Departments of Pharmacognosy and [2]Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

The effects of extracts and sub-fractions of *Avicennia marina*, *Crocus sativus* and sildenafil on the sexual behavior of male rats and their effects on the intracavernosal pressure (I.CV), intracavernosal cyclic GMP and dihydrotestosterone plasma level were examined. The sexual behavior was followed for four hours using infra-red video cameras to quantify the effects on various male sexual behaviours. The results revealed that the active sub-fraction in case of *A. marina* was the hexane fraction of the chloroform extracts (C/H) whereas that of *C. sativus* was the hexane fraction of the alcoholic extract (A/H). (C/H), (A/H) and sildenafil significantly increased the total sexual stimulation index from 53.8 ± 2.7 (control) to 406 ± 7.8, 225 ± 4 and 401 ± 30.1, respectively ($P < 0.001$, $N = 6$). They significantly increased the index of successful mounting and ejaculation from 2.6 ± 0.5 (control) to 40 ± 2.7, 21 ± 2.3 and 18 ± 1.7, respectively ($P < 0.01$, $N = 6$). They significantly increased the cyclic GMP level from 0.94 ± 0.07 (control) to 3.1 ± 0.13, 1.59 ± 0.11 and 3.66 ± 0.19 ng/mg wet tissue, respectively ($P < 0.05$, $N = 7$). They did not affect dihydrotestosterone plasma level. (C/H), (A/H) and sildenafil increased the (I.CV) pressure by 4.8 ± 0.3, 1.4 ± 0.8 and 4.2 ± 0.9 mmHg. The (C/H) seemed to be more active than sildenafil and twice active than (A/H). Both extracts and sildenafil acted via an increase in cyclic GMP.

P-2

Quality Control of Seeds of guarana by pharmacopoeia methods and chemical profile analysis by HPLC-DAD

Aguiar BAA, Fernandes FR, Panizzon GP, Marques LLM, Longhini R, Mello JCP

Universidade Estadual de Maringá, Avenida Colombo 5790, BR- 87020-900, Maringá, Brazil

Paullinia cupana var. *sorbilis* (Mart.) Ducke is a native Amazon plant found in Brazil, popularly known as guaraná. Its seeds are widely used in pharmaceuticals and foods. A wide range its pharmacological actions have been described from the use of the crude extract and semipurified fractions of guaraná [1,2]. The potential for the use of guaraná justifies the interest in the quality control and standardization of its preparations for the reproducibility and reliability of studies chemical and biological. The aim of the present study was to evaluate the quality of two lots of seeds of guaraná. Seeds were tested in regard to physicochemical aspects: extractives content (EC), determination of total ash content (AC), loss on drying (LD), methylxanthine contents (MX) and total polyphenols (TP), and chemical profile analysis by HPLC-DAD [3]. The results of physicochemical analyzes for lots 1 and 2 were: 32,75% and 29,71% for EC; 1,87% and 1,42% for AC; 8,84% and 9,94% for LD; 6,37% and 5,13% for MX, and 9,62% and 9,25% TP, respectively. The analysis by HPLC-DAD showed similarity in the chemical profile for two lots. Comparison of sample UV spectra with standards profile indicated the presence of procyanidin B1 signal with higher intensity in lot 2. Characteristic peaks of polyphenols and methylxanthines, such as catechin, epicatechin, procyanidin B2, and caffeine were observed in the two lots.

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P-3

Determination of gallocatechin and epigallocatechin by high performance liquid chromatography of the roots of *Limonium brasiliense*

Blainski A[1], Antonelli-Ushirobira TM[1], Isolani RG[1],Gaburo F[1], Lopes GC[1], Aguiar BAA[1], Leite-Mello EVS[2], Mello JCP[1]

[1]Departamento de Farmácia, Programa de Pós Graduação em Ciências Farmacêuticas;
[2]Departamento de Ciências Morfológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, BR-87020-900, Maringá, PR, Brazil

Limonium brasiliense L. is a common plant on the southern coast of Brazil. The roots are traditionally used for treatment of premenstrual syndrome, menstrual disturbances and genito-urinary infections [1]. Pharmaceutical preparations used for these purposes were marketed in Brazil in the 1980s and 1990s, but their use was discontinued because of a lack of scientific studies. The aim of this study was develop and validate an analytical method to determine the chromatographic profile and the content of gallocatechin (GC) and epigallocatechin (EPG) in an ethyl-acetate fraction by HPLC*. L. brasiliense* roots were extracted in acetone: water (7:3, v/v; 10% w/v). The method was validated according to national and international guidelines, and showed suitable results, besides a characteristic profile for this sample [2, 3]. The calibration equation was y=1.4.108x+1.2.107(n=5, r^2=0.9980) for GC and y=1.4.108x+1.0.107(n=5, r^2=0.9950) for EPG. The method was specific when the sample was submitted in acid, base, and oxidative conditions. The repeatability and intermediate precision for sample were, respectively, 2.71% ± 0.086 [3.18%] and 2.80% ± 0.046 [1.49%] for GC, and 2.18% ± 0.126 [5.77%] and 2.27% ± 0.085 [3.74%] for EPG. The accuracy test showed a percentage recovery 103.5, 102.4, and 100.9% for GC, and 99.8, 100.2, and 101.2% for EPG, respectively, for the lowest, intermediate, and highest levels. There is robustness with small variation of flux and temperature oven in chromatographic conditions, but the sample needs careful with exposition of temperature and light.

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P-4

Variation in levels of coumarin and chlorogenic acid of two species of guaco (*Mikania glomerata* and *Mikania laevigata*) grown under different intensities of light

Almeida CL, Sawaya ACHF

Instituto de Biologia, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz, Rua Monteiro Lobato, 255 - Campinas - SP - Brasil

Mikania glomerata Sprenguel and *Mikania laevigata* Schultz Bip. ex Baker, both popularly called *guaco*, are used in Brazilian traditional medicine for respiratory diseases. [1, 2]. Syrup based on *Mikania glomerata* is presently furnished by the Brazilian Health System (SUS) as medicine. Its properties are due to the presence of coumarin (chemical marker) and other compounds [2, 3]. Both species are found in the 1st Brazilian Phytotherapic Formulary and apparently may be used indiscriminately. Variations in the cultivation conditions of medicinal plants may result in different concentrations of active components [4, 5], affecting the safety, quality and efficiency expected of

herbal medicines. We evaluated how coumarin and chlorogenic acid content was influenced by different intensities of light: full sunlight, 25% shade and 50% shade levels. After three weeks of treatment, the hydro alcoholic extracts of the leaves were evaluated by liquid chromatography mass spectrometry (UPLC-MS) and compared by ANOVA. The results indicate no presence of coumarin in *M. glomerata*. Moreover, *M glomerata* has large quantities of chlorogenic acid, which significantly changed ($p < 0.05$) with the variation of the radiation (higher in full sunlight). This variation was also found in *M. laevigata* ($p < 0.05$). Although coumarin was predominant in *M. laevigada*, this compound's levels did not change significantly with variation of the radiation. We conclude that plants grown in full light show an increase in the levels of chlorogenic acid, but the same does not occur for coumarin. Furthermore, the two species must not be used indiscriminately since they have different phytochemical profiles.

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P-5

Hydroethanolic stem-bark extract from *Cedrela odorata* (HeECo) decreases serum triglycerides and VDL-c in diabetic streptozotocin (STZ) rats

Giordani MA, Andrade CMB, Almeida FJS, Souza DLP, Mezaroba DF, Kawashita NH

Universidade Federal de Mato Grosso. Avenida Fernando Corrêa da Costa, nº 2367, CEP: 78060-900, Bairro Boa Esperança. Cuiabá - Mato Grosso - Brazil

C. odorata, popularly known as cedro-rosa, is used by people from several regions in Brazil in the treatment and control of Diabetes. Diabetes is a pathology that causes alterations in the metabolism of carbohydrates, proteins and lipids and consequently causes alterations in the biochemical parameters in the blood. Thus, our objective in this work was to evaluate the effect of the HeECo on serum glucose, urea, lipids and lipoprotein concentration in the blood. Male *Wistar* rats weighing about 190 g were used. Non diabetic rats (N) received vehicle – citrate buffer 0,01M, pH 4.5 iv; and the diabetic rats (D) received streptozotocin 40 mg/Kg iv. After five days of diabetes induction, the diabetic rats with postprandial glycemia between 300-450mg/Kg b.w were divided in four groups (6 rats/group): diabetic control (DC), diabetic treated with 250 and 500 mg of EHE*Co* (DT250 e DT500, respectively) and diabetic treated with 500 mg of metformin (DM). After 21 days of treatment, the rats were sacrificed and blood samples were collected for analysis. The diabetic rats in our experimental model showed the values of lipid parameters similar to non-diabetic rats. However, the administration of the HeECo to diabetic rats reduced the levels of triglycerides (N= 132.0 ± 15.1, DC= 122.8 ± 15.3, DT250 = 73.6 ± 6.2, DT500 = 77.8 ± 8.1, DM= 142.3 ± 10.4 mg/dL) and VLDL-c (N= 26.4 ± 3.0, DC= 24.6 ± 3.1, DT250= 14.7 ± 1.2, DT500= 15.6 ± 1.6, DM= 28.5 ± 2.1) in the both doses. Although the subchronic administration of HeECo has not showed a hypoglycemic effect, the results showed that it had an important hypolipidemic effect in diabetic rats that deserves further investigation. These effects may be relevant to minimizing the risk factors of cardiovascular diseases, an important problem of public health in the world.

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P-6

Genotype influence in the *in vivo* anti-inflammatory activity of murtilla leaves

Arancibia-Radich J[1], Peña-Cerda M[1], Silva X[2], Delporte C[1]

[1]Laboratorio de Productos Naturales Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Santos Dumont 964, Santiago, Chile; [2]Instituto de Salud Pública de Chile

Ugni molinae (murtilla, Myrtaceae) is a native species whose leaves have been used in Chilean folk medicine to treat inflammation and pain [1]. The aim of this work was to study the leaves of ten murtilla genotypes and study the anti-inflammatory properties comparatively. To carry out this study, serial extracts of leaves of different genotypes murtilla were prepared and evaluated: ethyl acetate extracts (EAE) and ethanol (EET). To evaluate the oral anti-inflammatory activity *in vivo* the model mouse ear edema with phorbol ester (TPA) as the inflammation-inducing agent was used. Differences in the anti-inflammatory effect of the different genotypes were observed, EAE showed the highest anti-inflammatory effects: 36.6% and 32.1 % for the genotypes 23-2 and 19-2, respectively. The EET genotypes with the highest anti-inflammatory effects were 31-1 and 22-1, with 35.5 % and 35.3%, respectively. Statistical significance was evaluated using the Kruskal - Wallis test and Dunnett 's multiple test .The triterpenoids identified in their leaves [2] are part of the anti-inflammatory and analgesic active principles [3]. The chemical study shows significant differences between genotypes in terms of the profile of triterpenoids, partly explaining the differences in anti-inflammatory activities.

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P-7

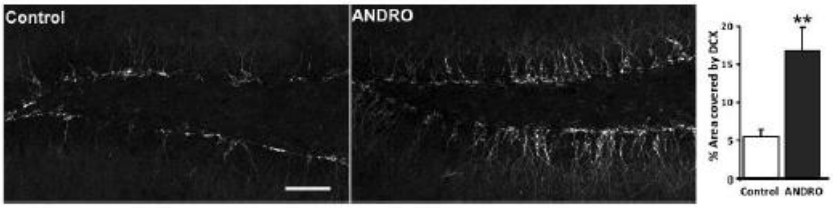
Andrographolide, a component of *Andrographis paniculata*, stimulates neurogenesis in the adult mouse brain

Arredondo SB[1,2], Tapia-Rojas C[1], Inestrosa NC[1], Varela-Nallar L[2]

[1]Centro de Envejecimiento y Regeneración Celular (CARE) Departamento de Biología Celular y Molecular, Facultad de Ciencias biológicas, Pontificia Universidad Católica de Chile, casilla 114-D, Santiago Chile; [2]Centro de Investigaciones Biomédicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello, 8370146, Santiago, Chile

Andrographolide [(3E,4S)-3-[2-[(1R,4aS,5R,6R,8aS)-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]ethylidene]-4-hydroxyoxolan-2-one) (ANDRO) is a labdane diterpenoid widely used for its anti-inflammatory properties. Previously, we determined that ANDRO is a competitive inhibitor of glycogen synthase kinase-3□, a key enzyme of the Wnt/□-catenin signaling cascade, which in turn has been implicated in the generation of new neurons (neurogenesis) in the adult brain. Here, we studied the effect of ANDRO on neurogenesis in the adult mouse hippocampus. In 2-month-old mice injected 3 times a week for 4 weeks with 2 mg/kg ANDRO, there was an increase in cell proliferation and in the density of immature newborn neurons (positive for doublecortin, DCX) in the hippocampus as compared to control mice injected with saline solution (Figure). The effect in proliferation was also observed in cultured adult

hippocampal progenitor cells isolated from adult mouse brain. Moreover, ANDRO increased cell proliferation and the number of immature neurons in the hippocampus of the double transgenic APPswe-PS1E9 mouse, an *in vivo* model of Alzheimer's disease. Our findings indicate that ANDRO stimulates neurogenesis in the adult mouse hippocampus in normal conditions and in AD brain, suggesting that this drug could be used as a therapy in neurodegenerative diseases in which neurogenesis is affected.



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P-8

Effect of ethanolic seed extract of *Croton penduliflorous* Hutch. on loperamide-induced constipated male Wistar rats

Ashafa AOT

Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State-QwaQwa Campus, Phuthaditjhaba 9866, South Africa

The laxative effect of ethanolic seed extract of *Croton penduliflorous* was evaluated in loperamide-induced constipated male rats on daily basis for 7 days. The loperamide significantly ($p<0.05$) reduced the feed and water intake and the fecal parameters (number, water content and weight). The feed and water intake, number, water content and weight of fecal pellets were significantly reduced in both the constipated and 200 mg/kg body weight treated animals whereas these parameters increased in the 50 mg/kg body weight. The extract at 100 mg/kg body weight produced values that compared with the unconstipated and senokot-treated animals. The body weight was not significantly altered in the 50, 100 and senokot-treated group whereas it was increased significantly in the constipated animals. The GIT ratio decreased in both the loperamide and 200 mg/kg body weight of the extract whereas the ratio at the 50 and 100 mg/kg body weight of the extract compared well with control animals. Overall, the ethanolic seed extract of *C. penduliflorous* produced a profound laxative activity against loperamide induced constipated rats.

P-9

Fungicidal and fungistatic effectiveness of polyphenols a propolis of southern Chile on isolates molecularly characterized of *Botrytis cinerea*

Balboa Ponce N[1, 2, a], Carvajal Caiconte F[1], Parada M[2], Paredes M[3], Salazar L[4], Alvear M[1]

[1] Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias; [2]Facultad de Ciencias Agropecuarias y Forestales; [3]Departamento de Ciencias Básicas; [a]Alumna de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada. Universidad de La Frontera. Av Francisco Salazar 01145. Casilla 54-D, Temuco, Chile

In Chile and other countries around the world *Botrytis cinerea* causes serious economic losses.

During the last few years, considerable efforts have developed to identify compounds of natural products like as polyphenols of propolis for controlling the fungal diseases. The main goals of this study were a) to determine the chemical characteristics of a propolis of southern Chile b) to evaluate antifungal activity of the ethanolic extract on four isolates of *B. cinerea* isolated from commercially important fruits (blueberry, cherry, raspberry and strawberry) due to the high variability in the genome and c) to identify four fungal isolates by Polymerase Chain Reaction (PCR). The chemical characteristics of an ethanolic extract of propolis were evaluated. The total polyphenols were quantified by the method of Folin-Ciocalteu and flavonoids (flavones and flavanones) by $AlCl_3$ to yield concentrations of 58.32 mg mL -1 equivalents of gallic acid standard and 9.40 mg mL-1 equivalents of quercetin, respectively. A high-performance liquid chromatographic (HPLC) analysis allowed the identification of apigenin, caffeic, acid phenyl ester (CAPE), galangin and pinocembrin. The antifungal and fungistatic activity were performed by agar diffusion method, where can be evidenced fungistatic differential response of each isolated with treatment. The best antifungal effectiveness was obtained at 5% of ethanolic propolis extract. Four isolated were confirmed through the amplification the IGS region that corresponds to *B. cinerea*. The results obtained in this study are promising and support the importance of further *in vivo* investigations into the antifungal capacity of a propolis of southern Chile.

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P-10

Are animal oils from the Peruvian Amazon anti-inflammatory crude drugs?

Delporte C[1], Vargas-Arana G[2], Valenzuela–Barra G[1], Silva X[3], Schmeda-Hirschmann G[4]

[1]Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Santos Dumont 964, Santiago, Chile; [2]Universidad Científica del Peru. Avda. Abelardo Quiñones Km 2.5, Iquitos, Peru; [3]ISP de Chile, Marathon 1000, Santiago, Chile; [4]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, 3460000 Talca, Chile.

The aim of the study was to assess the topic anti-inflammatory effect of the oils/fats and fatty acid composition of animal oils commercialized as crude drugs in Iquitos, Peru. The oils/fats were purchased from a traditional store at the Iquitos market of Belen. *Materials and Methods*: the topic anti-inflammatory effect was evaluated by the mice ear oedema induced by arachidonic acid (AA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) at the dose of 3 mg oil/ear. Indomethacine and nimesulide were used as reference anti-inflammatory drugs [1]. The fatty acids composition of the different animal oils was assessed by GC analysis. *Results*: all oils/fats showed topic anti-inflammatory activity, with better effect in the TPA-induced mice ear oedema assay. The most active drugs were *Potamotrygon motoro*, *Melanosuchus niger* and *Geochelone denticulata*. In the AA-induced assay, the best activity was found for *P. motoro* and *Electrophorus electricus* oil. The main fatty acids in the oils were oleic, palmitic and linoleic acids. *Conclusions*: Topical application of all the oils/fats investigated showed anti-inflammatory activity in the mice ear oedema assay. The effect can be related with the identity and composition of the fatty acids in the samples as oleic acid and linoleic acid [2, 3, 4]. This study gives support to the traditional use of animal oils/fats as ant-inflammatory agents in the Peruvian Amazon.

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P-11

Sonoran propolis and some of its chemical constituents inhibit the *in vitro* Growth of *Giardia lamblia*

Alday S[1], Diaz G[1], Rascon L[1], Quintero J[1], Robles-Zepeda R[1], Garibay-Escobar A[1], Velázquez-Contreras E[1], Astiazarán-García H[2], Hernández J[3], Velázquez C[1]

[1]Departamento de Ciencias Químico Biológicas, Universidad de Sonora, [2]Centro de Investigación en Alimentación y Desarrollo, [3]Universidad Veracruzana.

Propolis is waxy resins with large complex composition and biological activities. The nature of the chemical composition of propolis has a significant influence on the biological properties. The main aim of this study was to evaluate the *in vitro* antiparasitic activity of propolis from Sonora, México (Caborca propolis [CP]), (Pueblo de Alamos propolis [PAP] and Ures propolis [UP])), and some of its chemical constituents (caffeic acid phenethyl ester (CAPE), naringenin, hesperetin, pinocembrin, chrysin and rutine). Additionally, the seasonal effect of Sonoran propolis on growth of *G. lamblia* trophozoites was analyzed. To evaluate the antiparasitic activity of propolis and some of its constituents, axenic cultures of *G. lamblia* trophozoites (GS/M-83-H7) were used. The Sonoran propolis and its constituents were evaluated at different concentrations (0, 25, 50, 100 and 200 ug/mL). Propolis collection was performed during the four seasons (spring [SP], summer [SuP], fall [FP], and winter [WP]) in the region of Ures, Sonora. The UP showed the highest growth inhibition activity on *G. lamblia* trophozoites, compared with PAP and CP. The constituents of Sonoran propolis CAPE, naringenin, hesperetin and pinocembrin showed significant antiparasitic activity on *G. lamblia* trophozoites. Chrysin and rutine showed no antiparasitic activity at the concentrations evaluated. Propolis collected in summer (SuP) showed the highest inhibitory effect (92.1%) on *G. lamblia* cultures. These findings indicate that Propolis from Ures, Sonora, and some of its chemical constituents have antiparasitic activity *in vitro* against *G. lamblia* and this activity is dependent on the season.

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P-12

Present and future potential of Basidiomycetes products to control *Staphylococcus aureus* of medical significance

Barneche S[1], Cerdeiras MP[2], Vázquez A[1]

[1]Cátedra de Farmacognosia y Productos Naturales, Facultad de Química, Universidad de la República Av. General Flores 2124, Montevideo,11800, Uruguay; [2]Cátedra de Microbiología, Facultad de Química, Universidad de la República Av. General Flores 2124, Montevideo, 11800, Uruguay

In spite of the great advances in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. Although there seems to be a great array of antibacterial and antifungal drugs in clinical use, the appearance of resistant organisms makes them sometimes ineffective or lead to recurrence [1]. Amongst some of the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most

difficult bacteria to treat [2]. Fungi share with plants and animals certain infectious agents such as bacterial and fungal pathogens and therefore have the potential to produce biologically active metabolites to fight them. Microfungi are an important source, already established, of antimicrobial compounds. However, for macrofungi only a few isolated compounds are in the stage of clinical tests [3,4]. *Gymnopilus spectabilis*, the most abundant edible mushroom in Uruguay was submitted to antimicrobial assays and phytochemical characterization. Extracts that were positive in the qualitative diffusion tests against *S. aureus* were selected for a more detailed study. Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to the Clinical and Laboratory Standards Institute using sensitive (ATCC 6538p) and resistant (ATCC 43300 and ATCC 700699) strains. The promising results obtained encourage us to work in the isolation and structural elucidation of the active compound(s).

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P-13

Microencapsulation of red propolis using Coalho cheese whey through spay drying

Andrade VHO[1], Nascimento TG[2], Basílio JAD[3], Lira GM[2], Silva MCD[2], Andrade MNSC[1], Vieira DA[2], Nascimento NM[2], Silvino JNO[2], Basílio Jr ID[2]

[1]Institute of Chemistry and Biotechnology, Federal University of Alagoas, Av. Lourival Melo Mota, s/n, Campus A. C. Simões, Tabuleiro dos Martins/ Maceió/Alagoas-Brazil,57072-900,Brazil; [2]School of Nursing and Pharmacy, Federal University of Alagoas, Av. Lourival Melo Mota, s/n, Campus A. C. Simões, Tabuleiro dos Martins/ Maceió/Alagoas-Brazil,57072-900,Brazil; [3]Nursing Unit, Cesmac University Center, Rua Cônego Machado, 918, Farol. Maceió/Alagoas-Brazil,57072-900, Brazil

The cheese whey was considered for a long time a residue generated from the manufacture of cheese, but this concept has been lost due to the possibilities of reuse. One of the alternatives is its use in spray drying processes due to their properties provided by proteins, among them the ability to form emulsions. Making it possible to use in the drying process of propolis extract ethanolic, which is rich in bioactive compounds [1], but show several limitations as to use as food [2,3]. Through the mixture of between the whey of Coalho cheese added of excipients (malt dextrin, gum arabic, starch and gelatine) and ethanolic extract of propolis (EEP) were obtained two formulations F01 and F02, the F01 were added of starch and F02 of gelatine. The formulations were analysed as to the composition, morphology, distribution, and particle size. The results regarding the composition of the formulations had demonstrated a significant difference ($p < 0.05$) for the t test, to the parameters of moisture, protein and ash and not significant for dry matter and total flavonoids. The F01 formulation showed a lower average particle size (12.02 μm) compared to formulation F02 (42.13 μm) and also showed the best morphology of the particles. The addition of cheese whey with excipients to the propolis extract, obtaining a dehydrated product, expands the possibilities of use of propolis which is rich in total flavonoids, combined with whey proteins, makes it an intermediate product innovator that can used in the development of other products.

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P-14

Study of seasonal variation of some phenolic compounds from Brazilian red propolis using LC-DAD and LC-Orbitrap-FTMS and biological methods

Nascimento TG[1,2], Basílio Jr ID[1,2], Porto ICCM[1], Escodro PB[1],Oliveira JMS[1], Souza NS[1], Azevedo LF[1], Silva PF[1], Vieira DA[1], Arruda RES[1], Barboza APF[2], Albuquerque ALI[2], Basílio JAD[1], Oliveira EJ[3], Macedo RO[3], Watson DG[4]

[1]School of Nursing and Pharmacy, [2]College of Nutrition, Federal University of Alagoas, Av. Lourival Melo Mota, s/n, Campus A. C. Simões, Tabuleiro dos Martins/ Maceió/Alagoas-Brazil,57072-900,Brazil; [3]College of Pharmaceutical Sciences, Federal University of the Paraíba, Campus I, Castelo Branco, João Pessoa/Paraíba-Brazil, 58051-900,Brazil; [4]Strathclyde institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 cathedral street, Glasgow, G4 0RE, Glasgow-UK

Red propolis presents differences from the others Brazilian propolis due to the presence of isoflavones, chalcones, pterocarpans, isoflavans, terpens and guttiferones. Phenolic compounds in red propolis have shown in vitro anticancer activity, antibacterial activity and antioxidant activity. The aim of the present work was to perform the study of seasonal variation of phenolic compounds of the Brazilian red propolis using LC-DAD and LC-Orbitrap-FTMS and its correlation with biological methods. Propolis was donated by three apiaries between the periods of march/2011 to february/2012. Crude extracts of red propolis (36 samples) was obtained. A mass of 100mg was exactly weighed, solubilized in ethanol, diluted and submitted to quantification in UV method, LC-DAD and LC-Orbitrap-FTMS. A total of 12 flavonoids and 2 guttiferones were quantified. All samples were also monitored by microbiological method (MIC) using *S. aureus and Pseudomonas aeruginosa* and trypomastigotes activity method using *Trypanosoma brucei*. It was observed increase of flavonoids concentrations which were associated to the period of intense raining in north east region (months of may to august) and decreasing in guttiferones concentrations in same period. A increasing of guttiferones concentrations was observed in the period of summer and normalization of flavonoids concentration. A mechanism of upregulation and downregulation for flavonoids and guttiferones can be associated to double secondary metabolic pathway for these classes of phenolic compounds. All samples in all months presented trypomastigotes activity however only one apiary presented inverse correlation in MIC associated to decreasing in both concentrations of flavonoids and guttiferones in months of march, april, june.

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P-15

Prooxidant activity of norbixin in acute model of gastric ulcer induced by ethanol in rats

Bauermann LF[1], Rovani BT[1], Freitas RB[1], Araldi IC[2], Augusti PR[3], Somacal S[4], Emanuelli T[4]

[1]Post-Graduate Program in Pharmaceutical Sciences, Federal University of Santa Maria, Camobi Campus, 97105-900, Santa Maria, RS, Brazil; [2]Undergraduate Program in Biological Sciences, Federal University of Santa Maria, Camobi Campus, 97105-900, Santa Maria, RS, Brazil; [3]Institute of Food Science and Technology, Federal University of Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil; [4]Integrated Center for Laboratory Analysis Development (NIDAL), Federal University of Santa Maria, Camobi Campus, 97105-900, Santa Maria, RS, Brazil

Reactive oxygen species (ROS), free radicals production and oxidative stress play a central role in

injuries caused by ethanol (EtOH) on gastric mucosa, by example, gastritis and gastric ulcers [1,2]. Strategies to counteract EtOH toxicity in addition to the endogenous antioxidant defenses are highly desirable, such as the carotenoids (obtained from the diet) [3]. However, under certain conditions, carotenoids may exhibit prooxidant activity [4]. This study was aimed to evaluating the effects of the norbixin (NBIX), used in the food industry as a colorant, in the acute model of gastric ulcer induced by EtOH in rats. Male Wistar rats received norbixin (at doses 10 and 25 mg/kg, by gavage) after 1 hour of EtOH administration (75%, 1mL/200g of animal, by gavage), being euthanized one hour after extracts administration. The stomachs were removed to perform the macroscopical and histopathological analysis, protein quantification, quantification of non-protein sulfhydryl groups (NPSH), determination of catalase activity (CAT) and quantification of lipid peroxidation (LPO). Norbixin showed a pro-oxidant activity at the concentrations used, increasing LPO and damage in the gastric mucosa, as well causing CAT inhibition and NPSH depletion in EtOH treated animals.

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P-18

Trypanocidal activity of extracts of Argentinean Asteraceae species

Selener M[1], Cazorla S[2], Elso O[1], Frank F[2], Beer MF[1], Laurella L[1], Malchiodi E[2], Giberti G[1], Martino V[1], Sülsen V[1]

[1]Cátedra de Farmacognosia, IQUIMEFA (UBA-CONICET), Facultad de Farmacia y Bioquímica-UBA, Junín 956 2piso-CABA, 1113, Argentina; [2]Cátedra de Inmunología, IDEHU (UBA-CONICET), Facultad de Farmacia y Bioquímica-UBA, Junín 956 2piso-CABA, 1113, Argentina; IMPaM (UBA-CONICET), Facultad de Medicina-UBA, Paraguay 2155 13piso-CABA, 1121, Argentina

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a serious health problem mainly in Latin America. According to the World Health Organization, this parasitosis affects 7-8 million people worldwide [1]. Treatment is limited to two drugs, nifurtimox and benznidazole, that are effective only on acute Chagas' disease and have serious side effects. Natural products have provided useful drugs that are used nowadays to treat different health problems. Asteraceae species have been a rich source of active compounds and have been attractive for drug discovery. In this sense, the aim of the present study was to evaluate the trypanocidal activity of dichloromethane and methanol extracts of twelve Argentinean Asteraceae species on epimastigotes of *T. cruzi* (RA strain). Dichloromethane extracts of *Aspilia sulphioides*, *Viguiera tuberosa*, *Verbesina subcordata*, *Gymnocoronis spilanthoides*, *Gaillardia megapotamica*, *Thelesperma megapotamicum* and *Zexmenia buphtalmiflora* were active against *T. cruzi* with inhibitions higher than 60% at a concentration of 10 µg/mL. Only the methanolic extracts of *Helenium radiatum* and *G. megapotamica* have shown trypanocidal activity (inhibitions of 70.1 and 77.7%, respectively at 10 µg/mL). These results encourage further investigation of Asteraceae species as source of lead molecules for Chagas' disease.

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P-19

Cytotoxic effect of natural pseudoguainolides against lymphoma cells

Sülsen V[1,2], Martino R[1], Beer MF[1,2], Elso O[2], Martino V[1,2], Anesini C[1]

[1]Instituto de Química y Metabolismo del Fármaco (IQUIMEFA) UBA-CONICET, Junín 956 2piso-CABA, 1113, Argentina; [2]Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica-UBA, Junín 956 2piso-CABA, 1113, Argentina

Cancer is one of the most important causes of death worldwide. According to the World Health Organization, lymphoma stand at the fifth place of cancer related death over the world. It is characterized by abnormal lymphocyte proliferation and appears as a solid tumor most commonly in the lymph nodes of the neck, chest, armpit or groin [1]. Chemotherapy is one of the best options in the treatment of cancer. However, currently used drugs have serious side effects. For this reason, it is necessary to search for new and more selective drugs with fewer adverse effects. Sesquiterpene lactones (STLs) are natural terpenoid compounds that have received considerable attention due to their antiparasitic, antiinflamatory and antitumoral activities. In this sense, the aim of the present study was to evaluate the effect of psilostachyin, psilostachyin C and peruvin, three STLs of the pseudoguaianolide type, on a lymphocityc leukaemia cell line (BW 5147). Psilostachyin C was the most active compound with a 50% effective concentration (EC_{50}) of 4.89 µg/mL when tested for its *in vitro* antiproliferative activity. This compound exerted cytostatic and cytotoxic activity in relation to apoptosis, but was non toxic when tested on normal murine lymphocytes (50% cytotoxic concentration (CC_{50}) higher than 50 µg/mL). These findings show the importance of STLs as potential candidates for lymphoma and leukemia treatment.

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P-20

Acute toxicity of two compounds (chalcone and Pinostrobin Sakuranetin) isolated from *Renealmia alpinia* (Rottb.) Maas

Gómez-Betancur IC[1], Benjumea D[1], Osorio E[2], Jimenez N[2]

[1]Ophidism / Scorpionism Program, University Research Center, Tower 2 Lab 631. Faculty of Pharmaceutical Chemistry, University of Antioquia; 70 Street No. 52-21, Medellin, Colombia; [2]Research Group for Bioactive Substances, University Research Center, Tower 2 Lab 229, University of Antioquia. Medellin, Colombia

Renealmia alpinia, is known as "matandrea" and has been used by the Chocó indians against *Bothrop*s (mapaná X) snakebite [1]. Furthermore, it is used in traditional medicine to treat pain [2]. It has been significantly used as febrifuge (to lower fever, antipyretic) and antiemetic, to treat wounds and malignant ulcers [3]. In addition, in Suriname, it is also used to treat epilepsy [4], heartburn and stomach pain [5]. The popularity of its ethnomedicinal use has drawn our attention to further investigate its medicinal properties. Given the high consumption of *R. alpinia* and that their safety has not been established scientifically, our recent studies have focused on the investigation of the acute toxicity of extracts *in vivo* to determine the safety of this plant for consumption. Patiño et al. [6] showed that ethanol extract from *R. alpinia* is safe for consumption. *In vivo* studies showed no significant changes in body weight of mice treated with *R. alpinia*. In addition, all histopathological parameters used to evaluate the effect of toxicity showed no adverse changes [6]. In this work, we reported the evaluation of acute toxicity of two isolated compounds (chalcone and-Pinostrobin

Sakuranetina) from *R. alpinia* leaves which was carried out according to standard 423 of the Organization for Economic Cooperation and Development (OECD) adopted in 2001 [7]. Both compounds did not caused death or apparent signs of toxicity during the 14 days of observation in any of the experimental animals treated with a single dose (300 mg / kg) during the experiment.

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P-21

New flavonoids and anti-dengue activity of *Arrabidaea chica* extracts

Brandão GC[1], Soares GMA[1], Kroon EG[2], Oliveira AB[3]

[1]Universidade Federal de Ouro Preto - Departamento de Farmácia; [2]Universidade Federal de Minas Gerais - Microbiologia; [3]Universidade Federal de Minas Gerais - Departamento de Produtos Farmacêuticos

Arrabidaea chica (Bonpl.) Verl. (Bignoniaceae) is climbing plants popularly used in the treatment of infections, diarrhea, anemia, intestinal pain, uterine inflammation and for the healing of skin wounds. Chemically, bignoniaceous plants are characterized by the presence of flavonoids, terpenoids, quinones, mainly naphthoquinones, and aromatic compounds such as lignans, cinnamoyl, benzoyl and acetophenone derivatives. Several species of this family are reported as used to treat diseases possibly related to viral infections what has motivated the evaluation this species [1]. The *in vitro* antiviral activity against *Dengue virus* 2 (DENV-2) was carried on by the MTT colorimetric method (n =4); the cytotoxicity was determined *in vitro* in $LLCMK_2$ cell lines (n=4); interferon was used as positive controls (2.5×10^3 UI/ml) [2,3]. Ethanol extracts from stems, leaves and fruits were prepared and analyzed by TLC and RP-UPLC-DAD-MS using previously validated methodology [1]. Only the ethanol extract of leaves showed anti-dengue activity (EC_{50} 39.6 ± 1.1 µg/mL). The extracts presented low cytotoxicity (CC_{50} > 500 µg/mL). UPLC-MS analyses of extracts, allowed the identification of the majority of flavonoids present in those extracts, the known compounds, apigenin, kaempferol, scutellarein, scutellarein-7-O-rhamnoside, scutellarein-7-O-rutinoside and six new flavones derivatives 5-methoxy-6,7,4'-trihydroxyflavone, 5-methoxy-6,4'-dihydroxy-7-O-hexosyl-flavone, 5-methoxy-6,4'-dihydroxy-7-O-rhamnosyl-flavone, 5,6,3',4'-tetrahydroxy-7-O-hexosyl-flavone, 5,4'-dimethoxy-6-hydroxy-7-O-hexosyl-flavone, 5-methoxy-6,4'-dihydroxy-7-O-rutinosyl-flavone. The results of the phytochemical studies here described suggest that flavonoids are the substances that contribute to the antiviral activity of the ethanol extract of this species.

Acknowledgments: Financial support by FAPEMIG and CNPq.
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P-22

LC/MS profiling of xanthones and flavonoids of the anti-dengue extracts of *Arrabidaea samydoides* (Bignoniaceae)

Brandão GC[1], Calvichon EG[1], Kroon EG[2], Oliveira AB[3]

[1]Universidade Federal de Ouro Preto - Departamento de Farmácia; [2]Universidade Federal de Minas Gerais - Microbiologia; [3]Universidade Federal de Minas Gerais - Departamento de Produtos Farmacêuticos

Arrabidaea samydoides (Cham.) Sandwith belongs to the Bignoniaceae family that is represented by more than 100 genera and about 800 species in Brazil. Chemically, bignoniaceous plants are characterized by the presence of flavonoids, terpenoids, quinones (mainly naphthoquinones), and aromatic compounds such as lignans, cinnamoyl, benzoyl and acetophenone derivatives. Several species of this family are reported as used for treatment of diseases possibly related to viral infections what has motivated the evaluation of *A. samydoides* [1]. The *in vitro* antiviral activity against *Dengue virus* 2 (DENV-2) was carried on by the MTT colorimetric method (*n* =4); the cytotoxicity was determined *in vitro* in $LLCMK_2$ cell lines (*n*=4); interferon was used as positive control (2.5×10^3 UI/ml) [2, 3]. Ethanol extracts from stems and leaves were prepared and analyzed by TLC and RP-UPLC-DAD-MS using previously validated methodology [1]. Results are presented as cytotoxic concentration to 50% (CC_{50}), antiviral effective concentration to 50% (EC_{50}) and Selectivity Index (SI) values. The extracts presented low cytotoxicity (CC_{50} > 90 µg/mL). All ethanol extracts were active against DENV-2, EC_{50} < 25.0 µg/mL and SI > 3.0. UPLC-MS analyses of extracts, allowed the identification of most flavonoids and xanthones present in the extracts. The compounds identified included the flavonoids vitexin, isovitexin, orientin, isoorientin, chrysin, O-methylchrysin, and xanthones as mangiferin, isomangiferin, 2'-*trans*-O-caffeoylmangiferin, 2'-trans-O-coumaroylmangiferin, 2'-*trans*-O-cinnamoylmangiferin, muraxanthone, and 2'-O-benzoylmangiferin. The results of the phytochemical studies here described suggest that flavonoids and xanthones are the substances that contribute to the antiviral activity of the ethanol extract of this species.

