PharmacologyOnLine

2016 - Supplementary Issue - vol. 1

SILAE 2016

25° SILAE CONGRESS

"Paolo Ruffini"

11th - 15th September 2016
Modena, Italy

Abstract Book

SILAE

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Pharmacologyonline is the journal of SILAE - Società Italo-LatinoAmericana di Etnomedicina

http://www.silae.it

http://pharmacologyonline.silae.it

version 1.3
13/01/2017 07:46:55

ISSN: 1827-8620

WELCOME TO SILAE 2016

As President of SILAE 2016 and Director of the Department of Life Science at the University of Modena and Reggio Emilia, I have the privilege to welcome all participants to the XXV Italo-Latinamerican Congress of Ethnomedicine, which will take place in Modena from September 11th to September 15th 2016.

The overall goal of the meeting is to shed light on the importance of medicinal plants, and to disclose, by innovative models and techniques, new potential applications for treatment or prevention of diseases. Special emphasis will be given to scientific breakthroughs in the area of traditional medicine unveiling new using perspectives of native plants that have been poorly studied so far.

Thanks to the contribution of worldwide recognized experts, a multidisciplinary approach will be the *file rouge* connecting sessions on anthropology, ethnobotany, phytochemistry, food chemistry and nutrition, green and analytical chemistry, pharmacognosy and medicinal chemistry, experimental and clinical studies on natural products within a unique and exciting congress.

As previous meetings of the Italo-Latinamerican Society of Ethnomedicine, this conference will represent an excellent opportunity to establish new scientific partnerships, to share results from the most recent studies in the field of traditional medicine and to update the current knowledge and applications of bioactive and/or natural compounds.

Furthermore, the meeting will also offer the chance to spend some spare time in a region universally known as the land where art, culture and tradition are fused with the passion for motors, music and cuisine.

Prof. Daniela Quaglino

ISSN: 1827-8620

The 25th SILAE Congress is dedicated to the memory of Paolo Ruffini, one of the scientific personalities that along the past centuries linked his life to the history of our University. He developed many interests and was capable of solving scientific problems with multidisciplinary approaches. His broad concept of science was sustained by the same *file rouge* wich will connect, in the next few days, researches from different countries, different cultural backgrounds and different technical expertise and skills through the numerous scientific sessions of this 25th Ethnomedicine Congress. Starting from studies aiming to understand the present from the experience of the past, we will search for new compounds with high-throughput techniques disclosing innovative properties and characteristics to be used for clinical applications and sustainable food chains.

Paolo Ruffini (Valentano, 1765 - Modena 1822) is recognized as an eminent Italian mathematician and physician, who made numerous studies on equations anticipating the algebraic theory of groups. At the University of Modena he studied medicine, philosophy, literature, and mathematics, including geometry and infinitesimal calculus. Ruffini obtained his degree in philosophy and medicine in 1788 and, soon afterwards, in mathematics. He gained a permanent position at the University of Modena as a professor of mathematics, although, never forgetting his passion for health issues, he received in 1791 a license to practice medicine from the Collegiate Medical Court of Modena.

Paolo Ruffini

Following the conquest of Modena by Napoleon Bonaparte, Ruffini was barred from teaching and public office, but in 1814, after Napoleon's defeat, Francesco IV appointed Ruffini as Rector for life and, at the same time, professor of Practical Medicine and Applied Mathematics. During the typhus epidemic of 1817 he sacrificed himself for his citizens and, although he recovered, he never regained his strength.

As a mathematician, Ruffini's name is inseparably associated with the proof of the impossibility of solving algebraically the quintic equation, having written several treatises on this subject. He also published an important medical dissertation on typhus ("Memoria sul tifo contagioso"), dealing with symptoms and treatment of the disease on the basis of his own experience. His scientific and medical background lead him to apply a mathematical outlook to philosophical questions as in "Della immaterialità dell'anima", in which he enunciated the "theorem" stating that a being endowed with the faculty of knowledge is necessarily immaterial.

ISSN: 1827-8620

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Program

September 11, 2016

15.00 · **Registration**

17.00 · **Opening Cerimony**

Lectiones Magistrales

Chairs: Quaglino D. and Rastrelli L.

18.00 · **Chocarro ML. (Spain),** Plants and people: a long history of human-environment interaction through archaeobotanical research

18.45 · **Duncan S. (United Kingdom),** The impact of diet, pre- and probiotic strategies on gut microbiota composition and health

19.30 · **Welcome reception**

September 12, 2016

Session 1 · Archeobotany and Ethnobotany

Chairs: Vit P. and Mercuri AM.

9.00 · **Invited Lecture:**
Caneva G. (Italy), Plant patterns for ethnobotanical food and nutraceutical uses: the case study from traditional villages of Bali (Indonesia)

9.30 · **Re T. (Italy),** Material and immaterial constitutive elements of healing sites - The Mayantuyacu case study in the Peruvian Amazon

9.45 · **Mariotti MG. (Italy),** The role of familiar gardens in preserving the ethobotanical knowledge of a Peruvian native community

10.00 · **Costa RC. (Brazil),** Biodiversity Use: Productive Chain and Ethnoknowledge (Itacoatiara, Amazonas-Brasil)

10.15 · **Vit P. (Venezuela),** Biodiversity of *Meliponini bees* in Equador: an intangible eritage to protect genuine honey

10.30 · **Mercuri AM. (Italy),** Pollen as indicator of human behaviour

10.45 · **Crescenti C. (Italy),** A preliminary study of methodologies for an Investigation about Ichthyology and gastronomy in classical sources

11.00 · **Coffee and Poster Session 1**

Chairs: Costa RC. and Castelletti L.

11.45 · **Castelletti L. (Italy),** The paleonutrition from 500 BC to 500 AD: case studies between that Adda and Ticino river (Northern Italy)

12.00 · **Riso FM. (Italy),** Not only for beauty: three ritual plants in classical times

12.15 · **Martinelli E. (Italy),** *Persicaria lapathifolia* (Polygonum lapathifolium; pale smartweed) seeds as food during the Iron Age South of the Alps? New data from the site of Padova-S.Eufemia (NE-Italy)

12.30 · **Moreno MI. (Colombia),** Wayuu traditional medicine and the use of medicinal plants in the control and

prevention of hyperglycemia

12.45 · **Levuok Mena KP. (Colombia),** Ethnopharmacological study of some plants traditionally used as antidiabetic in the Colombian Pacific region.

13.00 · **Lunch**

Session 2 · Phytochemistry

Chairs: Haba H. and Benvenuti S.

14.30 · **Invited Lecture:**
Appendino G. (Italy), *Helichrysum italicum*: waking up the sleeping giant of mediterranean herbal medicine

15.00 · **Borges WS. (Brazil),** Alkaloids from *Hippeastrum canastrense* J. Dutilh & R. S. Oliveira (Amaryllidaceae)

15.15 · **Costa R. (Italy),** Phytochemical investigation of ethanolic extracts from *Kigelia africana* (LAM) Benth. Fruits

15.30 · **Malagon Aviles O. (Ecuador),** Novel secondary metabolites extracted from the Ecuadorian plant *Macrocarpaea lenae* J.R. Grant

15.45 · **Coffee and Poster Session 1**

Chairs: Borges W. and Archbold R.