Acknowledgements: Financial support by FAPEMIG and CNPq.
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P-23

Chilean propolis: antioxidant and antibacterial activity

Atala E[1], Nuñez G[2], Montenegro G[2], López-Alarcón C[1], Bridi R[1]

[1]Facultad de Química, [2]Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Avda. Vicuña Mackenna 4860, Macul - Santiago - Chile

The most important components of Chilean propolis are polyphenols, which depending on their chemical structure, display powerful antioxidant and biological activities. We evaluated the antioxidant and antibacterial activity of six samples of propolis from two areas of the VI region (Chile). Studies involved reactive species such as peroxyl radicals (ORAC-FL and ORAC-PGR), hypochlorite (ORAC-PGR), DPPH, and nitrous acid. The antibacterial activity was determined against *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The botanical analysis evidenced a predominance of pollen from *Galega officinalis* and in a minor degree from native species, as *Quillaja saponaria* and *Aristotelia chilensis.* Total phenolic content, determined by the Folin–Ciocalteu, varied between 25 and 105 mg of gallic acid

equivalents/g propolis, while flavonoids content varied between 21 and 77 mg of quercetin equivalents/g propolis. DPPH antiradical activity varied between 17 and 85%, while ORAC-FL and ORAC-PGR values varied between 0.2 and 4.1 mM Trolox equivalents/g propolis. Towards hypochlorite, obtained values of the PGR-HOCl index varied between 0.7 and 13.7 mM Trolox equivalents/g propolis. When samples were exposed to nitrous acid, nitric oxide was generated in the 3.1 to 6.9 µM range. Propolis samples also presented a high antibacterial activity against *S. pyogenes* and *S. aureus* with an average inhibition halo between 14 and 29 mm. Amongst the studied samples, two of them, from the valley area, presented the highest antioxidant and antibacterial activity. In these samples, employing HPLC-DAD technique, phenolic compounds such as caffeic, coumaric and cinnamic acid, and flavonoids such as quercetin, pinobanksin and apigenin, were identified. Some these compounds present high reactivity towards hypochlorite and peroxil radicals (quercetin) [1, 2], ability to generate NO• (caffeic acid) [3], and antibacterial action (cinnamic acid) [4].

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P-24

ORAC index of the Chilean cv "*Pica"* mango (*Mangifera indica* L.) byproducts

Dorta E[2*], Blanco A[1], Fuentes-Lemus E[2], Atala E[2], López-Alarcón C[2], Bridi R[2]

[1]Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile; [2]Facultad de Química, Pontificia Universidad Católica de Chile

The use of fruit residues as source of bioactive compounds is an emerging area, the mango byproducts being well-recognized by their high phenolic content and antioxidant activity. "*Pica"* mango (*Mangifera indica cv piqueño or Pica*) is an endemic variety from Atacama desert, which antioxidant activity, assessed by the ORAC (Oxygen Radical Absorbance Capacity) assay, has not been studied. Therefore, the present work was undertaken to evaluate the ORAC index of Pica mango and also to compare this activity with other mango varieties widely employed in food industry (cv *"Kent"*, cv *"Keitt"* and cv *"Tommy Atkins"*). Mango fruits (around 10-15 units) were obtained from local markets, harvested at physiological maturity and allowed to ripen, until the consumption stage, at 18◦C and 80-90% relative humidity. Fruit texture, measured as deformation varied between 67 and 76º Durofel, while penetration force varied between 9 and 14 N.
Independently of the variety, mango seed showed higher total phenolic content, determined by the Folin–Ciocalteu method (38-89 g gallic acid equivalents / 100g dry weight), than mango peel (9-36 g gallic acid equivalents/ 100g dw). By contrast, similar ORAC values were determined between mango seed and peel (between 1.9 and 3.5 mmol Trolox equivalents /g dw). Comparatively, cv *"Pica"* mango byproducts showed similar antioxidant capacity and phenolic content than cv *"Kent"*, cv *"Keitt"* and cv *"Tommy Atkins"*. These results evidenced that mango cv "*Pica*" byproducts could be considered as an excellent candidate for using in food industry as a source of phenolic compounds with antioxidant properties.

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P-25

Isolation of terpenoids from *Nolana ramosissima I. M. Johnst* using high speed countercurrent chromatography (HSCCC)

Brito A, Bórquez J, Simirgiotis MJ

[1]*Laboratorio de Productos Naturales, Departamento de Química, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile*

Paposo is a small town in northern Chile, located 50 km from Taltal and 200 km from Antofagasta with unique plant species, including *Nolana ramosissima*, *N. leptophila, and N. aplocarioides*. High speed countercurrent chromatography (HSCCC) is a liquid-liquid technique which allows fast isolation with total recovery of all compounds present in an organic sample [1]. Several terpenoids were easily isolated using HSCCC from the petroleum ether extract of the aerial parts of *Nolana ramosissima*. The machine employed was a Quattro MK7 (AECS inc, Bridgend UK), equipped with four stainless steel multilayer coils of 2.16 mm ID tubing with a total capacity of 435 mL. The revolution speed of the planetary coils was set at 850 rpm, the machine was run in a reverse phase-Head to Tail mode, at 27 ^{o}C and pumping was performed with a Series II SSI model HPLC pump (LabAlliance, PA, USA) at a flow rate of 7 mL-minute, using a two-phase non aqueous solvent system composed of **n**-hexane: ethyl acetate: acetonitrile 5:2:5 v/v/v, which is a system previously used for the separation of kaurenoic acids [1, 2]. The fractions eluted were collected every minute with a Gilson FC 203B model fraction collector (Middleton, MI, USA) and analysed by TLC (F_{254} Silica gel Plates, developed with hexane:EtOAc, 8:2 v/v, and spots visualized by spraying with vanillin:sulfuric acid 2 % in ethanol and heating. Using this liquid-liquid technique several terpenoids including labdanes [3, 4] have been isolated.

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P-26

Isolation of mulinane and azorellane diterpenoids from *Mulinum crassifolium Phil.* using high speed countercurrent chromatography (HSCCC)

Brito A[1], Bórquez J[1], Ramirez JE[1], Simirgiotis MJ[1]

[1]*Laboratorio de Productos Naturales, Departamento de Química, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile*

"Chuchikan" or "Chukikaylla" are the Aymara common names for *Mulinum crassifolium* Phil. a native Chilean plant whose infusion is used in Northern Chile for the treatment of cold and lung diseases [1]. High speed countercurrent chromatography (HSCCC) is a liquid-liquid technique which allows fast isolation with total recovery of all compounds present in an organic sample [2]. Several diterpenoids were easily isolated using HSCCC from the acetone extract of the aerial parts of "Chuchikan". The machine employed was a Quattro MK7 (AECS inc, Bridgend UK), equipped with four stainless steel multilayer coils of 2.16 mm ID tubing with a total capacity of 435 mL. The revolution speed of the planetary coils was set at 850 rpm, the machine was run in a reverse phase-Head to Tail mode, at 27 ^{o}C and pumping was performed with a Series II SSI model HPLC pump (LabAlliance, PA, USA) at a flow rate of 7 mL-minute, using a two-phase non aqueous solvent system composed of **n**-hexane: ethyl acetate: acetonitrile 5:2:5 v/v/v, which is a system previously used for the separation of kaurenoic acids [2, 3]. The fractions eluted were collected every minute

with a Gilson FC 203B model fraction collector (Middleton, MI, USA) and analysed by TLC (F_{254} Silica gel Plates, developed with hexane:EtOAc, 8:2 v/v, and spots visualized by spraying with vanillin:sulfuric acid 2 % in ethanol and heating. Using this liquid-liquid technique the known compounds mulinic, 17-acetoxy-mulinic, isomulinic, mulinolic and mulinenic acids [4, 5] were isolated together with two new diterpenoids.

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P-27

Biological activity and intracellular alterations of 4-nitrobenzaldehyde thiosemicarbazone derived from *S*-limonene against *Trypanosoma cruzi*

Britta EA[1], Scariot DB[1], Falzirolli H[2], Ueda-Nakamura T[1], Silva CC[2], Dias Filho BP[1], Borsali R[3], Nakamura CV[1]

[1]Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil; [2]Departamento de Química, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá - PR, Brazil; [3]CERMAV - Centre Macromolecules Vegetales, BP 53, 38041 Grenoble cedex 9, Grenoble - France

Trypanosoma cruzi is the causative agent of Chagas' disease that represents a major worldwide health problem. Current treatment is based on benzonidazole and nifurtimox, both displaying high toxicity and being ineffective in patients during the chronic phase of the disease [1, 2]. The present study report the *in vitro* activity of 4-nitrobenzaldehyde thiosemicarbazone (BZTS) against epimastigotes of *T. cruzi,* as well as morphological and intracellular changes in the parasite. The effects of BZTS in the protozoan growth was assessed by direct counting of the cells in a Neubauer chamber. The 50% inhibition concentration (IC_{50}) was determined. The morphological and ultrastructural alterations were determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. Flow cytometry assays were employed to evaluate the cytoplasmatic membrane and mitochondrial membrane potential. BZTS showed an IC_{50} value of 9.2 ± 1.7 µM. Analysis by SEM and TEM showed important alterations in the shape and size of the parasites, presence of autophagic process, vacuolization of the cytoplasm, and mainly mitochondrial damage. By flow cytometry it was possible to observe that BZTS did not caused cytoplasmatic membrane alterations, but produced a decrease in the mitochondrial membrane potential. These results encouraged us to continue the experiments with the BZTS to identify the compound action mechanism(s).

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P-28

Natural insecticides safe for human health and the environment: Evaluation in field conditions of extracts of *Ovidia andina* on *Caliroa cerasi* larvae

Bado S[1], Tarcaya V[2], Cufre I[2], Clemente SV[3], Broussalis AM[2]

[1]Instituto Nacional de Tecnología Agropecuaria Estación Experimental Chubut. Ex ruta 2 5 km 1480 Trelew, Chubut, Argentina; [2]Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica, Junín 956, Ciudad Autónoma de Buenos Aires, Argentina; [3]Área Protección Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Av San Martín 4453, Ciudad Autónoma de Buenos Aires. Argentina

Caliroa cerasi L. (Hymenoptera: Tenthredinidae), "babosita del peral" is the main pest of cherry trees in Southern Patagonia, Argentina. Currently, their population levels decrease only by use of synthetic pesticides. At present, the search for insecticides is oriented to plant compounds that are safe for human health and the environment. With the aim to develop a less toxic insecticide formulation and given the promising results found in the laboratory [1-4], the efficacy of a dichloromethane extract of *Ovidia andina* (P. et E.) M. (Thymeleaceae), "pillo-pillo" [5] was assessed on larvae of *C. cerasi* under field conditions. The assay was performed in a cherry orchard located in the northern Lower Valley of the Chubut River. Three control trees and three trees treated with dichloromethane extract were compared. Before starting the experiments, a larval count was carried outh with removal of 50 leaves from each tree. Spraying was performed with the *O.andina* extract at a concentration of 7000 ppm (7 g/L). The vehicle (water, mineral oil and Tween 80) was applied on the control plants. Observations were made after 24 and 96 hours, collecting 50 leaves from each tree at each observaion time to count larvae. The average mortality after 24 hours of spraying with the extract was 95% and 99% at 96 hours, whereas in the control was 0% at 24 hours, and 2% at 96 hours (ANOVA and Tukey test, $p \leq 0.05$). These findings are relevant to the development of a novel organic formulation intended to manage this pest.

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P-29

Exploring metabolic profile and antioxidant capacity of wild edible berries from south Patagonia

Ruiz M, Bustamante L, von Baer D, Vergara C, Mardones C

Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Casilla 160-C, Concepción, Chile

In the extreme south of Chile, wild edible berries are produced by several vascular plants, which are not used widely because their nutritional properties have not been studied in depth. Samples of 9 different berry species were collected in Magallanes region. The anthocyanin, hidroxycinnamic acid derivates (HCADs) and flavonol profiles and their respective concentrations were studied using HPLC-DAD-ESI-MS/MS. Antioxidant capacity (TEAC and CUPRAC) and ascorbic acid contents were also determined. All concentrations were expressed in fresh weight. In *Berberis empetrifolia*

and *Berberis ilicifolia* a predominance of delphinidin, petunidin and malvidin 3-glucoside was observed. Their total anthocyanin concentrations were 17.80 and 4.51 µmol/g, respectively. In *Ribes magellanicum* and *Ribes cucullatum*, the 3-glucoside and 3-rutinoside derivates of cyanidin predominated, showing total anthocyanin concentrations of 5.08 and 13.76 µmol/g. *Gaultheria mucronata*, *Gaultheria antarctica*, *Rubus geoides, Myrteola nummularia* and *Fuchsia magellanica* presented as main anthocyanidins cyanidin and/or delphinidin derivates, with lower total concentrations in comparison with the other studied species. Caffeoylquinic acid isomers were the most abundant HCADs as well as quercetin derivates were the main flavonols found in the fruits. *Berberis* and *Ribes* genera showed a high diversity and concentration of these 2 families, between 0.68 to 2.03 µmol/g and 1.12 to 3.53 µmol/g for flavonols and HCADs respectively. Ascorbic acid concentration was between 2.08 to 8.78 µmol/g, being higher for *M. nummularia* and *F. magellanica*. *Rubus* and *Ribes* genera had high cupric reducing antioxidant capacity, while *Ribus* and *Berberis* genera showed high ABTS reducing capacity, having interesting antioxidant power.

Acknowledgement: Fondecyt 1100944.

P-30

Cholinesterase inhibitory effect alkaloid of *Rhodophiala pratensis* (Poepp.) Traub.

Bustos E[1], Uribe Matilde[2], Salazar C[2], Pérez C[3], Gutiérrez M[4], Becerra J[3]

[1]Laboratorio de Química de Productos Naturales; [2]Centro de Biotecnología; [3]Departamento de Botánica. Universidad de Concepción, Casilla 160-C, Concepción, Chile; [4]Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 841, Talca, Chile

Plants from the Amaryllidaceae family occur worldwide and are pharmacologically relevant for its significant cholinesterase inhibitory effect [1-3]. *Rhodophiala pratensis* is an endemic Chilean species, distributed from the Region de Antofagasta to Los Lagos. No previous studies have been carried out on the plant constituents. The aim of the present work was the alkaloid characterization in bulbs of *Rhodophiala pratensis* collected at three locations of the Región del Bio-Bio: Chillán, Antuco and Concepción, using GC-MS techniques. The acetylcholinesterase inhibitory effect of the bulb extracts was assessed enzymatically according to the Ellman method. The following alkaloids were identified. Concepción sample: nerinine, galanthan, crinine, tazettine, 3-epymacronine; Chillán sample: galanthamine, lycoramine, crinan-3-ol, tazettine, galathan, pancracine, dihydrolycorine, 3-epymacronine; Antuco sample: lycoramine, acetyl-pancracine, pancracine, lycorenan-7-one and tazettine. The activity of the extracts towards the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) (reported as IC_{50} values in µg/mL) was higher for the Concepción (Hualpén) (AChE 255.5 ± 2.2, BuChE 149.0 ± 2.8) and Antuco samples (AChE 277. 2 ± 1.8, BuChE 245. 4±2. 5), while the sample of Chillan was not relevant as cholinesterase inhibitor, with IC_{50} values > 500 µg/mL.

Acknowledgements: Proyecto Fondef-IDEA-CA13I10272. Universidad de Concepción.
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P-31

Seasonal variation of anticandidal activity of *Zuccagnia punctata* Cav. (Fabaceae) DCM exudate and quantification of active and analytical markers in each period of the year

Butassi E[1], Svetaz L[1], Feresin G[2], Tapia A[2], Zacchino S[1]

[1]Farmacognosia, Fac. Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Suipacha 531, Argentina; [2]Inst. de Biotecnología- Inst. de Cs Básicas, Univ. Nac.de San Juan, San Juan, Av. Libertador Gral San Martín 1109, Argentina

The DCM of *Zuccagnia punctata* Cav. (Fabaceae), a monotypic species found in arid and semiarid areas of Argentina at 900-2200m osl [1] traditionally used for wound healing purposes [2] showed in previous studies anti *Candida albicans* activity, being 2(,4(-dihydroxychalcone (1) the active marker and 2(,4(-dihydroxy-3-methoxy chalcone (2) the analytical marker [3,4]. Studies on mechanism of action showed that *Z. punctata* exudate and its most active component have a different mode of action than the polyene and azole class existing drugs, since they did not disrupt membranes at concentrations as amphotericin B does and are fungicide and not fungistatic, an important difference with azoles [5]. In this work, we report the antifungal activity of *Z. punctata* exudate in the four seasons of a year against the clinically important *C. albicans* (Ca) and *C. glabrata* (Cg) strains by determining the minimum concentrations that inhibit 50, 80, 95 and 95 % growth (MIC_{50}, MIC 80, MIC 90 and MIC 95). The different MICs were obtained from non-linear regression dose-response curves of mixed-effects constructed for each extract with MixLow software from properly designed plates (in triplicate) in which 10 different extract concentrations (in sextuplicate = 60 wells) were mixed with inoculum along with 36 control wells [5]. Results showed that the best effects were observed in the end of Spring (MIC_{50}-MIC_{95} ranges 27.33- 38.01 µg/mL) and of Summer (28.63-39.96 µg/mL) for Ca. In turn, Cg was less sensitive with higher values of MIC ranging between 58.73 and 100.09 %, being the activities slightly higher in Summer. From the HPLC quantification of the two markers named above, it could be seen that the statistical differences in activity is not correlated with the active marker which maintain a statistical similar concentration in all seasons of the year. Synergism studies of the different principal compounds are in progress and will allow the determination of the best proportion of them that could be correlated to the seasonal variation of the antifungal activity.

Acknowledgements: SZ, GF and AT to ANPCyT PICT 0608; EB and LS acknowledge CONICET.
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P-32

Schaftoside from *Costus spiralis* (Jacq.) Roscoe with anti-inflammatory activity

Garcia T, Amorim J, Braga F, Coelho M, Castilho R

Universidade Federal de Minas Gerais, Pharmacy Faculty, Department of Pharmaceutical Products, 31270-901, Minas Gerais, Brazil

Costus spiralis (Jacq) Roscoe (Costaceae), popularly named “cana do brejo”, is traditionally used in Brazil to treat kidney affections. Previous phytochemical studies have revealed the occurrence of flavonoids from leaves and saponins from rhizomes of *Costus spiralis* with potential to treat inflammatory diseases [1, 2]. This study aimed to evaluate the anti-inflammatory activity of extract

and fractions of *C. spiralis,* isolate and identify the chemical constituents. The crude ethanol extract from *C. spiralis* leaves was fractionated by silica gel column chromatography, using hexane, ethyl acetate and methanol as eluent, affording 7 fractions. The anti-inflammatory activity of the ethanol extract from *C. spiralis* and fractions were evaluated by using the model of carrageenan-induced paw edema in Swiss mice. The extract and the fraction ethyl acetate:methanol (1:1) showed anti-inflammatory activity. This fraction was rechromatographed by preparative reverse phase high performance liquid chromatography resulting in the isolation of a solid named FLAV1. Analyses of spectrometric data recorded for FLAV1 were compatible with schaftoside. The anti-inflammatory activity of schaftoside, at the doses of 0.5, 1.0 and 2.0 mg/kg administered by intraperitoneal injection was evaluated in the same model. Schaftoside ellicited anti-inflammatory response at the higher doses (1 and 2 mg/Kg), suggesting its participation in the anti-inflammatory activity of *C. spiralis* and, therefore, that it may represent a chemical marker for the species.

Acknowledgements: FAPEMIG and CNPq for financial support.
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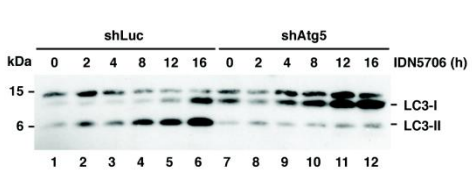
P-33

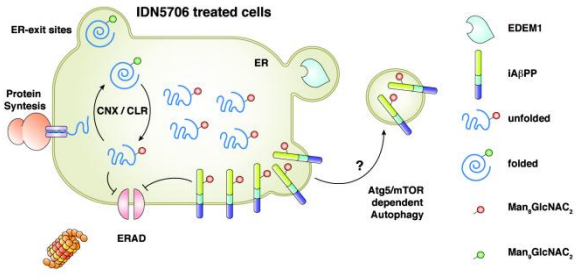
Inhibition of ERAD by tetrahydrohyperforin enhances autophagic degradation of immature amyloid precursor protein

Cavieres VA, González A, Yefi C, Bustamante HA, Muñoz VC, Caster JF, Salamín ME, Otth C, González CB, Mardones GA, Inestrosa NC, Burgos PV

Laboratory of Cell and Molecular Biology, Department of Physiology, School of Medicine, Universidad Austral de Chile, Valdivia 511-05666, Chile and Centro de Envejecimiento y Regeneración (CARE), Pontificia Universidad Católica de Chile, Santiago, Chile

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of amyloid-βeta (Aβ). We have previously shown that tetrahydrohyperforin (IDN5706), a stable hyperforin derivative isolated from the St John's Wort plant, prevents the accumulation of Aβ species in an in vivo model of AD. In this report, we show that IDN5706 decreases the levels of EDEM1, a key chaperone protein that regulates the disposal of improperly folded glycoproteins via Endoplasmic Reticulum Associated Degradation (ERAD). Low levels of EDEM1 correlated with the accumulation of immature amyloid precursor protein (APP) in the endoplasmic reticulum, indicating that APP is a substrate of ERAD. In addition, we observed that IDN5706 inhibits the mammalian target of rapamycin (mTOR), increases the number of autophagosomal structures, and enhances the levels of microtubule-associated protein light chain 3 (LC3-II), in an Atg5-dependent manner, suggesting that IDN5706 is also able to activate autophagy. Finally, cycloheximide-chase experiments in cells treated with IDN5706 showed that immature APP could also be degraded in an Atg5-dependent manner. Taken together, our findings imply that IDN5706 promotes an early degradation of immature APP by autophagy when ERAD is impaired, revealing a crosstalk between these protein degradation pathways at the ER, and shedding light on the mechanism that may contribute to the reduction of Aβ production in vivo.





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P-34

Caenorhabditis elegans as a model for toxicology screening of natural products

Chapula-Iñiguez M, Peraza-Campos AL, Parra-Delgado H, Garcia-Ruelas MC, Guzmán-López B, Saldivar-Ramírez D

Facultad de Ciencias Químicas, Universidad de Colima, Campus Coquimatlán, Km 9, Carr. Colima-Coquimatlán, CP 28400, Colima, México

The nematode *Caenorhabditis elegans* represents an attractive bio-model because of its well-characterized and evolutionarily conserved biology, and ability to be used in high throughput screening. Nowadays, the nematode is used for studies of obesity, aging and Alzheimer's disease. In fact, it was proposed as a toxicological model for heavy metals as well as pharmaceuticals [1]. Taking this information into account, we employed the model for the toxicology screening of extracts of medicinal plants used in Colima, Mexico [2]. Subsequently, methanol extracts from species *Swietenia humilis, Brosimum alicastrum* and *Ampelocissus acapulcencis* were evaluated from 10-3 to 1 mg/mL. Therefore, we found that methanol extract from seeds of *S. humilis* did not have any toxicity. On the other hand, methanol extract of *A. acapulcencis* induced changes in nematode's behavior such as sudden movements followed by lethargy. According with Swatlosky et al. [3] this extract can be considered like "not acutely toxic" because its lethality after 24h (1mg/mL) was only 35%. Finally, although methanol extract of *B. alicastrum* leaves caused 52.3% of lethality at 1mg/mL, an extract obtained from its bark was more toxic (76%).

Acknowledgements: H. Parra-Delgado thanks financial support from PRODEP.
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P-35

Brosimum alicastrum: From ethnomedical use to chemical analysis

Garcia-Ruelas MC[1], Parra-Delgado H[1], Peraza-Campos AL[1], González-González R[1], Chapula-Iñiguez M[1], Ventura-Salazar B[1], Torres-Colín R[2]

[1]Facultad de Ciencias Químicas, Universidad de Colima, Campus Coquimatlán, Km 9, Carr. Colima-Coquimatlán, CP 28400, Colima, México; [2]Herbario Nacional de México (MEXU), Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, Distrito Federal, México

Brosimum alicastrum Swarts, known as "mojo" in Mexico, is a multi-purpose species used as forage, food and medicine (Table 1) [1].Because of there is no chemical knowledge about their bioactive metabolites, we prepared the ascending polarity extracts (hexane, ethyl acetate and methanol) of seeds, leaves and bark of *Brosimum alicastrum.* Chromatographic analysis of the methanol extract seeds allowed the isolation of sucrose, fructose and glucose besides glycerol. Phytochemical screening of the bark showed the presence of tannins and coumarins. Furthermore, flavonoids tannins, and sterols were detected in leaves and β-sitosterol was isolated from its hexane extract. Each isolate was identified by ^{1}H and ^{13}C-NMR, IR and MS. On the other side, antioxidant activity, measured by DPPH method, was detected in methanol extracts from leaves

and bark [2].

Table 1

Part of tree	Ethnomedical uses
Seeds	Lactogen
Leaves	Treatment of diabetes as well as respiratory diseases
Bark	Treatment of diabetes, tonic, anti-inflammatory remedy

Acknowledgements: H. Parra-Delgado thanks financial support from PRODEP.
References: [1] Sánchez V.L. et al. (2004). Forest Ecology and Management. 198: 401–404. [2] Argumedo DR, Parra-Delgado H et al. (2003) Rev. Soc. Quím. Méx. 47: 167-172.

P-36

Intestinal absorption and metabolism of *Epimedium* flavonoids in osteoporotic rats

Chen Y[1], Zhou J[1], Wang Y[1,2], Qu D[1], Liu C[1]

[1]Key Laboratory of New Drug Delivery System of Chinese Materia Medica, Jiangsu Provincial Academy of Chinese Medicine, 100 Shizi Road, Nanjing 210028, Jiangsu, China; [2]College of Pharmacy, Jiangsu University, Zhenjiang 212013, Jiangsu, China

Herba Epimedii is a famous Traditional Chinese Medicine for treatment of osteoporosis in the Orient [1–3]. In the perspective of modern medicine, the main pharmacological ingredients were *Epimedium* flavonoids [4]. According to the previous research in our group, *Epimedium* flavonoids were able to transform into secondary glycosides mediated by intestinal flora and enzymes, leading to enhanced absorption and anti-osteoporosis activities. Current studies seemed over-focus the health status animal model, however, the pathologic models still underemphasized. Herein, we investigated the intestinal absorption and metabolism of five *Epimedium* flavonoids in osteoporosis rats. In order to compare the metabolic rates of the parent drug in various models, intestinal absorption and metabolism of flavonoids was evaluated by *in vitro* co-incubation with rat intestinal enzyme and intestinal flora at 37 °C and *in situ* intestinal perfusion. In addition, UPLC/Q-TOF-MS technology was also employed to determine the chemical structures of corresponding metabolites. The results showed that the metabolic rates of icariin, epimedin A, epimedin B, and epimedin C in osteoporosis group were noticeably lower than that of normal group both in intestinal flora and enzyme incubation. More importantly, compared to the normal group, the permeability coefficients and elimination percentages in intestinal perfusion of icariin, epimedin A, epimedin B, and epimedin C in osteoporosis group were decreased significantly. The metabolites of icariin, epimedin A, epimedin B, epimedin C in intestinal flora and enzyme incubation and infuse samples were unanimous identical. In summary, the intestinal absorption and metabolism of *Epimedium* flavonoids glycosides in the pathological state are slower than that in the healthy state. The potential mechanism might be resulted from affecting the intestinal flora composition and intestinal enzyme activity, which further influence intestinal absorption and metabolism of *Epimedium* flavonoids.

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P-37

Protective effects of extracts from South African *Agathosma* and *Helichrysum* species against aflatoxin B1 mutagenicity

Chokoe PKP[1,2], Botha CJ[2], Elgorashi EE[1]

[1]Toxicology Section, Agricultural Research Council - Onderstepoort Veterinary Institute, Private Bag X05, 0110, RSA; [2]University of Pretoria, Faculty of Veterinary Science, Department of Paraclinical Sciences, Private Bag X04, Onderstepoort, 0110, RSA

Few natural compounds have been found to inhibit aflatoxin-induced mutagenesis and this has fuelled research to find extracts/pure plant compounds with more potent protective effects from plants endemic to South Africa [1-3]. The potential of crude methanolic extracts of 10 *Agathosma* and 10 *Helichrysum* species to lower the genotoxic effect of aflatoxin in bacterial and mammalian cells was evaluated using the Vitotox® assay, Ames test and micronucleus assay. None of the *Agathosma* plant extracts showed strong antimutagenic activity in any of the genotoxicity assays employed. Extracts from 3 *Helichrysum* species exhibited good antigenotoxic activity in the Vitotox® assay and Ames test (using *Salmonella typhimurium* TA98 and TA100, with and without metabolic activation). However, only 2 of these extracts were found to decrease the occurrence of micronuclei in the C3A hepatocellular carcinoma cell line by more than 50% in the highest concentration of extract used. From these results, only one plant (*Helichrysum odoratissimum)* exhibited excellent bioactivity that mitigates the genotoxic effect of aflatoxin B1 in both bacterial and mammalian cell cultures, with no evidence of cytotoxicity or subsequent genotoxcity. This plant is a good candidate for further exploitation and isolation of antigenotoxic compounds that hinder the mutagenicity of aflatoxin B1, which can be used as parent compounds for animal feed additives and/or nutraceuticals for human use.

Acknowledgments: The National Research Foundation, Department of Science and Technology and Agricultural Research Council are duly acknowledged for funding the study.
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P-38

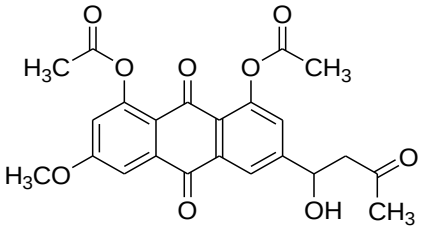
A new anthraquinone from the Peruvian Andean lichen *Xanthomendoza mendozae*

Condor-Cuyubamba EA[1], Soriano MDPC[2], Garcias-Morales C[3], Ariza-Castolo A[3], Schmeda-Hirschmann G[2]

[1]Laboratorio de Productos Naturales, Instituto de Química, Facultad de Ciencias, Universidad de Ingeniería, Lima, Perú; [2]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [3]Departamento de Química, Cinvestav, Av. IPN 2508, Zacatenco, México D.F., 07360, México

Recently, the number of lichen species worldwide has been estimated to include about 28,000 taxa. Parmeliaceae is regarded as the largest lichen family with more than 2,726 species distributed in southern Africa, South America and Australia [1]. Peru is known as one of the world´s 12 “megadiverse” countries, and it hosts about 10 % of the world plant species. The geographical and climatological conditions in Peru are unique and the biodiversity include little investigated and still unrecorded species. A new anthraquinone (**1**) and physcion (**2**) have been isolated from the Peruvian lichen *Xanthomendoza mendozae* (Räsänen) S. Kondr. & Kärnefelt collected in the Andean region of Jauja, Junin, at 3,200 m.o.s.l. The compounds were isolated after acetylation and the new compound structure was elucidated using multidimensional NMR spectroscopic methods and confirmed by single crystal X-ray diffraction [**2**: orthorhombic, *Pnna*, *a* = 856.8(10) *b* = 2122.7(4)

c = 2174.3(2) pm]. The chemical structure of Physcion was compared with literature data [3]. *X. mendozae* is recorded as a new species for Peru.



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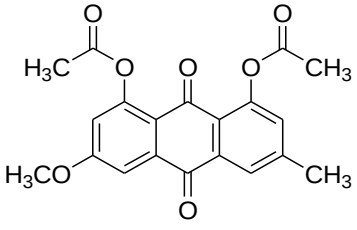

2

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P-39

Chemical variability of the *Croton funckianus* based in phytoplasma infection and collection sites

Coy A, Gómez D, Castiblanco A, Cely W, Guerrero C, Coy ED

School of Science, Universidad Militar Nueva Granada, AA 49300, Cajicá, Colombia. InQuiBio Group Research

The genus *Croton* (Euphorbiaceae), reports several uses in traditional medicine and has been studied in recent years due to the variety of biosynthesized chemical compounds, highlighting the presence of labdane (type of diterpene), cyclitols, triterpenoids, steroids, flavonoids and phenolic substances, their main feature is a wide range of biological activities [1], justifying the importance of their study in terms of bioprospecting [2]. Furthermore, trees of the genus *Croton* located in Bogotá, Colombia, presented symptoms associated with the presence of phytoplasma that dramatically affect their morphology and alter the biochemical composition of plants by stimulating the synthesis of secondary metabolites, as part of defense responses or as a result of infection [3]. The present study showed the preliminary phytochemical analysis, and results from fourteen ethanolic extracts (from healthy and host plants collected in three sites near to Bogotá), using the HPLC technique in order to study the possible changes in the production of secondary metabolites according to profiles obtained.

Acknowledgements to Universidad Militar Nueva Granada, project 1473 by financial support.
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P-40

Antioxidant capacity based in total phenolic compounds and total flavonoids from *Croton funckianus*

Coy A, Gómez D, Castiblanco A, Cely W, Guerrero C, Coy ED

School of Science, Universidad Militar Nueva Granada, AA 49300, Cajicá, Colombia. InQuiBio Group Research

Croton genera are characterized by content mainly flavonoids and phenolic compounds with promising biological activities, highlighting isoflavones and flavones hydroxylated wich are the compounds with verify antioxidant activity [1]. *Croton funckianus* is a tree located in sites near to Bogotá, with easy access, and a lot of individuals. This research focuses on finding target species with high antioxidant capacity, fourteen extracts collected in three sites were evaluated by the conventional method of DPPH [2], obtaining results with IC_{50} values (between 10 and 18ppm), using a statistical package PRISM 6.0, moreover present us the total content of flavonoids and phenolic compounds using conventional methodologies, showed similar values for all the extracts. This study confirms that *croton* can be a species that produces compounds with potential antioxidant activity and that can be used as alternative therapeutic agents in diseases currently affecting the world population.

Acknowledgements to Universidad Militar Nueva Granada, project 1473 by financial support.
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P-41

Evaluation of antiproliferative and antioxidant effect of isoprenoids for potential chemopreventive and/or chemotherapy use

Crespo R, Lavarías S, Soberon V, Galle M, Rodenak Kladniew B, Montero Villegas S, Castro A, Polo M, M García de Bravo

INIBIOLP (CCT-La Plata-CONICET-UNLP) Calles 60 y 120, CP 1900, La Plata, Argentina

Isoprenoids are naturally occurring organic chemicals, derived from five-carbon isoprene units, and they are present in the essential oils (EOs) of many aromatic plants. They have attracted interest for its potential antitumor and antioxidant properties. The objective of our work is to evaluate the effect of several monoterpenes as geraniol, limonene, cineole and linalool, and *Citrus reticulate* and *Lippia alba* EOs, in the cell cycle progression of tumor cells, as well as its antioxidant enzymatic activity. Several assays were performed on lung epithelial (A549) and hepatoma (HepG2) human cell lines. Cell viability and proliferation was evaluated by MTT assays and trypan blue exclusion method, cell cycle progression was analyzed by flow cytometry, effects on DNA integrity was assessed with the comet assay and lipid peroxidation by TBARS assay. Superoxide dismutase, catalase and glutathione - S -transferase activities were determined by spectrophotometric analyses. Our results showed that at low levels of these natural compounds, cell viability was increased while DNA damage, lipoperoxidation and the activity of the three enzymes analyzed were not induced. But at high doses, we found that they imposed oxidative stress, damaged DNA and lipid peroxidation, and inhibit cell cycle progression with an arrest occurring at the G0/G1 interphase. These data suggest that isoprenoids possess both pro- and antioxidative effects and they should be used as cytoprotective and/or cytotoxic agents.

P-42

Screening and identification of neuroprotective compounds relevant to Alzheimer's disease from medicinal plants of S. Tomé e Príncipe

Currais A[1], Chiruta C[1], Goujon-Svrzic M[1], Costa G[2], Santos T[2], Batista MT[2], Paiva J[3], Madureira MC[3], Maher P[1]

[1]The Salk Institute for Biological Studies, Cellular Neurobiology Laboratory, 10010 N. Torrey Pines Rd., La Jolla, 92037 CA, USA; [2]Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, and Center for Neurosciences and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal; [3]Centre for Functional Ecology, Faculty of Sciences and Technology, University of Coimbra, 3001-455 Coimbra, Portugal

Alzheimer's disease (AD) is strongly associated with brain inflammation, and long-term use of anti-inflammatory drugs reduces the risk of the disease. In S. Tomé e Príncipe (STP), several medicinal plants are used both for their positive effects in the nervous system and their anti-inflammatory properties. The goal of this study was to determine whether a phenotypic cell-based screening approach can be applied to identify natural compounds of interest for AD therapeutics from selected plants of STP (*Voacanga africana*, *Tarenna nitiduloides*, *Sacosperma paniculatum*, *Psychotria principensis*, *Psychotria subobliqua*). Plant extracts were tested in a panel of screening assays that reflect multiple neurotoxicity pathways relevant to AD - glutamate toxicity in hippocampal cells, in vitro ischemia, intracellular amyloid toxicity, inhibition of microglial inflammation and nerve differentiation. HPLC fractions from the extract that performed the best were tested and the most protective fraction was analyzed by mass spectrometry. The predominant compound was purified, its identity confirmed by ESI mass spectrometry and NMR, and then tested in the screening assays. Our data show that an extract from *V. africana* was more protective than any other plant extract. The most protective HPLC fraction of the extract revealed the alkaloid voacamine as the predominant compound. Voacamine was very protective at low doses in all of the assays. The findings validate the use of our phenotypic screening cell-based assays to identify potential compounds to treat AD from plants with ethnopharmacological relevance. Our study identifies voacamine as a major compound in *V. africana* with potent neuroprotective activities.

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Study of antiproliferative activity of extracts C*aryocar brasiliense* cambess

de Paula Jr W[1,2,3], Santos CAM[3], Weffort-Santos AM[3]

[1]Department of Pharmacy, Santo Agostinho College, Montes Claros, MG, Brazil; [2]State University of Montes Claros, Montes Claros, MG, Brazil; [3]Pharmaceutical Sciences Post-graduation Program, Federal University of Paraná, Curitiba, PR, Brazil

Caryocar brasiliense Cambess is a widely distributed Brazilian cerrado tree species. Its fruits are popularly known as piqui. The oil extracted from the fruits has several applications in the cosmetic industry and has been used against influenza and lung diseases. Leaves infusion has been used for liver disorders. Molluscicidal activity of the crude extract against *Biomphalaria glabrata*, trypanosomicidal activity of the ethanol extract against *Trypanosoma cruzi* and antifungal activity of the oil have also been reported. Its constituent oleanolic acid inhibits the growth of mouse sarcoma 180. The objective of this study was to investigate the activity of *Caryocar brasiliense* leaf extracts on two cell lines: Mc Coy and Hela. *C. brasiliense* leaves were collected in the region of Montes Claros, dried at room temperature and in an oven at 50°C, crushed and subjected to percolation in 70% ethanol. The material obtained was taken to dryness under reduced pressure and then lyophilized. The extract was stored in the refrigerator protected from light until ready to use. The

following crude extracts concentrations were prepared in RPMI medium 0.1, 1, 10, 100, 250, 500 and 1000µg/mL. The solutions were filtered through 0.22 µM filter and used for the tests. Mc Coy cells and Hela cells at the concentration of 106 for 100 µl of culture were distributed in 96-well plates. After 24 hours, cells were treated with different concentrations of *C. brasiliense* extract. After 24 hours of treatment, cell viability was assessed by the Neutral Red method. Untreated cells and ells treated with DMSO were used as controls, negative and positive, respectively. All assays were performed in triplicate. The results show that any concentration tested showed activity against Mc Coy cells. These cells are fibroblasts strains, adherent and constitute themselves as normal cells. The extracts showed activity against Hela cells at all concentrations; the effect was dose-dependent. Hela cells are derived from cervical cancer while Mc Coy cells are considered as normal. These results suggest selectivity of extracts against malignant cells and may be useful in developing a therapeutic agent without affecting normal cells of the human body.

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ESI-MS metabolic fingerprinting for classification of plants known as “malva” in Brazil by PLS-DA

Martins CAF, de Paula Jr W, Ribeiro RP, Zaramella IF, Piantavini MS, Pontarolo R

Universidade Federal do Paraná, Avenida Pref. Lothário Meissner, 632, 80210-170, Curitiba-PR, Brazil

Malva sylvestris, popularly known as “malva” in Brazil, is one of the most widely used anti-inflammatory herbal medicine [1]. However, according to the local name, 21 other plants are also know as ”malva” in Brazil, such as *Sida cordifolia* (“malva-branca”) and *Pelargonium graveolens* (“malva-cheirosa”). Thereby, it is necessary adequate identification methods to prevent that different species are provided as *M. sylvestris*. Direct injection mass spectrometry coupled with chemometric analysis can be a useful method for the recognition of species, providing a fast characterization of complex mixtures [2]. The aim of the study was to apply this approach to classified commercial samples of “malva” using PLS-DA. Commercial samples (dried leaves) of “malva” (n=60) were purchased and were originally labeled as: *M. sylvestris* or “malva” (n=49), *S. cordifolia* or “malva-branca” (n=7) and *P. graveolens* or “malva-cheirosa” (n=4). The samples were milled and the powder was extracted with methanol 70% (25 mg/mL), for 90 min in ultrasonic bath. The extract was filtered (0.22 µm pore), diluted 1/20 with methanol and injected in the ESI-MS (API 3200, Applied Biosystems®). Positive ion mass spectra were recorded in the range m/z 100–900 and the MS parameters were: IS: 4500 V; EP: 6 eV; DP: 40 eV; CUR: 10 psi; GS1: 15 psi. Chemometric analysis was using PLS Toolbox, version 6.5 (Eigenvector Technologies) under MATLAB environment, version 7.13 (The Math-Works, Natick). The identity of all samples was confirmed by microscopic morpho-anatomical analysis and compared with reference standards (voucher material was retained in the Museu Botânico de Curitiba-Brazil). The spectra showed no significant differences among the samples; however, PLS-DA analysis applied to spectral data identified three clusters. Among the samples labeled as M. sylvestris, only 37% were classified as *M. sylvestris* by chemometrics analysis and morpho-anatomical confirmation. Overall, the results show the application of ESI-MS and chemometrics in the differentiation of “malva” species and verification of unconformity in commercial samples.

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Traditional herbal remedies used by women in a rural community in South Africa for the treatment of gynaecology and obstetrics complaints

De Wet H, Ngubane SC

[1]Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa

According to the World Health Organization, there are annually 350 000 maternal deaths in sub-Saharan Africa, with obstetric haemorrhage the most common cause of death [1]. Although maternal mortality can be reduced by health-care interventions such as the provision of family planning, maternity care and access to safe abortion practice, it is not happening in rural areas. Previous studies in a rural community in northern Maputaland (South Africa) had indicated the importance of medicinal plants in their primary health care-system [2-4]. However, no survey has been done in this region to document the medicinal plants used to treat various gynaecological and obstetric problems. The aim of the study was to conduct an ethnobotanical survey, focussing on lay people's knowledge on plants used to treat gynaecological and obstetrics complaints. A total of 70 lay people were purposively interviewed using structured questionnaires. Thirty two plant species from 21 families were recorded for the treatment of 19 different gynaecological and obstetric disorders. Sixteen new plant species were found to be recorded for the first time in literature to treat gynaecological and obstetric disorders, and twelve new Zulu vernacular names were documented. The three most treated gynaecological conditions were dysmenorrhoea by 21 plant species, infertility (14 species) and menorrhagia (10 species), whereas blood purification (14 species), to ease labour (9 species) and to induce abortion (2 species) were the most mentioned for obstetric conditions. *Bridelia cathartica* was the most cited plant species for treating both gynaecological and obstetric problems. This wealth of new knowledge gained with the current survey reinforces the importance of documenting lay people's indigenous medicinal plant knowledge in rural communities.

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Identification of murtilla genotypes that inhibit the activity of glycogen phosphorylase A

Delporte C, Arancibia-Radich J, Bugueño I, Peña-Cerda M

Laboratorio de Productos Naturales, Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Santos Dumont 964, Santiago, Chile

The aim of this study was to demonstrate the influence of the genotype on the inhibition of glycogen phosphorylase a (GPa), activity carried out by ethanolic extracts (EETs) obtained from *Ugni molinae* (Myrtaceae) leaves. Methodology: GPa (85 ng) activity was measured in the direction of glycogen

synthesis by the release of phosphate from glucose-1-phosphate at 22 °C in 100 mL of buffer containing 50 mM Hepes (pH 7.2), 100 mM KCl, 2.5 mM EGTA, 2.5 mM MgCl2, 0.5 mM glucose-1-phosphate, and 1 mg/mL glycogen. Phosphate will be measured at 620 nm, 20 min after the addition of 150 mL of 1 M HCl containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green. The significance of the results was determined by Tukey test. The results show that there are significant differences at the fifty inhibitory concentrations (IC_{50}) of the GPa between leaves of different genotype. Of the ten genotypes evaluated, the EET of the 19-1 genotype showed the highest inhibitory effect on GPa (IC_{50} = 1.03 µg/mL). By contrast, the EET of 19-1 ha genotype had the lowest effect (IC_{50} = 3.53 µg/mL). Differences in the triterpenoids profile between genotypes could explain the results obtained.

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Characterization of *Jatropha curcas* seed oil and biodiesel obtained in Cuba

Díaz M[1], Milián YE[1], Cepero L[1], Martin GJ[1], Baena LM[2], Guerrero GE[2]

[1]Experimental Station of Pastures and Forages "Indio Hatuey". Central España Republicana, CP 44280, Matanzas, Cuba; [2]Universidad Tecnológica de Pereira, Colombia

Jatropha curcas is a multipurpose species, with uncountable attributes and remarkable potential. It is a plant of tropical origin, from the *Euphorbiaceae* family [1] and has been considered one of the most promising oilseeds for biofuel production [2, 3]. Also is used as a folk remedy in many countries for the treatment of various diseases [4]. The aim of the present work was the characterization of *Jatropha curcas* seed oil and biodiesel obtained in Cuba. Contents on saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of the seed oil and biodiesel were determined using Gas Chromatography/Mass Spectrometry (GC-MS) after methyl derivatization. PUFA contents were almost the half of the fatty acid methyl esters detected in Biodiesel. In seed oil, levels of MUFA were higher (42.5 % oleic acid) than other type of fatty acids. Furthermore, biodiesel presented high amount of linolenic acid (36.5%). Refractive index at 25°C was 1,4690 for seed oil and 1,4601 for biodiesel. Acidity index was 2,1964 and 1,4402 mg KOH/g for oil and biodiesel, respectively. According to the fatty acid composition of the oil, jatropha is the most promising oil seed for biodiesel production in Cuba and the plant or its constituents can be used directly as a model of pharmacologically active compounds.

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Identification of fatty acids in extracts from *Morus alba* roots

Díaz M[1], Lugo Y[1],Takahashi JA[2]

[1]Experimental Station of Pastures and Forages "Indio Hatuey". Central España Republicana, CP 44280, Matanzas, Cuba; [2]Universidade Federal de Minas Gerais. Departamento de Química. Laboratório de Biotecnologia e Bioensayos. Av. Antonio Carlos, 6627 CEP 31270-901, Belo Horizonte, MG, Brazil

The *Morus* genus constitutes an important source of bioactive substances of diverse chemical nature [1-5]. For the study, 7 varieties and hybrids of *Morus alba* were used (Tigreada, Indonesia, IZ-40, IZ-64, IZ-13/6, IZ-15/7 and IZ-56/4). The dried roots were pulverized and the secondary metabolites were extracted with hexane. The composition of fatty acids was determined in the extracts through gas chromatography. The fatty acids which constituted majority were linoleic acid (41.55-49.69%) and palmitic acid (20.10-30.25%). The saturated fatty acids oscillated between 31.41 and 41.19%. The Tigreada variety was the one with higher values, palmitic acid being the most abundant. The monounsaturated fatty acids were found in the range from 7.07 to 13.07% and the variety IZ-40 showed the highest percentage, with predominance of omega 9 (oleic acid). The polyunsaturated fatty acids were present between 48.28 and 55.01% and the abundance of omega 6 (linoleic acid) stood out in the Indonesia variety. The hybrid IZ-56/4 showed the highest value of omega 3 (linolenic acid) with 10.90%. In all the varieties and hybrids minority fatty acids were found, such as lauric acid, myristic acid, pentadecanoic acid, heptadecanoic acid, arachidic acid and stearic acid. The hexanic extracts of mulberry are concluded to show essential fatty acids important in human nutrition (Omega 3, 6 and 9), mainly linoleic acid (omega 6). The utilization of *Morus alba* is suggested as a healthy alternative to enrich the animal and human diet, as a nutritional supplement or a nutraceutical.

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Ethnobotany, phytochemistry and pharmacological activity of *koas*, aromatic plants used as incense (*sahumerios*) in northern Chile

Echeverría J, Santos P, Niemeyer HM

Facultad de Ciencias, Universidad de Chile, Casilla 653, Las Palmeras 3425, Santiago, Chile

Plant-derived smoke is used in medicinal as well as ritual contexts by many cultures worldwide. These two aspects of smoke, pharmacological and cultural, can blend or intertwine, for example in the ancient use of tobacco as incense, medicine, and for religious purposes. Ethnographical studies in the Chilean Andean highlands have shown the use of a variety of aromatic plant species, generically denominated *koas* as incense or *sahumerios* by Atacameño and Aymara communities [1]. The *koa* concept in the central Andes integrates various aromatic and resinous bushes of the Asteraceae and Solanaceae whose smoke is used during certain ritual ceremonies. The aims of this work were: i) to establish the aromatic nature of the species by obtaining and analyzing their

essential oils, ii) to determine the composition of the smoke generated by the combustion of different *koas* using a dynamic headspace sorption method for the collection of smoke samples, and to relate it to the pharmacological activity of its components, and iii) to analyze the combustion residues to identify chemical markers for the use of different *koas* in archaeological residues. Chemical results will be presented and the pharmacological properties of the substances identified discussed.

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Plants smoked by pre-Hispanic populations of central Chile: chemical and pharmacological aspects

Echeverría J, Espinoza S, Niemeyer HM

Facultad de Ciencias, Universidad de Chile, Casilla 653, Las Palmeras 3425, Santiago, Chile

Several plant species are known to have been used in smoking practices by prehispanic inhabitants of central Chile. Archaeobotanical studies have shown the presence of seeds and plant micro-remains attributed to *Datura* sp. and *Nicotiana* sp., and chemical studies the presence of nicotine, both in residues of smoking pipes from various archaeological sites [1]. Besides the use of these psychoactive alkaloid-containing plant species, ethnohistorical and ethnographical studies have reported the use of species that act as adjuvants in the smoking practices [2-3]. The purpose of these elements is unclear; they have been interpreted as conferring softening and flavouring characteristics, or otherwise enhancing the effect of alkaloidal and/or non-alkaloidal active compounds. The aims of this work were: i) to assess the aromatic nature of the species reported as adjuvants by obtaining and analyzing their essential oils; ii) to identify the components of the smoke generated by the combustion of these species and to explore correlations with their reported pharmacological activities; iii) to study their alkaloidal content to judge their potential for enhancement of psychoactivity, iv) to analyze their combustion products to identify chemical markers for their presence in archaeological residues, and v) to use these markers to assess their use in artifacts of the smoking complex from various archaeological sites in central Chile. Chemical results will be presented and the pharmacological properties of the substances identified discussed.

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Chachacoma (*Senecio graveolens*): an altiplanic plant with anticancer and antimicrobial properties

López N[1], Alfaro-Lira S[1], Salas C[2], Cuellar M[3], Santander J[4], Brown N[5], Echiburú-Chau C[1]

[1]Centro de Investigaciones del Hombre en el Desierto (CODECITE-CIHDE), Arica 1000007, Chile; [2]Departamento de Química Orgánica, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago 6094411, Chile; [3]Ciencias Químicas y Recursos Naturales, Facultad de Farmacia, Universidad de Valparaíso, Gran Bretaña 1093, Playa Ancha, Valparaíso, Chile; [4]Nucleus for Microbiology and Immunity, Faculty of Sciences, Universidad Mayor, Santiago 8580745, Chile; [5]Escuela de Medicina, Universidad de Talca, Talca 3600000, Chile

The incidence of breast cancer has increased worldwide during the last decade [1]. At present, the development of resistance to anti-cancer drugs is the main driving force for the continuous search of novel therapeutic alternatives [2]. We became interested in the potential chemotherapeutic benefits of phytochemical compounds derived from *Senecio graveolens,* known as Chachacoma. Chachacoma is a herb belonging to the Asteraceae family occurring in the Andean highlands at altitude over 3500 masl. It is frequently used to ameliorate high-altitude-associated sickness. Ethanolic extract (200 µg/mL) and fractions (50 µg/mL) from *S. graveolens* were tested on breast cancer cell lines ZR-75-1, MCF-7, MDA-MB-231 and the normal counterpart MCF-10F, under hypoxia (1% O_2) and normoxia. The crude extract (LD_{25}) led to a dramatic reduction of the viability of cancer cells (5% survival) compared to normal cells (75% survival). Interestingly, extract-associated cytotoxicity was increased in MCF7 and ZR-75-1, cells with low expression of MnSOD. In contrast, MDA-MB-231 which were resistant to the extract, displayed high expression of MnSOD. Electron microscopy and western blot analysis revealed that the cytotoxicity elicited was associated with a mixture of autophagy, apoptosis and necrosis. The most polar fraction was identified as the responsible of exert such effect. On the other hand, we also found that main compound of *Chachacoma*, 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone, has antibacterial activity against Gram-positive but not against Gram-negative bacteria. This compound seems to interrupt cell division through disruption of the cell wall and division septum. The anti-cancer properties displayed by the crude extract and antibacterial activity showed by 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone, point to *S. graveolens* as a potential source of new drugs.

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Phenolic compounds and antioxidant capacity of grape cane extract produced at bench scale

Escobar D[2], Bustamante L[1], Riquelme S[2], Ruiz MA[1], Jara P[3], von Baer D[1], Lamperti L[3], Fuentealba C[2], Vergara C[1], Berg A[2], Mardones C[1]

[1]Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Casilla 160-C, Concepción, Chile; [2]Unidad de Desarrollo Tecnológico UDT, Universidad de Concepción. Av. Cordillera Nº 2634 - Parque Industrial Coronel - Coronel. Código Postal: 4191996. Casilla 4051, Correo 3, Concepción - Chile; [3]Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción, Casilla 237, Concepción, Chile

The evidence of the health benefits of *trans*-resveratrol has increased during the last twenty years. This fact has triggered the interest in studying the levels of this and other stilbenes in grapes, wine

and winemaking residues. In Chile, more than 100.000 tons of winemaking residues are produced each year, including pomace, seeds, stalks and canes. In this research, profiles and levels of stilbenes in these waste products are studied by HPLC-DAD-ESI-MS/MS. Main results show that □-viniferin is the main stilbene in these matrixes, followed by *trans*-resveratrol, except for canes, where *trans*-resveratrol was higher. Total stilbenes (expressed as *trans*-resveratrol equivalents in dry matter) were 22.81±10.59 mg/kg; 5.17±2.02 mg/kg; 0.93±0.44 mg/kg and 4485.87±200.28 mg/kg in stalks, pomace, seed and cane, respectively. Considering these results, a stilbene rich extract was produced at bench scale (7 L reactor) using canes. The extract obtained under optimal conditions was lyophilized and showed 38.7 % of total lignin, 27.5% of carbohydrates, 6.7% of proteins, 1.03% of metals, and 11.80 % of phenolic compounds. Between these, mainly stilbenes, flavan-3-ols, procyanidins and some flavonols were predominant. The concentration of these families was respectively 4.60%, 4.84%, 2.11% and 0.25% in basis dry matter. The antioxidant capacity of this extract was studied using cell free and cell based antioxidant assays (TEAC, ORAC, CUPRAC and DCF-DA).The results showed that the crude extract have reducing and protection capacity, however, synergic and antagonistic behaviors are evidenced due to the complex composition of the extract.

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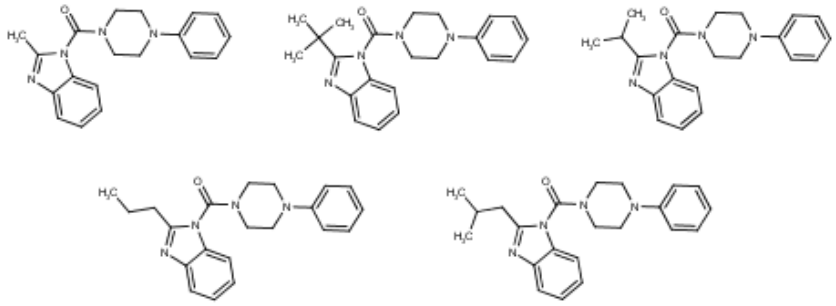
Synthesis of Benzo [d] imidazoles with potential activity in FAAH enzyme

Escobar-Valderrama R, Faúndez-Cáceres M, Pessoa-Mahana D

Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile. Vicuña Mackenna 4860, Casilla 306, Santiago 22, Chile

Fatty Acid Amide Hydrolase (FAAH) is an intracellular serine hydrolase responsible for anandamide degradation which is a potent agonist of the CB_1 receptor, and other endogenous ligands. It is secreted in postsynaptic terminals and their inhibition is linked to relief of pain, inflammation, depression, anxiety disorders, etc. Inhibition of this enzyme, as a therapeutic strategy, decreases central effects of exogenous ligands that activate the CB_1 receptor [1-2]. Based on this, isosteric changes to FAAH cognate ligands containing a benzisoxazol ring bioactive core was proposed. The design of a new series of five benzoimidazole-based non-competitive FAAH inhibitor compounds is presented.



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Toxicological evaluation of aqueous extract of *Aloe ferox* Mill. in loperamide-induced constipated rats

Wintola OA, Sunmonu TO, Afolayan AJ

Medicinal Plant and Economic Development Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa

Aloe ferox Mill. is a widely used medicinal plant in South Africa for the treatment of many ailments including constipation [1]. Herbal remedies are commonly employed in developing countries for the treatment of various diseases including constipation. The rationale for utilization of medicinal plants rested largely on the belief that they are safe and free of side effects [2].The present study evaluated the toxicological effect of aqueous leaf extract of the herb at 50, 100 and 200 mg/kg body weight for 7 days on the haematological parameters as well as liver and kidney function indices in loperamide–induced constipated rats. The extract did not cause any significant ($p > 0.05$) effect on the kidney and liver–body weight ratio as well as the kidney function indices including serum levels of creatinine, uric acid, urea, calcium and potassium ions at all the dosages investigated. Whereas the serum levels of total protein, albumin, bilirubin and gamma glutamyl transferase (GGT) were not affected, the elevated activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in the untreated constipated animals were normalized following treatment with extract. The data obtained with respect to the haematological analysis indicated that the extracts had no significant ($p > 0.05$) effect on the haematological parameters with the exception of lymphocyte count which was increased in the untreated constipated rats. This was however attenuated after administering the herb. The available evidence in this study suggests that *A. ferox* may be safe as an oral remedy for constipation. Generally, the effect of the extract compared favourably well with senokot, a recommended drug for the treatment of constipation.
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Cardiovascular effects of (E) - methyl isoeugenol isolated from *Pimenta pseudocaryophyllus*

Fajemiroye JO, Silva EF, Oliveira TS, Oliveira LP, Ghedini PC, Pedrino GR, Costa EA

Department of Physiological Sciences, Federal University of Goiás, Campus Samambaia, 74001-970, Brazil

Leaf preparations of *Pimenta pseudocaryophyllus,* popularly known as cataia, are being used as diuretics in Brazilian communities. Diuretics decrease blood volume, venous pressure, cardiac filling, ventricular stroke volume, cardiac output, systemic vascular resistance to elicit a fall in arterial pressure. Since (E) - methyl isoeugenol (MIE) constitutes 93.7 % of *Pimenta pseudocaryophyllus* leaf essential oil, we sought to investigate hypotensive property of MIE and the involvement of central and/or peripheral mechanism (s). The effects of MIE on parameters like mean arterial pressure (MAP), heart rate (HR), systolic blood pressure (SBP), baroreflex sensitivity of normotensive rats and vascular reactivity were evaluated. MIE (1.11, 2.25 or 4.50 mg/kg, iv) elicited dose-dependent decrease in MAP (-16.9 ± 1.13; -19.0 ± 4.18 or -27.2 ± 3.65 mmHg,

respectively) and an increase in HR (17.4 ± 1.79; 24.4 ± 5.11 or 29.9 ± 6.62 bmp, respectively). MIE 25 or 50 mg/kg (p.o) decreased the SBP (-13.6 ± 4.18 or -16.6 ± 5.60 mmHg, respectively) without altering baroreflex function. The hypotensive effect of MIE remained unaltered by WAY100635 (antagonist of 5-HT1A) and L-NAME (NO synthase inhibitor). Intracerebroventricular injection of MIE did not change MAP. MIE induced endothelium independent vasorelaxation (endothelium-intact vessels, Emax 92.5 ± 1.75%; Endothelium-denuded vessels, Emax 91.4 ± 2.79%). $CaCl_2$ or BAY K8644 (L-type voltage gated calcium channel activator)-induced vascular contractions were attenuated by MIE. The hypotensive-vasorelaxant effects and calcium channel blockade by MIE could be a consequent of diuretic effect of sodium-water balance.

P-56

Natural food flavour (E) - methyl isoeugenol: Central effects and neural mechanisms

Fajemiroye JO, Danillo, Dayane, Martins JLR, Joelma PAM, Costa EA

Department of Physiological Sciences, Federal University of Goiás, Campus Samambaia, 74001-970, Brazil

Consumption of (E) - methyl isoeugenol (MIE) in the preparations of *Pimenta pseudocaryophyllus* leaf as a calming agent and aphrodisiac has been reported in Brazil. The present work evaluated anxiolytic - antidepressant effects and possible mechanisms of MIE's actions. Male Swiss mice were exposed barbiturate sleep, light dark box - LDB, elevated plus maze - EPM, wire hanging, open field - OF and forced swimming test – FST following MIE's treatment. Pretreatment of mice with WAY100635 (antagonist of 5-HT1A), α-methyl-p-tyrosine (AMPT; depletor of catecholamine) or p - chlorophenylalanine (PCPA; depletor of serotonin) were used to evaluate the participation of monoamine system. MIE potentiated hypnotic effect of sodium pentobarbital and elicited anxiolytic like effect in the LDB, EPM and OF. This effect was blocked by WAY100635 pretreatment. In the FST, MIE induced antidepressant like effect. Unlike AMPT, pretreatment with PCPA reversed antidepressant like effect of MIE. These results showed evidences of anxiolytic and antidepressant like properties of (E)-methyl isoeugenol and suggested the participation of serotonergic pathways.

P-57

Antiparasitic extracts from Amazonian Lauraceae

Fernandes N[1], Dias A[1], Desoti V[2], Nakamura CV[2], Veiga Jr VF[1]

[1]Programa de Pós Graduação em Biotecnologia, Universidade Federal do Amazonas Av. Rodrigo Octávio, CEP 69077-000, Manaus, Amazonas, Brazil; [2]Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo, CEP 87020-900, Maringá, Paraná, Brazil

Trypanosoma cruzi is the responsible for Chagas diseases, affecting around 15 million people throughout the American continent [1]. The development of drugs to treat this disease is a difficult task and is still a great epidemiological problem. Secondary metabolites from natural products become a necessary tool to find potential drugs against Chagas disease. Lauraceae species produce substances with a huge variety of biological activities, such as antiviral, antibacterial, antioxidant and antitumor, being commonly found to be used in human medicine [2]. *Laurus nobilis* and *Cinnamomum tamala* are Lauraceae species used in traditional medicine as antiseptic, anti rheumatic stimulant and antigonorrhoeic [3]. *Nectandra* species also inhibit growth of *T. cruzi* epimatigote forms [4]. At this study, antiparasitary activity of ethanolic extracts from leaves and stems obtained from seven species of Amazonian Lauraceae were evaluated. *T. cruzi* infective forms (trypomastigote) were obtained from the supernatants of previously infected LLCMK2 cells monolayer. The Lauraceae extracts were diluted in DMSO and DMEM for final concentration (1, 5,

10, 100, 500 and 1000 µg/mL), and incubated for 24 h at 37 oC. The viability was evaluated using the Pizzi-Brener method [5]. All extracts affect ability of trypomastigote at major concentrations (1000 and 500 µg/mL). The extracts with the highest activities (EC_{50} < 10 µg/mL) were obtained from *Aniba panurensis* (stems), *Aniba parviflora* (leaves), *Mezilaurus duckei* (stems), *Rhodostemonodapne peneia* (stems), *Paraia bracteata* (stems), *Ocotea leucoxylon* (stems) and *Licaria martiniana* (stems).These high activity extracts could be helpful in the search of new active compounds against *T. cruzi*.

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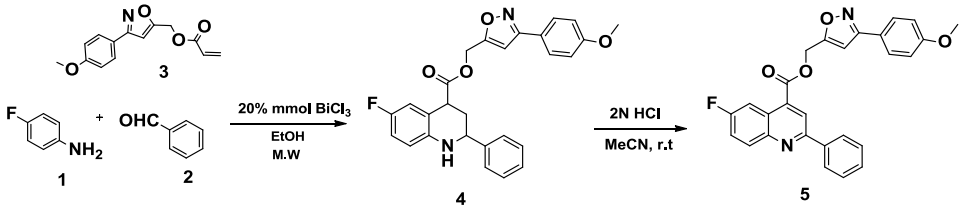
P-58

Synthesis of new tetrahydroquinolines and quinolines as potential anti-tubercular agents

Fernández CM, Astudillo LA

Laboratorio de Síntesis Orgánica, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile

Tuberculosis (TB) is a major infection that causes thousands of deaths every year. *Mycobacterium tuberculosis* is the predominant causal agent, leading to approximately 1.3 million deaths in 2012. Despite the fact that no new drug has been discovered to treat TB in the last 40 years, quinoline-based compounds are known to exhibit anti-TB properties. Moreover, fluoroquinolones, such as gatifloxcin and moxifloxacin, which target DNA topoisomerase IV and DNA gyrase, constitute potential anti-TB agents. The aim of this study is the synthesis of new chemical entities based on quinoline-isoxazol-core, by employing imino Diels-Alder reaction, which allows the use of aromatic anilines **1**, aromatic aldehydes **2** and electron-rich alkenes **3** to deliver tetrahydroquinolines **4** and quinolines **5**.



In order to obtain **(4)** in a microwave tube, the compounds **1** (1.0 equiv, 0.02 mL), **2** (1.1 equiv, 0.02 mL), **3** (1.5 equiv, 0.075 g) and $BiCl_3$ (20% mmol, 0.01 g) were added in ethanol. The compound **3** was previously synthesized through 1,3-dipolar cycloaddition reaction. The reaction was carried out under microwave radiation (P= 300 W, T^{o}_{max} = 150 °C, t = 15 min). After this course the reaction was filtered, washed with EtOH and then was characterized by mass spectrometry. To obtain **(5)**, a mixture of **4** in 2N HCl solution was carried out to stirring at room temperature for 12 h.

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P-59

In vitro antiviral activity of selected Paraguayan plant extracts and essential oils

Sotelo PH[1], Chnaiderman J[2], Martínez MJ[2], Degen de Arrua, R[1], Burgos A[1], Bazán N[1], Alvarenga N[1], Ferro EA[1]

[1]Facultad de Ciencias Químicas Universidad Nacional de Asunción, Campus San Lorenzo, San Lorenzo, Paraguay; [2]Programa Disciplinario de Virología, ICBM, Universidad de Chile, Independencia 1027, Santiago, Chile

In Paraguay, several plants are employed for medicinal purposes. Their traditional use for the treatment of infectious diseases is widely recognized by the population, but very little knowledge is available concerning the activity of medicinal and aromatic natural resources on viral pathogens.
In order to evaluate the potential antiviral activity, methanolic extracts of *Baccharis trimera* (jaguarete ka'a), *Acanthospermum hispidum* (toro râti), *Buddleja* sp (cambará) and the essential oil from *Baccharis trimera* (jaguarete ka'a), *Bulnesia sarmientoi* (palo santo), and *Piper regneli* (jaguarundi) have been studied. Plants were collected from the Jardín de Aclimatación de Plantas Mecinales, of the FCQ; and subsequently identified at the Herbarium FCQ, where voucher specimens are deposited. Methanolic extracts were obtained by cold maceration and essential oils by hydrodistilation. The citotoxicity and maximal nontoxic concentration in Vero and Ma104 cells was analyzed for all materials. The antiviral activity was measured against rotavirus, an ARN nonenveloped and herpes simplex 1 (HSV-1) a DNA enveloped virus, using plaque reduction assay. All essential oils showed antiviral activity against HSV-1, but only *B. sarmientoi* exhibited activity against rotavirus. This result could be related with the presence of a lipidic envelope in HSV-1. The extract of *A. hispidum* presents the stronger antiviral activity against HSV-1 (90% inhibition) and *B. trimera* extract against rotavirus (79% inhibition). Interestingly, *B. trimera* extract shows activity against both viruses, with a plaque inhibition of 48% for HSV-1, and 88% for rotavirus.

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P-60

Evaluation of radical scavenging capacity, LC-DAD-MS profiling and phenolic composition of extracts obtained from *Copaifera officinalis*, *Jatropha curcas*, *Cnidoscolus aconitifolius* and *Solanum nigrum* from Agua Azul, Casanare.

Velásquez Y, Plazas D, Fierro J, Orduz L, Coy-Barrera E

Bioorganic Chemistry Laboratory, Universidad Militar Nueva Granada, Cajicá-Cundinamarca, Colombia

The present study is focused on the species *Copaifera officinalis*, *Jatropha curcas*, *Cnidoscolus aconitifolius* and *Solanum nigrum* which exhibit great interest as medicinal plants by their ethnopharmacological uses and their several reported biological activity. Some of them are as antimicrobial, anti-ischemic, anti-inflammatory, antileishmanial and effect antitumor for *Copaifera officinalis [*1]; to treat cancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic to *Jatropha curcas* [2]; diabetes treatment, rheumatism, gastrointestinal disorders and inflammation-related diseases for *Cnidoscolus aconitifolius* [3] and as antiperiodic, antiphlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative, sedative, analgesic, antispasmodic, anti-inflammatory and vasodilator to *Solanum nigrum* [4]. The free radical scavenging capacity (determined by the DPPH method), phenolics and flavonoids content, LC-DAD-MS profiling of extracts from leaves, petiole, wood, steams, fruits and seeds of the four mentioned plants collected in Agua Azul, Casanare, was determined [5]. The best radical scavengers were found to be *C.*

officinalis seed-derived extract, followed by *J. curcas* and *C. aconitifolius* leaves, and *S. nigrum* steam and leaves-derived extracts, all with an IC_{50} values in the 1-10 mg/mL range. The extract with higher phenolic content was *C. officinalis* seeds (34% dry extract basis). A close relation was observed between antioxidant activity and total phenolic content, since the main antioxidant activity was observed in the high-containing phenolics extracts. The extract with higher flavonoids content was *C. aconitifolius* leaves (4% dry extract basis). Additionally, a large variability on chemical constituents was observed by LC-DAD-MS profiling between test extracts.

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P-61

LC-Based Profiling and Acaricidal Activity of *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) of Piper-derived extracts

Numa S, Rodríguez L, Fierro J, Rodríguez D, Coy-Barrera E

Universidad Militar Nueva Granada, Km 2 vía Cajicá-Zipaquirá,Cundinamarca

An important exportation-type product for Colombia is rose, and the phytophagous mite *Tetranychus urticae* is the main pest for this plant. This mite is traditionally controlled by chemical-synthesized acaricides and this fact generates negative impacts on the human health and environment as well as acaricide-resistant populations. Therefore, alternative approaches have been pursued for the management of this pest such as the use of biocontrol agents and plant extracts. As part of our research on natural acaricides, the present study was conducted in order to evaluate the effect of ethanol-soluble extracts from leaves of two piper species (*P. aduncum* and *P. elongatum*) on mortality of eggs and adults of *T. urticae* under laboratory conditions. Five treatments (two leaves-extracts at 0.6 µg/µmL, and a positive, an absolute and a relative controls) were evaluated. The administration of the treatments was conducted by direct spraying and leave-dipping. The mortality in egg and adult stages of *T.* urticae was evaluated. Both extracts exhibited pretty low activity against eggs, but the mortality on adults was >25%. Each extract was chemically characterized by measuring the total phenolics and flavonoids contents and by RP-UFLC-DAD. *P. elongatum* exhibited major contents (>1.5 mg/g DE) to that than *P. aduncum*. The LC-derived profiles of both extracts exhibited common main compounds, but *P. elongatum* showed distinctive metabolites. Although the acaricidal activity was found to be modest, the LC profiles exhibited interesting compounds that could serve in further studies.

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Berberis microphylla induced-glucose uptake stimulation in HepG2 involves AMPK signaling

Furrianca MC[1,2], Alvear M[2], Barrientos L[2], Fajardo V[1], Salazar LA[2]

[1]Departamento de Ciencias de la Salud, Universidad de Magallanes, Av. Bulnes 01855, Punta Arenas, Chile; [2]Centro de Biología Molecular y Farmacogenética, Núcleo Científico- Tecnológico en Biorecursos (BIOREN), Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco, Chile

Plants belonging to the genus *Berberis* (Berberidaceae) are of particular phytochemical interest due to their alkaloid content and composition. Folk medicine uses different species of *Berberis* to treat a variety of diseases, highlighting its hypoglycaemic value. The purpose of this study was to determine glucose consumption in HepG2 cells stimulated with a root extract from *Berberis microphylla* by AMPK activation (AMPK-activated protein kinase). Glucose consumption rate was assessed in HepG2 cells stimulated with the extract of the root of *Berberis microphylla*, using the glucose-oxidase method. To determine the extent of AMPK phosphorylation, we used the EnzyFluoTM AMPK phosphorylation assay kit (EAMPK-100). Data suggest that the extract of *Berberis microphylla* stimulates glucose uptake in HepG2 cells, by activating AMPK protein at concentrations of 10 and 1.25 x 10^{-3}µg/µl. Results of this study indicate that the analyzed extract of *Berberis microphylla* root possesses hypoglycaemic effects in HepG2 cells.

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P-63

Antileishmanial activity of a dibenzylideneacetone derivated (A3K2A3) is related to mitochondrial dysfunction in *Leishmania amazonensis*

Garcia FP[1], Din Khan Z[2], Rodrigues-Filho E[2], Ueda-Nakamura T[1], Nakamura CV[1]

[1]Departamento de Ciências Básicas da Saúde, Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil; [2]Departamento de Quimica, LaBioMMi, Departamento de Química, Universidade Federal de São Carlos, CP 676, 13.565-905 São Carlos, SP, Brazil

Leishmaniasis is a neglected disease caused by parasites of the genus *Leishmania.* An estimated 12 million people are currently infected and up to 350 million people are at risk of infection [1]. Current drugs available for this infection have many limitations including various toxicities. In order to find new potential biological agents, we have described the antileishmanial activity of A3K2A3, a sinthetic compound derivated from 1,5-diaryl-3-oxo-1,4-pentadienyl [2]. The aim of this work was to investigate the mechanism of action induced by this substance in *L. amazonensis*. For this, promastigotes treated with IC_{50} (3.4 µM) and IC90 (9.3 µM) of A3K2A3 for 48 h were fixed in glutaraldehyde, dehydrated with acetone and embedded in Epon. Ultrathin sections were obtained, stained with uranyl acetate and lead citrate, and observed on TEM. To analyse mitochondrial membrane potential, promastigotes treated were loaded with rhodamine 123 and analyzed in flow cytometer. Additionally, promastigotes treated with A3K2A3 were exposed to the fluorescent O2•−-sensitive probe MitoSOX (5 µM/ 10 min), washed with KH buffer and fluorescence was measured in a fluorimeter. Treated parasites showed various ultrastructural alterations, including mitochondrial swelling, lipid bodies accumulation and desorganization in flagelar pocket. Moreover, A3K2A3 caused a decrease in Rh123 fluorescence of 28% for 3.4 µM and 40% for 9.3 µM indicating mitochondrial depolarization and exhibited significant augmentation in superoxide anions production. It is possible to suppose that the antileishmanial action of A3K2A3 maybe involve its

effect on the mitochondrial function leading to cell death of the parasite.

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P-64

In vitro antileishmanial and cytotoxicity activities of crude extract, fractions and isolated compound of the stem bark of species *Aspidosperma macrocarpon* Mart. - Apocynaceae

Pessini GL[1], Garcia FP[2], Oliveira AJB[2], Mello JCP[2], Sant’Ana AEG[1], Nakamura CV[2], Araújo-Jr JX[1]

[1]Institute of Chemistry and Biotechnology, Universidade Federal de Alagoas, UFAL, Campus A. C. Simões. Tabuleiro dos Martins CEP 57072-970, Maceio-AL, Brazil; [2]Universidade Estadual de Maringá, UEM, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil

Species of the genus *Aspidosperma* (Apocynaceae) are restricted in Americas [1], and its stem bark is used in infusion by Amazon as folk medicine [2]. *A. macrocarpon* is known in traditional medicine as “guatambu” or “peroba-do-campo”. The aim of this study was to evaluate the *in vitro* cytotoxicity and activity against *Leishmania amazonensis* of crude extract, fractions and isolated compound from the stem bark of *A. macrocarpon*. The ethanol crude extract was prepared by maceration, and the hexane, chloroform, ethyl acetate and methanol fractions were obtained by partition. They were evaluated against promastigotes and intracellular amastigotes using several concentrations and the antiproliferative effect was determined. Additionally, red blood cell lysis assay was performed and expressed by percentage of hemolysis. The crude extract, hexane, chloroform, ethyl acetate and methanol fractions of *A. macrocarpon* showed activity against promastigotes with IC_{50} of 151.5 µg/mL; 1000 µg/mL; 29 µg/mL; 29.5 µg/mL and 173.5 µg/mL, respectively. Chloroform fraction was fractioned by chromatography on silica "flash". The isolated compounds 203 and 213 showed the best activity against promastigotes (IC_{50} 9.79 µg/mL) and intracellular amastigotes (IC_{50} 4.32 µg/mL), respectively. Aditionally the compound 203 caused only 12.4% hemolysis at 1000 µg/mL (highest concentration tested). The present study revealed that the two isolated compounds from the stem bark of *A. macrocarpon* showed activity against both forms of *L. amazonensis*.

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P-65

Analgesic activity of aqueous and methanolic extract from wild growing and *in vitro* propagated *Renealmia alpinia* (Rottb.) Maas.