16.30 · **Haba H. (Algeria),** Chemical composition and biological activities of *Helianthemum sessiliflorum*

16.45 · **Nunez CV. (Brazil),** Proanthocyanidin A2 from *Diplotropis purpurea* leaves

17.00 · **Benkhaled M. (Algeria),** Cycloartane glycosides from *Astragalus gombo*

17.30 · **Derita MG. (Argentina),** Natural and semisynthetic compounds from Argentinian *Poligonum acuminatum* against human pathogenic yeasts

17.45 · **Castilho CVV. (Brazil),** Comparative phytochemical profiles of *Lippia origanoides* grown wild and in vitro

18.00 · **Akkal S. (Algeria),** Identification of phenolic compounds from *Carduncellus helenioides* by HPLC-ESI-MS (TOF)

18.15 - **Goleniowski ME (Argentine),** Profile characterization of volatile compounds on wild and "in vitro" plants of *Clinopodium odorum* (Lamiaceae) using HS-SPME/GC-MS analysis

20.00 · **A glass of wine in the shadow of the "Ghirlandina"**

September 13, 2016

Session 3 · Food Chemistry and Nutrition

Chairs: Melo Ruiz V. and Plessi M.

9.00 · **Invited Lecture:**
Dugo G. (Italy), The role of the food chemist in the enhancement of the food farming chain

9.30 · **Colín Cruz MA. (Mexico),** *Capsicum annum* seed as source of food: physicochemical and microbiological characterization

9.45 · **Amaretti A. (Italy),** The peptide profile of Parmigiano Reggiano cheese: evolution through the gastrointestinal transit and utilization by bifidobacteria and lactobacilli

10.00 · **Ramiréz Aristizabal LS. (Colombia),** Evaluation of the antoxidant capacity and content of polyphenols obtained from tea (*Camellia Sinensis*) of four brands sold in Colombia by extraction at room

temperature

10.15 · **Gonçalves Rodrigues R. (Brazil),** Chemical characterization of Jatobá: oil, fruit and flour

10.30 · **Coffee and Poster Session 1**

Chairs: Nunez Selles AJ. and Pulvirenti A.

12.00 · **Rossi M. (Italy),** Polyphenols, microbiota and bifidobacteria: recent insights

12.15 · **Corradini C. (Italy),** Characterization of fos and inulins by HPAEC-PED analyses

12.30 · **Melo-Ruiz V. (Mexico),** Macronutrient composition of giant water bug (*Lethoserus Sp*) edible insect

12.45 · **Albergamo A. (Italy),** Profiling of major and trace elements of the Mediterranean sepia ink iductively coupled-mass spectrometry

13.00 · **Lunch**

Session 4 · Green Chemistry and Sustainable Food Chain

Chairs: Franco L. and Rossi M.

14.30 · **Invited Lecture:**
Riva S. (Italy), Biocatalysis: the green side of chemistry

15.00 · **Silva NCB. (Brazil),** Effects of jasmonic acid and indole -3 acetic acid in the volatile profile of *Anemia Tomentosa* in vitro plants, and aromatic fern

15.15 · **Tropea A. (Italy),** Transformation of food agriculture waste into value added products: bioethanol and SCP

15.30 · **Gil JH. (Colombia),** Bioconversion of alkaline hydrolysates obtained from agroindustrial waste to produce flavoring agents

15.45 · **Garcia Martinez MR. (Mexico),** To the rescue of cofee fruit (*Coffea Arábica*) as a functional food

16.00 - **Coffee and Poster Session 2**

17.00 A tour through the flavours of Modena

September 14, 2016

Session 5 · Analytical and Medicinal Chemistry

Chairs: Gonzales-Lavaut JA. and Quaglino D.

9.00 · **Invited Lecture:**
Wolfender JL. (Switzerland), Efficient analytical strategies for an early identification of bioactive natural products at the herbarium sample scale

9.30 · **Pellati F. (Italy),** Metabolite fingerprinting of *Punica granatum l.* (pomegranate) polyphenols by means of HPLC-UV/DAD,ESi-MS and MS2

9.45 · **Robayo MAT. (Colombia),** Typification of avocado fruit (*Persea americana Mill.*) cultivated in Colombia by Multivariate statistical analysis with a view towards industrialization

10.00 · **Fonseca-Bazzo YM. (Brazil),** Validation of analytical method by HPLC-DAD for identification and quantification of caffeoylquinic acid derivatives in *Cynara scolymus L.* tablets and capsules

10.15 · **Tardugno R. (Italy),** Essential oils: phytochemical composition, cytotoxycity and antimicrobial activity

10.45 · **Coffee and Poster Session 2**

Chairs: Zepeda R. and Corradini C.

11.45 · **Osorio C. (Colombia),** Chemical studies on *Psidium friedrichsthalianum Nied*. Fruit and their precursors
12.00 · **Cicero N. (Italy),** Determination of squalene in e.v.o.o by different analytical techniques
12.15 · **Linciano P. (Italy),** Flavonoids as a scaffold for the development of antitrypanosomatidic agents
12.30 · **Escobar G. (Colombia),** Synthesis and evaluation Leishmanicidal of analogues ent-Beyer-15-en-18-ol and isoesteviol
12.45 · **Gonzalez Lavaut JA. (Cuba),** Gangliosides N-Acetyl and N-Glicolyl GM3. Strategy to support the platform VSSP in vaccines against cancer.

13.00 · **Lunch**

14.30 · **SILAE Assembly**

16.00 · **At the discovery of Modena's treasures**

20.00 · **Social Dinner at Palazzo dei Musei**

September 15, 2016

Session 6 · Pharmacognosy

8.30 · **Coffee and Poster Session 2**

Chairs: Sepulveda Arias JC. and Pellati F.

9.30 · **Invited Lecture:**
Cech N. (United States), Untargeted metabolomics to identify bioactive compounds and synergists from botanical medicines
10.00 · **Espinoza JS. (Chile),** Effect of secondary metabolites of Canelo bark fractions (*Drimis winteri Forst*) on the viability of *Helicobacter pylori*
10.15 · **Machado AC. (Brazil),** Aroeira's (*Myracrodruon urundeuva*) extract bactericidal activity and FNC1, FNC2, FNE1, FNE2 nanoparticules in *Streptococcus aureus* and *Esterichia coli* strains.
10.30 · **Quartieri A. (Italy),** Esters of fatty acids as alternatives or adjuvants of antibiotics in animal feed
10.45 · **Hernández Ortega M. (Mexico),** Effect of a mixture of allergens as oral immunotherapy on a murine model of allergen induced asthma
11.00 · **Capataz-Tafur J. (Mexico),** Hypoglycemic activity in vitro plants of *Azadirachta indica*
11.15 · **Lopez O. (Spain),** Biological asessment of *Pistacia lentiscus* extract: phytopharmacological properties
11.30 · **Echeverri F. (Colombia),** Do you really think antioxidants are effective and safe?

11.45 · **Short Break**

Chairs: Echeverry F. and Vita Finzi P.