Gómez-Betancur IC[1], Benjumea D[1], Fernández M[1], Cortés N[2]

[1]Ophidism / Scorpionism Program, University Research Center, Tower 2 Lab 631. Faculty of Pharmaceutical Chemistry, University of Antioquia; 70 Street No. 52-21, Medellin, Colombia; [2]Research Group for Bioactive Substances, University Research Center, Tower 2 Lab 229, University of Antioquia. Medellin, Colombia

Renealmia alpinia is a herb from the *Renealmia* genus belonging to the Zingiberaceae family. *R.*

alpinia is widely found in Neo-tropical countries where it is commonly used in ethnomedicinal preparations [1-2]. This study evaluated the analgesic activity of methanolic and aqueous extracts of *R. alpinia* prepared from wild growing plants and from plants propagated *in vitro*. Analgesic activity was examined using two models in mice: induction of pain by a chemical agent (Siegmund test) [3] and applying heat (Tail flick test) [4]. A comparative analysis of the extracts was done by High Performance Thin Layer Chromatography (HPTLC). It was shown that aqueous and methanolic extracts from *R. alpinia* cause significant dose-dependent antinociception, when evaluated using the chemical model of pain induction. Aqueous extracts obtained from wild plants at concentrations of 1 %, 5%, 10%, 15% m/v showed pain inhibition percentages of 29.7, 50.6, 55.3 and 64.7%, respectively. At the same doses of the plant obtained by *in vitro* propagation, the values were 30.6, 41.1, 58.1 and 73.1%, respectively. Percent inhibition of methanolic extract (wild) at doses of 50, 100, 200 and 300 mg/kg were 36.1, 72.2, 62.1 and 94.6%, respectively while for *in vitro* propagated *R. alpinia* the values were 25.0, 36.3, 40.4 and 54.2%, respectively. Tail flick test showed no protection from pain. It may suggest that methanolic and aqueous extracts of *R. alpinia*, obtained from wild plants and by *in vitro* propagation, have peripheral analgesic effect in mice. The HPTLC patterns allow a differentiation of the extracts. The presence of flavonoid-type compounds was detected in the extracts.

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Activity of *Passiflora caerulea* on gastrointestinal tract

Anzoise ML[1], Marrassini C[2], Bach H[3], Gorzalczany S[1]

[1]Cátedra de Farmacología; [2]Cátedra de Farmacognosia; [3]Cátedra de Farmacobotánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires, C1113AAD, Argentina

Passiflora caerulea (Passifloraceae) is a medicinal plant commonly used in traditional medicine in South America as sedative, antispamodic, diuretic, and anti-bacterial agent (1). Since other species belonging to the same family have demonstrated antiinflammatory and antidiarrhoeal activities (2,3,4), the aim of the present study was to assess the effect of ethanolic extract of *P. caerulea* on gastrointestinal tract. The extract non-competitively inhibited the cumulative concentration-response curves induced by acetylcholine and $CaCl_2$ and significantly reduced the maximal response in a concentration–dependent manner, tested at different concentrations (0.1, 0.3, 1 and 3 mg/mL). *P. caerulea* was effective against castor-oil induced diarrhoea, reducing the induction time of diarrhoea and the amount of semi solid and watery stools. Taking into account this information, *P. caerulea* (125 mg/kg, p.o) was tested on the acetic-acid induced colitis model in rats. The extract decreased colon weight and lesion score, reducing significantly the colonic myeloperoxidase activity (*P. caerulea:* 0.53 OD/mg tissue, control group: 0.79 OD/mg tissue) and TBARs levels (*P. caerulea:* 1.04 nmol/mg protein, control group: 6.83 nmol/mg protein). The phytochemical analysis indicated that the total phenol content as GAE/g of extract was 61.5 ± 0.17. These results suggest that *P. caerulea* produced a protective activity on gastrointestinal tract.

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P-67

Antihyperalgesic effect of *Lithraea molleides*

Riedel R[1], Anzoise L[1], Catalano A[2], López P[2], Gorzalczany S[1]

[1]Cátedra de Farmacología; [2]Cátedra de Farmacognosia. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires, C1113AAD, Argentina

Lithrea molleoides (Vell.) Engl. (Anacardiaceae) is a medicinal plant commonly used in traditional medicine in South America for its properties to treat inflammation of respiratory and digestive tract, as antiarthritic, diuretic and tonic (1). In previous investigations, *L. molleoides* showed a significant anti-inflammatory and antinociceptive activity in experimental models (2, 3). So, it is expected that this species possesses effect on hyperalgesic model in rats. In this sense, intraperitoneal treatment of *L. molleoides* (300 mg/kg) reduced carrageenan-induced mechanical hyperalgesia (26 % of inhibition), without any effect at dose of 100 mg/kg i.p. Mechanical withdrawal thresholds of the paw were evaluated using von Frey filaments. In the same model, only higher dose produced a significant inhibition of myeloperoxidase activity in rat paw, representing the activity of extract on neutrophil recruitment in paw rat. Furthermore, western blotting of paw rat revealed that 300 mg/kg i.p. of *L. molleoides* decreased carrageenan induced cyclooxygenase-2 expression without any effect at dose of 100 mg/kg. On the other hand, lipid oxidation products assessed by the TBARS assay in paw were significantly modified when the animals were treated with *L. molleoides*, showing that the extract possess antioxidant effect in vivo. These results suggest that *L. molleoides'* aqueous extract produced antinociceptive activity in hyperalgesic model.

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P-68

Inhibition of lipid peroxidation of red blood cells and human low-density lipoprotein oxidation by an extract of *Pitavia punctata* (R. *et* P.) Molina, an endangered and endemic Chilean tree species

Guzmán L[1], Castro R[2], Forero O[2], Soto-Cerda L[3], Lamperti L[4], Leiva E[1]

[1]Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, Talca, Chile; [2]Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [3]Facultad de Ciencias Forestales, Universidad de Talca, Casilla 747, Talca, Chile; [4]Departamento Bioquímica Clínica , Universidad de Concepción, Chile.

Pitavia punctata (Ruiz & Pav.) Molina, belongs to a monotypic genus from the Rutaceae family. It is an endemic tree from the Chilean Coast Range in Central Chile [1]. Its common names are "*pitao*", "*canelillo*" and "*pitrán*" [2]. Nowadays, subpopulations of *P. punctata* are fragmented, being this situation a threat for the conservation of this species. It is also known that in its natural distribution area, there are no more than 1,000 reproductive individuals [3]. There are records about medicinal uses of *P. punctata*. For the Araucanian people, the species is a medicinal resource and the leaves infusions have anthelmintic properties [2]. A large number of polyphenols and antioxidant activity has been reported in species of the family Rutaceae [4]. Given its potential as a natural source of polyphenols and as the only Rutaceae representative in continental Chile, the aim of this work was to determine the total polyphenols and the *in vitro* antioxidant properties of a methanolic extract of *P. punctata* leaves on biological markers of oxidative stress as the oxidation of human LDL and lipid peroxidation of red blood cells. The results showed that together with the high concentration of total

polyphenols (170.3 mg GAE/g sample) and the inhibition of the DPPH radical (IC_{50} 0.07 mg/mL), *P punctata* extract significantly inhibited the *in vitro* oxidation of human LDL (6 µg/mL) and the lipid peroxidation of red blood cells (13.6 µg/mL), which was also evidenced by scanning electron microscopy (50 µg/mL).

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P-69

Evaluation of plant extracts from Amazonian biodiversity in French Guiana for the discovery of new natural insecticides

Falkowski M[1], Jahn-Oyac A[1], Boulogne I[1], Odonne G[2], Hérault B[1], Touré S[3], Flora C[1], Pulvar J[4], Eparvier V[3], Stien D[3], Robinson JC[4], Béreau D[4], Petit P[5], Girod R[6], Dusfour I[6], Houël E[1]

[1]CNRS-UMR EcoFoG, Campus Agronomique, F-97379 Kourou, French Guiana; [2]USR 3456 CNRS-Guyane, [2]Avenue Gustave Charlery, F-97300 Cayenne, French Guiana; [3]CNRS-ICSN, 1 Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France; [4]UMR QUALITROP, Campus Saint-Denis, BP792, F-97337 Cayenne, French Guiana; [5]UMR QUALITROP, Campus de Fouillole, BP 250, F-97157 Pointe- à-Pitre, French West Indies; [6]Institut Pasteur de la Guyane, 23 Avenue Pasteur, BP6010, F-97306 Cayenne, French Guiana

The exceptional biodiversity encountered in the Amazonian forest, along with a rich entomofauna and various herbivores leads to a promising chemodiversity. This has prompted us to search for new natural insecticides against *Aedes aegypti* in the flora of French Guiana. To improve our chances to isolate new compounds, we collected plant species growing in less studied environments as inselbergs, savannahs, river banks or white sands forests. We collected more than 80 different plant species from 36 different families, performed solvent extraction on 159 different plant parts, and assessed the larvicide activity of the extracts on a susceptible laboratory strain. Among the 22 extracts exhibiting more than 50% mortality at 100 µg/mL, 10 were also strongly active against a local pyrethroid resistant strain. One of the extracts also gave interesting results on adult mosquitoes. Eventually, we investigated the role of the different parameters (environment, taxonomy, organs, etc.) on larvicidal activity. The active compounds originating from these extracts are currently under study and may represent a promising alternative in the control of *Aedes*-transmitted diseases such as dengue or chikungunuya, in the context of a growing resistance against insecticides in the tropical areas of the globe.

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P-70

Phenolic composition and antioxidant activity of *Tristerix aphyllus* (Loranthaceae), a parasite from native Cactaceae in northern Chile

Jimenez-Aspee F

Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Región del Maule, Chile

Plants of desert and semi-desert climate live under harsh environmental stress conditions. Water shortage, strong radiation, extreme temperature variations and poor soil are factors accounting for natural selection pressures, leading to unique plant-plant relationships. The Chilean endemic mistletoe *Tristerix aphyllus* (Miers ex D.C.) van Tiegh. ex Barlow et Wiens, is a holoparasite of Cactaceae, including *Echinopsis* and *Eulychnia* species[1]. It is known under the common name "fosforito" and it is long shaped and red colored[2]. The objective of this work was to describe the main phenolic constituents and the antioxidant potential of the plant extractives. A sample of *T. aphyllus* was collected in the IV Region of Coquimbo, Chile, growing as a parasite on *Eulychnia acida*. The antioxidant activity of the methanolic extract was assessed for its ability to scavenge DPPH and ABTS radicals, and its ferric reducing antioxidant power (FRAP). An HPLC-DAD-MS/MS^n method was developed for phenolic profiling. High total phenolic content (14.66 ± 0.11 g gallic acid equivalents/100 g MeOH extract) and total flavonoid content (1.79 ± 0.10 g quercetin equivalents/100g MeOH extract) were found in the sample. Scavenging activity against DPPH radical of the crude extract was similar than that of the positive control quercetin (SC_{50} 9.17 and 7.82 µg/mL, respectively). The main phenolic compounds were identified as gallic acid derivatives. This is the first report of the phenolic composition of *T. aphyllus*, and set the basis for further studies, including a comparison of constituents according to the host and more specific environmental conditions.

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P-71

Anti-inflammatory activity of Copao fruits (*Eulychnia acida* Phil., Cactaceae)

Jimenez-Aspee F[1], Alberto MR[2], Quispe C[1], Soriano MDPC[1], Theoduloz C[3], Isla MI[2], Schmeda-Hirschmann G

[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Productos Naturales, Universidad de Talca, Casilla 747, 33460000 Talca, Chile; [2]Instituto de Química del Noroeste Argentino CONICET, Universidad Nacional de Tucumán, Tucumán, Argentina; [3]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, 3460000 Talca, Chile

Copao (*Eulychnia acida* Phil., Cactacea) is an endemic species occurring in arid areas of northern Chile. The fruits are commercialized within the Elqui and Limari valleys, and are appreciated for its refreshing and acidic taste. A comparison of the anti-inflammatory effect of the enriched fruit phenolics from the pulp and epicarp was undertaken in samples from the Elqui and Limari valleys, main sources of supply and commercialization of the fruits. The anti-inflammatory activity of the XAD-copao extracts was assessed by the inhibition of the pro-inflammatory enzymes lipooxygenase (LOX) and cyclooxygenase (COX-1 and COX-2) *in vitro* at 100 µg extract/mL. The inhibitory capacity of LOX by extracts from the Limari valley was between 1.46-64.3% for pulp and 23.0-

35.6% for epicarp. The COX-2 inhibition afforded values of 20.2-57.6% for pulp and 0-44% for epicarp. The COX-1 was not inhibited. For the Elqui valley samples, LOX inhibition ranged between 34.4-38.4% for pulp and 3.3-33.8% for epicarp. The COX-2 inhibition was between 0-27% for pulp and 1.1-39.9% for epicarp. The COX-1 inhibition ranged between 0-43% for pulp and 0-15.1% for epicarp. The main flavonoids found in this fruit are isorhamnetin-3-*O*-[α-rhamnopyranosyl-(1→6)-β-glucopyranoside] and quercetin-3-*O*-rutinoside[1]. Positive correlation was found between the anti-LOX activity and the content of rutin in pulp (R= 0.8235, $p<0.05$) and epicarp (R= 0.9120, $p<0.05$). Also, the anti-COX-2 activity and the content of isorhamnetin in epicarp showed a positive correlation (R=0.9422, $p<0.01$). The anti-inflammatory activity of isorhamentin and rutin have been reported[2,3]. The findings add value to the fruits as nutraceuticals.

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P-72

Ethnobotanical survey of medicinal species in Nossa Senhora Aparecida farm, Santa Barbara D'Oeste city, São Paulo State, Brazil

Juliani BC, Capellari L, Aleoni G, Ricci M

Luiz de Queiroz College of Agriculture - University of São Paulo, Pádua Dias Avenue, 11, Piracicaba, 13418-900, Brazil

In the domains of Nossa Senhora Aparecida farm, located in the rural neighborhood of Santa Barbara D'Oeste city, in Northwest of the state of São Paulo, there are remnants of Atlantic Florest. This is one of the biomes with greatest biodiversity in the world, presenting an enormous variety of plants species. Many of them are considered medicinal plants and are used by the farm owner Rosivaldo Pereira Santos, who is an indigenous descendant and has a rich knowledge of the use of these plants. As many species are not properly identified, there is need of a survey, aiming to document the identity and way of use of the crude drugs in a modern scientific context. Through this project an ethnobotanical survey was performed in which 146 species indicated as medicinal by Mr. Santos were documented and identified. The plants belong to 55 botanical families, being Asteraceae and Fabaceae most representative.The project presents the therapeutic indications and information about the used parts of the medicinal plant. Brazilian species such as *Solidago chilensis* Meyen (Asteraceae) and *Varronia curassavica* Jacq. (Boraginaceae) are in the survey. Ethnobotanical studies contribute to the management and conservation of natural resources [1], then it is expected that the results obtained will serve as a stimulus for further studies about the chemical constituents and pharmacological properties of the Brazilian medicinal plants, increasing the use of these species and encouraging its cultivation.

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P-73

Traditional knowledge on medicinal plants used by the community of Pilolcura, Valdivia, Chile: documentation and consensus

Jürgens K, Valencia E, Villatoro JM

Instituto de Farmacia, Universidad de Austral de Chile, Casilla 567, Valdivia, Chile

The people in Chile still depend upon the use of medicinal plants to treat simple health problem, like the ones who lives in rural areas as Pilolcura [1, 2]. This area is located near to Valdivia and surrounded by the "Selva valdiviana", a tipe of rain forest, which is relatively well conserved. Ethnobotanical studies are quit relevant to document the uses of medicinal species used for the treatment of ailments or diseases [3]. This study sought to collect information from local population about the use of medicinal plants, parts used and preparation of herbal drugs. Data was obtaining from 13 informants using semi-structured forms. For analysis of general use of plants, factor informant consensus (Fic) was used [4]. The study revealed the use of 26 species, 13 native, belonging to 21 families. These plants were used to treat different ailments grouped under 12 broad disease categories. The result of factor informant consensus showed that the urological category had the greatest agreement, followed by the cardiovascular and pain/fiber categories. Herbs (50%) were the main source of medicine. Leaves (88%) were the main plant parts used in remedy preparation while infusion was the major form of preparation (77%). This study provides basic data for further pharmacological studies and conservation of the most important native species.

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P-74

Ultrastructural and biological effects of quinoxaline 2,3-diarylsubstitued derivatives on promastigote forms of *Leishmania amazonensis*

Kaplum V[1], Cogo J[1], Sangi DP[2], Côrrea AG[2], Ueda-Nakamura T[1], Nakamura CV[1]

[1]Departamento de Farmácia, Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil; [2]Departamento de Química, Universidade Federal de São Carlos, Rodovia Washington Luis Km 235, 13565-905 São Carlos-SP, Brazil

Leishmaniasis is endemic in 98 countries worldwide, with about 350 million people at risk of infection and 12 million currently infected [1]. Cutaneous leishmaniasis is the most common clinical form, caused by *Leishmania* amazonensis [2]. Currently, the treatments of leishmaniasis have severe side effects that encourage the search for therapeutic alternatives [3]. Compounds 6,7-dichloro-2,3-diphenylquinoxaline (**5i**) and 2,3-di-(4-methoxyphenyl (**5k**) have antiproliferative activity in promastigotes of *Leishmania amazonensis*. The aim of this study was to investigate ultrastructural changes and biological effects of these compounds. Promastigotes treated with compounds **5i** and **5k** were evaluated by transmission electron microscopy and biochemical assays (mitochondrial membrane potential (01m), mitochondrial superoxide anion (O2•−), cell volume, cell membrane integrity). Ultrastructural changes in promastigotes treated with **5i** included vesicles within the flagellar pocket and membrane structures within the mitochondria. Similar changes occurred with **5k**, plus intense mitochondrial swelling. The biochemical changes identified in

promastigotes were the increase in 01m and O2•− in the concentration of 20 7M for **5i** and above 75 7M for **5k**. Cell volume did not change in promastigotes treated with **5i**, but with **5k** there was reduction in cell volume above 75 7M. The integrity of the cell membrane of promastigotes treated with both compounds did not change. Ultrastructural changes and the biochemical assays suggest abnormalities in the mitochondria and the reduction of cell volume in promastigotes, which are indicative of cell death by apoptosis.

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P-75

Anti-HSV-1 Evaluation of native Brazilian species

Oliveira LA[1], Kaplum V[1], Nocchi SR[1], Oliveira MM[1], Daré RG[1], Nakamura CV[1,2], Ueda-Nakamura T[1,2], Truiti MCT[1,3]

[1]Programa de Pós-Graduação em Ciências Farmacêuticas. Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900, Maringá, Paraná - Brazil; [2]Departamento de Ciências Básicas da Saúde. Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900, Maringá, Paraná - Brazil; [3]Departamento de Farmácia, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900, Maringá, Paraná - Brazil

The high resistance rates to current anti-HSV-1 agents, mainly among immunocompromised patients, is a serious medical problem leading to the need for development of novel antiviral prototype molecules that may be effective in the treatment of herpes. Natural products with anti-viral constituents from native Brazilian species have been reported [1, 2]. In the present work, the crude ethanolic extracts obtained from *Campomanesia guaviroba* (DC.) Kiaersk., *Heliocarpus popayanensis* Kunth, *Psychotria myriantha* Müll.Arg, *Nectandra cuspidata* Ness, *Nectandra hihua* (Ruiz & Pav.) Rohwer and *Senegalia polyphylla* (DC.) Britton & Rose were evaluated for their inhibitory effects on the replication of the *Herpes simplex* virus type 1 (HSV-1, KOS strain) and cytotoxicity on the Vero cells by the sulforhodamine B colorimetric assay. Among the plants investigated, *C. guaviroba* (EC_{50} 17.7 ± 7.3 µg/mL; CC_{50} 128 ± 46.0 µg/mL), *H. popayanensis* (EC_{50} 100 ± 10.8 µg/mL; CC_{50} 310 ± 5.0 µg/mL), *P. myriantha* (EC_{50} 90 ± 0.0 µg/mL; CC_{50} 300 ± 17.5 µg/mL) and *N. hihua* (EC_{50} 65 ± 1.7 µg/mL; CC_{50} > 500 µg/mL) showed anti-HSV-1 activity, and *C. guaviroba* and *N. hihua* were the most effective, presenting the best SI (Selectivity Index). Additional studies, especially phytochemistry analysis, experiments to elucidate the mechanism of anti-HSV-1 action by which these plants act and in vivo toxicity studies must be conducted.

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P-76

Cytotoxicological effects and antimicrobial activity of *Pithecellobium dulce* (Guamúchil) extracts

Villarruel JB[1], López Z[1], Solís-Padilla DB[1], Rico J[2], Salazar J[1], Knauth P[1]

[1]Cell Biology Laboratory, Centro Universitario de la Ciénega, Universidad de Guadalajara, Av. Universidad 1115, 47810 Ocotlán Jalisco, México; [2]Laboratorio de Catálisis, Facultad de Ingeniería Química, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, 58060 Morelia, Michoacán, México

Pithecelobium dulce is an endemic tree of Mexico, which is widely used in Mexican folk medicine to treat different diseases; as possible anticancerogenic effects have not been published so far [1], this plant is a good initial screening candidate. The leaves and stem were collected from branches of 20-30 cm in length, dried at RT for 15 d and grinded to particles of 0.6-2.0 mm. 25 g of each were extracted with 250 ml of ethanol or water (the latter briefly heated to 80 °C) shaked at 80 rpm for 24 h at RT. Under reduced pressure (ethanol at 40 ºC and water at 60-65 ºC) the extracts were concentrated to about 2 ml. 10 µl of each, Guamuchil Stem (or Leaf) Ethanolic Extract (GSEE or GLEE) exhibited antimicrobial activity against the Gram-positive strains of *Enterococcus faecalis*, *Bacillus cereus* as well as the yeast *Candida* spp., while the watery extract did not present any antimicrobial activity. The cancerous cell line HeLa exhibited up to 0.3 µl/ml GLEE normal metabolic activity (WST-1), which was reduced to 25% at 3 µl/ml; the cell membrane remained intact (LDH-release) at 0.1 µl/ml GLEE and was seriously disrupted at 10 µl/ml. At the critical concentration of 1 µl/ml GLEE only few cells were dead (trypan blue), while with Annexin V (green) and PI-counterstaining (red) roughly the half of the cells seems to be necrotic (green & red) and the rest was still alive (not stained). The aqueous extracts as well as GSEE did not exhibit any cytotoxic effects up to 10 µl/ml.

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P-77

Evaluation of the potential toxicity of crude root extract of *Limonium brasiliense*

Antonelli-Ushirobira TM[1], Blainski A[1], Krzyzaniak LM[1], Fernandes HG[2], Leite-Mello EVS[3], Mello JCP[1]

[1]Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá Avenida Colombo, 5790, BR-87020-900, Maringá, PR, Brazil; [2]Graduate Student of Medicine, Universidade Estadual de Maringá, Avenida Colombo, 5790, Maringá, PR, BR-87020-900, Maringá, PR, Brazil; [3]Departamento de Ciências Morfológicas, Universidade Estadual de Maringá, Avenida Colombo, 5790, Maringá, PR, BR-87020-900, Maringá, PR, Brazil

Roots of *Limonium brasiliense*, known as baicuru, are employed in relieving menstrual cramps, regulating the menstrual cycle, and mood swings [1, 2]. The objective of this work was to evaluate the subchronic toxicity and mutagenic potential of the crude roots extract (CE) of the plant. Male and female Swiss mice received a single oral dose of 2.0, 1.5, 1.0 and 0.5 g/kg CE (n=10/group), water (negative control) and cyclophosphamide at the dose of 50 mg/kg (positive control). After 24 h of administration the animals were sacrificed and the pair of femur removed for mutagenic evaluation [3]. Male and female Wistar rats received CE for 90 days by gavage at doses of 200, 100 and 50 mg/kg (n=20/group) or water (control). During the test period ponderal evaluation was held, and the animals were submitted to the open field test. At the end of the period, animals were

sacrificed and organs removed, weighed and the results expressed in relative weight [4]. Statistical analysis of the mutagenic effect showed a significant increase in the number of micronucleated polychromatic erythrocytes in the group treated with cyclophosphamide (26.7±7.0), characteristic of the positive control and the group treated with 2.0 g/kg of *L. brasiliense* CE (17.9±.7). The pre-clinical trial showed no statistical difference between the groups of animals for weight assessment, in the open field test, and organs evaluated. Toxicology tests showed preliminary results of low toxicity, absence of anatomical change and genotoxic effects at doses above 2.0 g/kg.

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P-78

Application of simplex-centroid design in optimization of extraction from flavonoids from *Tagetes patula* study in *Aedes aegypti* larvicidal activity

Krzyzaniak LM[1], Munhoz VM[1], Longhini R[1], Zequi JAC[2], Leite-Mello EVS[3], Souza JRP[4], Novello CR[5], Lopes GC[1], Mello JCP[2]

[1]Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo, 5790,BR-87020-900, Maringá, PR, Brazil; [2]Instituto Nacional de Pesquisas da Amazônia (INPA), BR-69067-971, Manaus, AM, Brazil; [3]Departamento em Ciências Morfológicas, Universidade Estadual de Maringá, Maringá, PR, Brazil; [4]Programa de Pós-graduação em Agronomia, Centro de Ciências Agrárias, Universidade Estadual de Londrina, BR-86057-970, Londrina, PR, Brazil; [5] Coordenação de Engenharia Química, Universidade Tecnológica Federal do Paraná–UTFPR, Câmpus de Francisco Beltrão, BR- 85601-970

Tagetes patula L. (Asteraceae) is traditionally known as “cravo-francês”, “cravo-de-defunto” or “botões-de-solteirão” [1]. In popular medicine the flowers and leaves have been used as an antiseptic, diuretic, depurative, and insect repellent [2]. The objective of this work was to evaluate the larvicidal activity of *T. patula* on *Aedes aegypti* [3], using extracts of *T. patula* flowers obtained with a factorial simplex-centroid design type. Simplex-centroid design with $2q^{-1}$ for three components with five replicates at the center point was used. The conditions were: solvents: acetone, ethanol, and water; extraction: ultra-turrax T25 (Ika Works) at the proportion of 2.5 % (m/v), and 9 min of extraction. The results showed acetone as the best liquid extractor, based on the flavonoid content, which showed higher antioxidant capacity. This suggests that the content of flavonoids may be directly related with the antioxidant activity. When tested against mosquito larvae, the acetone extract obtained from the flowers of *T. patula*, previously prepared with *n*-hexane, at 1 ppm showed a mortality rate of 100% in 48 h. The ethanol: water (1:1, v/v) extract (flowers were not prepared with *n*-hexane) at the concentration of 10 ppm caused 100% mortality of *A. aegypti* larvae in 48 h. It was concluded that acetone and ethanol:water extracts were active to control mosquito larvae.

Acknowledgements: CNPQ, Fundação Araucária e CAPES.
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P-80

MAPA Platform: Computational management system for pharmaceutical bioprospecting research in forest environment

Rody H[1], Simão MVRC[2], Almeida AA[1], Fernandes IG[1], Escudeiro MC[2], Silva AF[3], Leite JPV[1]

[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa, Av. P.H.Rolfs, Viçosa, Minas Gerais, Brazil; [2]Departamento de Engenharia Florestal; [4]Departamento de Biologia Vegetal, Av. P.H.Rolfs, Viçosa, Minas Gerais, Brazil; [3]Empresa de Pesquisa Agropecuária de Minas Gerais, Avenida José Cândido da Silveira, 1647, Belo Horizonte, MG, Brazil

According to current regulations of the Convention on Biological Diversity, countries with high biodiversity should propose goals and challenges for the conservation, monitoring and sustainable use of their natural resources [1,2]. The MAPA Platform and database were created in order to optimize access to the genetic biome of the Atlantic Forest and for investigation of natural products in the searching of bioactive molecules. This program is interdisciplinary, with participation of researchers from diverse fields of knowledge whose goal is to implement a computerized database of plant extracts from species of the Atlantic Forest (Extract library), associated with georeferencing resources and databases with ethnopharmacological, chemical and biological information. The MAPA Plataform was developed by using PHP "Hypertext Preprocessor", an open source scripting language suited for web development. Geographic coordinates and other detailed information about species from fragments of Atlantic Forest biome, belonging to the Federal University of Viçosa, Brazil, are stored in a single MySQL database. Data from a global positioning system (GPS) are obtained by a Garmin GPSmap 60CSx unit, which are listed for assessment of species located in Atlantic Forest fragments. Currently MAPA consists of approximately 230 registered samples that can generate around 900 extracts, considering the production of two extracts from different parts of the plant. PHP and MySQL together permit an complete integration between the data from MAPA with Google Maps API, and provide an user friendly interface to searching for bioactive natural products from Atlantic Forest.

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P-81

Phytochemical screening and comparative antimicrobial potential of different extracts from *Strychnos pseudoquina* St.Hill. (Loganiaceae)

Xavier AA[1], Farias LM[1], Carvalho ASS[2], Mendonça RCS[2], Silva AF[3], Leite JPV[1]

[1]Departamento de Bioquímica Agrícola, Universidade Federal de Viçosa, Av. P.H.Rolfs, Viçosa, Minas Gerais, Brazil; [2]Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Av. P.H.Rolfs, Viçosa, Minas Gerais, Brazil; [3]Empresa de Pesquisa Agropecuária de Minas Gerais, Avenida José Cândido da Silveira, 1647, Belo Horizonte, MG, Brazil

Strychnos pseudoquina (Loganiaceae), a native Brazilian tree, has been widely used in traditional Brazilian medicine as antipyretic, liver and gastric protective, wound healing, depurative, hypolipidemic, and vermifuge [1,2]. In previous studies for our groups, flavonoids compounds purified from stem bark extract, quercetin 3-O-methyl ether and strychnobiflavone, have shown remarkable antileishmanial activity [3]. In the present investigation, the antimicrobial activities of

different extracts (ethyl alcohol, defatted ethanol, ethyl acetate and water) obtained from the leaves and stem bark of *S. pseudoquina* were evaluated against 3 bacterial strains, isolated from food samples. The Gram-positive bacteria was: *Staphylococcus aureus*; for the Gram-negative were: *Escherichia coli* and *Salmonela enteritidis*. Futhermore, we performed a phytochemical analysis by TLC and HPLC of these extracts. The ethyl acetate extract from *S. pseudoquina* stem bark was the most active, showing bacteriostatic and bactericidal activity towards Gram-positive and Gram-negative microorganisms, with significant inhibitory activity against *S. aureus* (3,12 mg/mL) and *E. coli* (0,78 mg/mL). Phytochemical analysis showed this extract consist triterpenes, alkaloids, tannins and flavonoids. The chromatographic profiles showed differences between the extracts, indicating the influence of the solvent and extraction method on the antibacterial activity of the plant. This result showed that these by-products can be successfully valorized with solvent extraction of valuable compounds. The antibacterial properties of these extracts make them suitable to be employed in areas like medicines and food.

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P-82

Botany as a linchpin of ethnobotanical surveys in Cambodia

Leti M[1], Hul S[2], Cheng SK[3], David B[1]

[1]Institut de Recherche Pierre Fabre, 3 avenue Hubert Curien – BP13562, 31035 Toulouse, France; [2]Museum national d'histoire naturelle, Departement de Systematique et Evolution - UMR 7205 OSEB, 16, Rue Buffon, 75231 Paris Cedex 05, France; [3]Faculty of Pharmacy, 73 Bd Monivong, Phnom Penh, Cambodia

The Joint Laboratory of Phytochemistry USS-IRPF of Phnom Penh is a scientific training and research structure dedicated to plant studies and governed by a public-private partnership. It is located in Cambodia, which shows various vegetation types, from lowland evergreen rain forests to mangrove swamps [1]. This large plants reservoir constitutes a source of food, medicines, and income for a population still mainly rural [2]. But still today, available scientific data regarding Cambodian flora remains quite unavailable. Nevertheless, in-depth knowledge of local plants is a key point for phytochemical studies, and incorrect determination is often the source of misunderstandings [3]. Thus, a large ethnobotanical inventory of traditionally used plants has been conducted from 2005 to 2011 in selected Cambodian provinces well-known for species richness. Today, more than 1250 medicinal or useful plants specimens have been collected and pictured. Thanks to the assistance of botanists from numerous renowned Herbariums (Paris, Leiden, Singapore, Edinburgh, Kew…) the collected species have been formally identified and some new species described (*Solanum sakhanii* Hul, *Curculigo fabrei* Hul). The research studies performed in the laboratory now rely on trustful data. To make this inventory available to a large audience, the first Cambodian Photographic Flora was published in 2013 in which 523 Cambodian medicinal and useful plants are described and illustrated by more than 2000 pictures [4].

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UHPLC-PDA-QTOF-MS/MS based chemical profiling approach to evaluate the influence of preparation methods on the global quality of traditional complex herbal medicine Qiong-Yu-Gao

Xu JD[1], Mao Q[1], Shen H[1], Zhu LY[1], Li SL[1], Yan R[2]

[1]Department of Pharmaceutical Analysis and Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing, China; [2]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

Qiong-Yu-Gao (QYG), consisting of Rehmanniae Radix (RR), Poriae (PO) and Ginseng Radix (GR), is a commonly used tonic traditional complex herbal medicines (CHM). So far, three different methods have been documented for preparation of QYG, i.e., method 1 (M1): mixing powders of GR and PO with decoction of RR; method 2 (M2): combining the decoction of RR and PO with the decoction of GR; method 3 (M3): decocting the mixture of RR, GR and PO. In present study, an UHPLC-PDA-QTOF-MS/MS based chemical profiling approach was developed to investigate the influence of the three preparation methods on the holistic quality of QYG. All detected peaks were unambiguously identified by comparing the UV or mass spectra and retention times with those of reference compounds, and/or tentatively assigned by matching empirical molecular formula with that of known compounds, and/or elucidating quasi-molecular ions and fragment ions referring to information available in literatures [1-4]. In total 103 components, mainly belonging to ginsenosides, phenethylalcohol glycosides, iridoid glycosides and triterpenoid acids, were identified, of which 5 degraded ginsenosides were putatively determined to be newly generated during preparation of QYG. Triterpenoid acids and malonyl-ginsenosides were detected only in M1 samples, while degraded ginsenosides were merely detectable in M2/M3 samples. The possible reasons for the difference among chemical profiles of QYG samples were deduced to be hot-induced degradation or transformation of the original components [2,4]. It could be concluded that preparation method do significantly affect the holistic quality of QYG. The influence of the altered chemical profiles on the bioactivity of QYG needs further investigation. The present study demonstrated that UHPLC-PDA-QTOF-MS/MS based chemical profiling approach is efficient and reliable for evaluating the holistic quality of CHM.

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P-84

Ethnobotanical survey of medicinal species in “São Paulo” farm (Sud Mennucci City) and “Araçatuba Botânico” Hotel (Araçatuba City), São Paulo, Brazil

Lima AG, Capellari L, Martin LF, Melli JLC

Luiz de Queiroz College of Agriculture - University of São Paulo, Pádua Dias Avenue, 11, Piracicaba, 13418-900, Brazil

Located in the Northwest of São Paulo State, Sud Mennucci and Araçatuba Cities are placed in the transition of Atlantic Forest and Savannah (“Cerrado”) biomes. Those biomes are some of the most

biodiverse in the world. However, there are serious conservation threats in function of agricultural activities and urbanization that endanger the survival of countless species, many of them still little studied by science. In function of the very high plant biodiversity occurring in those biomes and looking for the preservation of popular knowledge on medicinal plants, the present project developed on a rural property of Sud Mennucci City, called “São Paulo Farm”, an ethnobotanical survey of medicinal species in the remaining ecosystem of the property. Some 80 medicinal plants were collected and identified. The results will serve as a basis for collecting seeds with the aim to establish a sustainable commercial production of medicinal plants, studies about genetic improvement and for a guide of medicinal species of the property.
In Araçatuba City, an ethnobotanical survey was conducted in the greenhouse at the Araçatuba Botânico Hotel, which uses medicinal plants in its own products. In this property, 26 medicinal plant species were collected and identified. Both properties belong to the same businessman and project, serving as a model of sustainability and valorizing of medicinal plants scientific studies, mainly on Brazilian medicinal plants.

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P-85

Alkaloids from Chilean species of the genus *Rhodophiala* C. Presl (Amaryllidaceae)

Lizama I, Becerra J

Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

The Family Amaryllidaceae is widely distributed from temperate to tropical regions. Amaryllidaceae species can biosynthesize alkaloids with important physiological effects which include antitumor, antiviral, antimalarial and immunostimulant activity; as well as inhibition of the acetylcholinesterase enzyme [1]. *Rhodophiala* C. Presl is one of the native genera of Amaryllidaceae of Chile, Argentina and Brazil [2]. There are more than 20 species of this genus in Chile. However, despite this diversity, the phytochemistry has been studied previously in only two Chilean species. The aim of this work was to analyze the alkaloids profile of Chilean species of *Rhodophiala*. Extracts from bulbs were analyzed by means of GC-MS. Alkaloids were characterized by their retention time and fragmentation pattern. The alkaloids skeleton types detected were lycorine, crinine, galanthamine, homolycorine, tazettine and montanine. A total of 14 alkaloids were found. All analyzed species showed different alkaloids profiles, therefore these compounds can be used as a chemotaxonomic tool. Furthermore, the alkaloids detected in this genus have multiple biological properties reported and these species can constitute new sources of products of medicinal importance.

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Ethnobotany of and antioxidants from *Gerbera piloselloides*

Long C[1], Wang J[1], Shi Y[2], Hong L[1], Wang Y[1], Petrova V[3], Wu S-B[1,3], Kennelly EJ[1,3]

[1]*College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China;*
[2]*Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650205, China;*
[3]*Department of Biological Science, Lehman College, City University of New York, Bronx, NY 10468, USA*

In indigenous communities of southwest China, *Gerbera piloselloides* (Asteraceae) is a very important ethnobotanical and ethnomedicinal plant used by different ethnic groups. The local people in SW China like Miao, Dong, Shui and Zhuang have been using this plant as a spice when they cook meat. The most interesting use is that the Miao and Shui people grind its dry plant to make distillers' yeast, and believe the flavor of such distilled liquor might be much tasty. *Gerbera piloselloides* is usually used as a traditional herbal medicine to treat cold, fever, acute conjunctivitis, and virtual. Ten compounds were obtained using activity-guided isolation, including two caffeic acid derivatives, two coumarins, a parasorbosid derivative, and five flavonoids and were identified from the whole plant of *G. piloselloides*. Their antioxidant activity was evaluated by ABTS assay. Eight of the compoundsd showed very strong antioxidant activities. Our ethnopharmacological results supported the indigenous uses of *G. piloselloides* in the local communities.

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The effect of sodium salicylate on the biology of MDA MB-231 breast cancer cells

Madunic J, Majstorovic I, Matulic M

Faculty of Science, Horvatovac 102, 10000 Zagreb, Croatia

Sodium salicylate (NaS) is a derivative of natural compound acetylsalicylic acid (aspirin) which is commonly used as a non-steroidal anti-inflammatory drug (NSAID). NaS lacks aspirin's inhibitory effect on prostaglandins, but still has anti-inflammatory functions and shares with aspirin certain downstream interactions. At present, mechanisms of NaS action are still not fully explained. In vitro it was shown that NaS targets intracellular signaling mechanisms such as mitogen activated MAP kinases cascade and influences several transcription factors such as NF-kB and AP-1. Recently it was found that it can activate AMPK, central cellular metabolic regulator. The aim of our study was to elucidate the effect of NaS on the morphology, proliferation and urokinase plasminogen activation (uPA) system of MDA MB-231 breast cancer cells. Cells treated for 24 h with NaS exhibited morphology changes and cell proliferation was inhibited in concentration range 5 to 20 mM. We also investigated effect of NaS on uPA system. Its main component is uPA, an extracellular protease involved in extracellular matrix remodeling. By plasmin activation it can regulate tissue degradation. These processes regulate normal tissue reconstruction, but also tumor invasiveness and metastasis. In our experiments, NaS decreased extracellular uPA activity of treated cells up to a 90 % of control cells in dose- and time-dependent manner. To examine the nature of uPA activity decrease, components of the uPA system, such as uPA, its inhibitors PAI-1 and PAI-2 and receptor uPAR were analyzed. We observed change in uPA/PAI-1 ratio in treated cells on RNA and protein level. Furthermore, we investigated the influence of NaS on signaling pathways involving NF-kB, β catenin and MAP kinases. Morphology changes lead us to analyze integrin expression. We found selective decrease in αv and β5 expression. Our results suggest that NaS could be an interesting modulator of uPA activity in certain types of cancer cells.

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Phenolic compounds and antioxidant capacity of grape cane extract produced at bench scale

Riquelme S[2], Bustamante L[1], Escobar D[2], Ruiz MA[1], Jara P[3], von Baer D[1], Lamperti L[3], Fuentealba C[2], Vergara C[1], Berg A[2], Mardones C[1]

[1]Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Casilla 160-C, Concepción, Chile; [2]Unidad de Desarrollo Tecnológico UDT, Universidad de Concepción. Av. Cordillera Nº 2634 - Parque Industrial Coronel - Coronel. Código Postal: 4191996. Casilla 4051, Correo 3, Concepción - Chile; [3]Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción, Casilla 237, Concepción, Chile

The evidence of the health benefits of *trans*-resveratrol has increased during the last twenty years. This fact has triggered the interest in studying the levels of this and other stilbenes in grapes, wine and winemaking residues. In Chile, more than 100.000 tons of winemaking residues are produced each year, including pomace, seeds, stalks and canes. In this research, profiles and levels of stilbenes in these waste products are studied by HPLC-DAD-ESI-MS/MS. Main results show that ε-viniferin is the main stilbene in these matrixes, followed by *trans*-resveratrol, except for canes, where *trans*-resveratrol was higher. Total stilbenes (expressed as *trans*-resveratrol equivalents in dry matter) were 22.81±10.59 mg/kg; 5.17±2.02 mg/kg; 0.93±0.44 mg/kg and 4485.87±200.28 mg/kg in stalks, pomace, seed and cane, respectively. Considering these results, a stilbene rich extract was produced at bench scale (7 L reactor) using canes. The extract obtained under optimal conditions was lyophilized and showed 38.7 % of total lignin, 27.5% of carbohydrates, 6.7% of proteins, 1.03% of metals, and 11.80 % of phenolic compounds. Between these, mainly stilbenes, flavan-3-ols, procyanidins and some flavonols were predominant. The concentration of these families was respectively 4.60%, 4.84%, 2.11% and 0.25% in basis dry matter. The antioxidant capacity of this extract was studied using cell free and cell based antioxidant assays (TEAC, ORAC, CUPRAC and DCF-DA).The results showed that the crude extract have reducing and protection capacity, however, synergic and antagonistic behaviors are evidenced due to the complex composition of the extract.

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Supercritical fluid extraction of compounds from guaraná (*Paullinia cupana*) seeds

Marques LLM[1], Cardozo Filho L[1], De Paula F[2], Guedes TA[1], Mello JCP[1]

[1]Universidade Estadual de Maringá, Avenida Colombo 5790, BR- 87020-900, Maringá, Brazil; [2]Water Technologies, Alameda Tocantis, BR-06455-020, Barueri, Brazil

Guaraná (*Paullinia cupana* var. *sorbilis* (Mart.) Ducke - Sapindaceae) is a Brazilian native Amazonian plant widely used in beverage industry, cosmetics, and medicine because of the versatility of its biological activities. Its seeds have caffeine, theophylline, theobromine, and also tannins like catechin, epicatechin and procyanidins, and saponins, starch, oils, and pigments [1,2]. The tannins present in guaraná have favorable pharmacological effects to human health as stimulants of the nervous and cardiovascular system, antidiarrheal, diuretic [3], panicolytic, tonic, antidepressive [4], antineuralgic, febrifuge, antipyretic, analgesic, aphrodisiac [5], among others. This study aimed to investigate means of orthogonal array design, four factors at three different levels that affect the extraction using a supercritical fluid extractor MV10 (Waters ®). The factors and levels were: modifiers (ethanol, methanol and ethanol:methanol (1:1) in concentrations of 10,

20, and 40%), extraction temperature (40, 50, and 60 °C), extraction pressure (100, 200, and 300 bar) and extraction time (20, 40, and 60 min). Three blocks of nine experiments were performed in duplicate. The yield of each extraction was calculated and the samples were analyzed in Acquity UPLC-PDA-MS (Waters®). The results showed that the yield of extraction is proportional to the percentage of modifier used, and the high percentage of this modifier facilitates the extraction of polar compounds. The better extraction conditions assayed were: 40% ethanol, 40 °C, 200 bar, and 40 min. In this condition, several compounds such as methylxanthines, (epi) catechin, and procyanidins were identified.

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P-91

Development and modification of pH responsive hydrogels based on sodium alginate of *Desmarestia menziesii* and poly(vinyl alcohol) (PVA)

Guerrero J[1], Mansilla A[2], Martínez-Gómez F[3], Matsuhiro B[3]

[1]Departamento de Química de los Materiales, Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 40, Santiago, Chile; [2]Departamento de Ciencias y Recursos Naturales, Universidad de Magallanes, Punta Arenas, Chile; [3]Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 40, Santiago, Chile

The major structural polysaccharide of brown seaweed is sodium alginate, a linear copolymer of β-mannuronic acid and α-guluronic acid [1,2]. Sodium alginate possesses excellent properties for hydrogels preparations. The dynamic and mechanical properties of alginate hydrogels can be optimized by combining physical and covalent crosslinking with other non-toxic polymers, like poly(vinyl alcohol) (PVA). Sodium alginate/PVA hydrogels have immense potential for use as functional biomaterials [3-5]. Sodium alginate (Mn=87,000 g/mol) obtained by alkaline extraction of Desmarestia menziesii (Heterokontophyta) from South Shetland Islands was characterized by FT-IR, NMR and HPLC techniques. The extracted sodium alginate was composed mainly of guluronic acid residues. The mixture solution of sodium alginate and PVA was firstly crosslinked with freeze-thaw cycles and then with Ca^{2+} or glutaraldehyde. The swelling degree decreased with increasing PVA content of the hydrogels and the crosslinking degree. The results indicate that the best swelling and pH-sensitive properties of hydrogels was obtained when the ratio of sodium alginate:PVA is 1:1, without Ca^{2+} or glutaraldehyde treatment. The swelling ratio in the equilibrium for this formulation reached a value of 19.

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Effect of *Salvia elegans* Vahl over cytokines levels associated to damage induced by high fat diet and chronic administration of AG II

Martínez-Hernández G [1, 2], Jiménez-Ferrer E[1], González-Cortázar M[1], Botello-Amaro C[1], Ramírez- Serrano C[1], Herrera-Ruiz M[1]

[1]Centro de Investigación Biomédica del Sur-IMSS. Av. Argentina No. 1 Col. Centro, Xochitepec, Morelos C.P. 62790. México; [2]Facultad de Ciencias Biológicas- UAEM. Av. Universidad No. 1001, Col. Chamilpa, Cuernavaca, Morelos C.P. 62209, México

Salvia elegans Vahl, known in Mexico as "Mirto" is used as traditional medicine for CNS, hypertension and inflammatory diseases (1, 2). The aim of this work was to evaluate the effect of hydroalcoholic extract (SeHA), and two fractions (FAcOEt, F23), over cytokines in a high fat diet (HFD)/Angiotensin II (AGII) chronic model. ICR male mice (n=12). They were given high fat diet (HFD) for a period of 12 weeks, 8 weeks with AG II (DAMAGE), clinical drugs and *S. elegans* extracts and fractions (100 mg/kg) for 4 weeks. Cytokines levels of IL-10, IL-1β y TNF- α were measured by ELISA test on right kidney (RK), left kidney (LK) and brain (B). The results indicate that the levels for the RK on TNF-α are significantly high on the HFD/AGII and AGII compared with the basal group. Meanwhile for the LK, the basal is significantly high compared with HFD/ AGII and AGII. For the B, the basal group has high levels as well as AGII. The HFD/AGII is significantly different, since the level is low. *S. elegans* has activity in all three organs which is more obvious in SeF23. For the IL- 10, RK has high levels in all groups including *S. elegans* groups. LK for the basal, the levels are significantly low compared with HFD/AGII. For the B, the levels are low in all groups, also in *S. elegans*. With these results we can conclude that *S. elegans* has inmmunomodulatory activity over the cytokine levels, in the model used.

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P-93

Leptocarpin, a sesquiterpen lactone isolated from *Leptocarpha rivularis*, induces programmed cell death in cancer cells

Bosio C[1], Martínez R[1], Villena J[2], Carrasco H[3], Olea A[3]

[1]Universidad Andrés Bello, Facultad de Ciencias Exactas, Quillota 910, 252000, Viña del Mar, Chile; [2]Universidad de Valparaíso, Facultad de Medicina, Hontaneda 2664, 2340000 Valparaíso, Chile; [3]Universidad Autónoma de Chile, Facultad de la Salud, Carlos Antúnez 1920, 7520000, Providencia, Santiago, Chile

Previous studies have shown that Leptocarpin, a sesquiterpen lactone of Heliangolides type isolated from *Leptocarpha rivularis*, exhibit interesting biological activity [1,2]. In this study, the cytotoxic effect of leptocarpin over cell lines HT-29, PC-3, DU 145, MCF7, MDA-MB-231 has been determined by cell viability assays with sulforhodamine B. In all examined cell lines, condensation and/or fragmentation of chromatin was detected by using Hoechst 33342 stain, which suggest a possible process of cell death by apoptosis. The occurrence of an apoptotic process is corroborated by registration of increasing permeability of the mitochondrial membrane, which is measured by flow cytometry and increased caspase activity [3], observed after treatment with leptocarpin. Leptocarpin is also capable of generating a G0/G1 cell cycle arrest phase that can be attributed to the participation of p21WAF-1 [4]. In addition, leptocarpine induces a reduction of the clonogenic capacity of the treated cells.

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P-94

Mechanisms involved in the gastroprotective action *Celtis iguanaea* (Jacq.) Sargent (Cannabaceae)

Martins JLR[1], Rodrigues ORL[1], Galdino PM[1], Fajemiroye JO[1], Vaz BG[2], Ghedini PC[1], Costa EA[1]

[1]Department of Physiological Sciences, Federal University of Goiás, Campus Samambaia, 74001-970, Goiânia-GO, Brazil; [2]Institute of Chemistry, Federal University of Goiás, Campus Samambaia, Goiânia-GO, Brazil

The leaf preparation of *Celtis iguanaea* (popularly known as “esporão-de-galo”), are being used to treat colic and poor-digestion [1]. The aim of the present study was to investigate the chemical composition and the gastroprotective mechanisms of hexane leaf extract of *Celtis iguanaea* (HE) in different gastric ulcer-induced models. The leaves were collected in Hidrolândia (GO) and a voucher specimen was deposited in the Herbarium of the Federal University of Goiás - UFG (N° 40.110). The HE was obtained by exhaustive extraction in Soxhlet apparatus and the chemical characterization of HE was perfomed with electrospray Fourier transform ion cyclotron mass spectrometry (ESI FT-ICR MS) analysis. Male Swiss mice (n=8), provided by Central Animal House of UFG were used in this research. In the HCl/EtOH-induced ulcer, the treatments with HE (100 or 200 mg/kg, p.o.) reduced the ulcerated area by 34.3 % and 43.2 % respectively. The HE (100 mg/kg, p.o.) reduced the lesion index by 49.2 % and ulcerated area by 68.4 % in hypothermic-restraint stress and acetic acid-induced gastric lesions, respectively. In the mucus quantification, the treatment with HE (100 mg/kg, p.o.) enhanced the Alcian blue binding capacity of gastric wall mucus in 161.33%. The pretreatment with yohimbine 2 mg/kg, s.c. (an α2-adrenergic antagonist), *L*-NAME 20 mg/kg, s.c. (an inhibitor of nitric oxide synthase) or indomethacin 10 mg/kg, s.c. (an inhibitor of prostaglandin synthesis), reversed the gastroprotective activity of HE (100 mg/kg, p.o.). The mechanism of gastroprotective activity suggest increased mucus as well as the involvement of α2-adrenergic receptors, NO and prostaglandins. The substances responsible for the protection are probably hydroxyl-linolenic and linoleic acids and conjugated oxo-linoleic acids.

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P-95

Gastric lesions: antiulcer and antisecretory activity of *Eugenia uniflora* L. in mice

Martins JLR[1], Carvalho AG[2], Ferreira AL[3], Santos SC[2], Costa EA[1]

[1]Department of Physiological Sciences, Federal University of Goiás, Campus Samambaia, 74001-970, Goiânia-GO, Brazil; [2]Institute of Chemistry, Federal University of Goiás, Campus Samambaia, Goiânia-GO, Brazil; [3]Biological Sciences Department, Federal University of Goiás (UFG), Catalão-GO, Brazil

The leaves of *Eugenia uniflora* L. (Myrtaceae), popularly known as “pitangueira”, are being used to treat stomach disorders in Brazil [1]. The aim of this study was to evaluate the gastroprotective

activity of the aqueous fraction from the hidroacetonic extract from *Eugenia uniflora* L. leaves (FAHP) in gastric ulcer models induced in mice. The leaves were collected in Anápolis (GO) and a voucher specimen was deposited in the Herbarium of the Federal University of Goiás (UFG) (N° 25.481). Male Swiss mice (n=8), provided by Central Animal House of UFG were used in this research. In the indomethacin-induced gastric ulcer, the treatments with FAHP (100, 300 and 1000 mg/kg, p.o.) reduced the lesions index by 32.7 %, 38.7 and 42.9 %, respectively in relation with control group (filtered water 10 mL/kg). In the HCl/EtOH-induced and hypothermic restraint stress-induced gastric ulcer, the treatment with FAHP (300 mg/kg, p.o.) reduced the ulcerated area by 58.9% and lesions index by 43.8 %, respectively. At this dose (300 mg/kg, i.d.), FAHP, also reduced the total acidity, increased the pH. The FAHP (300 mg/kg, p.o.) also increased mucus production in 28.74% and glutathione concentration by 48.5%. The pre-treatment with *L*-NAME (20 mg/kg, s.c.), did not reversed the gastroprotective activity of FHAP. The results suggest gastroprotective activity of FAHP. This effect could be associated with antisecretory activity, an increase in the mucus production and sulfhydryl groups.

Reference: [1] Lorenzi, H. (1998) São Paulo: Instituto Plantarum de Estudos da Flora, 262p.

P-96

The trypanocidal action of synthetic compound 5-[4-methylphenyl]-N-{2-[(1R)-4-methylcyclohex-3-en-1-yl]propan-2-yl}-1,3,4-thiadiazole-2-amine may be related with production of reactive oxygen species

Martins SC[1], Falzirolli H[2], Lazarin-Bidóia D[1], Desoti VC[1], Silva CC[2], Ueda-Nakamura T[1], Nakamura CV[1]

[1]Departamento de Ciências Básicas da Saúde, Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil; [2]Departamento de Química, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil

Chagas' disease, caused by the protozoan *Trypanosoma cruzi* affects about 7-8 million people in 21 endemic Latin American countries. The available treatment cause serious adverse reactions and have low cure rate in chronically infected individuals (1). Consequently, the search for new therapeutic options is a priority. Thus, the goal of this study was to investigate the trypanocidal activity and possible mechanisms of action of the synthetic compound 5-[4-methylphenyl]-N-{2-[(1R)-4-methylcyclohex-3-en-1-yl]propan-2-yl}-1,3,4-thiadiazole-2-amine (TDZ 02) involved in the cell death of the parasitic forms of *T. cruzi*. For this, were evaluated the activity, the production of mitochondrial-derived superoxide anion (O2•−), the total reactive oxygen species (ROS) and the lipoperoxidation in parasites treated with concentrations different of TDZ 02. The TDZ 02 showed IC_{50} values of 15.0 ± 3.0 μM and 1.33 ± 0.52 μM for epimastigotes and amastigotes, respectively, and EC_{50} of 170.0 ± 22.5 μM to trypomastigotes. Additionally, were observed a significant increase in O2•− production and in total ROS production in three forms of parasites treated with TDZ 02, when compared with parasites untreated. Our results showed also, that epimastigotes and amastigotes treated with the same compound showed a significant increase in lipoperoxidation, compared to the untreated parasites. Moreover, trypomastigotes showed no difference in the concentrations and time tested. Therefore, it is possible to suppose that the tripanocidal action of TDZ 02 involves an oxidative imbalance leading cell death. These findings might contribute to the comprehension of the mechanism of action of TDZ 02 in *T. cruzi*.

Acknowledgements: This study was supported through grants from CNPq, Fundação Araucária, FINEP, and CAPES.
Reference: [1] World Health Organization (WHO)., Chagas disease (American trypanosomiasis) (2014) (http://www.who.int/mediacentre/factsheets/fs340/en/).

P-97

Mitochondrial dysfunction induced by compound (1E,4E)-2-methyl-1-(4-nitrophenyl)-5-phenylpenta-1,4-dien-3-one is required in cell death by apoptosis in *Trypanosoma cruzi*

Lazarin-Bidóia D[1], Desoti VC[1], Martins SC[1], Nakamura CV[1], Ueda-Nakamura T[1], Din ZU[2], Rodrigues-Filho E[2], Silva SO[1]

[1]Universidade Estadual de Maringá, Av Colombo 5790, Maringá-PR, Brazil; [2]Universidade Federal de São Carlos, São Carlos-SP, Brazil

The current chemotherapy for the treatment of patients with Chagas' disease based on old drugs has serious side effects and ineffectiveness in the chronic phase of the disease [1]. Consequently, the development of new pharmacologic agents is urgently needed. The compound (1E,4E)-2-methyl-1-(4-nitrophenyl)-5-phenylpenta-1,4-dien-3-one (A3K2A1), a dibenzylideneacetone, has already been described as a trypanocidal agent, making this compound a new alternative for Chagas' disease treatment [2]. Thus, as a way to understand the *Trypanosoma cruzi* death induced by A3K2A1, the goal of this study was to investigate the effect of this compound on mitochondrial membrane potential, cell volume and phosphatidylserine exposure. For this, epimastigotes were treated with 3.5 and 9.0 µM; trypomastigotes with 17.5 and 27.8 µM and amastigotes with 3.9 and 29.5 µM of A3K2A1 for 24 h. After, the cells were incubated with rodamine 123 (Rh123) or annexin-V FITC/PI. All experiments analyses were performed using a BD FACSCalibur flow cytometer. The parasites treated with different concentrations of A3K2A1 exhibited a decrease in fluorescence intensity total of Rh123 (71–92% Rh123-positive parasites) compared with the control group, indicating mitochondrial depolarization. Additionally, the treated with A3K2A1 exhibited an increase in annexin-V fluorescence intensity (10-58% annexin-V-positive parasites), compared with the untreated parasites, indicating phosphatidylserine exposure. Moreover, a decrease in cell volume of greater than 18% was observed in parasites treated compared with the control group. It is possible to suppose that the trypanocidal action of A3K2A1 maybe involve its effect on the mitochondrial function leading to cell death of the parasite by apoptosis.

Acknowledgements: This study was supported through grants from CAPES, CNPq, FINEP and Fundação Araucária.
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P-98

Enzymatic synthesis of heterodimeric flavonoids with antifungal activity

Vásquez J, Castro P, Cotoras M, Mendoza L, Melo R

Laboratorio de Micología, Facultad de Química y Biología, Universidad de Santiago de Chile, Av. Libertador O'Higgins 3363, Casilla-40, Correo-33, Santiago, Chile

Enzyme-mediated synthesis of new compounds presents advantage in comparison to the traditional chemical method, because activation, protection and deprotection of functional groups are not necessary. One of these enzymes is the laccase, a Cu^{+2} containing polyphenol oxidase, which catalyzes oxidation of aromatic compounds and the reduction of molecular oxygen to water. The oxidation of phenolic compounds followed by heteromolecular coupling with non-substrate compounds has been used to synthetize biological active compounds [1,2]. The aim of this work was to synthetize a heterodimer among the flavonoid 3-methyl galangin and *p*-chloro aniline using laccase and to evaluate the antifungal activity against *Botrytis cinerea.* This fungus is a phytopathogen that infects to 250 plant species, causing important losses in Chile [3]. The laccase-

mediated synthesis was carried out in two phases. To good yields were: molar ratio of aniline:flavonoid 2:1, 16.8 mM of flavonoid, 200 rpm, 15 h, and 2.15 U of enzyme. In the reaction, several products were obtained, which were isolated and, characterized by ^{1}H and ^{13}C NMR. Three products were formed: two homomolecular and one heteromolecular. The homomolecular of p-chloroaniline (Benzidine) was generated by a substitution and presented a lesser IC50 (276,8) than aniline (219,7). The heteromolecular compound formed corresponds to 3-(p-Chlorophenylimino)-5,7-dihydroxy-2-phenyl-4-chromanone, the effect of a mixture of these compounds at 60 ppm against mycelia growth of *B. cinerea* was determined. The mixture inhibited in a 34% the mycelial growth of the fungus.

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P-99

Antimicrobial activity and chemical composition of essential oil of *Eucalyptus globulus* Labill. from Arauco, Chile

Morales Rivera MF[1], González-Rocha G[1], Hernández V[2], Domínguez M[1], Bello H[1]

[1]Facultad de Ciencias Biológicas, Universidad de Concepción, casilla 160-C, Concepción, Chile; [2]Facultad de Ciencias. Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Eucalyptus globulus is one of the main forest resources in Chile, its logging generates tons of biomass available for use [1]. Several tests have determined the antimicrobial and antibiofilm properties of *Eucalyptus* oils and their various components [2-4]. Furthermore, the currently available antibiotics are becoming less effective as bacteria have evolved mechanisms that allow them to resist their action. In recent decades, resistance has become a most pressing worldwide public health problem [5]. In the search for new antimicrobials, the activity of *E. globulus* oil from adult leaves, young leaves and fruits was evaluated against various bacterial species. The chemical composition and antimicrobial activity of oils of *E. globulus*, collected in Arauco, was determined by GC/MS and broth microdilution assay, respectively. Forty-nine compounds were identified from the oils. The main identified compounds from adult leaves were 1R-α-pinene (24.9%) and eucalyptol (29.6%). 1R-α-pinene (26.2%), eucalyptol (19%), globulol (9.5%) and α-terpineolacetate (9.5%) from juvenile leaves. Finally, 1R-α-pinene (10.1%), α-phellandrene (21.8%), aromadendrene (10%) and globulol (11%) were the main compounds in the oils from fruits. The oils displayed a variable degree of antimicrobial activity (ANOVA $p<0.0001$). The highest activity, with a minimum inhibitory concentration (MIC) of 2.3 mg/mL, was found in oils from adult leaves against *Acinetobacter baumannii* and the lowest one, with a MIC between 28.8-115 mg/mL was exhibited against *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterococcus faecalis* for all the oils tested. Interestingly, the growth of carbapenemase-producing *K. pneumoniae* ATCC BAA-1705 was inhibited with 24 mg/mL of oils from adult leaves.

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P-100

Evaluation of antileishmanial activity of the fruits pericarps of Brazilian *Sapindus saponaria* L.

Moreira AL, Scariot DB, Nakamura CV, Ferreira ICP

Departamento de Farmácia, Laboratório de Fitoquímica e Tecnologia de Fitoterápicos e Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil

The fruit of *Sapindus saponaria* L. (Sapindaceae), a tropical tree found principally in America and India, is used by population as soap, for curing ulcers, external wounds and inflammations [1]. Scientific works has shown antimicrobial activity [2-4], which may be attributed to the presence of glycosides in fruits pericarps. Some of these structures were described previously [5], but few biological studies have been carried out with these substances. The aim of this study was to obtain hydroalcoholic extract (HE) and glycosides enriched fractions from Brazilian *S. saponaria* and investigate its activity against Leishmania amazonensis and also its haemolytic activity. The HE was obtained by maceration of S. saponaria fruits pericarps with hydroalcoholic solution, and the glycosides present in HE were efficiently fractionated in two separated classes: acyclic sesquiterpene oligoglycosides and saponins (ASOG and SAP) using solid phase extraction in a reversed phase (ODS) cartridge. The compounds in these two classes were identified by ESI-MS/MS and NMR methods. It was found that HE, ASOG and SAP were active against promastigote forms of L. amazonensis with 50% inhibition (IC_{50}) of cell growth at concentrations of 153.70±3.20 µg/mL, 100.92±1.56 µg/ml and 25.41±2.88 µg/ml respectively. As regards haemolitic activity, at 100 µg/mL, HE, ASOG and SAP showed 22.51±1.07%, 0% and 100% of haemolysis. In conclusion, acyclic sesquiterpene oligoglycosides from S. saponaria showed to be active against *L. amazonensis* with low citotoxicity, being a potential compound for the development of antileishmanial drugs.

Acknowledgements: This study was supported through grants from CNPq, Fundação Araucária, FINEP, and CAPES.
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P-101

Antinociceptive effects and toxicity evaluation of the Amazon oil - Pp-oil

Mota A[1], de Lima AB[1], Albuquerque TLF[1], Silveira TS[2], do Nascimento, JLM[3], da Silva JKR[1] , Maia JGS[1], Riberio A[4] , Bastos GNT[1]

[1]Laboratório de Neuroinflamação, Instituto de Ciências Biológicas, Universidade Federal do Pará 1, Rua Augusto Corrêa,01-Guamá, 66075-900 Belém, PA, Brazil; [2]Universidade do Estado do Pará, Av. Plácido de Castro - 1399, 68040-090 Santarém, PA, Brazil ;[3]Laboratório de Neuroquímica Molecular e Celular,Instituto de Ciências Biológicas, Universidade Federal do Pará; [4]Universidade Federal do Sul e Sudeste do Pará,2, Folha 31, Quadra 7, Lote especial s/n, 68507-590 Marabá, PA, Brazil

Pp001 is an Amazon plant and its seed oil (Pp-oil) is used in folk medicine in order to fight against inflammatory processes such as rheumatism. Since Pp-oil mechanism is not well known the aim of this study was to investigate the acute, sub cronical toxicity and antinociceptive effect of Pp-oil. Male swiss albino mice were observed for toxic symptoms and mortality daily for 14 days and were

also submitted to thermal and chemical stimulation at the hot plate test, acetic acid -induced abdominal writhing test and formalin test to evaluate the analgesic activity. No toxicological problems were observed at the doses of 2000 mg/kg and 5000 mg/kg as well in 100 mg/kg and 200 mg/kg body weight in both acute and sub cronical tests, respectively. In the hot plate test (55 ± 0.5° C), animals receiving 200 mg/kg of the oil presented no alterations in latency time when compared to control. However, in the writhing test at the doses of 25, 50 and 100 mg/kg, animals presented a significant decrease in abdominal writhes. Furthermore, the oil at 50 and 100 mg/kg reduced significantly the second phase of the algic stimulus in the formalin test. Moreover, its nociception was reversed by naloxone in order to evaluate its mechanism of action. Therefore the results suggest that Pp-oil has analgesic activity which tests demonstrated to be of putative peripheral origin. The mechanisms are not completely understood even though these results suggest that opiod receptors are involved in the antinociceptive action of Pp-oil.

P-102

Copaiba oil and isolated compounds as chemotherapeutic agent against dermatophytes

Nakamura MT[1], Endo EH[2], Souza JPB[1], Ueda-Nakamura T[2], Dias-Filho BP[2], Freitas O[1], Lopes NP[1], Nakamura CV[2]

[1]Faculdade Ciências Farmacêuticas de Ribeirão Preto - USP, Av. Do Café s/n, CEP 14040-903 Ribeirão Preto-SP, Brazil; [2]Departamento de Ciências Básicas da Saúde, Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil

Copaíba oil, extracted from plants of the *Copaifera* genus was fractionated by liquid chromatography in silica gel 60. The compounds caryophyllene oxide, copalic acid and acetoxy-copalic acid were isolated and their structures were confirmed by ^{1}H and ^{13}CNMR analysis. The aim of present study was to investigate the activity of copaiba oil and isolated compounds against the dermatophyte species *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum gypseum*. It was found that oil and copalic acid were active against dermatophytes with minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) tests [1]. MIC values for oil were 125, 500 and 250 µg/mL while for copalic acid the values were 50, 100 and 50 µg/mL against *T. rubrum, T. mentagrophytes* and *M. gypseum*, respectively. Fungicidal concentrations were the same or just one two-fold dilution above the inhibitory concentration. Acetoxy-copalic acid was not active against the tested strains and caryophyllene oxide showed weak inhibition of fungal growth at 1000 µg/mL. Strong reduction in hyphal growth occurred in fungi treated with oil and copalic acid at sub-inhibitory concentrations when compared with control cells. Irregular growth pattern, short and stubby hyphae and conidia not germinated were observed by Fluorescence Microscopy and Scanning Electronic Microscopy analysis. In conclusion, copaiba oil and its biomarker, copalic acid showed great activity against dermatophytes, being potential compounds for the development of antifungal drugs.

Acknowledgements: This study was supported through grants from CNPq, Fundação Araucária, FINEP, and CAPES.
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P-103

Chemical profiling in Bolivian propolis

Nina V[1,2], Quispe C[1], Gimenez A[3], Schmeda-Hirschmann G[1]

[1]Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [2]Facultad de Ciencias de la Salud, Programa de Magister en Ciencias Biomédicas, Universidad de Talca; [3] IIFB, Universidad Mayor de San Andrés, La Paz, Bolivia

Propolis is a resinous material that honeybees collects from bud and exudates of the plants and is mixed with bee enzymes for use in construction and adaptation of their hives. The chemical composition and bioactivity of propolis samples from several South American countries including Argentina, Brazil, Chile and Uruguay has been described, but there is no scientific information about the chemical composition of Bolivian propolis. The aim of the present work was the chemical characterization of different samples of Bolivian propolis, covering the biomes of the country, from the highlands to the tropical forests. The samples were collected in the Bolivian highland (La Paz, 2 samples), the tropics (Santa Cruz, 3 samples), Cochabamba (2 samples) and the valleys (Tarija, 2 samples and Chuquisaca, 1 sample). The constituents profile was analyzed by HPLC-DAD and HPLC-DAD-MS/MS to get a first insight into the diversity of Bolivian propolis. Clear differences were observed and associated with the different biomes. The propolis from the highlands contained mainly triterpenes belonging to the cycloartane skeleton. By GC-MS and NMR analysis, four triterpenes were identified in the samples from the Departamento de La Paz while the samples from the valleys yielded mainly flavonoids and phenolics. Further work is in progress to characterize the constituents in the Bolivian samples and to associate the composition with possible applications in cosmetics and health.

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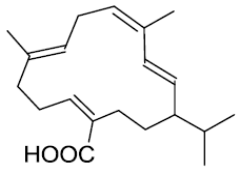
P-104

A cembrane diterpene as chemical marker for propolis from the Andean slopes of the Region del Maule, Chile

Nina V[1,2], Quispe C[1], Leiva E[2], Schmeda-Hirschmann G[1]

[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [2]Facultad de Ciencias de la Salud, Programa de Magister en Ciencias Biomédicas, Universidad de Talca

Propolis is a bee product with increasing demand for its therapeutic properties and potential in cosmetics. In a study with the aim to characterize propolis from the Region del Maule, samples from the central valley, the coastal dry zone and the western Andean slopes were compared by HPLC-DAD. While several of the main compounds were flavonoids, the HPLC pattern of the western Andean slopes showed a constituent with longer Rt and UV spectrum not compatible with this class of constituents. The compound was isolated by column chromatography on silica gel and Sephadex LH-20 and the structure was elucidated by spectroscopic and spectrometric means. The compound, with a molecular formula C20H30O2 showed a conjugated carboxylic acid and two conjugated double bonds. Extensive NMR analysis allowed the identification of the product as the cembrane diterpene poilaneic acid, previously reported from the Asian Euphorbiaceae *Croton poilanei*. This is the first report of a cembrane diterpene in propolis. The finding is a valuable contribution to characterize propolis from the Region del Maule and open the question about the biological origin of this rare diterpene in bee products.



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P-105

Protective effects of South African plants against mutagenicity of aflatoxin B1

Nkala BA[1,2], Botha CJ[2] Elgorashi EE[1, 2]

[1]Toxicology Section, ARC- Onderstedpoort Veterinary Institute, Private Bag X05, 0110, RSA; [2]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04,Onderstedpoort, 0110, RSA

Aflatoxin B1 (AFB1) is the most carcinogenic, hepatotoxic, directly and indirectly-acting mutagen; which poses serious health risk in both livestock and humans [1, 2]. Naturally occurring plant compounds inhibit the mutagenic effect of AFB1 against *Salmonella* strains [3, 4]. The aim of this study was to identify plants which are endemic to SA with potential chemo-protective effect against the genotoxicity of AFB1. The antimutagenic effect of 42 plant extracts was evaluated using Ames test (TA98, TA100 & TA102) with or without metabolic activation [5] and micronucleus test [6] using C3A human liver cell line [7] against mutagenicity of AFB1. None of the extracts had genotoxic effect in Ames test, with or without metabolic activation. 15 extracts possessed some degree of antimutagenic effect against mutagenicity of AFB1. However, extracts of *A. polyacantha* (bark) and *P. nitida* (bark) exhibited strong antimutagenic effects against mutagenicity of AFB1 in both Ames and micronucleus assays. Extracts of *A. polyacantha* (bark) and *P. nitida* (bark) are promising chemo-preventative agents against mutagenicity of AFB1.

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P-106

The root alkaloid extract of *Berberis darwinii* H (Berberidaceae), inhibits innate cellular responses in murine macrophages and monocytes *in vitro*

Núñez D[1], Paredes M[1], Díaz R[2], Yuri L[1]

[1]Research Laboratory of Animal Biotechnology, Universidad de La Frontera, Francisco Salazar # 01145, Temuco, Chile; [2]School of Environmental Sciences, Universidad Católica de Temuco, Prieto Norte # 371, Temuco, Chile

Berberis darwinii is a species that inhabits southern Chile and Argentinian Patagonia [1, 2]. It has been used by the Mapuche for the treatment of inflammation, stomach pain, indigestion and colitis [3,4]. These properties may be associated with the presence of bioactive compounds such as

alkaloids. The purpose of this study was to evaluate *in vitro* the properties of *B. darwinii* alkaloids on cell viability, superoxide anion production and phagocytic activity. Alkaloids were isolated from the dried and crushed roots by reaction of interconversion between salt and free base soluble alkaloid using non polar organic solvents. Concentrations of 100, 1,000, and 10,000 ng/mL were used to assess the cell viability, production of superoxide anion and phagocytic activity in splenic macrophages and blood monocytes of rat. These cells were isolated by discontinuous Percoll gradient and were used at $1x10^6$ cells/mL. Cell viability was evaluated by the trypan blue assay. The superoxide anion was determined by nitroblue tetrazolium reduction (NBT) and the phagocytic activity of phagocytosis by direct counting in the presence of stained yeast. We used bacterial lipopolysaccharide (LPS) and phorbol myristate acetate to activate defense responses in macrophages and monocytes treated *in vitro*. The data indicate that the extract is not toxic to cells at concentrations than 100 ng/mL. Extracts inhibited the phagocytic activity and superoxide anion production in macrophages and monocytes. The results indicate that *B. darwinii* total alkaloids inhibit cellular defense mechanisms and possibly signal transduction pathways that modulate expression of genes involved in immune responses.

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P-107

Variability in the resin composition of the Cupressaceae *Austrocedrus chilensis*

Olate VR[1], Soto A[2], Schmeda-Hirschmann G[1]

[1]Instituto de Química de Recursos Naturales, Laboratorio de Química de Productos Naturales, Universidad de Talca, Casilla 747, Talca, postal code 3460000, Chile; [2]Instituto de Matemática y Física, Universidad de Talca, Casilla 747, Talca, postal code 3460000, Chile

The chemical composition of resins from South American native gymnosperms is an interesting but poorly explored field. The investigations in this area include members of the families Cupressaceae, Araucariaceae and Podocarpaceae, among others [1, 2]. One of the most important native species in the southern part of our continent is the Cupressaceae *Austrocedrus chilensis.* It is a dioecious species covering a wide area in both sides of the Cordillera de los Andes, in Chile and Argentina, and it is locally known as “ciprés de cordillera”. The chemical composition of the resin of this species has been described as a mixture of labdane, abietane and isopimarane diterpenes [3]. When resins of female and male trees were separately analyzed, some differences in its profiles were detected. The aim of the study was to evaluate a possible variability in resin profiles of *A. chilensis*. Single resin drops from different trees were collected in different seasons of the year (spring, summer and winter), including samples from male and female individuals. Resins were analyzed by GC-MS and ^{1}H-NMR techniques. The results were compared by multivariate statistical analysis [4]. The resin profiles showed differences in its composition according to the season of the year. This variability was verified by statistical means. The results also suggested a variation in the relative composition of the resin diterpenes according to the sex of the trees.

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P-108

Study of salmonid diseases using MALDI-MS techniques

Olate VR[1], Nachtigall FM[2], Santos LS[1,2], Ríos M[3], Oyanedel S[4], Díaz V[4], Jakob E[3]

[1]Instituto de Química de Recursos Naturales, Laboratorio de Síntesis Asimétrica, Universidad de Talca, Casilla 747, Talca, postal code 3460000, Chile; [2]Fraunhofer Chile Research Foundation, Nanobiotechnology Division 2 Norte 685, Talca, postal code 3460000; [3]Fraunhofer Chile Research Foundation, Aquaculture Division, Carretera Austral Km 25, Puerto Montt, postal code 5480000; [4]Fundación Chile, Estación Experimental Quillaipe, Carretera Austral Km 25, Puerto Montt, postal code 5480000

Aquaculture is an important activity for Chilean economy, especially salmonid farming. As reported, the salmon industry has been affected by many environmental and biological agents, including infectious diseases. In consideration to the different diseases affecting salmon fishes in the world, the information about salmonid biomarkers could be helpful for salmon industry. The implementation of new diagnostic methodologies is essential in the detection and control of those diseases. Matrix-assisted laser desorption/deionization (MALDI) technique is a good alternative to evaluate its potential applications in the study of salmonid diseases, because it provides useful tools to determine the mass of a large number of biomolecules, allowing the detection and identification of different biomarkers in biological samples as profiling experiments (MALDI-MS) [1,2]. The purpose of this study is to compare spectrometric profiles of farmed salmonid samples to search potential biomarkers of disease. The work includes profiles of healthy and diseased individuals. The samples correspond to different fish tissues, organs and/or fluids from Atlantic salmon. The samples were extracted by a simple extraction method and the corresponding extracts were analysed by a MALDI-TOF equipment (Bruker Daltonics). MALDI-MS profiles were different according to the type of sample (tissue, organ, fluid) and also show slight differences between healthy salmons and fishes in early stages of disease.