12.00 · **Munoz DR. (Colombia),** Cytotoxic alkenylphenols from *Piper eriopodon* (Piperacae)
12.15 · **Scher R. (Brazil),** Chemical characterization and cytotoxic effect of leaf essential oil of *Lippia gracilis* and Lippia sidoides
12.30 · **Frion-Herrera Y. (Cuba),** Comparative assessment of the apoptotic potential of Cuban and Brazilian

propolis in human laryngeal epidermoid carcinoma cells.

12.45 · **Martinez-Vazquez M. (Mexico),** Masticadienonic and 3α-hydroxy masticadiedonic acids inhibit proliferation prostate cancer in vivo and in vitro by inducing apoptosis.

13.00 · **Pezzani R. (Italy),** The usefulness of oregano's phytocomplex crude extract: novel anti-proliferative effects in adrenocortical tumor cells

13.15 · **Montejano-Rodriguez JR. (Mexico),** Antitumor effect of aqueous extract of *Jatropha dioica Sessé* ex Cerv lymphoma L5178Y in CD1/C mice

13.30 · **Lunch**

Session 7 · Natural Products: from basic research to clinical applications

Chairs: Quesada S. and Pellegrini M.

14.45 · **Invited Lecture:**
Santos-Buelga C. (Spain), Evaluation of the biological activity of phytochemicals using in vivo model organisms

15.15 · **Jha V. (India),** Herbal drug Interactions and remedies: a challenging problem in therapy

15.30 · **Milella L. (Italy),** *Echinacea angustifolia DC.* extract quali-quantitative analysis and fibroblast cell growth evaluation

15.45 · **Casamayor Laime Z. (Cuba),** Effect of *Spirulina platensis* in patients with metabolic syndrome

16.00 · **Waksman N. (Mexico),** *Turnera diffusa* (Damiana) as source of hepatoprotective and hypoglycemic compounds

16.15 · **Russo R. (Italy),** ROSSOPURO™: a red yeast rice extract for the management of hypercholesterolemia

16.15 · **Rosado JR. (Colombia),** Effects of Paralejo (*Curatella americala l.*) in the expulsion of kidney stones, using traditional methods

16.45 · **Short Break**

Chairs: Malagon Aviles OG. and Romussi G.

17.00 · **Round Table:**
Nunez Selles AJ. (Rep. Dominicana), Antoxidant therapies through signaltransduction and gene regulatory pathways

17.20 · **Sepulveda-Arias JC. (Colombia),** Nrf2-mediated antioxidant activity of *T. rosea* (Bertol) DC and *T. chrysantha* (Jacq) G. Nicholson extracts

17.30 · **Quesada S. (Costa Rica),** Antioxidant activity and apoptosis induction assessed by flow cytometry of a *Bactris guineensis* polyphenol extract

17.40 · **Plazas EA. (Colombia),** In Vitro antiooxidant and anticholinesterase activities of Colombian plants as potential neuroprotective agents

17.50 · **Bello-Martinez J. (Mexico),** Antioxidant and antiproliferative activity of *Haematoxylom brasiletto Karst*

18.00 · **Kadri F. (Algeria),** Antioxidant, hypoglycemic and antimicrobial activity of two Algerian tannin sorghums crud extracts correlated with polyphenols and tannin contents

18.15 · **Closure of the Meeting**

18.30 · **Farewell party looking forward to the next meeting**

POSTER SESSION 1

September 12th – 13th

(Posters should be mounted in the afternoon of September 11 and removed in the afternoon of September 13)

1. **NUTRACEUTIC EFFECT OF CUETLAS *ARSENURA ARMIDA* C EDIBLE INSECT LOCAL FOOD AT IXCAQUIXTLA, MEXICO.** Melo-Ruíz V, Quirino-Barreda T, Macin-Cabrera S, Sánchez-Herrera K, Díaz-García R. (Mexico)

2. **ARGENTINIAN MEDICINAL PLANTS AGAINST POSTHARVEST PHYTOPATHOGENICS FUNGI ISOLATED FROM ORANGES, STRAWBERRIES AND PEACHES.** Di Liberto MG, Svetaz LA, Derita MG. (Argentina)

3. **CACAO CO-PRODUCTS: AN ALTERNATIVE TO OBESITY CONTROL.** Hidalgo-Pérez Tejada EI, Aguirre-López AE, Mena-López JA, Plazola-Jacinto CP, Valadez- Carmona L, Ortiz-Moreno A, Hernández- Navarro MD, Ceballos-Reyes GM, Hernández-Ortega M. (Mexico)

4. **BOTRICIDE ACTIVITY OF DRIMENOL AND DERIVATIVES.** Carrasco H, Olea A, Silva E, Robles-Kelly C,' Martínez R, Sedan A, Thomas M. (Chile)

5. **A NEW IRIDOID AND OTHER CONSTITUENTS FROM *PTEROCEPHALUS NESTORIANUS NAB.*** Abdullah FO, Hussain FHS, Vita Finzi P, Vidari G. (Iraq, Italy)

6. **BOTRICIDE ACTIVITY OF EUGENOL AND DERIVATIVES.** Carrasco H, Olea A, Silva E, Barraza D, Robles-Kelly C, Martínez R, Sedan A, Thomas M. (Chile)

7. **THE LONG HISTORY OF PASTORALISM IN SOUTHERN ITALY AS SHOWN BY ARCHAEOPALYNOLOGY.** Florenzano, A. (Italy)

8. **MULTIDISCIPLINARY INVESTIGATION ON EARLY-MID HOLOCENE WILD CEREALS FOUND AT TAKARKORI (CENTRAL SAHARA).** Fornaciari R, Arru L, Mercuri AM, di Lernia S. (Italy)

9. **BIOACCESSSIBILITY AND BIOACTIVITY OF POLYPHENOLS EXTRACTED FROM SIX CHERRY CULTIVARS.** Martini S, Tagliazucchi D, Conte A. (Italy)

10. **INFLUENCE OF THE GELATO BASE FORMULATION ON THE SURVIVAL OF *LACTOBACILLUS CASEI* STRAINS DURING GELATO PRODUCTION AND STUDY OF THEIR IMPACT ON THE RHEOLOGICAL PROFILE.** Chiari P, Belmonte AM, Pulvirenti A, Giudici P. (Italy)

11. **HERBAL REMEDIES IN THE NORTH OF ALGERIA: POTENTIAL ADVERSE INTERACTIONS WITH ANTICANCER AGENTS.** Zitouni H, Benguedda FZB, Benhamida W, Benalla L, Baira M, Toumi H. (Algeria)

12. **PORTULACA OLERACEA L. IN THE ERA OF GLOBALISATION: A SPECIES OF GREAT NUTRACEUTICAL VALUE.** Mantovani V, Buldrini F, Bosi G, Volpi N. (Italy)

13. **ANTIOXIDANT ACTIVITY AND APOPTOSIS INDUCTION ASSESSED BY FLOW CYTOMETRY OF A *BACTRIS GUINEENSIS* POLYPHENOL EXTRACT.** Quesada S, Quesada MS, Azofeifa G, Pérez AM. (Costa Rica)

14. **ASSESSMENT OF THE HEAVY METAL CONTENT IN AROMATIC SPICES BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY.** Bua DG, Annuario G, Albergamo A, Bartolomeo G, Cicero N, Dugo G.mo (Italy)

15. **ANTI-INFLAMMATORY EFFECT AND PHYTOCHEMICAL SCREENING OF *FICUS PERTUSA.*** Fabian Medina D, Orejon Gomez, Herrera Hernández N. (Perù)

16. **VISCERAL ADIPOSE TISSUE REDUCTION THROUGH CACAO CO-PRODUCTS CONSUMPTION.** Hidalgo-Pérez Tejada EI, Valadez-Carmona L, Plazola-Jacinto CP, Hernández-Ortega M, Ceballos-Reyes GM, Ortiz-Moreno A. (Mexico)

17. **ANTIMALARIAL ACTIVITY OF XANTHONES ISOLATED FROM *GARCINIA MANGOSTANA.*** Quiñones W, Echeverri LF, Robledo SM, Torres LF, Gil JF, Escobar GA, Archbold R. (Colombia)

18. **UNCOVERING ARTIFICIAL HONEYS FROM ECUADORIAN MARKETS.** Vit P, González I, Maza F. (Venezuela, Ecuador)

19. **ANTI-INFLAMMATORY SCREENING OF PLANT EXTRACTS USED IN FOLK MEDICINE OF COLOMBIAN CARIBBEAN COAST.** Castro JP, Ocampo YC, Mejia NM, Bolívar S, Díaz F, Franco LA. (Colombia)

20. **BIOGUIDED FRACTIONATION OF *TRICHILIA HIRTA* SEEDS EXTRACT WITH CYTOTOXIC ACTIVITY AGAINST A BREAST CANCER CELL LINE.** Caro D, Ocampo Y, Bolivar S, Diaz F, Aristizábal FA, Franco LA. (Colombia)

21. **CAPTURING THE DIVERSITY OF FUNGAL POPULATION IN HUMAN COLONIC MICROBIOTA THROUGH CULTURE-DEPENDENT AND INDEPENDENT APPROACHES.** Gozzoli C, Raimondi S, Somone M, Orsi CF, Peppoloni S, Blasi E, Cavalieri D, Rossi M. (Italy)

22. **SUBDOMINANT BUT CONSTANT *LACTOBACILLUS* POPULATION IN THE HUMAN GUT MICROBIOTA AS REVEALED BY WHOLE GENOME SEQUENCING.** Rossi M, Martinez-Martinez D, Amaretti A, Ulrici A, Raimondi S, Moya A. (Italy, Spain)

23. **PRELIMINARY STUDY OF PESTICIDE RESIDUES IN PETITGRAIN DISTILLATED FRACTIONS.** Bartolomeo G, Rando R, Tropea A, Cicero N, Recupero S, Dugo G.mo (Italy)

24. **PRODUCTION OF SINGLE CELL PROTEIN BY *SACCHAROMYCES CEREVISIAE* FROM FOOD WASTE.** Gervasi T, Cicero N, Russo E, Naccari C , Dugo G.mo (Italy)

25. **DEVELOPMENT OF A NEW MULTIRESIDE METHOD TO ANALYZE 67 CONTAMINANTS IN FOOD WASTE BY GC-MS/MS.** Fede MR, Di Bella G, Potortì AG, Lo Turco V, Mottese A, Dugo G. (Italy)

26. **MOLECULAR CHARACTERIZATION OF HYBRID STRAINS OF *PLEUROTUS* BY ISSR.** Aguilar DL, Valencia TG, Zarate SPB, Villanueva AR, Guadarrama MPC, Sánchez HA, Garín AME. (Mexico)

27. **SERJANIC ACID OBTAINED FROM CECROPIA TELENITIDA REGULATES BLOOD LIPID LEVELS AND REDUCES THE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES.** Balcazar N, Guillen A, Muñoz DL, Ramirez-Pineda JR, Montoya G, Acín S. (Colombia)

28. **PRELIMINARY STUDY ON THE PHENOLYC PROFILE OF *BOLETUS AEREUS.*** Pellizzeri V, Lo Turco,V, Di Bella G, Saitta M, Gervasi T, Dugo G.mo (Italy)

29. **ETHNOBOTANICAL INVESTIGATION IN THE ITALIAN CENTRAL ALPS: HEALTH BENEFITS FROM EDIBLE PLANT SPECIES.** Vitalini S, Puricelli C, Iriti M. (Italy)

30. **EFFECT OF COMBINED ADMINISTRATION OF *ARTHROSPIRA* (SPIRULINA) *MAXIMA* AND WASTE OF *COCOS NUCIFERA L.* ON LIPID PROFILE IN MALE MICE FED WITH HYPERCHOLESTEROLEMIC DIET.** García GE, Martínez E, Chamorro G, Ortíz A, Garduño L. (Mexico)

31. **BIOLOGICAL ACTIVITIES OF *LAVANDULA ANTINEAE MAIRE*. ENDEMIC SPECIES IN ALGERIA.** Krimat S, Dob T, Lamari L, Ksouri A, Metidji H, Nouasri A. (Algeria)

32. **PRIMARY SCREENING OF BIOLOGICAL ACTIVITIES IN EXTRACTS OBTAINED FROM AROMATIC AND MEDICINAL PLANTS GROWN IN COLOMBIA.** Flechas MC, Barrios SX, Cuadros AJ, Stashenko EE, Fuentes JL, Ocazionez RE. (Colombia)

33. **ETHNOBOTANICAL SURVEY ON THE USE OF TRADITIONAL MEDICINE FOR THE TREATMENT OF OSTEOARTHRITIS IN ORAN, ALGERIA.** Lardjam A, Mazid R, Sadaoui A, Bensahaila S, Khalfa, Khitri W, Azaiz A, Djebli N, Toumi H. (Algeria)

34. **GREEN SYNTHESIS OF NEW LIGANDS STARTING FROM CHIRAL p-HALOGENATED AMINES.** Hernández Téllez MG, Moreno Morales GE, Gutiérrez Argüelles D, Portillo Moreno Ó, Gutiérrez Pérez R. (Mexico)

35. **GREEN SYNTHESIS OF A NEW CHIRAL LIGAND HALOGENATED AND ITS Pd(II) COMPLEX.** Hernández Téllez MG, Moreno Morales GE, Gutiérrez Argüelles D, Portillo Moreno Ó, Gutiérrez Pérez R. (Mexico)

36. **EFFECTIVENESS AND SAFETY OF NUTRISOL AS NUTRITIONAL SUPPLEMENT IN THE TREATMENT OF ADOLESCENTS WITH NUTRITIONAL DEFICIENCY.** González S, Domínguez MI, Casamayor Z, Hernández B, Conde E, Leal A, Filgueira I, Agramonte G, Casas MJ, Povea E, Hevia D, Díaz M, Álvarez B, Pérez IM, Esplugan A, Bejerano CJ, Albertine M. (Cuba)

37. **ANTI-STAPHYLOCOCCAL ACTIVITY IN VITRO AND IN VIVO OF ZINNIA PERUVIANA. HISTOLOGICAL STUDY.** Echenique DR, Mattana CM, Laciar, AL, Aguilera Merlo C, Cruceño AM, Satorres SE. (Argentina)

38. ***AZORELLA TRIFURCATA* AND *ZINNIA PERUVIANA* EXTRACTS: ACTIVITY AGAINST CLINICAL ISOLATES *STAPHYLOCOCCUS AUREUS.*** Echenique DR, Mattana CM, Laciar AL, Alcaráz LE, Satorres SE. (Argentina)