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P-109

Anti-inflammatory activity of *Vismia guianensis* (Aubl.) Pers. in the infection induced by *Sporothrix schenckii*

Oliveira AH, Portuondo DL, Duharte AB, Placeres MCP, Ferreira LS, Manente FA, Gonçalves AC, Carlos IZ

Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Farmacêuticas de Araraquara, Departamento de Análises Clínicas. Rua Expedicionários do Brasil, 1621, Centro, 14801902 - Araraquara, SP - Brazil

Vismia guianensis (Aubl.) Pers. (Clusiaceae), known as “lacre”, was chosen for this study because it is popularly described by Amazonian peoples and other tropical regions in the treatment of various diseases such as rheumatism and skin diseases, especially fungal infections. Tests for cell viability were performed using peritoneal exudate cells and spleen cells culture from male Balb/c mice[1]. The analysis of immunological activity was carried out by determining the production and inhibition of NO [2]. Production activities of IL-10, IL-1β, IL-12 and TNF-α were also made. All procedures were authorized by the Ethics Committee CEUA/FCF/CAr No. 03/2014. The extract of the stem bark of *V. guianensis* showed less toxicity to the cells at different concentrations (100

µg/mL, 50 µg/mL, 25 µg/mL), showing cellular viability of 98-100% for exudate cells peritoneal and spleen cell culture. The concentration of 100 µg/mL was used in the other studies. Indicating an anti-inflammatory activity greater inhibition of NO by the extract of the stem bark (81.36% – 8.94 ± 4.51 µmoles nitrito/5x10^6cells) was observed. The anti-inflammatory activity was also observed by the low production of TNF-α and IL-1β and through increased production of IL-10 in animals infected with *S. schenckii* and treated with the extract at the dose of 10mg/kg body weight.

Acknowledgements: CNPq, INPA.
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P-110

Cytotoxic activity and inhibition of P-glycoprotein by 19 synthetic derivatives of Curcumin towards human CCRF-CEM and multidrug resistant CEM/ADR 5000 leukemia cells

Ooko Opany E[1], Titinchi S[2], Efferth T[1]

[1]Institute of Pharmacy and Biochemistry, Johannes Gutenberg University Mainz, Staudinger Weg 5, 55128 Mainz, Germany; [2]Department of Chemistry, University of Western Cape P/B X17 Bellville, 7535 Cape Town South Africa

Human leukemia is one of the commonly diagnosed neoplasms and a major leading cause of human death. Multidrug resistance (MDR) is a phenomenon that is associated with decreased intracellular drug accumulation in the tumor cells. Curcumin has been considered as one of the most promising chemopreventive agents against a variety of human cancers. However the utility of curcumin is limited by its lack of water solubility and relatively low *in vivo* bioavailability. The study focuses on 19 synthetic derivatives of curcumin to assess their cytotoxic activity and their ability to inhibit P-glycoprotein (P-gp) function in parental drug-sensitive CCRF-CEM and multidrug resistant CEM/ADR5000 cell lines in comparison to curcumin. The cytotoxcicity was assessed using the resazurin assay, while the modulation of P-gp function was analyzed by flow cytometry. Among the 19 synthetic derivatives of curcumin 1A11 and 1A6 were the most cytotoxic to CCRF-CEM and CEM/ADR 5000 cells with IC_{50} values in a range of 1.2 µM to 1.4 µM. Flow cytometric analyses showed that the synthetic derivatives significantly increased doxorubicin accumulation in CEM/ADR5000 cells. 1A11 and 1A6 showed the highest increase in doxorubicin accumulation in CEM/ADR5000 cells. This study clearly showed that synthetic derivatives of curcumin inhibit the efflux function of P-gp and are able to overcome some of the limitations of curcumin as they also show lower IC_{50} values and are able to increase doxorubicin accumulation in comparison to curcumin.

P-111

Extracts of plants of the *Virola* genus from the Colombian Amazon: their chemical profiles and the promising antifungal activity against *Fusarium oxysporum*

Orduz-Diaz L[1,2], Cuca LE[1], Jiménez P[2], Coy-Barrera E[2]

[1]Department of Chemistry, Universidad Nacional de Colombia, AA 14490, Bogotá, Colombia; [2]School of Science, Universidad Militar Nueva Granada, AA 49300, Cajicá, Colombia

In the search for antifungal bioactives, natural extracts are a promising opportunity. Plants belonging to the genus *Virola* are widely used by indigenous communities in the Colombian Amazon [1], and they have also been reported as potential antifungals [2]. Eighteen ethanol-soluble

extracts from different parts of plants belonging to the genus *Virola* (*V. carinata, V. elongate, V. peruviana, and V. callophhylla*) were evaluated using the micro-scale supplemented medium method against *Fusarium oxysporum*. The extracts were profiled using RP-UFLC-DAD chromatographic techniques, and total phenolics and flavonoids were also quantified [3] in order to get fingerprints and determine similarities in the composition thereof. The results indicated that extracts exhibited dose-dependent inhibition of fungal mycelium at different levels. The UFLC-derived profiles allowed determining that the chemical composition of the extracts considerably varies between species, although they showed some similarities between extracts from the same part of a plant. Additionally, a principal component analysis (PCA) with antifungal activity and total contents data and a partial least squares (PLS) analysis with chromatography data clustered the extracts by common chemical and biological features, indicating a composition-activity relationship useful for further studies focused on rational isolation of antifungals from *Virola* species.

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P-112

Radical scavenging capacity and LC-based profiling of highbush blueberry fruits from Colombia

Prada J, Orduz-Diaz L, Coy-Barrera E

Bioorganic Chemistry Laboratory, Universidad Militar Nueva Granada, AA49300, Cajicá, Colombia

Highbush Blueberry (*Vaccinium corymbosum*) is a shrub fruit species belonging to the Ericaceous family. It is characterized by fruits valued for their flavor, being also very renowned for their content of anthocyanin-related compounds (1). Hence, its fruits have been an objective in several studies (2), but there is a lack of chemical information related to the fruits commercialized and cultivated in Colombia. However, several varieties of *V. corymbosum* fruits are cultivated in different regions throughout Colombia without any control of chemical composition and/or biological potency. Therefore, the present work shows the antioxidant capacity by methods DPPH and FRAP for methanol-soluble extracts of commercial and cultivated samples in Colombia. Reducing power (by FRAP) was found to be proportional to the anthocyanin content. Hence, extracts from a commercial and two cultivated samples were found to have good reducing power. Radical scavenging capacity by DPPH was observed to be good for commercial and Legaxi cultivar (IC_{50} <30 µg/mL). A variability of antioxidant capacity and LC-based anthocyanin profiles between samples was evidenced, indicating the importance of quality controlling for cultivar conditions of these fruits in order to offer and ensure the better medicinally-attributed properties. Our results constitute the first report on chemical analysis of *V. corymbosum* fruits cultivated and commercialized in Colombia with the aim to highlight the requirement for authentication and quality controlling on these materials.

Acknowledgments: The present work is a product derived by the Project IMP-CIAS-1567 financed by Vicerrectoría de Investigaciones at UMNG - Validity 2014.
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P-113

Ancient medicinal plants as sources of new antibacterial compounds

Orrego E

Saint Thomas University, Basic Sciences Department, Río Copiapó Sur 2351, Copiapó, Atacama region, Chile

In Chile there is an old and interesting tradition of medicinal plants use. In Copiapó (northern Chile) and rural areas, the traditional knowledge is much better preserved than in urban areas with less access to the native resources (1). The aim of this work was to determine *in vitro* the antimicrobial activity of hydroalcoholic and organic extracts of ethnic medicinal plants against bacteria. The medicinal plants were selected on the basis of traditional use for infectious diseases. The plant material was cleaned and dried at 55ºC for 72h, powdered and submitted to Sohxlet extraction with 50% v/v ethanol solution during 3.5 hours. The hydroalcoholic solution was dried under reduced pressure at 45ºC to obtain a dry residue that was suspended in ethanolic solution to obtain a hydroalcoholic extract with concentration of 50 mg/mL. A similar procedure was carried out with other sample of the same plant using methanol-acetone as a solvent. The plant material was extracted in a Soxhlet with methanol-acetone for 3.5 hours. Then, the extract was taken to dryness under reduced pressure. Extracts were tested against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas* sp. by the diffusion method. Culture dishes were incubated at 37ºC for 24h. The inhibitory effect was determined measuring the diameter of inhibition of bacterial growth. Seven out of twelve plants presented activity, being the most active the extracts from *Geoffroea decorticans*. Compared with gentamicin and cirpofloxacin their activity was 52.1 and 35.5% against Gram-positive, and 50.8 and 55.9% against Gram-negative microorganisms, respectively. The phytochemical analysis showed flavonoids and tannins detected in the ethanol extract, and alkaloids in the methanol-acetone extracts of *G. decorticans* (2).

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P-114

Chemical modifications of agarose from *Ahnfeltia plicata* (Rhodophyta, Ahnfeltiales) and synthesis of graft copolymers with *N*-isopropylacrylamide

Ortiz JA, Matsuhiro B, Zapata PA

Universidad de Santiago de Chile, Avenida Libertador Bernardo O´Higgins 3363, 9170022, Chile

During the last years, natural polysaccharides have been widely studied and modified for the preparation of hydrogels, which can encapsulate and control the release of drugs, cells or proteins under specific conditions as pH and temperature. Some modifications of polysaccharides that conduce to the hydrogels synthesis are crosslinking (chemical or ionic) and graft copolymerization with a monomer or synthetic polymer [1]. Many studies indicated that the conjugation of synthetic polymers with a polysaccharide enhanced the pH and temperature responsive properties, close to the physiological conditions of human body values [2]. In this study, agarose from *Ahnfeltia plicata* collected in the Magellan region [3], a neutral linear polysaccharide of Dgalactose and 3,6-anhydro-L-galactose was modified by carboxymethylation with monochloroacetic acid in 2-propanol, formamide and dimethylsulfoxide and by esterification with maleic anhydride to enhance the solubility in aqueous medium. The normal and second derivative FT-IR, and 1D and 2D NMR

studies showed a good incorporation of carboxymethyl and maleate groups on C-6 of β-D-galactopyranosyl residues. The modified derivatives from agarose were copolymerized with *N*-isopropylacrylamide using ceric nitrate as initiator. These copolymers were characterized by spectroscopic methods. NMR spectroscopic data confirm that the grafting of *N*-isopropylacrylamide has taken place on the polysaccharide backbone. Modification of agarose by carboxymethylation, esterification and graft copolymerization produced derivatives with potential biotechnological applications.

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P-116

Is *Hypericum perforatum* most appropriate therapeutic modality for the treatment of diabetic patients with mild-to-moderate depression?

Öztürk Y, Can ÖD, Demir-Özkay Ü

Anadolu University, Faculty of Pharmacy, Departments of Pharmacology, Eskisehir, Turkey

St.-John's Wort (*Hypericum perforatum* L.) is well-known antidepressant plant which has been used as both therapeutic drug and OTC product in all around the world. There are many ethnomedical, experimental and clinical studies demonstrating its antidepressant activities. It seems to be quite effective on the treatment of mild-to-moderate depression, as reported in various controlled clinical trials. In addition to its well-known antidepressant activities, St.-John's Wort has been reported to be used in folkloric medicine for the cure of diabetes mellitus. We have quite recently reported that extracts of aerial parts of *Hypericum perforatum* L. normalize blood glucose and pain perception [1] and depression/anxiety levels [2] in streptozotocin diabetic rats.
On the other hand, clinical management of depression is a dilemma in diabetic patients. Most of antidepressant agents may interfere with glycaemic controls by increasing or decreasing blood glucose levels in depressive patients with diabetes mellitus. On the other side of this clinical problem, there are drug-drug interactions interfering both antidepressant and antidiabetic treatments. Hence, preparations of St.-John's Wort seem to be most appropriate cure for the diabetic patients having mild-to-moderate depression. However, it should not be combined with other antidepressant drugs to avoid dramatic herb to drug interactions, i.e. serotonin syndrome. Further, implications are discussed on the basis of literature data.

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P-117

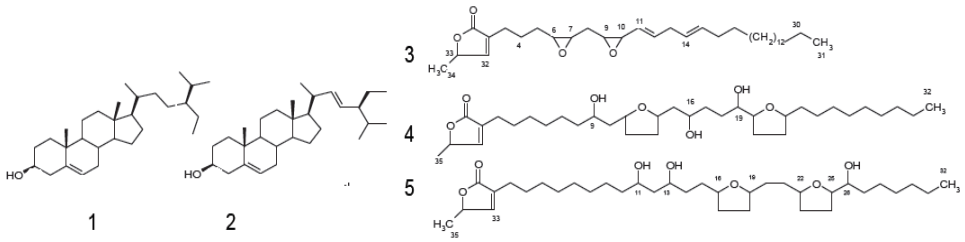
Secondary metabolites from roots of *Annona purpurea*

Hernández-Fuentes GA, Parra-Delgado H, Silva-Zamora F

Facultad de Ciencias Químicas, Universidad de Colima, Campus Coquimatlán, Km 9, Carr. Colima-Coquimatlán, CP 28400, Colima, México

Annona purpurea is a tree native to America. Its fruit known as "cabeza de negro" is edible and several parts of the tree are used in traditional medicine. In previous chemical studies, alkaloids and acetogenins were isolated from bark, leaves and seeds [1]. Taking into account the long worldwide

ethnomedical history, it was proposed, the chemical study of the root, with the aim of carrying out the isolation and structural elucidation of secondary metabolites of *Annona purpurea*. Therefore, several ascending polarity extracts (hexane, dichloromethane and methanol) were prepared. After, each extract was chromatographed by further Si gel column, preparative TLC or reversed-phase HPLC. Then, β-sitosterol (1), stigmasterol (2) and three acetogenins (3-5) were isolated from hexane and dichlorometane extracts. All isolates were identified by 1H and 13CNMR, IR and MS. Additionally, treatment of methanol extract under basic/ acid conditions followed by HPLC allowed to detect 13 alkaloids.



Acknowledgements: H. Parra-Delgado thanks financial support from PRODEP.
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P-118

Phytochemical study of *Ampelocissus acapulcensis*

Guzmán-Lopez B[1], Parra-Delgado H[1], Medrano-García MR[1], Torres-Colín R[2]

[1]Facultad de Ciencias Químicas, Universidad de Colima, Campus Coquimatlán, Km 9, Carr. Colima-Coquimatlán, CP 28400, Colima, México; [2]Herbario Nacional de México (MEXU), Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, Distrito Federal, México

Roots of *Ampelocissus acapulcensis,* known as wild grape in Colima, Mexico, are used in Mexico for treatment of Diabetes as well as for kidney`s illness [1]; however there are no information about its chemistry. Taking that into account, a dried sample of roots was extracted with ascending polarity solvents (hexane, EtOAc, MeOH) and three extracts were obtained by evaporation under vacuum. MeOH extract showed antioxidant activity in DPPH model [2]. Phytochemical screening indicated the presence of sterols in hexane extract, phenolic metabolites in EtOAc extract, and saponins, tannins and carbohydrates in MeOH extract. A conventional analysis of the hexane extract by column chromatography allowed the isolation of β-sitosterol and stigmasterol, which were identified by ^{1}H and ^{13}C-NMR as well as by comparison with authentic sample.

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P-119

The comparative action of two vehicles of *Copaifera langsdorffii* leaves extract in skin wound healing

Gushiken LFS[1], Hussni CA[2], Bastos JK[3], Lemos M[3], Polizello Jr M[3], Padovani CR[1]; Takahira RK[2], Pellizzon CH[1]

[1]Instituto de Biociências, Unesp - Campus de Botucatu, Caixa Postal 510, Botucatu, Brazil; [2]Faculdade de Medicina Veterinária e Zootecnia, Unesp - Campus de Botucatu, Caixa Postal 560, Botucatu, Brazil; [3]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ribeirão Preto, Brazil

Skin wound healing is a dynamic process which involves injury edge contraction, connective tissue remodeling and increase of vessels, resulting in the formation of a scar until total skin remodeling. We use the hydroalcoholic extract of leaves of *Copaifera langsdorffii* (EC) due to its related anti-inflammatory, antimicrobial and wound healing properties [1]. This study aims to analyze the skin healing process in rats treated with two different vehicles (aqueous and ointment base) of EC. Male Wistar rats were submitted to injury of 2 cm diameter in the dorsal region. They were divided in groups (n=6) to daily treatment with EC at 1, 5 and 10%, negative control (S) and positive control (Colagenase) within 3 (M3) days. After sacrifice, total blood was collected for hepatic and renal enzymes and dorsal lesions were collected for histological analysis, to measure the cellularity in normal skin, edge and center of the lesion. EC didn´t show statistically change of weights or any hepatic and renal enzyme alteration. The percentage of retraction in M3 were 27% (S and EC1), 34% (EC5) and 36% (EC10) for aqueous extract and 18% (S), 19% (Colagenase) and 25% (EC1, EC5 and EC10) for ointment base and follows a trend to lesion retraction. The cellularity showed statistical difference between the three treated groups and controls in the edge of the lesions after M3. The data from this study stand out the role of EC in skin wound healing process during 3 days of treatment.

Acknowledgements: FAPESP.
Reference: [1] Paiva L.A.F. et al. (2002) Phytother. Res. 16:737-739.

P-120

Differences in peroxyl radical scavenger capacity of ethanolic extracts from five *Ugni molinae* genotypes

Peña-Cerda M[1], Arancibia-Radich J[1], Perez R[1], Valenzuela P[1], Seguel I[3], Zuñiga MC[2], García L[1], Delporte C[1]

[1]Laboratorio de Productos Naturales; [2]Laboratorio de Radicales Libres y Antioxidantes. Facultad de Ciencias Químicas y Farmacéuticas, U. de Chile. Santos Dumont 964, Santiago. Postcode 8380494, Chile; [3]INIA, Carillanca, IX Región

Murta leaves have been the focus of recent research that demonstrates genotype has an influence in pharmacological activity [1,2]. The aim of this study was to evaluate the effect genotype has on peroxyl radical scavenger capacity in five murta leaves ethanolic extracts. The murta leaves of different genotypes (accession numbers: 14-4, 19-1, 19-1ha, 22-1 and 27-1) were ceded by INIA Carillanca and the ethanolic extracts (EE) were obtained by successive maceration with solvents of increasing polarity. ORAC assay was used to detect differences in peroxyl radical scavenger activity of the EEs using fluorescein as the fluorescent probe [3]. The 22-1 and 14-4 genotypes show significant greater activity than the other genotypes; while 19-1ha showed the lowest activity. Significance ($p<0,05$) was determined using the One Way ANOVA and Tukey's multiple comparison test. The results of the ORAC assay allowed us to conclude that the antioxidant capacity of murtilla leaves is affected by their genotype, and this could be due to differences in chemical composition

(mainly quercetin and miricetin heterosides) [4]. The search for an improved and standardized culture with medicinal purpose is an important aspect in natural product research and it should be taken into account to achieve chemical and pharmacological standardization.

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P-121

A comparative study of the DPPH• radical scavenging kinetics of different *Ugni molinae* genotypes

Pérez-Arancibia R[1], Peña-Cerda M[1], Rivera-Gonzalez D[1], Valenzuela-Bustamante P[1], Seguel I[2], Delporte C[1]

[1]Laboratorio de Productos Naturales, Departamento de Farmacología y Toxicología, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. Santos Dumont 964, Santiago, Chile, postcode 8380492; [2]INIA, Carillanca, IX Región

Murtilla (*Ugni molinae,* Turcz*.)*, also known as “murta”, is a wild shrub that grows in south-central Chile that is commonly used in Chilean folk medicine for the treatment of diarrhea, dysentery and to lessen urinary tract pain [1,2]. Several studies have been performed to assess the chemical composition of murta leaves [1,2,3]. The aim of this study is to compare the radical scavenge ability of 5 different ethanolic leaves extracts (EEs) from different genotypes of murtilla cultivated in the same conditions and selected by the quality of their fruits. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) method studies the ability of the EEs to scavenge free radicals, and is based on the decrease of absorbance of the DPPH solution at 517nm when adding an antioxidant [6]. We found that there were no significant differences ($p < 0,05$) between the antioxidant capacities of the different EEs, except for the EE 19-1 ha, which had the highest concentration needed to reduce 50% of DPPH• (EC_{50}) (12.45 ± 0.5 mg/mL). EE 27-1 had the lowest EC_{50} (10.13 ± 0.58 mg/mL), thus being the extract with the highest antioxidant capacity. Previous studies have described the presence of phenolic substances, such as quercetin, myricetin, kaempferol epicatechin, and some of their glycosylated derivatives, which are supposed to be responsible for the antioxidant capacity of EEs [1,2,5,6]. The variations between the antioxidant capacities of the EEs can be related to differences on their phenolic composition due to their different genotypes.

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P-122

Synthesis and cytotoxicity of new hybrid compounds combining terpenes and commercial anti-inflammatory drugs

Pertino MW[1], Theoduloz C[2], Schmeda Hirschmann G[1]

[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [2]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, Talca, Chile

Chemical diversity is required when looking for structure-activity relationships in natural and synthetic compounds. The diterpenes ferruginol **1** and imbricatolic acid **2**, and the triterpene oleanolic acid **3** present a strong gastroprotective activity in induced gastric lesion models in rodents. Another relevant activity reported for those terpenes is anti-inflammatory effect. The anti-inflammatory drugs ibuprofen **4** and naproxen **5** have a carboxylic acid function that can be used to prepare hybrid compounds, coupling the synthetic moiety with an alcohol or phenol function in terpenes. The aim of the present work was to synthesize a small library of compounds containing a naturally occurring terpene (**1**-**3**) and a synthetic anti-inflammatory moiety (**4** or **5**). The ibuprofenyl and naproxenyl esters of **1**, **2** and **3**, were synthesized via the acid chloride of **4** or **5**. The methylesters **9**, **11**, **13** and **15** were prepared treating the carboxylic acids **8**, **10**, **12** and **14** with diazomethane. All the compounds were assessed for cytotoxicity towards human lung fibroblasts and the human cancer cell lines AGS and HepG2. The synthetic compounds **4** and **5** were devoid of toxicity towards the three cell lines, with IC_{50} values > 1000 µM. The most toxic and less selective compound was ferruginol, with IC_{50} values in the range of 29-39 µM for the three cell lines. The toxicity was strongly reduced in the ferruginyl hybrids **6** and **7** with values closer to that of the synthetic part of the molecule. The hybrids from imbricatolic acid were more toxic than the starting diterpene. However, a strong decrease in cytotoxicity was observed after methylation for the diterpenyl derivatives **9** and **11**. For the oleanolic acid derivatives, all hybrids were less toxic than the parent terpene, showing that the new compounds present relevant changes in the selected parameter.

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Chilean *Prosopis* mesocarp flour: polyphenolic content, antioxidant activity and fingerprint analysis

Schmeda-Hirschmann G[1], Quispe C[1], Soriano MDPC[1], Theoduloz C[2], Jiménez-Aspee F[1], Pérez MJ[3], Cuello AS[3], Isla MI[3]

[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [2]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Chile; [3]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA-CONICET). Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina

In South America, the mesocarp flour of *Prosopis* species played a relevant role as a food resource in pre-hispanic times. The main local source of the pods is *P. chilensis,* but other species might occur. The aim of the present work was the characterization of the phenolic antioxidants occurring in the mesocarp flour of Chilean *Prosopis* occurring in areas associated with the traditional use of this resource. Large variations in pods shape, flour content and composition were observed. Best flour yield was found for a deep purple sample from Puquio (36.72%) followed by the pods accepted as edible from *P. chilensis* (15.5-31.79%). Overall, the phenolic content of the flour was

low. Highest phenolic-enriched flour extract yield was found for the Puquio sample (4.26%), with lower values for *P. chilensis* (0.10 to 2.08%). The phenolic content in the enriched flour extracts (PEFE) of *P. chilensis* morphotypes was 3.27-12.46%. The best antioxidant activity of the PEFE, measured by the DPPH assay was observed for samples from the Copiapo and Huasco valleys. HPLC-MS/MS^n analysis allowed the tentative identification of eight antocyanins in the deep purple sample from the Copiapó valley and 14 phenolics including flavonol glycosides, *C*-glycosyl flavones and ellagic acid derivatives in the different flour mesocarp samples analyzed. The antioxidant activity and other biological effects described for the compounds occurring in the flour suggest a nutraceutical potential for this ancient South American food resource.

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P-124

Activity of *Tilia americana* var. *mexicana* on memory loss of hypertensive mice

Ramírez-Serrano C[1], Jiménez-Ferrer E[2], Zamilpa A[2], Martínez-Hernández G[2], Herrera-Ruiz M[2]

[1]Facultad de Ciencias Biológicas - UAEM. Universidad Av.1001.Chamilpa. Cuernavaca, Morelos. P.C. 62209 Mexico;[2]Centro de Investigación Biomédica del Sur - IMSS. Argentina 1 Centro, Xochitepec, Morelos. P.C. 62790 Mexico

Hypertension induces loss of memory and executive functions (**1**), both crucial for the interaction between individuals. *Tilia americana* is widely used in Mexico to treat anxiety and hypertension (**2**). In this work the hydroalcoholic extract of *T. americana* (TaHA) was evaluated on the memory of Angiotensin II (AngII) and L-butionin-(S,R)-sulfoximine (BSO) hypertension-induced mice. ICR mice (35g, n=8) were used for the experiments. The groups included 1) Basal (untreated), 2) Hypertensive, 3) TaHA-treated mice. Group 2 and 3, received 400 mg/kg BSO and 0.4µg/Kg AngII (intraperitoneally)/21 days. From day 15 until day 29, group 3 received 100mg/kg TaHA; group 1 received Tween20, vehicle. The blood pressure was measured and animals were subject to Morris Water Maze (WM) and open field (OFT) tests. Results were analyzed using ANOVA and Tukey post hoc test. Results: BSO/AngII induced hypertension (HAS=153.78/111.62mmHg); TaHA causes a significantly decrement of this condition (116.20/ 67.29mmHg). In OFT, there was no difference between the groups. In WM test, the hypertensive group (2) showed an increment on latency to the Platform quadrant (13.77s) respect to Basal (9.81s), while mice with TaHA diminished this time (7.30s). Entries to the Platform quadrant, was higher in the TaHA group (4.33) and Basal (4.33) in comparison with hypertensive mice (3.44). These spent a total time in Platform quadrant of 11.81s while mice with TaHA increment its (13.65s) similar to the basal group (15.87s). Frequency of platform zone was lowest in hypertensive group (1.14), TaHA induced an increment (1.55). Time spent in the Platform zone was 0.22s in hypertensive mice, but this parameter was incremented with TaHA (0.37s). Conclusion: For the first time, it is shown that *T. americana* possesses an anti-hypertensive chronic effect and diminished the cognitive damage.

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P-125

Chemical characterization and antiproliferative activity of *Asclepias subulata*

Rascón-Valenzuela LA[1], Robles-Zepeda RE[1], Garibay-Escobar A[1], Velázquez-Contreras CA[1], Medina- Juárez LA[2], Jiménez-Estrada M[3]

[1]Departamento de Ciencias Químico Biológicas, Universidad de Sonora; [2]Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora; [3]Departamento de Productos Naturales, Instituto de Química, Universidad Autónoma de México

Several species of the genus *Asclepias* are part of traditional medicine since ancient times, and 14 out of 68 species found in Mexico are used as medicinal plants [1], including *Asclepias subulata*. *A. subulata* is a shrub occurring in the Arizona-Sonora desert. It is used by ethnic groups of Sonora for the treatment of gastrointestinal disorders, eye diseases and cancer [2]. There are few studies on their biological properties. The main aim of this project was to evaluate the antiproliferative activity of *A. subulata* methanol extract and its fractions on cancer cell lines (M12.C3.F6, RAW 264.7, HeLa, PC-3, A549 and LS180). In addition, we studied the mechanism of action of the active fractions and carried out the chemical characterization of the active compounds. The methanol extract of *A. subulata* and its solvent fractions showed strong antiproliferative activity against human cancer cell lines (IC_{50} 0.2 to 30 µg/mL). The polar fractions showed a higher cytotoxic activity and selectivity toward cancer cells. The polar fractions were separated chromatographically and most active subfractions (IC_{50} <0.2 µg/mL in A549 cells) were obtained. These subfractions induced apoptosis of cancer cells by the intrinsic apoptotic pathway. Four active compounds were characterized: calotropin, 12,16 dihydroxycalotropin, corotoxigenin and desglucouzarin. In conclusion, *A. subulata* has substances with strong antiproliferative activity and selectivity against human cancer cells. The mechanisms of action of these substances must be studied in detail.

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Biological and chemical activity of aqueous and methanolic extracts of *Asparagus officinalis* (Asparagus)

Rodríguez-Azúa R, Concha-Meyer A

Centro de Estudios en Alimentos Procesados, CONICYT-Regional, Gore Maule, R09I2001, Talca, Chile

Cardiovascular diseases (CVD) are the leading cause of death worldwide. Studies demonstrate the protective effect of fruit and vegetables [1]. In this study was evaluated the antioxidant and human antiplatelet activity of aqueous and methanolic extracts of asparagus. The *in vitro* evaluation of the antiplatelet activity of the aqueous and methanolic extracts of asparagus was done using ADP, arachidonic acid, collagen and TRAP-6 as agonists [2], while the antioxidant activity of the extracts was determined using the radical 2,2-diphenyl-1-picryl hydrated hidrazilo (DPPH, $C_{18}H_{12}N_5O_6$) violet discoloration test. Also crude fiber, fat, ash, and protein were determined using the proximal moisture content analysis. The results show a significant antioxidant capacity and antiplatelet activity *in vitro* of aqueous and methanolic extracts at a concentration of 1mg/mL. *In vitro* studies showed that both the edible part of the industrial waste as asparagus, show statistically significant

antiplatelet activity compared to the negative control when 2µg/mL collagen is used as agonist ($p < 0.05$, ~ 48 ± 7, ~ 1% and 53 ± 5%; centers and industrial waste respectively) and 1 mM arachidonic acid ($p < 0.05$, ~ 16 ± 4.1% and ~ 60 ± 2.5%, respectively centers and industrial waste).
Therefore, given the antiplatelet activity and antioxidant capacity, these extracts of asparagus and asparagus industrial waste could represent an important source for the prevention of atherothrombotic events.

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P-127

Preventive effect of *Myrcia bella* hydroalcoholic extract on gastric ulcer in rats

Rozza AL[1], César DAS[1], De-Faria FM[2], Souza-Brito ARM[2], Saldanha LL[3], Dokkedal AL[3], Pellizzon CH[1]

[1]Morphology Department, UNESP, Rubião Jr District, wn Botucatu/SP, Postal code 18618-970 Brazil; [2]Physiology Department, Unicamp, Zeferino Vaz Campus, wn Campinas/SP, Postal code 13083-970 Brazil; [3]Biological Science Department, Unesp, Eng Luiz EC Coube Avenue, 14-01 Bauru/SP, Postal code 17033-360, Brazil

Myrcia bella (MB) presents ethnopharmacological indication to treat gastrointestinal diseases such as gastric ulcer and diarrhea. The present work aims to evaluate the gastroprotective effect of MB hydroalcoholic extract. Male Wistar rats (n=7, 250 g) were orally treated with vehicle (0.9% saline), carbenoxolone 100 mg/kg or MB 50, 100 or 150 mg/kg. After one hour, the rats were treated with 1 mL of ethanol, and after one additional hour the rats were euthanized and the stomachs were removed. Ulcer area was measured (mm2). Samples of stomachs were destined to histological analysis, measurement of antioxidant markers (glutathione GSH, glutathione reductase GR, glutathione peroxidase GPx, superoxide dismutase SOD and myeloperoxidase MPO) and inflammatory cytokines (tumor necrosis factor-α TNF-α, interleukin IL-6 and IL-10) by ELISA. Hepatic and renal toxicity was evaluated in the rat's serum. The assay was approved by UNESP Institutional Animal Care and Use Committee (permit number 342-CEUA). Mean ± s.e.m., ANOVA, Tukey or Dunnett, $p<0.01$. MB 50 mg/kg was the lowest effective dose, presenting 69% of gastroprotection in comparison to the vehicle group. Histological analyses evidenced gastric mucosa preservation and mucus production in MB group. MB treatment increased GSH level and decreased MPO activity in comparison to vehicle group, besides decreasing the levels of pro-inflammatory cytokines TNF-α and IL-6 and increasing the level of anti-inflammatory cytokine IL-10. There was no sign of toxicity in the rat's serum. MB presented gastroprotective effect with antioxidant and anti-inflammatory activity without toxic effects in rats.

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P-128

Isolation and structure elucidation of anthocyanidin 3,7-*O*-β-diglucosides from calafate (*Berberis microphylla* G. Forst) berries

Ruiz A[1,5], Mardones C[1], Vergara C[1], von Baer D[1], Gomez-Alonso S[2,3], Gomez M[2,3], Hermosín-Gutiérrez I[2,4]

[1]Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, PO Box 160-C, Concepción, Chile; [2]Instituto Regional de Investigación Científica Aplicada, Universidad de Castilla-La Mancha, Campus Universitario s/n, 13071 Ciudad Real, Spain; [3]Fundación Parque Científico y Tecnológico de Albacete, Paseo de la Innovación, 1, 02006, Albacete, Spain; [4]Escuela de Ingenieros Agrónomos de Ciudad Real, Universidad de Castilla-La Mancha, Ronda de Calatrava 7, 13071 Ciudad Real, Spain; [5]Departamento de Ciencias Químicas y Recursos Naturales, Universidad de la Frontera, PO 54-D, Temuco, Chile

Calafate (*Berberis microphylla* G. Forst) is a native berry grown in the Patagonian area of Chile and Argentina, very rich in polyphenols and high antioxidant activity [1, 2]. The study of this phenolic composition has revealed this fruit contains unusual phenolic structures when compared to other berries. In the case of hydroxycinnamic derivatives, 20 acid derivatives were identified [(2)]. Regarding anthocyanins, 3 series of 5 derivatives has been found, but a fourth series of dihexosides was also identified and tentatively assigned as 3,5-diglucosides of the same 5 anthocyanidins [1]. However, the latter compounds did not chromatographically matched with authentic standards of these compounds. In this work, the aforementioned five anthocyanidin dihexosides have been isolated and their structure unequivocally elucidated. The isolation was achieved by a combination of fractionation by fast centrifugal partition chromatography (FCPC), SPE (C18 reversed-phase and/or mixed ion exchange and reversed phase), and semi-preparative HPLC purification. The structure elucidation was mainly based on ESI-MS/MS, ^{1}H-, and ^{13}C-NMR spectroscopic data. The main anthocyanidin dihexosides were fully characterized as the 3,7-*O*-β-diglucosides of delphinidin, petunidin and malvidin. The two other minor compounds were isolated in low amounts and not enough pure to perform a complete NMR study. However, the 3,7-*O*-β-diglucoside of peonidin was assigned on the basis of its chromatographic (retention time) and spectral (UV-vis, MS/MS, and ^{1}H-NMR) data that matched with those reported for a standard of this compound previously isolated from Garnacha Tintorera grapes [3]. Finally, the occurrence of cyanidin 3,7-*O*-β-diglucoside was suggested by its UV-vis and MS/MS spectral data.

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P-129

Pre-Columbian edible plants used for food and medicine by people from Williche community in southern Chile

Saavedra-Rodríguez R

Departamento de Ciencias Básicas, Universidad Santo Tomás, Los Carrera 753, Osorno, Chile

An ethnobotanical study was carried out from 2007 to 2008 to investigate traditional knowledge of native edible plants in the commune of San Juan de la Costa, Los Lagos Region, Southern Chile; an important biodiversity hotspot. Information was collected from 35 informants, using direct observation, botanical collections, and key informant interviews with semistructured questionnaires. A total of 77 edible plant species in 66 genera of 44 families were recorded. They were listed with

scientific name, family, indigenous name, and traditional uses. The best families represented were Rosaceae with 7 species, Myrtaceae (5), Asteraceae (5), Philesiaceae and Poacea (4). Most species were edible fruits (35) and vegetables (14). It was also recorded the culinary use of 11 species of fungi, spices (6), underground edible organs (5), flowers (2), and seeds (2). Most species (70%) have multiple uses (*i.e.* medicinal, basketry, ritualistic) besides food value. Fruits are mainly collected and eaten raw. Vegetables are usually boiled and served as side dishes. The most commonly eaten fruits are: *Ugni molinae*, *Aristotelia chilensis*, *Greigia sphacelata*, *Lapageria rosea*, and *Peumus boldus*. The most widely used vegetables are: *Gunnera tinctoria*, *Mimulus glabratus*, *Chusquea coleou*, *Brassica campestris*, and *Blechnum chilense*. Food plants species are abundant and diverse in this region; they provide food and nutrients to local people and are also a source of cash income. Thus, documentation of these species may provide information for conservation, sustainable utilization, and preservation of local traditional knowledge and cultural heritage.

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Effect of terpenes and flavonoids of *Serjania schiedeana* in the model of carrageenan/kaolin induced mono-arthritis

Sánchez-Sánchez V [1,2], Salinas-Sánchez DO[3], Jiménez-Ferrer E[1], Zamilpa A[1], Herrera-Ruiz M[1]

[1]Centro de Investigación Biomédica del Sur-IMSS. Argentina No. 1 Col Centro, Xochitepec, Morelos, 62790; [2]Facultad de Ciencias Biológicas-UAEM. Av. Universidad 1001. Col. Chamilpa. Cuernavaca, Morelos. C. P. 62209; [3]Centro de Investigación en Biodiversidad y Conservación. Av. Universidad 1001. Col. Chamilpa. Cuernavaca, Morelos. C.P. 62209

Serjania schiedeana is used in the Mexican traditional medicine for the treatment of inflammatory illness [1]. Few studies are related to this specie, so little is known about its chemistry and pharmacology. In this project, we assessed the effect of *S. schiedeana* in the carrageenan/kaolín induced mono-arthritis and identified chemical compounds responsible for this activity. The size of the joint of ICR female mice (28 g) with micrometer, anesthetized with pentobarbital (60mg/kg) and injected with kaolin (4 %, 40µl) and carrageenan (2%, 40µl) in the joint cavity was measured heel subsequently flexion and extension were performed [2]. The development of joint inflammation was measured. Oral treatment was initiated for 10 days: Methotrexate 1.0 mg/kg; SesAcOEt 200 and 400 mg/kg, F14 and F16 at 10 mg/kg. The vehicle group presented (animals with experimental arthritis) showed a slowest recovery velocity of joint damage induced by carrageenan -0.15 ± 0.033, this inflammatory effect was blocked by the administration of methotrexate (0.067 ± 0.026), ethyl acetate extract at 400 mg/kg (0.10 ± 0.03), fraction 14 (0.089 ± 0.032) and fraction 16 (0.139 ± 0.033). The dose of 200 mg/kg was not able to prevent joint inflammation (-0.123 ± 0.025). The index of spleen indicate to decrease in weight regarding the treatments F-14 (0.56% ± 0.1), 16 (0.4 ± 0.05), SesAcoEt 400 mg/kg (0.52 ± 0.07), Metotrexato (0.4 ± 0.06) and Meloxicam (0.64 ± 0.11) presented a significative difference with respect of goup Vehicle (0.65 ± 0.11), the treatment SesAcoEt 200mg/kg (0.7 ± 0.07) is not different with respect to Vehicle. Thin layer chromatography indicated that the fraction F14 and 16 contain mainly terpenes and flavonoids, some shared by both fractions. Conclusion: *Serjania schiedeana* is a plant with potential to be evaluated in biological models related to inflammation, between them Rheumatoid arthritis.