39. **ANTIPROLIFERATIVE ACTIVITY ON HUMAN PROSTATE CELLS OF ESSENTIAL OILS OF THREE LEBANESE *SALVIA* SPECIES.** Russo A, Cardile V, Graziano ACE, Formisano C, Avola R, Arnold NA, Senatore F, Rigano D. (Italy, Lebanon)

40. **ANTIMICROBIAL PROPERTIES OF GARLIC (*ALLIUM SATIVUM L.*) AND ONION (*ALLIUM CEPA L.*) EXTRACT ON COMMON CARP (*CYPRINUS CARPIO*) RESTRUCTURED MEAT DURING STORAGE IN 4 °C.** Dublán-García O, Rodríguez-Quiroz ML, Gómez-Oliván LM, López-Martínez LX, Díaz-Bandera D, Cira-Chávez LA, Hernández-Navarro MD, Jáuregui-Rodríguez B. (Mexico)

41. **EFFECT OF CHEMICAL COMPOUNDS AGAINST BACTERIAL BIOFILMS UNDER MONOSPECIES AND DUAL-SPECIES CONDITIONS.** Mohamed AM, Satorres SE, Novoa RE, Cifuentes DA, Mattana CM. (Argentina)

42. **EVALUATION OF GENOTOXIC ACTIVITY OF THREE AQUEOUS EXTRACTS OF NATIVE PLANTS FROM ARGENTINA.** Mohamed AM, Cangiano MA, Alcaráz LE, Satorres SE, Laciar AL, Mattana CM. (Argentina)

43. **MEDICINAL PLANTS OF COLOMBIA WITH POTENTIAL FOR TREATMENT INFECTIOUS DISEASES.** Hernández-Rodríguez P, Pabón LC, Rodríguez MF. (Colombia)

44. **INHIBITORY CAPACITY OF AQUEUS OREGANO EXTRACT (*ORIGANUM VULGARE L.*) ON MICROORGANISMS (*S. AUREUS, E. COLI AND SALMONELLA TYPHIMURIUM*) AND IT´S EFFECT ON THE SHELF LIFE OF A GEL-TYPE MADE OF PROTEIN FROM GIANT SQUID (*DOSIDICUS GIGAS*).** Dublán-García O, López-Medina FA, Gómez-Oliván LM, López-Martínez LX, Díaz-Bandera D, Cira-Chávez LA, Hernández-Navarro MD, Jáuregui-Rodríguez B. (Mexico)

45. ***ERYSIMUM CRASSIPES* AS NEGLECTED MEDICINAL PLANT: STORAGE AT EARLY BRONZE AGE KÜLLÜOBA/TURKEY.** Çizer O, Guse J. (Germany)

46. **LEVELS OF HEAVY METALS IN OCTOPUS (*OCTOPUS VULGARIS*) FROM SOUTH TYRRHENIAN: PRELIMINARY RESULTS.** Ariano A, D'Ambola M, Vassallo, A, Smaldone G, Velotto S, Severino L. (Italy)

47. **PHYSICOCHEMICAL EVALUATION OF THE OLEORESIN FROM *COPAIFERA PAUPERA* COLLECTED SEASONALLY IN ACRE, BRAZIL.** Nakamura MJ, Vaucher AS, A.S. Siani AC, Mazzei JL, Guarino ESG, Freitas O, Ramos MFS. (Brazil)

48. **ACETIC ACID BACTERIA AND CELLULOSE PRODUCTION: STRAIN SELECTION AND POLYMER CHARACTERIZATION.** Gullo M, Zanichelli G, Sola A, Montorsi M, Messori M. Giudici P. (Italy)

49. **AMARANTH GRAIN (*AMARANTHUS HYPOCHONDRIACUS*) AS SUSTAINABLE ALTERNATIVE PROTEIN SOURCES TO MEAT.** Vilchis-Perez A, Melo-Ruíz V, García-Serralde L, Gazga-Urioste C, Diaz-García R. (Mexico)

50. **AGRONOMIC AND PHYTOCHEMICAL STUDY OF *SALVIA SCLAREA L.* SICILIAN.** Tuttolomondo T, Napoli E, Ruberto G, Leto C, Virga G, Gennaro MC, Licata M, La Bella S. (Italy)

51. **EVALUATION OF THE HYPOLIPEMIANT AND HYPOGLUCEMIANT EFFECT OF A BASE POWDER TO ELABORATE FUNCTIONAL FOOD OBTAINED FROM TOMATO PEEL (*PHYSALIS IXOCARPA*) IN MICE.** Zambrano E, Pérez G, Pérez Pastén R. (Mexico)

52. **BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *ARTOCARPUS HETEROPHYLLUS LAM.*** Figueroa-Ibarrra LM, Hernández-Navarro MD, Chamorro-Cevallos GA, Hernández-Ortega MM, Gil-Escamilla JO, Jáuregui-Rodríguez B, Gómez-Oliván LM, Dublán-García O, Islas-Flores H. (Mexico)

53. **ANTIOXIDANT ACTIVITY OF PORIFERANS FROM THE COLOMBIAN CARIBBEAN ROCKY COAST BY DPPH AND ABTS METHOD.** Tovar BGP, Ramírez ALS, Valle-Molinares RH, Valencia JA, Arboleda VJW. (Colombia)

54. **SESQUITERPENES AND MONOTERPENES FROM *AMMOIDES ATLANTICA* (COSS. ET DUR.) WOLF.** D'Ambola M, Boudermine S, Vassallo A, Benayache S, Severino L, De Tommasi N, Malafronte N. (Italy, Algeria)

55. **PLANT CELL CULTURE AS EXPERIMENTAL MODEL APPLIED TO THE BIOSYNTHESIS PATHWAY STUDY OF COUMARIN AND CHLOROGENIC ACID IN *MIKANIA GLOMERATA* (ASTERACEAE).** Aranha Netto L, Andreazza NL, Sawaya ACHF, Salvador MJ. (Brazil)

56. **BIOACTIVITY AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM THE LEAVES OF *ANNONA PARVIFLORA* (A. ST. -HIL.) H. RAINER (ANNONACEAE).** Siqueira CAT, Aranha Netto LA , Lourenço CC, Serain AF, Mesquita JT, Tempone AG, Salvador MJ. (Brazil)

57. **ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SEVERAL AGRO-INDUSTRIAL WASTES FROM COFFEE AND COCOA PRODUCTION.** Ortiz AJF, Betancur P, Valle-Molinares RH, Valencia AJ, Arboleda VJW.(Colombia)

58. **EVALUATION OF ANTIOXIDANT PROPERTIES AND ACUTE TOXICITY OF CYANOBACTERIA PIGMENTS AND METHANOLIC EXTRACTS.** Galetović A, Neira I, Vega M, Ochoa P, Gallardo V, Núñez J, Seura F, Tapia C, Valdivia C, Gómez-Silva B. (Chile)