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P-131

Inhibitory potential of essential oils from the genus *Croton* and *Citrus* against acetylcholinesterase

Lima GS[1], da Camara CAG[1], dos Santos GF[2], Takahashi JA[2]

[1]Universidade Federal Rural de Pernambuco, R. Dom Manoel de Medeiros s/n, Recife, Brazil;
[2]Universidade Federal de Minas Gerais, Av. Antonio Carlos n° 6627, Belo Horizonte, Brazil

Alzheimer's disease (AD) is a degenerative and irreversible mental illness that affects a large proportion of the elderly population worldwide and is growing increasingly with increasing life expectancy. Anyone suffering with the disease suffers from gradual loss of memory in addition to decline in intellectual abilities [1]. This disease is characterized by the loss of cholinergic neurons which produce an essential acetylcholine (ACh) neurotransmitter in the neocortex and hippocampus. The deficiency of cholinergic neurotransmitters, especially the decline of ACh neurotransmitter level in the brain is intimately involved in memory loss in AD. Numerous approaches are being explored to restore central cholinergic function as the use of Acetylcholine (ACh) liberation agents, the stimulation of acetylcholine uptake, activation of cholinergic receptors and decreased metabolic breakdown of ACh by acetylcholinesterase (AChE) enzyme.The main form of treatment for AD is the administration of anticholinesterase drugs, which act inhibiting enzyme AChE [2,3]. This study explored the AChE inhibition activity by essential oil of some species of the genus *Croton* and *Citrus* tested for inhibition of AChE by the Ellman's spectrophotometric method [4]. All assays were performed in quintuplicate. The results showed significant AChE inhibition activity by most of the essential oil screened, however the essential oil isolated by genus *Croton* showed the most significant results with inhibitions reaching up to 90%.

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P-132

Tyrosinase inhibitors from plants of *Xylopia* genus

dos Santos GF, Takahashi JA

Chemistry Department, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627, Belo Horizonte, Brazil

Melanin plays an important role in protecting the skin against the harmful effects of ultraviolet rays. Increased or abnormal distribution melanin production leads to epidermis hyper pigmentation [1], a pathological alteration that can be treated inhibiting the tyrosinase, an enzyme that plays a key role in melanin biosynthesis. The tyrosinase catalyzes the tyrosine oxidation to dopaquinone forming melanin [2]. The inhibition of tyrosinase is essential to decrease melanin production and substances having this activity are being widely used in cosmetics[1]. This work explored the tyrosinase inhibition activity by different natural and semi-synthetic diterpenes with kaurane and trachylobane skeletons, isolated from plants of *Xylopia*. Diterpenes assayed for inhibition of tyrosinase by the spectrophotometric method described by Kim et al (2007) [3] in quintuplicate. The results showed that most of the active compounds showed a time-depending activity. The most active tyrosinase inhibitor was xylopic acid (98.4% inhibition). Kaurenol and trachyloanic acids showed tyrosinase inhibitions superior to 50% even after 2 h or reaction. Comparative analysis of inhibitory power of xylopic acid and its methyl ester showed that the later is 35% less active than xylopic acid after 2 h of exposition to tyrosinase. These results are interesting since most of the tested compounds are

major constituents widely found in plants of known safety for human use.

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Comparison of a treatment with Ulmo honey (*Eucryphia cordifolia*) alone and combined with ascorbic acid in healing burns in guinea pigs

Schencke C[1], Vasconcellos A[1], Acevedo F[1], del Sol M [1], Salvo J [2], Veuthey C[2]

[1]Facultad de Medicina, Universidad de La Frontera, Francisco Salazar 01145, codigo postal: 4811230, Casilla 54 D, Temuco, Chile; [2]Facultad de Medicina, Universidad Mayor, Avenida Alemania 0281, codigo postal: 4801043, Temuco, Chile

The literature shows beneficial healing, anti-inflammatory and antioxidant effects of topical use of honey and vitamin C in wound healing. There are however, few studies on the combined effect of vitamin C and honey. The objective of this research was to histologically evaluate the effect of Ulmo honey with ascorbic acid and compare its healing effect with Ulmo honey alone, in wounds caused by type B burns. This study was approved by the CEC at the Universidad de La Frontera. The guinea pig (*Cavia porcellus*) was used as an animal model for having a vitamin C dependent metabolism. We separated 15 guinea pigs into three groups: A (added honey), B (no added honey), and C (positive control). A burn of 0.5 cm diameter was caused on the dry back. Daily treatments were performed until biopsies were obtained at day 10 post-injury. 4 µm sections were stained for histological and histochemical techniques. Group A showed rapid debridement compared to groups B and control group. Complete regeneration of the epidermis was observed with advanced proliferative phase in the dermis, vascularized tissue and abundant presence of fibroblasts. Group B presented no epidermal layer and presented an initial proliferative stage at dermal level confirmed that adding antioxidants to honey enhances debriding and granulation properties for an effective, rapid and good quality healing.

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Role of diterpens in calcium and synaptic transmission: potential candidates for neuroprotection

Serrano FG[1], Ordenes D[1], Ureta R[1], Schmeda-Hirschmann G[2], Inestrosa NC[1]

[1]Laboratory of Molecular Neurobiology, Centro de Envejecimiento y Regeneración (CARE), Department of Molecular and Cellular Biology, Faculty of Biological Science, Pontificia Universidad Católica de Chile, Santiago +5626862722, Chile; [2]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile

The family of diterpenes present in a variety of plants has been related to many biological activities as antioxidant, antibacterial, anti-inflammatory, anticarcinogenic and promoting healing of subacute gastric lesions [1, 2, 3]. However, there is no evidence about the potential effects of diterpenes in the nervous system. We evaluated diterpenes (Ferruginol, Junicedric acid and Jatrophone) in hippocampal neurons culture and brain slice. Ferruginol, Junicedric acid and Jatrophone induce an increase in the synaptic transmission, which is consistent with an increase in the somatic calcium level in culture. Additionally, we evaluate the neuroprotective action of Ferruginol and Jatrophone in

brain slice mediating exposition to amyloid-β peptide (Aβ), observing a reduction of the active caspase-3 levels (pre-apoptotic state). With the same approach, we studied the neuroprotective action of these molecules by electrophysiological studies in synaptic plasticity as long-term potentiation (LTP), a functional process correlated to learning and memory. We observed that the pre-incubation of Ferruginol and Jatrophone prevent the damage induced by the Aβ, protecting and allow the induction of a robust LTP. Furthermore, we observed that Ferruginol decrease the 4-hydroxinonenal levels, an indicator of lipid peroxidation. To understand the possible neuroprotective mechanism of action of diterpenes, we evaluated components of the Wnt signaling pathway, which are associated to cellular viability and protection (GSK-3β and beta β-catenin) suggesting an activation of the Wnt signaling [4]. This is a new approach in the study of diterpenes with a protective role against the Aβ damage and a potential role in Wnt signaling in the nervous system.

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Development of different sample preparation for quantification of PB2 and EPI in crude extract of *Trichilia catigua* by HPLC

Longhini R, Fialho AJ, da Silva AOF, Mello JCP

Departamento de Farmácia, Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo, 5790, BR-87020-900, Maringá, PR, Brazil

The main constituents of the bark of *Trichilia catigua* are phenolic compounds, mainly condensed tannins [1]. This plant it is popularly known as “catuaba”, “catiguá”, and “catuama”, and is commonly used for medicinal purposes [2]. An HPLC method for the simultaneous quantification of phenolic compounds (procyanidin B2 (PB2) and epicatechin (EPI)) was developed and validated in semipurified extract (EAF) [1]. Quality control of extractives is especially important, because their chemical composition may vary according local, year season, and among others [3]. In order to check the quality of vegetable drug, the aim of the present work was developed a simple method of sample preparation. The method consists of a microextraction or micropartition (ME) with ethyl acetate from acetone crude extract according Lopes et al. [4]. The HPLC-chromatographic profile of the two methods was compared and the content of PB2 and EPI were determined. The results were to PB2 and EPI (µg/mL), respectively: EAF=8.53 and 14.39; ME=8.85 and 14.44. The mean concentrations were tested by ANOVA one way (p <0.05) and it was no found statistical difference. The developed method was more rapid. Thus, it is very important to produce the semipurified ethyl-acetate fraction. Thus, the advantages are economic, time, and may use for lower amounts of sample or not adequate amounts of the chemical constituents.

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HPLC-PDA-ESI-MS profiles of phenolic compounds and antioxidant activity of fruits from three *Citrus* species consumed in Northern Chile

Ramirez JE, Bórquez J, Brito A, Simirgiotis MJ

Laboratorio de Productos Naturales, Departamento de Química, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile

Peels and pulp from fruits of three species of *Citrus* including two varieties of lime fruits (*Citrus aurantifolia* varieties *pica* and *sutil*) besides *lemon* (*Citrus* x *lemon* var. Genova) widely cultivated and consumed in Northern Chile (I and II region) were analyzed for phenolic compounds and antioxidant activity. A high performance electrospray ionization mass spectrometry method (HPLC-PDA-ESI-MS) was developed for the rapid identification of phenolics in extracts from peels and juices of the three species. Results showed that fruit peels are significantly rich in phenolic acids, particularly flavone C-/O-glycosides [1], with rutin (MS= 609, MS^n 301) and leucenin II- 4′-methyl ether (MS= 623, MS^n 503, 383) as major compounds in the variety *Pica*. Other compounds detected were the flavonoids leucenin II (MS= 609, MS^n 591, 489, 399 and 369), Leucenin II- 4′-methyl ether (MS= 623, MS^n 503, 383), neohesperidin (MS= 609, MS^n 301), scoparin (MS= 461, MS^n 503, 383), chrysoeriol 7-O-neohesperidoside (MS= 607, MS^n 299, 284) and several other C-glycosides commonly found in Citrus fruits [2, 3]. The amounts of phenolics found in pulps and peels were correlated with the antioxidant activity measured by the DPPH method and FRAP assay. The HPLC method employed allow a clear differentiation between the three species, which is particularly important for the chemical authentication of the tart and tangy lime called *limón de Pica*, most famous for its use in Pisco Sour cocktails all around Chile.

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P-139

Taxonomical survey of medicinal plants from Cerrado of Ecologic and Experimental Stations, Itirapina, São Paulo, Brazil

Siqueira CC, Capellari L

Universidade de São Paulo - Escola Superior de Agricultura "Luiz de Queiroz", Pádua Dias, 11, Piracicaba, São Paulo, Brazil

The Cerrado (Savannah) is an exclusively brazilian biome, currently featuring 7% of its original area, which has great potential for studies in the fields of morphology, taxonomy, pharmacology, physiology and ecology. A research project was executed for a year involving bibliographic studies about medicinal properties, in addition to gathering and taxonomy of the plant species found in the area of Ecological and Experimental Stations in the city of Itirapina, countryside of São Paulo, southeastern Brazil, where the studies were conducted. This project aims, in addition to evidence the importance of preserving the Cerrado, provides a database that enables the collection of agronomic seeds for propagation and cultivation studies.
A hundred and five plant species were collected and studied, most of them medicinal and within the Asteraceae and Fabaceae families were the most representatives. The species found in the Cerrado have very particular active ingredients, making their survival possible in the environment they live, which is adverse to many life forms. Only the fittest plants are able to endure this

environment due to its soil, which has an acid pH, high temperatures and high levels of aluminum.

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P-140

Antioxidant and anti-inflammatory activity of Chilean *Geoffroea decorticans* fruits ("Chañar")

Soriano MDPC[1], Quispe C[1], Jiménez-Aspee F[1], Theoduloz C[2], Alberto MR[3], Costamagna M[3], Isla MI[3], Schmeda-Hirschmann G[1]

[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile; [2]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, Talca, Chile; [3]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA-CONICET) Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina

Geoffroea decorticans (Gill. *ex* Hook. *et* Arn.) is a native tree, popularly known as "Chañar" growing from I to IV regions of Chile. The distribution range of the species comprises the Chaco phytogeographic zone of South America, including Argentina, western Paraguay and Bolivia. The sweet and pleasant tasting fruits are much appreciated and are commonly used for both culinary and medicinal purposes. The fruits are consumed in different forms (chicha, arrope, hydroalcoholic beverage and jam). Arrope is very similar to honey or plant molasses and it is used instead.[1,2] The aim of the current work was to assess the antioxidant and anti-inflammatory activities of chañar fruits and to compare the HPLC pattern of the Amberlite-retained methanolic extracts (ARME) from different Chilean collections of fruits. An arrope sample was included for comparison. The samples comprised different collection places from the Copiapo and Limari valleys associated with pre-columbian cultures. Large differences were found in the HPLC patterns of phenolics, allowing a differentiation of fruit samples. The antioxidant activity was measured by scavenging of the DPPH free radical and by ferric reducing antioxidant power (FRAP). The anti-inflammatory activity was assessed by the inhibition of the proinflammatory enzymes lipoxigenase (LOX), ciclooxigenases 1 and 2 (COX-1 and COX-2). At 50 µg/mL, all ARME inhibited the LOX from the beginning of the incubation, with inhibition values between 17.3 and 82.6%. Large differences were found for the activity towards COX-1 and COX-2 with values of 0-80.8% for COX-2 and 0-93.3% for COX-1. The COX-1 was not inhibited by the fruits collected in Alto del Carmen and Camino a El Tránsito. This finding is relevant considering the side effects like gastrointestinal toxicity, derived from COX-1 inhibition.

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P-142

The use of "Barbatimão" (*Stryphnodendron adstringens* (Mart.) Coville, family Fabaceae) in pharmacy manipulation

de Souza CVCA[1], Accorsi WM[1], Capellari L[2]

[1]Prof. Accorsi Medicinal Plants Ltd. - Pharmacy of Manipulation, R. Governador Pedro de Toledo 568, Piracicaba, 13400-060, Brazil; [2]Luiz de Queiroz College of Agriculture - University of São Paulo, Av. Pádua Dias 11, Piracicaba, 13418-900, Brazil

The name "barbatimão" has an indigenous origin ("iba"-"timo"), which means "tree that tightens", or astringent. This name is used for some species of the genus *Stryphnodendron*, of the family Fabaceae (or Leguminosae), subfamily Mimosoideae, and are typical cerrado (brazilian savannah) plants. From these species, the most used in phytotherapy in Brazil is *Stryphnodendron adstringes* (Mart.) Coville. It is a small, very tortuous and grayish bark tree, deciduous, with reddish shoots; the flowers are small and grouped in dense spikes. The bark of this plant species has a high content of tannin that has a powerful healing action, prevents lipid peroxidation, degradation of nucleotides and accelerates the healing process1, in the form of ointments that are used on wounds to accelerate tissue regeneration2. The bark "barbatimão", in tincture, or soap is also very efficient in the treatment of bacterial diseases which have developed resistance to conventional antimicrobial drugs3, preventing and curing infections caused by golden-staphylococci (*Staphylococcus aureus*). It is also used in various pharmaceutical formulations with anti-inflammatory, antiviral and anti acneica properties with high healing power. It is a low-cost resource with very satisfactory effects, providing safety and effectiveness in its use. The raw material (bark) is only obtained through extraction of a plant species of the Cerrado, a very threatened biome, hence the need for its cultivation.

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P-145

Hydroethanolic extract of the *Cedrela odorata* stem bark (HeECO) reduces hyperglycemia after a glucose and sucrose load

Giordani MA, Souza de Almeida FJ, Bieski IGC, de Oliveira Martins DT, Andrade CMB, Kawashita NH

Universidade Federal de Mato Grosso. Avenida Fernando Corrêa da Costa, nº 2367, CEP: 78060-900, Bairro Boa Esperança. Cuiabá - Mato Grosso - Brazil

The inner stem bark of *Cedrela odorata* (Meliaceae), known as "cedro rosa", has been popularly used to treat diabetes in Brazil. Although fasting hyperglycemia is the parameter normally used for Diabetes diagnosis in Type 2 Diabetes, other alterations such as reduction in glucose tolerance, precedes that alteration. For this reason, we monitored the effect of HeECo on glycemia after a load of glucose and other carbohydrates derived from glucose. Male non-diabetic and diabetic (streptozotocin, 40 mg/kg b.w, iv) Wistar rats were used, with glycemia levels between 250-400 mg/dL in the beginning of the experiments. After 15 h fasting, the rats were treated with 250 mg/kg (DT250) or 500 mg/kg (DT500) of HeECo. The non-diabetic (C) and diabetic control (D) rats

received 2% DMSO (vehicle). The glucose positive control group was treated with phlorizin (DF – 100 mg/kg) and starch and sucrose positive control groups were treated with acarbose (DA – 3 mg/kg). After 30', glucose, or sucrose or starch was administered (2.5, 4 e 3 g/kg b.w, respectively). The HeECo reduced the glycemia in rats that received a load of glucose and sucrose. In reference to glucose, the reduction of glycemia was dose dependent (45' – DC= 402±22, DT250= 329±16, DT500= 254±14 mg/dL) and the under curve area (glycemia x time of evaluation) at 500 mg/kg dose had similar effect of the phlorizin (DC= 37666±2347, DT500= 27666±1423 and DF= 25033±1241 mg/dL.120'). In reference to sucrose, although HeECo administration had not promoted difference in the under curve area, there was about 25% reduction in the maximum level of glycemia in the rats of the DT500 group, similar to rats of the DA group (DC= 468±29, DT250= 362±73, DT500= 285±83 e DA= 280±44 mg/dL). HeECo can contribute to increase glucose tolerance, reduced in diabetic rats, and thus retard or prevent diabetes clinical manifestation.

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Antioxidant capacity of resveratrol in vitro assayed in human erythrocytes

Suwalsky M[1], Concha I[1], Gallardo MJ[2]

[1]Faculty of Chemical Sciences, University of Concepción, Casilla 160C, Concepción, Chile; [2]Center for Optics and Photonics, University of Concepción, Casilla 160C, Concepción, Chile

Resveratrol (RV) is a polyphenol nonflavonoid compound abundant in red grapes. It is a potent antioxidant, anticancer and anti-inflammatory agent. Although the main target of RV action is the cell membrane, its effect on them has been scarcely investigated. In order to better understand the molecular mechanisms of its interaction with cell membranes human erythrocytes and molecular models of its membrane were utilized. These consisted in bilayers of DMPC and DMPE, representative of phospholipid classes located in the outer and inner monolayers of cell membranes. The capacity of RV to perturb DMPC and DMPE bilayers was evaluated by X-ray diffraction. Intact human erythrocytes were observed by scanning electron (SEM) and defocusing microscopy (DM). Results by X-ray diffraction showed that RV produced a significant structural perturbation of DMPC, but no to DMPE. SEM and DM observations showed that RV changed the normal discoid shape of erythrocytes to echinocytes. These results imply that RV is located in the outer monolayer of the erythrocyte membrane. The antioxidant properties of RV were evaluated in erythrocytes and lipid bilayers in vitro exposed to the oxidative stress induced by hypochlorous acid. Results showed that RV neutralized the deleterious effect of HClO on DMPC and DMPE; SEM, DM and hemolysis assays showed similar results. It is concluded that RV would act by blocking access of the oxidant and free radicals into the lipid bilayer and scavenges them before they can penetrate the cell membrane.

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Correlation of gallic acid and other chemical compounds with anti-tyrosinase activity of some plant extracts

Corradi IF[1,2], Souza E[1], Takahashi JA[1]

[1]Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil;
[2]Faculdade de Farmácia, Universidade de Itaúna, Itaúna, MG, Brazil

Many common and commercial treatments used for smoothing skins stains and hyper-pigmentation are based on the inhibition of the tyrosinase, a key enzyme that acts on the melanogenesis. Several anti-tyrosinase formulations contain or are based on vegetal extracts or natural products [1]. The present work involved the experimental assembly of the HPLC profiles of extracts from various Brazilian vegetal species and quantification of some secondary metabolites (gallic acid, methyl gallate, caffeic acid, luteolin, quercetin and rutin) present in the extracts. Furthermore, employing *in vitro* procedures, the anti-tyrosinase activities of the pure compounds were determined and compared with the activities of the crude extracts. In general, extracts that contained high amount of gallic acid, one of the most effective anti-tyrosinase inhibitor, also present high anti-tyrosinase efficiency. On the other hand, in some cases, it was not found a direct correlation between the presence of the compounds quantified in the crude extracts and their actual anti-tyrosinase effects. For instance, methyl gallate was the most effective anti-tyrosinase inhibitor among the assayed compounds but it was present in low yield in two of the most active extracts. Therefore, methyl gallate content cannot, only by itself, justify the high anti-tyrosinase properties of these extracts. In resume, the anti-tyrosinase activity of some vegetal extracts herein studied does not keep an unequivocal relationship with the concentration of specific substances but seems to be caused by the concomitant presence of compounds that, separately, do not show remarkable anti-tyrosinase effect.

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P-148

Determination of conditions for expression of antimicrobial metabolites by marine fungi

Lyra FH, Costa, LM, Takahashi JA

Universidade Federal de Minas Gerais, Av. Antonio Carlos n° 6627, 31270-901, Belo Horizonte, MG, Brazil

Fungi have conquered prominence in the chemistry of natural products since they produce a great diversity of natural products currently used in the therapeutics. However, metabolites of biotechnological interest may not be expressed in readymade culturing media. Fungi have high capacity of adapting to the environment; therefore, small modifications in culturing conditions, carried by experimental design, usually lead to alterations in the expression of secondary metabolites [1]. This work aimed at determining suitable conditions for improved production of anti-infective secondary metabolites by the marine fungi *Aspergillus tubingensis*, *Fusarium solani* and *Microsphaeropsis arundinis*. These fungi were cultivated under different conditions, varying parameters such as nitrogen, carbon and salts source, time of growth and agitation using a fractional factorial planning (2n-2). Crude extracts were prepared and assayed against micro-organisms. The results showed great variation of the activity according to the culturing conditions

since microbial inhibitory activities of the extracts varied from 0.7 to 99%. Among the results, it was observed that fungal species *A. tubigensis* and *F. solani* need the presence of glucose and peptone for production of compounds able to inhibit the bacterium *S. sanguinis.* Under this condition, active compounds production was maximized after 21 days of fungal growth. By the other hand, the fungus *A. tubigensis* expressed metabolites with improved activity when sodium chloride was added to the medium and cultured by 35 days. In relation to *M. arundinis*, expression of antimicrobial metabolites showed to be yeast-extract dependent. These data were useful for setting up large scale experiments directed to the isolation of the bioactive metabolites.

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P-149

Chemical constituents from *Kageneckia oblonga* Ruiz & Pav. leaves

Tapia J[1], Cristen P[2] , Muñoz O[1]

[1]Departamento de Química, Facultad de Ciencias, Universidad de Chile; [2]School of Pharmaceutical Sciences,University of Geneva, University of Lausanne, Geneva, Switzerland

Kageneckia oblonga Ruiz & Pav., also known as “Bollen”, is an evergreen tree belonging to the Rosaceae family. It is endemic to Chile and grows from Coquimbo to Malleco (29° to 38°S). The tree can be found in central Chile within the La Campana National Park and Cerro La Campana forest areas. The species grows well in dry or semi humid soils, from the central valley and Andean slopes to an altitude of 1800 mosl [1]. In Chilean traditional medicine, the infusion of the aerial part is used as emetic, laxative and to treat fever, on spite of data suggesting some toxicity [2]. The wood is very hard and it is used for elaborating tools. Previous studies on *Kageneckia oblonga* reported the isolation of pentacyclic triterpenes acids [3], prunasin, several cucurbitacins [4] and cucurbitacins glycosides [5]. Oxygenated tetracyclic triterpenes and their derivatives have been found in many families of plants, including Rosaceae. The occurrence of this compounds and the biological activity of the isolated molecules has been reported [5]. As part of our ongoing studies on Chilean medicinal plants, we report here the isolation and identification of others chemical constituents in *K. oblonga*.

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P-150

Phytocosmetics used in Turkey

Tatli II[1], Ozbeycan A[2]

[1]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sihhiye, Ankara, 06100, TurkeyM; [2]Hacettepe University, Faculty of Pharmacy, 5th Grade Student, Sihhiye, Ankara, 06100, Turkey

In parallel to the development of mankind, the use of cosmetics continues from the first times until today increasingly. Being healthy and well-groomed are positive factors that influence the physical and mental health of human. Nowadays, the development of new cosmetic formulation and new active groups in formulation have opened up new applications and areas in cosmetology. Cosmetic preparations manufactured from herbal extracts using current technologies are called as "phytocosmetics". Consumers do not have enough information about the use of phytocosmetics. So many negative or adverse effects can stand out as a result of being sold cosmetics in an easily accessible place or being proposed by those who have not mastered on topic. Phytocosmetics are sometimes considered as 100% natural products by consumers, however these products have side effects. In these products, the use of standardized herbal extract defined with the chemical content is crucial. The raw materials used in the preparation of phytocosmetics are known to greatly influenced by various factors (geographic positioning, seasons, soil and environmental conditions, heavy metals, etc.). Thus, the production must be carried out with the Good Agricultural and Collection Practices as well as Good Laboratory Practices and Good Manufacturing Practices. In Turkey, about 12,000 plant species are naturally growing, one third of which are endemic. The richness of our flora is almost as that of the flora of whole Europe. Floristic diversity of Turkey is one of the most important values that needed to be. Therefore, herbal materials used in phytocosmetics, their interactions and adverse effects were investigated by our groups, and it has been presented as Graduation Project in Hacettepe University, Faculty of Pharmacy. The results will be discussed. Additionally, this presentation will cover phytocosmetic preparations sold in pharmacies in Turkey.

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The Patagonian wild raspberry (*Rubus geoides* J.E.Sm.): antioxidant activity and phenolic composition

Theoduloz C[1], Jiménez-Aspee F[2], Valdés ST[2], Mardones C[3], von Baer D[3], Schmeda-Hirschmann G[2]

[1]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Talca, Chile; [2]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile

The Chilean wild raspberry (*Rubus geoides*, Rosaceae) is a native species occurring from the Araucanía Region to Patagonia and Tierra del Fuego. It has a pleasant taste and an attractive red color. The fruits were consumed by Amerindians, such as Pehuenches, who called it "Frutilla de zorro" or "Miñe-miñe". The aim of this work was to describe the phenolic profiles and to determine the antioxidant activity of several fruit samples collected in Araucanía, Aysen and Magallanes regions of southern Chile. Total phenolic, total flavonoid and total anthocyanin content was determined. The antioxidant activities of methanolic and phenolic-enriched-extracts (XAD) were assessed for their scavenging capacity of DPPH and ABTS radicals, and their reducing power (FRAP). A commercial raspberry (*R. ideaus*) sample was included for comparison. The total phenolic content ranged from 2.89 ± 0.09 to 3.78 ± 0.11 g gallic acid equivalents/100 g methanolic extract. Total flavonoid content was found between 0.11 ± 0.02 to 0.25 ± 0.01 quercetin

equivalents/100 g methanolic extract. The total anthocyanin content ranged from 85.28 ± 6.46 to 142.61 ± 13.16 mg cyanidin equivalents/100g methanolic extract. A positive correlation between total phenolic and DPPH SC_{50} was found in methanolic extracts (R= 0.94991, p<0.05) and between total flavonoids and DPPH SC_{50} in XAD enriched extracts (R=0.91558, p<0.05). In general, high antioxidant activity was found for the fruits in agreement with other native berries and currants. Further work is underway to obtain a more complete picture of the phenolic antioxidants from this native species, including determination of antioxidant activities in cell culture models.

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Anti-inflammatory, antioxidant and analgesic activities of extracts and phenolic compounds from G*unnera tinctoria* Mol. (nalca)

Rodriguez-Díaz M[1], Rodriguez-Llamazares S[2], Delporte C[3], Miranda H[1], Torres F[1], Delgado JM[1], Muñoz C[1]

[1]Universidad Andres Bello, Republica 330,Santiago,Chile; [2]Centro de Investigación de Polímeros Avanzados (CIPA),Concepcion, Chile; [3]Universidad de Chile, Santiago 1058, Chile

Gunnera tinctoria Mol. is a chilean species has been widely used by native peoples for its medicinal properties such as anthelmintic, antitusive and antiinflammatory [1]. The aim of this research is to carry out pharmacological studies of extracts from leaves and petioles of *Gunnera tinctoria* Mol. The present work showed the analysis of the phenolic compounds from methanol extracts by HPLC-DAD. The *G. tinctoria* extracts was subjected to topical assays for the inhibition of inflammation elicited by arachidonic acid (AA) or phorbol ester (TPA) [2]. All samples were assessed in mice using the topical tail-flick and i.p. hot-plate tests [3]. Methanolic and ethyl acetate extracts from petiols of nalca showed topical anti-inflammatory activity in both AA and TPA models. The results of the present study demonstrated *G. tinctoria extracts* elicit antinociceptive effects in two murine thermal models. Our results provide data to support further investigations about nalca compounds as potential anti-inflammatory, antioxidant and analgesic agents.

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P-153

Antiherpetic activity and cytotoxicity of hydroethanolic extract, fractions and isolated compound obtained from aerial parts of *Tanacetum parthenium* (Schultz-Bip.)

Zanqueta EB, Marques CF, Pelegrini B L, Oliveira TZ, Nakamura CV, Ferreira ICP, Ueda-Nakamura T

Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, PR, Brazil

The Herpes simplex virus - type 1 (HSV-1) is a DNA enveloped virus, belonging to the

Herpesviridae family, with ability to establish latency in the host, with high prevalence in the global population. The anti-herpetic therapy is done with nucleoside analogues that inhibit the HSV-1 replication. Although specific, these drugs are not always effective, considering the emergence of resistant strains. This study aimed to determine the antiherpetic activity of hydroethanolic extract, fractions and the pure compound guaianolide obtained from aerial parts of *Tanacetum parthenium*. Cytotoxicity and antiviral activity (against HSV-1 - KOS, an acyclovir sensitive strain) was determined by MTT assay. Selectivity index (SI) was determined showing that the most selective are the hydroethanolic extract (SI=18.4) and the dichloromethane fraction (SI=15.4). The compounds were also tested against HSV-1 AR-29 strain (resistant to acyclovir), and its activity was evaluated by plaque reduction assay, showing similar results obtained against the other strain. To assess which step of viral infection the guaianolide was active, the compound, cells and viruses were placed in contact in different moments and this interaction was evaluated by plaque reduction method. The results showed that guaianolide was able to neutralize the viral particle, and also acted on viral adsorption and penetration steps. Therefore, we can conclude that the hydroethanolic extract, fractions and the isolated compound guaianolide possess anti-HSV-1 activity *in vitro* by preventing the internalization of the viral particle in the host cell, but further studies should be conducted to clarify its mode of action.

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Genotypic variation of cocktail tomato chilling tolerance after pre-storage heat treatment and its physiological mechanisms

Smit I[1], Valdés ST[2], Settelmeier M[1], Fischer SF[1], Horneburg B[3], Pawelzik E[1]

[1]Georg-August-University Göttingen, Department of Crop Sciences, Division Quality of Plant Products, Carl-Sprengel-Weg 1, 37075 Göttingen, Germany; [2]Universidad de Talca, Instituto de Química de Recursos Naturales, Casilla 747, Talca, Chile; [3]Georg-August-University Göttingen, Department of Crop Sciences, Division of Plant Breeding, Von-Siebold-Str. 8, 37075 Göttingen, Germany

With a world production of 161 million tons of fresh fruits in 2012 tomato is the most important vegetable [1]. Its fruits can be harvested in the immature breaker stage to increase the shelf life during storage [2]. However, tomatoes are chilling-sensitive fruits, and in order to avoid chilling injury, storage and transport of immature fruits at temperatures below 12°C is restricted [3, 4]. Postharvest treatments [heat treatment (HT) and low temperature conditioning (LTC) in comparison to a cold stored control without pre-treatment] to increase the chilling tolerance of cocktail tomatoes were tested. In 2011 three tomato genotypes selected in the Organic Outdoor Tomato Project [5] were grown in the field in a randomised block design and harvested in the immature 'breaker stage' for subsequent storage. 'Resi' showed the best potential among genotypes for cold storage. None of the genotypes reached full maturity without fungal infections or the formation of brown lesions after cold storage. Under HT the formation of lesions and fungal infections were reduced. However, in comparison to cold storage without pre-treatment HT and LTC did not improve fruit quality [sugar-acid-ratio, lycopene, phenolics]. Colour intensity and fruit weight were reduced by the pre-treatments. The physiological mechanisms increasing the chilling tolerance of 'Resi' after HT have been studied with a proteomic approach. Comparing HT with cold storage without pre-treatment 29 proteins have been changed significantly. Using MALDI-TOF-MS 27 proteins were identified: 8 stress-related proteins [mainly heat shock proteins (HSP)] were up-regulated and may explain the reduction of brown lesions. Furthermore, 7 proteins related to defence response were down-regulated and proteolysis, carbon and nitrogen metabolism have been affected by HT.

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P-155

Antioxidant activity and phenolic profiling of the Argentinian Patagonia currant *Ribes magellanicum* Poir.

Valdés ST[1], Ladio A[2], Jiménez-Aspee F[1], Theoduloz C[3], Schmeda-Hirschmann G[1]

[1]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile; [2]Laboratorio Ecotono, INIBIOMA (CONICET-Universidad Nacional del Comahue, Bariloche, Río Negro, Argentina; [3]Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Talca, Chile

The fruits of the native currant *Ribes* spp. (Grossulariaceae) were very appreciated by the Amerindian hunther-gatherers for their sweet and pleasant taste. *Ribes magellanicum* is distributed in the South American Patagonia, including Chile and Argentina. At present, in the Provincia de Río Negro, Argentina, the fruits are eaten fresh or used to prepare preserves, sweets and jams. The aim of our study was to assess the phenolic composition and antioxidant activity of *R. magellanicum* fruits. Representative samples were collected at Rio Casa de Piedra and Villa La Angostura, Argentina. *In vitro* antioxidant activity was evaluated in methanolic extracts of the fruits by their ability to scavenge free radicals (DPPH and ABTS) and ferric reducing antioxidant power (FRAP). Total phenolic (TP), flavonoid (TF) and anthocyanin (TA) content were determined. The TP content ranged from 5.1-5.7 g gallic acid equivalents/100 g MeOH extract, TF from 0.4-0.9 g quercetin equivalents/100 g MeOH extract and TA from 0.03-204.95 mg cyanidin equivalents/100 g MeOH extract. The fruits presented high antioxidant activity, namely: DPPH SC_{50} 18.3-66.2 µg/mL, FRAP 342.0-846.1 µmol trolox equivalents/g MeOH extract and TEAC 339.6-1173.6 µM trolox equivalents/g MeOH extract. Phenolic content was assessed by HPLC-DAD-MS/MS^n. Main flavonol glycosides, including quercetin and kaempferol derivatives, as well as caffeoylquinic acid and anthocyanidins were tentatively identified from the MeOH fruit extracts. Further studies are underway to get a better picture of the chemical composition of South American currants and to disclose its potential as nutraceuticals.

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Determination of phenolic and flavonoid content, and evaluation of the reductive capacity over Fe^{+3} of different *Ugni molinae* genotypes

Valenzuela-Bustamante P[1], Peña-Cerda M[1], Pérez-Arancibia R[1], García L [1], Seguel I [2], Delporte C[1]

[1]Laboratorio de Productos Naturales, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santos Dumont 964, Independencia, Santiago, Chile; [2]INIA, Carillanca, IX Región

Ugni molinae Turcz., Myrtaceae, is a chilean shrub also known as “murtilla”, “murta” or “uñi [1], used in folk medicine because of its health beneficial effects. The properties attributed to its leaves are due in part to the presence of different phenolic compounds that have been identified in its extracts, such as tannins and flavonoids [2], and its antioxidant activity [2, 3, 4]. Because this chemical composition can be affected by different factors (i.e. the genotype), the aim of this study was determine and compare the flavonoid content ($AlCl_3$ colorimetric assay), total phenolic content

(Folin-Ciocalteau assay) and the antioxidant activity (Ferric Reducing Antioxidant Power, FRAP assay) in ethanolic leaves extracts (EETs) of 5 different genotypes of murtilla cultivated under the same conditions and selected by their quantity of leaves. Significant differences ($p<0,05$) were found in the results of the assays, being the ZF-18 genotype the one that presented the highest phenolic content (259.59 ± 3.83 GAE g^{-1} dry matter (dm)) and the highest FRAP value (5.40 ± 0.12 mmol Fe^{+2} g^{-1} dm at 60 min). The 19-2 genotype presented the lowest values in the two assays (157.63 ± 2.96 GAE g^{-1} dm y 4.51 ± 0.14 mmol Fe^{+2} g^{-1} dm, respectively). No correlation was found in the total flavonoid content, being the 31-1 genotype the one that obtained the highest value (53.52 ± 0.79 mg quercetin g^{-1} dm). There are significant differences in the chemical composition and the antioxidant activity of murta leaves extracts that can be attributable to genotype.

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P-157

A ursolic/β-sitosterol fraction from *Lippia graveolens* induced a relaxant activity on uterus muscle by different mechanism

Vargas Villa G[1,2], Jiménez Ferrer E[1], González Cortazar M[1], Herrera Ruiz ML[1]

[1]Centro de Investigación Biomédica del Sur, CIBIS-IMSS. Argentina No.1, Col. Centro. Xochitepec, Morelos. CP 62790; [2]Facultad de Ciencias Biológicas-UAEM

Lippia graveolens used as abortive and menstrual cramps, caused by the increase in the concentration of prostaglandin E_2 (PGE_2) and oxytocin between others. Was demonstrated that hydroalcoholic extract (LgHA), diminished spontaneous contraction of isolated rat uterus in a concentration dependent manner and by blocking Ca^{++} action. In this work fractions were evaluated and selected one of these according its activity, and then measured its spasmolytic effect on smooth muscle uterine of rat, when the contraction was induced with different substances. Female Sprague Dawley rats (17-β Estradiol to 1mg/kg, sc), were sacrificed with overdose of urethane, the uterus was removed, placed in a camera with a Ringer-Krebs solution (37°C/O_2/CO_2). Concentration-response curves (2.5, 5, 10, 20, 40 mg/mL) of: acetone fraction (FAc) and its separation products obtained by chromatography (F4, F6, F13, F18 and F21). The most active fraction was selected for to investigate the mechanism of actions, by blocking contractions-induced by: high potassium, different Ca^{+2} concentrations, PGE_2 or oxytocin. FAc and its fractions were capable of inhibit the spontaneous contractions. However, F6 was the most active because induced the largest decrease of the contraction. Also F6, caused a blockage of the contraction induced by Ca^{++}, K^{+} and PGE_2. Contractions-induced by oxytocin, was blocked by to diminish the frequency, duration and intensity of contraction in a concentration-dose manner. This fraction contains mainly ursolic acid and β-sitosterol. Of the others active fractions, like F18 and F21, was isolated and elucidated the phloritzin and 3-hydroxy-phloritzin, the latter is a new compound never before described.

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P-158

Comparative chemical profiling of fungal endophytes associated to *Bursera simaruba* from Colombia

Fierro J, Vargas-Medina L, Jiménez P, Coy-Barrera E

School of Science, Universidad Militar Nueva Granada, AA49300, Cajicá, Colombia

Lately, fungal endophytes have been playing an important role in the development of new strategies to assess problems such as phytopathogens controlling [1] and production of interesting metabolites, *e.g*. taxol [2]. Nevertheless, the biological and chemical diversity of endophytes, especially in the tropics, is still poorly understood [3]. Fungal endophytes of *Bursera simaruba* (Burseraceae) from Casanare, Colombia, were isolated form leaves that were superficially disinfected with 70% EtOH and 1% sodium hypochlorite. Explants of 2 mm^2 were cultured in PDA, 1/10PDA and Walter agar at 25 °C. A total of 57 fungi were isolated to obtain axenic cultures. Starting from a single conidia or hyphae tip culture, the fungi were cultured in 20 mL PDB for 15 days. The mycelia were separated from the medium by filtering. The mycelia were recollected in filter disc grade 3hW and the medium were lyophilized. Both mycelia and medium were subjected to an ethyl acetate extraction for 12 h. The ethyl acetate-soluble extracts were used to chemically characterize, compare and highlight main components on each extract, within an UFLC-DAD-based profiling. The resulting chromatograms were used to construct a PCA model that clustering the endophyte-derived extracts that share chemical constituents, which is useful to discriminate endophytic sources through distinctive fungal metabolites.

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Antifungal activity of Lupin (Fabaceae) extracts against the phytopathogenic fungus *Fusarium oxysporum*

Vargas-Medina L, Bernal F, Jiménez P, Coy-Barrera E

School of Science, Universidad Militar Nueva Granada, AA 49300, Cajicá, Colombia

The Fabaceae family has been recognized to produce a high diversity of secondary metabolites to defend themselves against herbivores, competing plants and pathogens. *Lupinus* plants are legumes that form root nodules to fix atmospheric nitrogen and produce more nitrogen-containing secondary metabolites, especially alkaloids, than other plants [1]. Most of those compounds exhibit some biological, pharmacological or toxicological activity and they are synthesized, *e.g.,* as the result of fungal infection or stresses by other factors [2]. The aim of the present study was to assess the antifungal activity of ethanol-soluble crude extracts of four lupin species (*Lupinus bogotensis*, *L. guascensis*, *L. mirabilis* and *L. pubescens*). Extracts from leaves, stems, flowers and seeds were evaluated using a micro-scale supplemented medium methodology where the response to these extracts on *F. oxysporum* was determined, using different concentrations (0.1-10 µg/µL range). Lupin extracts exhibited antifungal activity through mycelial growth inhibition at different levels following a dose-response behavior. Lupin-derived extracts could be considered as potential sources of antifungal compounds. A bio-guided fractionation in order to obtain active compounds is currently underway.

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P-160

Antistaphyloccocal activity of *Xanthium cavanillesii*

Paris N, Olivaro C, Vázquez A

Cátedra de Farmacognosia y Productos Naturales, Facultad de Química, UdelaR, Montevideo 11800, Uruguay

Even when considering the huge leap made concerning the chemotherapy of infectious diseases, they still are one of the main causes of death around the globe. The rapid resistance against existing drugs presents a huge challenge when treating them. Nonetheless, higher plants have shown to be a significant source for bioactive compounds. Several extracts of *Xanthium cavanillesii ("Abrojo"),* considered medicinal, were analysed and *in vitro* antibacterial activity was observed. The present work shows the antimicrobial activity of isolated sesquiterpene lactones against, both MSSA and MRSA *Staphylococcus aureus*. The synergy between the *X. canavanillesii* extract and purified samples, with sodium oxacillin was also studied. Dichloromethane extract of aerial parts of *Xanthium cavanillesii* was put through different chromatographic techniques, isolating three pure compounds, which were studied through spectroscopic methods. The MIC was determined using the microdilution technique, according *CLSI*, M7-A7. The synergy was determined by using the CHECKBOARD method. The strains utilized where *S. aureus* ATCC 6538 p, 43300 and 700699. The bioguided fractioning of the *Xanthium cavanillesii* extract led to three pure compounds, later determined by spectroscopy. The results of analysis also confirmed the structure of these compounds as sesquiterpene lactones. The MICs obtained for these were only the fourth of those of the extract, proving that the sesquiterpene lactones are responsible for this activity. There exists synergy between the extract, and purified samples, with sodium oxacillin: ΣFIC=0.5 with 69 µg/mL ext. *Xanthium* and 23 µg/mL sodium oxacillin and with 38 µg/mL purified sample and y 16 µg/mL sodium oxacillin.

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Characterization of antimicrobial activity of methanolic extract of the Antarctic lichen *Himantormia lugubris* against clinical isolates of *Acinetobacter baumannii*

Villanueva X[1], Casanova-Katny A[1], Arnold N[2], Beck A[3], Gónzalez G[1]

[1]Antibacterial Agents Investigation Laboratory, Microbiology Department, Biological Sciences Faculty, Universidad de Concepción. Barrio Universitario, Concepción, Chile; [2]Leibniz-Institut für Pflanzen-biochemie, Abt. Natur- und Wirkstoffchemie, Weinberg 3, 06120 Halle/Saale, Germany; [3]Research Scientist and Curator of Lichens and Bryophytes, Scientific Head of the Molecular Laboratory, Botanische Staatssammlung München, Dept. of Lichenology and Bryology, Menzinger Str. 6, D-80638 München, Germany

The emergence of Multidrug Resistant Bacteria [1] has become a serious problem in Health Care

Centers, lefting only 1 or 2 antimicrobials available against a set of strains known as *ESCAPE* (*Enterococcus* spp., *S. aureus*, *C. difficile*, *A. baumannii*, *P. aeruginosa* and Enterobacteriaceae). These bacteria are associated with high mortality and great level of resistance to first and second line antibiotics used in hospital setting [2]. Of these strains, *Acinetobacter baumannii* is one of the most dangerous, because of its ability for surviving in clinical surfaces by the production of Biofilm [3-4]. In accordance with this scenario, our purpose is the discovery of new antimicrobials from Antarctic lichens [5-6-7] by assaying the antibacterial activity of the Total Extract from *Himantormia lugubris* by the Agar Diffusion Method [8-9] against strains of *A. baumannii* obtained from several Chilean hospitals, divided according to its sensitivity or resistance against Colistin. Our results show a good level of antimicrobial activity (with 6 mg of Total Extract per disc) against all the investigated strains in comparison with negative control ($P < 0.0001$, evaluated by Kruskal-Wallis Test), with an average inhibition halo of 16.1 mm and no statistical differences between the activities against Colistin-sensitive and Colistin-resistant *A. baumnannii* strains. Despite the need of complementary experiments, these results show a promissory source of new antimicrobial compounds.

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P-163

Vitis vinifera L. canes, a promissing source of stilbenoids: effect of post-pruning storage, comparison between Pinot Noir and Cabernet Sauvignon cultivars

Gorena T[1], von Baer D[1], Vergara C[1], Mardones C[1], Winterhalter P[2]

[1]Depto. Análisis Instrumental, Facultad de Farmacia, Concepción, Chile; [2]Institute of Food Chemistry, TU Braunschweig, Alemania

Numerous authors have pointed out the beneficial properties of *trans*-resveratrol and other bioactive stilbenoids on human health, increasing the interest in finding new sources of these compounds. The waste generated by annual grape pruning, usually is incorporated into the soil as fertilizer or burned. However, this type of agricultural residue has significant economic potential as a source of bioactive compounds, with concentrations far exceeding the other organs of the plant, wine and wine industry residues [1]. The main aim of this research was to determine the effect of post-pruning storage (3 months) on stilbene levels in Pinot Noir and Cabernet Sauvignon grape canes. Stilbenes were extracted with an ethanol/water mixture 80:20 (v/v), using an ultrasonic bar for 5 min. Then, the extracts were analyzed by HPLC-DAD-ESI-MS/MS. In all samples, the predominant stilbenoid was *trans*-resveratrol, followed by ε-viniferin. In non stored canes, the mean of total stilbenoids was 902 ± 95 mg kg^{-1} dry matter in Pinot Noir and 586 ± 106 mg kg^{-1} dry matter in Cabernet Sauvignon. After 3 months of storage at room temperature, stilbenoids presented a significant increase. The yield raised up 4.1 times, reaching 3730 mg kg^{-1} dry matter in Pinot Noir canes and raised up 3,8 times, reaching 2212 mg kg^{-1} dry matter in Cabernet Sauvignon canes. This effect does not occur in frozen, lyophilized or milled material [2]. One possible explanation is that storage at room temperature and injury caused by pruning triggers stilbenoid biosynthesis, but the involved biochemical mechanism is still unknown for grape cane. Another alternative is that in fresh non-aged grape cane stilbenenoids are bound to other components of canes and thus are less extractable. In both cases, storage of whole canes at room temperature for at least 3 months is

advisable to increase the extraction yield of these bioactive compounds.

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P-164

Anticancer effect of apigenin on human breast cancer cells MCF-7 and MDA MB-231

Vrhovac I[1], Madunic J[2], Gajski G[1], Breljak D[1], Matulic M[2], Garaj-Vrhovac V[1]

[1]Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia; [2]Faculty of Science, Horvatovac 102, 10000 Zagreb, Croatia

Breast cancer is one of the most prevalent malignant diseases in women and its occurrence is rapidly increasing due to modern life stress. Targeted therapy includes treatment with selective estrogen receptor modulators (SERM), such as tamoxifen, in cells that express estrogen receptors (ER-positive cells) or antibodies like trastuzumab in HER2/Neu expressing breast cancer cells. However, not all breast cancer cells express ER, progesterone or HER2/Neu receptor. Such triple-negative breast cancer cells responds poorly to therapy and is often associated with negative prognosis. Several studies have shown that consumption of cruciferous vegetables suppresses the progression of malignant tumors including breast cancer. One of such natural phytochemicals is apigenin, a non-mutagenic and low toxicity flavone existing in fruits and vegetables. The aim of our study was to investigate possible anticancer effect of apigenin on the morphology and viability of breast cancer cells. Our model cells were hormone responsive, ER-positive MCF-7 and triple-negative MDA MB-231. After the treatment with apigenin we observed changes in cell morphology in dose- (10-100 μM) and time-dependent manner. Moreover, apigenin caused cell death in both cell lines (with MCF-7 cells being more sensitive towards apigenin treatment) leading to significant toxicity, which was evaluated by MTT and XTT tetrazolium colorimetric assay. Differential staining using acridine orange/ethidium bromide on fluorescent microscope showed apoptosis as dominant type of cell death in both cell lines after 24 and 72 h treatment. This was further proved by Western blot analysis which detected cleaved PARP (poly (ADP-ribose) polymerase) in samples treated for 72 h with apigenin concentrations of IC_{50} and above. Observed cytotoxic and pro-cell death activities of apigenin coupled with its low toxicity towards normal cells, indicate that this natural product could be used as a future anticancer agent.

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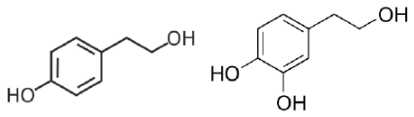
Identification of phenolic compounds and antioxidant capacity of alperujo extracts from Maule Region, Chile

Houy-Huang C[1], Guzmán L[2], Orrego R[2], Wehinger S[2], Zagmutt S[1], Leiva E[2]

[1]Facultad de Ciencias de la Salud, Programa de Magister en Ciencias Biomédicas, Universidad de Talca, postal code 3460000, Talca; [2]Programa de Investigación de Excelencia Interdisciplinario en Envejecimiento Saludable (PIEI-ES) Universidad de Talca Centro de Estudio de Alimentos Procesados (CEAP), Universidad de Talca, postal code 3460000, Talca

Alperujo, an olive by-product is the main residue of the olive oil industry and it represents a serious environmental problem and there is an increasing need to find a useful application to this residue. Olive cake has significant antioxidant properties due to the high content of polyphenols and especially the presence of tyrosol and hydroxytyrosol have been associated with these properties. In this study, we determinate the phenolic and flavonoid content in methanolic extracts from three

varieties: Arbequina, Frantoio and Barnea obtained from Maule region in Chile, followed by HPLC-UV analysis and the evaluation of antioxidant capacity. Barnea presented the highest content of phenols (41.15 mg/g) and flavonoids (8.45 mg/g). However, the free radical scavenging activity by DPPH method, showed a very similar activity in the three varieties, with a slightly major activity in Barnea. The presence of tyrosol and hydroxytyrosol was confirmed in the extracts by HPLC-UV, being found the highest concentration in Barnea (0.039 µg/mg and 4.93 µg/mg respectively). Finally, Barnea and Frantoio were evaluated to inhibit lipoperoxidation. In fact, both varieties were able to inhibit significantly lipoperoxidation in red blood cells. Taken together, these results suggest that alperujo extracts have characteristics to a potential therapeutic use as antioxidants.



Tyrosol Hydroxytyrosol

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Dihydromyricetin ameliorates the methylglyoxal-induced oxidative stress response via AMPK/GLUT4 signaling pathway in PC12 cell line

Xu L, Jiang B, Peng Y, Xiao P

Institution of Medicinal Plant Development, Chinese Academy of Medical Science, Peking Union Medical College, Beijing 100093

Dihydromyricetin (DMY) is the major bioactive flavonoid ingredient of the Vine tea (*Ampelopsis grossedentata*), it exhibits multiple pharmacological activities such as free radical scavenging capabilities, antitumor, anti-inflammatory, hepatoprotective effect and so on [1-3]. However, little is known about its biological activities in the treatment of diabetic encephalopathy. In the present study, the effect of DMY on diabetes-associated cognitive decline was investigated on methylglyoxal (MG)-induced PC12 cell line. The data show that DMY attenuated MG-induced changes in the levels of intracellular Ca^{2+}, reactive oxygen species (ROS), glutathione (GSH)/glutathione disulfide (GSSG), ATP and lactate production. DMY also reduced the translocation of glucose transporter 4 (GLUT4) and the uptake of glucose and increased glyoxalase I (GLO-1) activity and phosphorylated AMP-activated protein kinase (p-AMPK) expression. In conclusion, we found for the first time that DMY protected PC12 cells against MG-induced apoptosis and glycometabolic disorders. Our results also suggested that the mechanism at least partly through restraining the over-activation of p-AMPK activity, which led to the normalization of GLUT4 translocation and resulted in a balanced glucose uptake. These results indicate that DMY may become a potent leading compound for treating diabetic encephalopathy via reducing MG toxicity.

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P-167

In vitro photodynamic inactivation of orofaringeal *Candida spp.* by α-terthienyl isolated from *Porophyllum obscurum* (Spreng) DC (Asteraceae)

Postigo A[1], Petenatti E[2], Zacchino S[1], Sortino M[1]

[1]Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario (2000), Argentina; [2]Herbario de la Universidad Nacional de San Luis, Ejército de los Andes 950, San Luis, Argentina

Photodynamic therapy constitutes an alternative to conventional antifungal treatments, based on the utilization of compounds that inhibit or kill microorganisms only under the effect of light, process known as Photodynamic Inactivation [1]. *Porophyllum obscurum* is an aromatic sub-shrub, commonly known as "Yerba del venado" that inhabits the central and northern regions of Argentina. In previous works we found that *P. obscurum* hexanic extract showed photoactivity against *Candida albicans* ATTC 10231 under the influence of UV-A light. Bioassay-guided fractionation of this extract for antifungal activity with bioautography and microdilution assays [2] with UV-A light irradiation, led to the isolation of α-terthienyl (α-T) as the main active component of this extract. The optimal values of two variables, exposure irradiation time (ET) and distance to the irradiation source (DIS) were established against a panel of clinical *Candida* spp. strains (*C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*) isolated from patients with oropharyngeal candidiasis (OPC), by employing Design Expert Software (DES) [3]. Results showed that the optimal values of ET and DIS were 5 min and 6.06-6.43 cm with a Desirability factor of 0.989. In addition, time-kill assays and confocal microscopy after vital staining [4, 5] could demonstrate that α-T plus UV-A light, at the optimal conditions, caused a complete reduction of viable cells in 5 min. In another set of experiments, it could be demonstrated that the germ tube formation of *C. albicans* was inhibited by sub-inhibitory concentrations of α-T [6]. These data provide evidence that α-T plus UV-A light could constitute an alternative for OPC treatments at the optimal conditions determined with DES.

Acknowledgements: ANPCyT, PICT 0608 y 0049.
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Flour from *Prosopis alba* seeds could be used as functional food or dietary supplement

Cattaneo F[1], Costamagna MS[1], Zampini IC[1,2,3], Sayago J[1,2], Alberto MR[1,2,3], Ordoñez RM[1,2,3], Chamorro V[3], Pazos A[3] , Isla MI[1,2,3]

[1]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA.CONICET); [2]Facultad de Ciencias Naturales e IML. Universidad Nacional de Tucumán; [3]Fundación Miguel Lillo. (4000) S.M. de Tucumán. Tucumán. Argentina; [4]Instituto Tecnología de Alimentos, INTA, Buenos Aires, Argentina

In Argentina, the pods of *Prosopis alba* are used as a source of food products (syrup, flour, sweets such as arrope, patay and jam) and drinks (añapa, aloja and chicha). According to the "Código Alimentario Argentino", "algarrobo flour" is produced by grinding of whole mature pods of *Prosopis alba* and *P. nigra* but until this moment most of the seed is discarded and only the mesocarp is used in the grounding traditional process. The aim of this paper was to determine the nutritional

quality, phytochemical composition and biological properties of *Prosopis alba* seed flour to promote its use. Crude protein was the major component of cotyledon flour (62 %). Furthermore, the flour showed low level of carbohydrate and fat. The main fatty acids were linoleic (60.62%), oleic (18.08%) and palmitic (15.87%). As regards minerals, the flour was high in K and P and low in Na. Further, the cotyledon flour could be considered a "source of fiber" due to its high content (9%). Carotenoids (10%) and phenolic compounds (11%) were the dominant phytochemical. The extract enriched in free phenolic compounds obtained from flour exhibited ABTS•+ reducing capacity and scavenging activity of H_2O_2 and was able to inhibit three pro-inflammatory enzymes of the araquidonic acid metabolism. The extract was more effective to inhibit cyclooxigenase-2 and lipoxygenase enzymes than phospholipase A_2 enzyme. The results suggest that due to its nutritional and functional properties, *P. alba* cotyledon flour could be considered a new alternative in the formulation of foods or food supplements, alone or combined with cereal proteins.

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Cytotoxic effect of *Zuccagnia punctata* ethanolic extract and their flavonoids

Zampini IC[1,2,3], Francisco M[4], Nuño G[1], Ordoñez R[1,2,3], Isla M[1,2,3], Piloto Ferrer J[4]

[1]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA.CONICET); [2]Facultad de Ciencias Naturales e IML. Universidad Nacional de Tucumán; [3]Fundación Miguel Lillo (4000) S.M. de Tucumán. Tucumán. Argentina; [4]Departamento de Toxicología especial, Centro de Investigación y Desarrollo de medicamentos (CIDEM) La Habana (10400) Cuba

Zuccagnia punctata is a shrub from Fabaceae family which commonly grows distributed in arid and semiarid areas in western Argentina. This plant is used in ethnomedicine as antimicrobial and anti-inflammatory drug. Some biological properties were reported for *Z. punctata* extracts (antioxidant, anti-inflamatory, antigenotoxic, antimicrobial). The aim of the present study was to determine the *in vitro* cytotoxic effects of an ethanolic extract from *Z. punctata* aerial part and four of their flavonoids: 7-hydroxyflavanone (HF), 3,7-dihydroxyflavone (DHF), 2',4'-dihydroxy-3'-methoxychalcone (DHMC) and 2',4'-dihydroxychalcone (DHC) using mammalian cell cultures. The cytotoxicity of the natural products was tested using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) viability assay on MCF-7 human breast adenocarcinoma and CT26 mouse colon adenocarcinoma cells. The assays were performed at 24, 48 and 72 h. The results demonstrated that the *Z. punctata* ethanolic extract and their flavonoids induced cytotoxic effects on the two cell lines evaluated. The activity on cell lines after 72 h of exposing was in the following order: DHC >DHCM>DHF>Zp with IC_{50} values between 3.125 - 50 µg/mL and HF at the highest concentration (50 µg/mL) evaluated affected only 20% the cell viability in all cases. The active samples reduced CT26 cell viability in a concentration-dependent manner and the effect increased with the incubation time. However, the activity of the active flavonoids was stronger on MCF7 and started from 72 h of treatment. These results suggest that the studied natural product could be applied as natural anticancer drug.

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Anthocyanins and flavonoid glycosides as bioactive compounds of *Prosopis nigra* mesocarp flour

Perez J[1], Cuello S[1], Zampini IC[1,2,3], Sayago J[1,2,3], Alberto MR[1,2,3], Ordoñez RM[1,2,3], Quispe C[4], Schmeda-Hirschmann G[4], Isla MI[1,2,3]

[1]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA.CONICET); [2]Facultad de Ciencias Naturales e IML. Universidad Nacional de Tucumán; [3]Fundación Miguel Lillo. (4000) S.M. de Tucumán. Tucumán. Argentina; [4]Instituto de Química de Recursos Naturales, Universidad de Talca, Chile

The colour of foods and beverages is one of most important properties to obtain their acceptability. The purple colour of *Prosopis* pods and the different colours showed by the pod flour from different collections of *Prosopis nigra* draw our attention. The aim of this study was to determine the content of phenolic compounds and pigments responsible of the pod colours and their biological properties. The colour of the algarrobo pods is related to the content of anthocyanins. *P. nigra* pods having higher content of anthocyanins are darker (purple). The *P. nigra* flour showed a pattern characterized by the occurrence of anthocyanins, with cyanidin-3-glucoside as main compound as well as 14 flavonoid glycosides. The main flavonoids were quercetin *O*-glycosides and apigenin-*C*-glycosides. Furthermore, the polyphenolic ethanolic extracts of *P. nigra* as well as anthocyanin-enriched aqueous extracts from *P. nigra* showed free radical scavenging activity. Considering the simple extraction of pigments from *P. nigra* flour, its stability in aqueous system during storage as well as its biological properties, the extract enriched in anthocyanins could be added in food systems. Furthermore, the flour obtained from *P. nigra* mesocarp showed activity against cyclooxigenase, a pro-inflammatory enzyme. The results suggest potential of *P. nigra* mesocarp flour as a functional food.

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Mechanism of action of linalool involved in its antiproliferative effects on hepatocellular carcinoma cells

Rodenak B[1], Galle M[1], Castro M[1], Montero Villegas S[1], Crespo R[1], Polo M[1], De Saeger C[2], Stärkel P[2], García de Bravo M[1]

[1]INIBIOLP (CCT-La Plata-CONICET-UNLP) Calles 60 y 120 (1900), La Plata, Argentina; [2]Laboratoire d’Hepato Gastro-Entérologie-GAEN/IREC/UCL – Avenue Emmanuel Mounier 53 Bte B1.52.01 – 1200 Bruxelles, Belgique

Monoterpenes, like linalool (LN), are naturally occurring isoprenoids of ten carbons found in essential oils of many plants. It has been demonstrated that some isoprenoids have antiproliferative activities, phenomenon attributed to their multiple pharmacological effects on the mevalonate pathway (MP): the inhibition of the HMG-CoA reductase activity (HMGCR, the rate limiting step enzyme in the MP) and/or the inhibition of protein isoprenylation. Among these prenylated proteins, Ras family showed to be critical in human oncogenesis. They act as molecular switches controlling cell proliferation, apoptosis and survival. Prenylation of Ras enables it to associate with plasma membrane, which is required for its oncogenic activity. The aim of this work was to elucidate the potential mechanisms involved in the antiproliferative effects excerted by LN on human

hepatoblastoma (HepG2) cells. For this purpose, cells were treatred with LN at different times and/or concentrations and cell proliferation (MTT, cell counting and BrdU incorporation), apoptosis (TUNEL), cell cycle analysis (flow cytometry), HMGCR levels and Ras subcellular localization were studied. Our results showed that LN inhibited HepG2 cell proliferation, arrested cell cycle progression in G0/G1 phase, induced apoptosis and diminished Ras levels in the membrane fraction. Moreover, HMGCR levels became down-regulated. Added exogenous mevalonate failed to reverse the inhibition of proliferation exerted by LN, suggesting that HMGCR inhibition alone is not responsible for the antiproliferative activity of this compound. This work contributes to a clearer understanding of the mechanisms of action of LN suggesting that its use could provide significant health benefits as a chemopreventive and/or chemotherapeutic agent.

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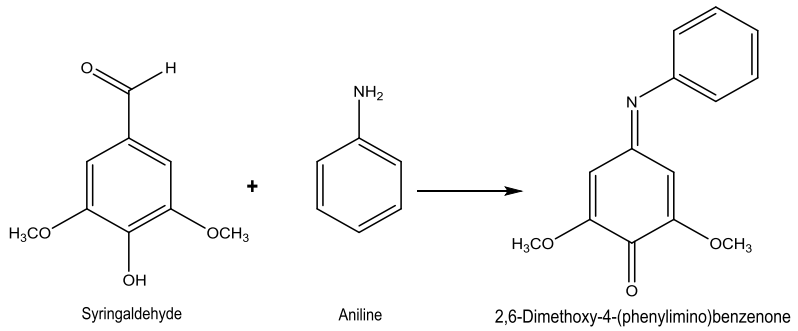
Enzymatic synthesis of compounds with antifungal properties against *Botrytis cinerea*

Cornejo P, Castro P, Cotoras M, Silva E, Mendoza L

Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

Botrytis cinerea is able to infect more than 235 species of plants, including important crops such as grapes, lettuce, tomatoes, tobacco, and strawberries, which produces disease grey rot [1]. The classic control is based on chemical method, which involves the use of synthetic fungicides [2]. Among the fungicides currently used it is possible to mention hydroxyanilides, anilinopyrimidines, carboximides and dicarboximides. However, despite to considerable progress into the chemical control of *Botrytis* this is still impeded by the development of resistance and the negative public perception regarding the safety of pesticides [3]. An alternative to synthesize new compounds with antifungal activity by means of a phenol oxidase enzyme, laccase (EC 1.10.3.2)[4]. It has been reported that laccase is used to synthesize a large amount of products with different applications [5] [6], which include antioxidants [7], antibiotic [8] and antitumor [4]. In this work, the enzymatic modification of syringaldehyde with aniline catalyzed by laccase was performed. The main product identified by means of spectroscopic analysis corresponded to 2,6-Dimethoxy-4-(phenylimino)benzenone. This product had already been identified in the reaction of aniline and syringic acid mediated laccase [9]. The antifungal activity of the reaction mixture against *B. cinerea* was determined and it was compared with the activity of pure substrates. It was founded that the mixture had higher antifungal activity than the reaction pure substrates (10 % and 0%, respectively of inhibition of mycelial growth); therefore the activity of the reaction mixture can be attributed to the product formed.



Syringaldehyde Aniline 2,6-Dimethoxy-4-(phenylimino)benzenone

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Antifungal activity against *Botrytis cinerea* of grape pomace extracts and a polymeric derivate obtained from phenolic acids and chitosan

Flores C., Fuentes N, Vidal A, Ramírez U, Mendoza L, Silva E, Cotoras M

Laboratorio de Micología, Facultad de Química y Biología, Universidad de Santiago de Chile, Av. Libertador O'Higgins 3363, Casilla-40, Correo-33, Santiago, Chile

Botrytis cinerea is a filamentous fungus that causes gray mould in more than 200 plant species. This disease is a problem in the wine industry because the wine organoleptic quality is affected. Therefore, *Botrytis* control is a constant challenge. Grape pomaces are wastes produced by wine industry and it has been described that are rich in phenolic compounds with antifungal activity.
The objective of this study was to evaluate the antifungal activity against *B. cinerea* of grape pomace extracts from Carménère and Cabernet Sauvignon varieties. The extraction was performed using extraction solid-liquid with methanol/HCl or with an aqueous solution containing hydrolytic enzymes. *In vitro* studies, the extracts, at tested concentrations, did not inhibit mycelial growth or conidial germination, despite to contain phenolic compounds with moderate activity against this fungus. To improve the antifungal activity of the extracts or pure compounds present in the extracts (syringic acid), a laccase catalyzed reaction among syringic acid or extracts and chitosan was performed. The reaction products were analyzed by thin layer chromatography and molecular exclusion chromatography. The formation of a chitosan-syringic acid polymer and of a syringic acid dimer was observed and the mixture of these compounds presented greater antifungal activity than chitosan.. Regarding to the reaction of the polymer with extracts, it was observed a colorimetric change in the resulting solutions and changes in the chromatographic profile; therefore, it is possible to assume that reaction among chitosan and the extracts occurred. Currently studies about the antifungal activity of these resulting polymers are being done.

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Phycella Lindl. and *Rhodophiala* C. Presl (Amaryllidaceae): Comparison between their Amaryllidaceae Alkaloids skeletal types

Lizama I, Becerra J

Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Phycella and *Rhodophiala* are two native genera of the Family Amaryllidaceae distributed in Chile mainly in North and Central zone. In this country both genera have more than 20 species with a wide altitudinal range [1]. Studies on the phytochemistry of these chilean species and on their chemical relationships are scarce. For this reason, the aim of this work was to identify and compare the skeletal types of the alkaloids from these genera. Extracts from bulbs were analyzed by means

of GC-MS. Alkaloids were characterized by their retention time and fragmentation pattern. The skeleton types alkaloids shared by these two genera were lycorine, crinine, galanthamine and homolycorine. The results indicate some alkaloids types potentially useful as chemotaxonomic tools to differentiate these Amaryllidaceae genera.

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Neuroprotective activity of *Acalypha diversifolia* and *Alchornea calophylla* (Euphorbiaceae) in cell line and against rotenone-induced toxicity in *Drosophila melanogaster*

Pedraza LM[1], Martínez-Cotacio, CA[2], Mosquera OM[1], Cardozo-Pelaez F[3], Nino J[1]

[1]Grupo de Biotecnología de Productos Naturales, Escuela de Tecnologia Química, Facultad de Tecnología, Universidad Tecnológica de Pereira. Pereira-Colombia; [2]Genetics Institute, building 426. National University of Colombia. Bogotá D.C-Colombia; [3]Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula-USA

Based on results of high antioxidant capacity in extracts from *Acalypha diversifolia* and *Alchornea calophylla* (Euphorbiaceae), we designed experiments to determine their neuroprotective capacities *in vitro* (cells lines) and *in vivo* (Drosophila melanogaster) models of neurotoxicity. The aerial parts of these 2 members of the Euphorbiaceae family were collected from the Ucumarí-Risaralda Natural Park Reserve in Pereira-Risaralda, Colombia. The plants were dried, crushed and extracted with MeOH. The methanolic extracts were analyzed by thin layer chromatography and evaluated for antioxidant activity by the DPPH and ABTS assay. The *in vitro* evaluations were done in two different brain-derived cell lines (CAD and MO3.13). Cell viability was determined in both cell lines after exposure for 6 or 24 hours to C2-ceramide. CAD cells were exposed to 12 or 25 μM levels in media, while the MO3.13 cells were exposed to 20 or 50 μM. Cell viability was assessed via MTT and LDH assays. Pre-exposure of the cell lines (one hour) to methanolic extract from both plants reduced the degree of cell loss at 6 hours, but not at 24 hours with all the C2-ceramide concentrations tested. The *in vivo* neuroprotective capacity was evaluated by the toxicological model with rotenone in *Drosophila melanogaster*. Flies were exposed to 1000 μM rotenone in food for 7 days. Rotenone exposure did no alter fly viability, but led to a significant decrease in motor behavior, as determined by negative geotaxis assay (i.e. climbing capability). Co-exposure to methanolic extracts to *A. diversifolia* or *A. calophylla* (10 mg/mL) in the food prevented the motor behavior deficiency seen with rotenone exposure alone. Our results suggest that chemical evaluation of antioxidant capacities in natural products may lead to biological evaluations that provide proof of potentially beneficial biological activities. Both toxicological models evaluated have been widely used to model neurodegenerative processes associated with Parkinson's disease (PD). Thus, the combination of chemical analyses and toxicological testing may generate lead compounds from natural products that could be relevant in neuroprotective strategies.

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Variation in the alkaloid content of *Peumus boldus* Mol. ("boldo")

Fuentes-Barros G[1], Castro-Saavedra S[1], Cassels BK[1,2]

[1]South American Phytochemistry (SAPHYCHEM); [2]Department of Chemistry, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile

Boldo leaves, from trees native to the central and south-central parts of Chile, have been used since time immemorial for medicinal purposes, presumably associated with their content of essential oil, alkaloids, and antioxidants such as catechin. Boldine, an aporphine, is the most characteristic and most studied alkaloid of at least 17 that have been isolated from the leaves although it is much more abundant in the bark, while laurolitsine is the major alkaloid in the wood.[1] Variations in the alkaloidal content of the leaves have been published, with the total alkaloid content showing genetic variation and high heritability.[2] We have now determined the boldine content in boldo leaves from three natural populations growing at different latitudes in Central Chile (32°30', 33°22-26', 34°30' S), comparing leaves harvested in summer or winter, and their northerly or southerly exposure on the tree. Significant differences were only found for the latitudinal variation, with increasing values for the more boreal populations. There was little correlation with the length, width, area or perimeter of the leaves. In a second stage, the variation of the boldine and total polyphenol content of the leaves, and of laurolitsine and boldine in the wood was studied in three natural stands at a similar latitude but located near the ocean or inland. In this case the only difference found was the lower polyphenol content in the leaves of the more maritime population. Finally, to determine if the age of the plant material affects its alkaloid concentration, samples were analyzed from two adjoining areas where the only difference is that in one the trees have not been subjected to human intervention and in the other the suckers have been harvested every five years in accordance with traditional practice. No difference was found in the laurolitsine concentration, but in the leaves and wood from intact trees the boldine content was significantly higher.

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Acute toxicity and LD50 of *Gunnera tinctoria* Mol. extracts by two bioassays: UMU-Chromotest and Daphtoxkit F magna

Torres F, Rodríguez-Dìaz M, López F

Universidad Andres Bello, Facultad de Medicina, Escuela de Química y Farmacia, Republica 330,Santiago, Chile

We implement and validate bio-analytical methodologies to determine the lethal dose 50 (LD50) of the methanol extract from *Gunnera tinctoria* Mol., in order to supplement the required pharmacological studies for the use of the plant extract for medicinal purposes [1]. This assay will be developed using two bioassays: "DAPHTOXKIT F" and "Salmonella typhimurium UMU-ChromoTest". From the bioassay UMU-Chromotest specific data on chronic toxic and dose dependent effects are obtained. Through this assay we can also obtain the rank of extract concentration which can be detected acute toxic effects.
Once the relationship effect/concentration is established, we proceed to implement the

bioassay Daphtoxkit F to determine accurately the LD50. Using this type of in vitro assay has a long practice and study in human use fresh water tests, being a strong indicator of the quality of this item and the safety of its use. Notably, these bioassays are regulated under norms ISO 13829 and 6341, respectively, and adhere to OECD guideline 202 [2]. Both methods were validated in order to ensure representativeness and reproducibility of results, as an approach to the use of new standardized in-vitro toxicological methods.

Acknowledgements: The authors thank the Public Health Institute (Chile) for providing the experimental rooms.
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Chronic toxicity and Endocrine Disruption by a standardized extract of Buddleja globosa Hope (matico) and its main component (verbascoside) in Daphnia magna

Mirtha Parada[1,2], Gabriela Valenzuela[1], Yuri Carvajal[3], Carla Delporte[1]

[1]Laboratorio de Productos Naturales, Facultad de Ciencias Químicas y Farmacéuticas Universidad de Chile, Código postal 8380492, Santiago, Chile; [2]Instituto de Salud Pública. Chile, Casilla 48, Santiago, Chile 3 Facultad de Medicina, Universidad de Chile, Código postal 8380453, Santiago, Chile.

Buddleja globosa Hope, Budlejaceae (matico) is a native species in Chile which used their leaves of its multiples therapeutic properties. The ethanol extract (EMATst) obtained from leaves and with an standardized main component (verbascoside) presented anti-inflammatory, analgesic and scarring effect [1,2]. Verbascoside has shown through in vitro assays concentration dependent estrogenic and antiestrogenic effects [3]. In this work we studied the estrogenic property and possible endocrine disruptor (ED) effect of EMATst and verbascoside using as a model the evaluation of chronic toxicity in Daphnia magna by determining the survival, reproduction and molt frequency of two generations at 14 days of exposure. Statistical analysis was performed using test Kruskal-Wallis (KW), followed by Poisson regression, the data were analyzed using STATA software version 11.0, $p<0.05$.The results show that there is a concentration dependent decrease in reproduction in both generations, this is more pronounced in the second generation and the ED effect of both samples was expressed because of the altered reproduction.

Acknowledgements: Financial support: FONDECYT 1130155 (CDV).
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Characterization of industrially defatted *V. vinifera* grape seeds as a source of procyanidins with antioxidant capacity

Mardondes C[1], Saez V[1], Apablaza E[1], von Baer D[1], Vergara C[1], Riquelme S[2], Escobar D[2], Fuentealba C[2], Berg A[2], Lamperti L[3], Jara P[3]

[1]Departamento de Analisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Casilla 160-c, Concepcion, Chile; [2]Unidad de Desarrollo Tecnologico – UDT, Universidad de Concepción, Av. Cordillera N°2634- Parque Industrial Coronel- Coronel, Chile; [3]Departamento de Bioquimica Clinica e Inmunología, Facultad de Farmacia, Universidad de Concepcion, Casilla 160-C, Concepción, Chile.

Grape seeds are appreciated because their procyanidin content and the health benefits associated with their consumption. Procyanidins are polyphenols with high structural diversity and different degree of polymerization. During the winemaking process, residues like grape seeds are generated, which are collected for oil extraction and later on the residue is burned or discarded. The procyanidin level, polymerization degree (mDP) and antioxidant capacity are evaluated in industrially defatted grape seeds (IDGS) collected between 2011 and 2013 by RP-HPLC-ESI-MS/MS, HILIC-ESI-MS/MS and antioxidant capacity 2',7'-diclorodihidrofluorescein diacetate assay. Monomers to hexamers are found in the IDGS, being catechin and epicatechin the highest in proportion, whereas mDP above pentamers are only in trace levels. The total procyanidin concentration found in IDGS ranged from 254 ± 21 mg/kg up to 723±12 mg/Kg catechin equivalents, which are much lower than the concentrations found in fresh pomace (6559-7004 mg/Kg catechin equivalents). Despite the low procyanidin levels, the exposure of endothelial cellular line ECV-304 to extracts from IDGS showed a decreased production of ROS, comparable to the extracts of pomace and native grape seeds.
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