59. **ADVANCES IN KNOWLEDGE OF *CHILIOTRICHUM DIFFUSUM* (ASTERACEAE), A NATIVE SPECIES FROM PATAGONIA USED IN TRADITIONAL MEDICINE OF THE ONAS.** Alcalde Bahamonde SM, Weisntein--Oppenheimer C, Flores ML, Córdoba OL. (Argentina, Chile)

60. **ESI-MS AND UHPLC-MS ANALYSIS OF RESIDUES OF GREEN PROPOLIS AND EVALUATION OF THEIR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES.** Corrêa WR, Rueda RYR, Polanco IM, Hernandez-Tasco AJ, Marinho JVN, López BGC, Prado SC, Cunha IBS, Sawaya ACHF, Salvador MJ. (Brazil)

61. **DEVELOPMENT AND CHARACTERIZATION OF ACTIVE ALGINATE-BASED EDIBLE FILMS FOR FOOD PACKAGING APPLICATIONS.** Bignardi C, Carà D, Cavazza A, Corradini C, Grimaldi M, Salvadeo P (Italy)

62. **ANTIMICROBIAL ACTIVITY OF TWO NEW TETRAHYDROQUINOLINES.** Valle-Molinares RH, Marsiglia-Lans MA, Arboleda VJW, Del Olmo-Fernandez E, Melendez-Gómez CM. (Colombia, Spain)

63. **PHENOLIC PRODUCTION AND ANTIRADICALAR ACTIVITIES OF IN VIVO PLANTS AND IN VITRO CULTURES OF SEVERAL HYPTIS SPECIES COLLECTED IN CERRADO AREA (GOIÁS, BRAZIL).** Rios RM, Silva FG, Pereira, PS, Dias AP. (Portugal, Brazil)

64. **APOPTOTIC EFFECT OF CARDENOLIDE GLYCOSIDES FROM *ASCLEPIAS SUBULATA*.** Robles-Zepeda RE, Velázquez C, Garibay-Escobar A, Vilegas W, Medina-Juárez LA, Gámez-Meza N, Rascón-Valenzuela LA. (Mexico, Brazil)

65. **ANTIPSORIATIC ACTIVITY OF LATIN AMERICAN NATIVE PLANTS.** Pinto NCC, Mendes RF, Silva JB, Duque APN, Castañon MCMN, Scio E. (Brazil)

66. **STUDY OF HEAVY METALS, TAA, POLYPHENOLS IN TRITICUM DURUM WHEAT SAMPLES GROUND WITH ANCIENT MILLSTONE.** Oliveri F, Brancato P, Gottuso V, Cosenza M, Pitti R, Bua DG, Annuario G, Reale S, Cicero N, Dugo G.mo (Italy)

67. **ETNOPHARMACOLOGICAL INVESTIGATION OF PLANTS USED BY THE RIVERINE POPULATION ON THE MICROREGION OF NORTH ARAGUAIA, MATO GROSSO, BRAZIL.** Ribeiro RV, Martins DTO. (Brazil)

68. **SCREENING OF ANTIBIOTIC PRODUCING ACTINOMYCETES FROM THE SEDIMENTS OF UNDISTURBED FOREST AREAS OF ASELLA, ETHIOPIA AND ITS HYPER ACTIVITY AFTER MUTATION.** Ashok Kumar P, Karpagam P. (Nigeria)

69. **CHARACTERIZATION AND EVALUATION OF SAPONINS FROM *YUCCA BACCATA* AGAINST *GIARDIA INTESTINALIS* IN VITRO.** León-Trujillo RC, Astiazarán-García H, Robles-Zepeda R, Hernández-Martínez J, Quihui-Cota L. (Mexico)

70. **CHARACTERIZATION OF ANTHOCYANINS IN BILBERRY (*VACCINIUM MYRTILLUS L.*) FOOD DERIVATIVES.** Benvenuti S, Brighenti V, Ranieri C, Pellati F, Bonacini C. (Italy)

71. **POLIACETILENS EXTRACTION IN *DAUCUS CAROTA.*** Spera DM, Napolitano TR, Venettacci N, Di Giammatteo V. (Italy)

72. **NUTRACEUTICAL PROPERTIES OF *PRUNUS DULCIS* EXTRACTS OBTAINED BY SUPERCRITICAL FLUID EXTRACTION.** Carlucci G, Spera DM, Anselmi A, Carlucci M, Ferrone, V. (Italy)

73. **IN VITRO POTENTIAL OF TWO LICHENS FROM DEPARTMENT TOLIMA-COLOMBIA AS ANTIOXIDANTS AND INHIBITORS OF DIGESTIVE ENZYMES.** Ortiz LT, Prieto JA. (Colombia)

74. **HEAVY-METAL BIOACCUMULATION CAPACITY OF WILD HERBACEOUS PLANTS IN THE PALERMO URBAN AREA.** Tuttolomondo T, Leto C, Virga G, Gennaro MC, Licata M, La Bella S, Gentile F, Dugo G. (Italy)

75. **CAROTENOIDS EXTRACTED FROM LYCIUM SPP PROMOTED THE ACTIVATION OF ARYL HYDROCARBON RECEPTOR.** Montesano D, Cossignani L, Scalisi G, Turco A, Simonetti MS, Blasi F. (Italy)

76. **ANTIOXIDANT CAPACITY AND PHENOL PROFILES OF SOME BEEHIVE PRODUCTS FROM UMBRIA (ITALY).** Blasi F, Cossignani L, Simonetti MS, Montesano D. (Italy)

77. **SECONDARY METABOLITES FROM CHILEAN PLANTS AND THEIR BIOLOGICAL ACTIVITY.** Alarcón JE, Cespedes CL, Muñoz E, Quiroz S. (Chile)

78. **DETECTION OF PANCREATIC LIPASE AND ACETYLCHOLINESTERASE INHIBITORS IN EXTRACTS FROM *Piper cf. asperiusculum* AND *Piper pertomentellum* BY TLC-AUTOGRAPHY METHODS.** Patiño OJ, Prieto JA, Patiño WR. (Colombia)

79. **CHEMICAL-FUNCTIONAL CHARACTERIZATION OF *COUROUPITA GUIANENSIS* BARK.** Rizzo D, Re T, Ventura C, Fanizzi FP, Miceli A. (Italy)

80. **COMPARISON OF TWO EXTRACTION TECHNIQUES FOR THE COMPREHENSIVE CHARACTERIZATION OF BIOACTIVE PHENOLIC COMPOUNDS IN ONION WATREUSING BY UHPLC-DAD-ESI-HRMS.** Campone L, Celano R, Piccinelli AL, Ibañez E, Carabetta S, di Sanzo R, Rastrelli L, Russo M. (Italy, Spain)

81. **A FULLY AUTOMATED METHOD FOR DETERMINATION OF OCHRATOXIN A IN WINE AND BEER BY ULTRA HIGH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY.** Campone L, Celano R, Rastrelli L, Russo M, Piccinelli AL. (Italy)

82. ***IN VITRO* EFFECT OF BRAZILIAN PROPOLIS ON THE EXPRESSION LEVELS OF MIR-27A-3P AND MIR-19A-3.** Daglia M, Curti V, Zaccaria V, Di Lorenzo A, Meneghini S, Fachini A. (Italy)

83. **AUTHENTICATION OF HONEYS BOTANICAL ORIGIN BY UHPLC-MS/MS AMINO ACIDS DETERMINATION. A PRELIMINARY STUDY.** Carabetta S, Campone L, Di Sanzo R, Cefaly V, Rastrelli L, Russo M. (Italy)

84. **CUCHAMÁ LARVAE *(PARADIRPHIA FUMOSA)* EDIBLE INSECT RICH IN NUTRIENTS.** Vilchis-Perez A, Melo-Ruíz V, Marquez-Cruz B, Gazga-Urioste C, Diaz-García R. (Mexico)

POSTER SESSION 2

September 14th – 15th

(Posters should be mounted in the late afternoon of September 13 and removed in the afternoon of September 15)

1. ***SECHIUM EDULE* ALKALOID EXTRACT APOPTOSIS INDUCE ON HELA CELLS CERVICAL CANCER.** Rivera PIA, Figueroa AP, Luna TAL, Silva TR. (Mexico)

2. **ANTITUMOR EFFECT AND TOXICITY ACUTE ETHANOL EXTRACT OF *GANODERMA CURTISII* (BERK.) MURRILL LYMPHOMA L5178Y IN BALB / C MICE.** Montejano-Rodríguez JR, Ríos-Ramírez MI, Romero-Bautista L, Islas-Santillán MA, Bautista-Avila M, Almaguer-Vargas G. (Mexico)

3. **SYNTHESIS AND EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF OPTICALLY ACTIVE TRIAZINES.** Arteaga DV, Abonía R, Sepúlveda-Arias JC, Veloza LA. (Colombia)

4. **SYNTHESIS AND STUDY OF PHYSICO-CHEMICAL PROPERTY OF DITHIOL-ONE'S DERIVATIVES FROM DITHIOL-THIONE.** Laifa El-Adoui (Algeria)

5. **COLON-AVAILABLE DIETARY MELANOIDINS EXHIBIT CYTOTOXIC ACTIVITY ON HUMAN COLON ADENOCARCINOMA CELL LINES.** Tagliazucchi D, Martini S, Conte A. (Italy)

6. **A FACILE SYNTHESIS OF 6-AZASTEROIDS.** Martínez Pascual R, Montiel Smith S,Vega Baez JL. (Mexico)

7. **REACTIVITY OF CEMBRENES AND VERTICILLENES FROM *BURSERACEAE* UNDER ACID TREATMENTS.** Hernández-Hernández JD, del Río-Chávez AA, Escobar Flores KD, Román-Marín LU, García-Gutiérrez HA, Cerda-García-Rojas CM, Joseph-Nathan P. (Mexico)

8. **ANTI-INFLAMMATORY AND ANALGESIC EVALUATION OF FLOWERS *SEDUM PRAEALTUM DC.*** Márquez-Flores YK, Ortega FLF, Silva-Torres TR, Meléndez-Camargo ME. (Mexico)

9. ***MORUS NIGRA L*. LEAVES EXTRACTS STANDARDIZED IN CHLOROGENIC ACID, RUTIN AND ISOQUERCITRIN: TYROSINASE INHIBITION.** Freitas MM, Fontes P, Homem-de-Mello M, Souza PM, Silveira D, Fonseca-Bazzo Y, Simeoni LA, Magalhães PO. (Brazil)

10. **ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY OF *SALVIA LERIIFOLIA* BENTH: EXTRACTS.** Loizzo MR, Rastrelli L, Falco T, Tundis R. (Italy)

11. **CHEMICAL COMPOSITION AND ENHANCEMENT OF TECHNOLOGICAL PROPERTIES OF *HALIMIUM HALIMIFOLIUM* EXTRACT WITH ANTIOXIDANT ACTIVITY.** Kerbab K, Mekhalfi T, Zaiter L, Benayache S, Benayache F, Picerno P, Mencherini T, Sansone F, Aquino RP, Rastrelli L., Smith AB, Brown ABC, Taylor A, Evans AB. (Algeria, Italy)

12. **SYNTHESIS OF (E)-1-(FURAN-2-YLMETHYLENE)-2-PHENYLHYDRAZINE.** Cabrera-Vivas BM, Ramírez-García JC, Aguirre-Cabrera C, Flores-Castillo ZA, Toledano-Magaña Y, García-Ramos JC. (Mexico)

13. **SYNTHESIS OF (E)-1,1-DIPHENYL-2-(THIOPHEN-2-YLMETHYLENE)HYDRAZINE AND CELL EVALUATION AGAINST AMIBIASIS.** Meléndez-Balbuena L, Cabrera-Vivas BM, Aguirre Cabrera C, Ramírez Juan C, García-Ramos JC, Juárez-Posadas JR. (Mexico)

14. **SYNTHESIS OF (E)-2-((5-(2-NITROPHENYL)FURAN-2-YL)METHYLENE)-1,1-DIPHENYLHYDRAZINE AND ANTICANCER CELL EVALUATION.** Cabrera-Vivas BM, Aguirre Cabrera C, García-Díaz IM, Ariza-Ramírez J, Ramírez-García JC, Orea-Flores ML. (Mexico)

15. **INFLUENCE OF THE GRANULAR PREPARATION METHOD AND THE TABLET HARDNESS IN THE DISSOLUTION OF A PHYTOPHARMACEUTICAL PRODUCT.** Archbold R, Quiñones W, Echeverri LF, Concesa Caballero, Torres LF, Escobar GA. (Colombia)

16. **IN VITRO VALIDATION OF ANTI-INFLAMMATORY, HEALING AND ANTI-LEISHMANIAL ACTIVITY OF *TABEBUIA CHRYSANTHA, TABEBUIA ROSEA CALENDULA OFICINALIS MATRICARIA CHAMOMILLA, THYMUS VULGARIS AND OREGANUM VULGARE.*** Robledo SM, Rios YK, Jurado DS, Echeverri F. (Colombia)

17. **ANTI-INFLAMMATORY ACTIVITY OF A *PHYSALIS ANGULATA* L. DICHLOROMETHANE FRACTION IN DSS-INDUCED COLITIS.** Rivera DE, Ocampo YC, Franco LA. (Colombia)

18. **CYTOTOXIC EFFECT OF ETHANOLIC EXTRACT AND FRACTIONS FROM *CROTON MALAMBO* BARK AGAINST MDA-MB-231 CELL LINE.** Caro D, Ocampo Y, Bolivar S, Diaz F, Salas RD, Aristizábal FA, Franco LA. (Colombia)

19. **ANTICONVULSANT EFFECT OF *TANACETUM PARTHENIUM (L.) SCHULTZ-BIP* ON PENTYLENETETRAZOLE-INDUCED SEIZURES IN MICE.** Reyes-Pérez VI, Hernández-Navarro MD. (Mexico)

20. **LC/MS ANALYSIS AND HPLC QUANTIFICATION OF MAIN COMPOUNDS OF FIVE *HYPERICUM* SPECIES INDIGENOUS IN THE PELOPONNESE.** Zeliou K, Koulakiotis NS, Vogiatzoglou A, Iatrou G, Tsarbopoulos A, Lamari FN. (Greece)

21. **OPTIMIZATION AND VALIDATION OF TWO METHODS OF ENZYME INHIBITION RELATED TO ANTI-HYPERGLYCEMIC ACTIVITY.** Granados-Guzmán G, Castro-Ríos R, Waksman de Torres N, Salazar-Aranda R. (Mexico)

22. **THEORETICAL STUDY OF THE CONVERSION (E)-□,□-UNSATURATED OXAZOLIDINONES.** Ramírez - García JC, Cabrera – Vivas BM, Aguirre C, Pineda FP, Márquez CG. (Mexico)

23. **COMPUTATIONAL ANALYSIS ON VITAMIN D RECEPTOR.** Ramírez - García JC, Cabrera – Vivas, BM, Aguirre C, Pineda FP, Márquez CG. (Mexico)

24. **MEDICAL APPLICATIONS OF OZONIZED OIL. AN UP-DATE.** Martínez-Sánchez G, Magistrelli D. (Italy)

25. **BIOSAFETY ASPECTS IN THE REGULATION OF MODERN BIOTECHNOLOGY IN PLANTS.** Borges BJP, Abreu PMV, Carminati LS, Arantes OMN, Fernandes AAR, Fernandes PMB. (Brazil)

26. **ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *LEPIDIUM SATIVUM* EXTRACTS.** Bouamra D, Baki CA, Touabet L, Meliani S, Bouchebour A, Dahamna S, Harzallah D. (Algeria)

27. **ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST *SALMONELLA* AND *STAPHYLOCOCCUS AUREUS*.** Serracca L, Rossini I, Battistini R, Imberciadori M, Corsi M, Terarolli A, Tomei L, De Montis G, Ercolini C. (Italy)

28. **TRITERPENES-ENRICHED FRACTIONS OF *EUCALYPTUS TERETICORNIS* MODULATE GENE EXPRESSION ON HUMAN ADIPOSE TISSUE CELL LINES MODELS.** Balcazar N, Guillen A, Castaño A, Fernando Echeverri LF, Acín S. (Colombia)

29. **ACTIVITY AGAINST THE FUNGUS *COLLETOTRICHUM LINDEMUTHIANUM* OF PHASEOLLIN ALONE OR IN COMBINATION WITH STRUCTURALLY RELATED COMPOUNDS, PHENYLPROPANOIDS AND AROMATIC MONOTERPENES.** Durango D, Quiñones W, Escobar G. (Colombia)

30. ***CORDIA CURASSAVICA: PRIMARY SOURCE FOR DISCOVERING A NATURAL MEDICINE FOR DENGUE TREATMENT.*** Velandia SA, Lopera IA, Sepúlveda-Arias JC, Veloza LA, Stashenko EE, Ocazionez RE. (Colombia)

31. ***ESSENTIAL OILS AND THEIR COMMON METABOLITE B-CARYOPHYLLENE AS PRIMARY SOURCE FOR DISCOVERING AN ANTIVIRAL AGENT AGAINST DENGUE VIRUSES.*** Velandia SA, Flechas MC, Stashenko EE, Ocazionez RE. (Colombia)

32. **PHARMACOLOGICAL INVESTIGATIONS ON *KIGELIA AFRICANA* (LAM) BENTH., FRUITS EXTRACT.** Micheli V, Sanogo R, D'Angelo V, Occhiuto F. (Italy, Mali)

33. **ANTILEISHMANIAL ACTIVITY AND IN VIVO TOXICITY OF FOUR CHEMOTYPES OF *LIPPIA* (FAM. *VERBENACEAE*) ESSENTIAL OILS.** Neira LF, Hernandez JC, Escobar P, Stashenko EE. (Colombia)

34. **OXIDATIVE STRESS IN DIFFERENT CLINICAL CONDITIONS, USING REDOX INDEXES OF DIAGNOSTIC VALUE.** Gil del Valle L, Guevara M. (Cuba)

35. **ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *LEPIDIUM SATIVUM* EXTRACTS.** Bouamra D, Baki CA, Touabet L, Meliani S, Bouchebour A, Dahamna S, Harzallah D. (Algeria)

36. **QUANTITATIVE HPLC- UV/DAD ANALYZES OF THE MAIN C-GLYCOSIDE FLAVONOIDS IN THREE DIFFERENT METHANOLIC EXTRACTS OF *ALTERNANTHERA TENELLA COLLA* AERIAL PARTS.** Marinho JVN, Soma R, Hernández-Tasco AJ, Salvador MJ. (Brazil)

37. **SPECTROSCOPY CHARACTERIZATION OF (E)-2-(2,6-DICHLOROBENZYLIDENE)-1,1-DIPHENYLHYDRAZINE.** Cabrera-Vivas BM, Ariza-Ramírez J, Aguirre Cabrera C, Ramírez JC, García-Ramos JC, Toledano-Magaña Y. (Mexico)

38. **ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *ORIGANUM GLANDULOSUM* ON BACTERIAL STRAINS OF HOSPITAL ORIGIN MOST IMPLICATED IN NOSOCOMIAL INFECTIONS.** Mazid R, Lardjam H, Khitri W, Boudghene SY, Izarouken A, Djebli N, Toumi H. (Algeria)

39. **PHYTOTHERAPY IN DIABETIC PATIENTS: ETHNOBOTANICAL SURVEY, PHYTOCHEMICAL ANALYSIS AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF FIVE MEDICINAL PLANTS.** Lardjam H, Mazid R, Boudghene SY, Izarouken A, Moussaoui N. (Algeria)

40. **IN VITRO AND IN VIVO ANTILEISHMANIAL ACTIVITY OF *ARTEMISIA ANNUA* LEAVES POWDER AND POTENTIAL UTILITY IN THE TREATMENT OF UNCOMPLICATED CUTANEOUS LEISHMANIASIS.** Mesa LA, Vásquez DA, Lutgen P, Vélez ID, Restrepo AM, Ortiz IC, Robledo SM. (Colombia)

41. **ANTILEPTOSPIRAL POTENTIAL OF MEDICINAL PLANTS COMMONLY USED FOR THE TREATMENT OF INFECTIOUS DISEASES.** Hernández-Rodríguez P, Pabón LC, Rodriguez MF, Gómez A. (Colombia)

42. **HYPOGLYCEMIC EFFECT OF ETHANOLIC EXTRACT OF *PARMENTIERA ACULEATA (KUNTH)* SEEM FRUIT AND BARK IN MALE WISTAR RATS.** Muñoz-Muñiz OD, Alcántara-López MG, Vázquez-Hernández M, Domínguez-Ortiz MA. (Mexico)

43. **DEVELOPMENT OF REPELLENT WITH ESSENTIAL OILS FROM MEDICINAL PLANTS.** Vázquez-Hernández M, Muñoz-Muñiz OD, Alcántara-López MG, Pérez-Gutiérrez RA. (Mexico)

44. **EFFECT OF COMBINATION OF CHIA (*SALVIA HISPANICA*) AND SPIRULINA (*ARTHROSPIRA MAXIMA*) ON DIABETES.** Vargas-Chávez S, Martínez-Galero E, Chamorro-Cevallos GA, Quevedo-Corona L, Ortega-Nava ME, García-Gutierrez GE, Garduño-Siciliano L. (Mexico)

45. **ORAL ANTI-INFLAMMATORY ACTIVITY OF DICHLOROMETHANE FRACTION OF *LACISTEMA PUBESCENS MART.* LEAVES.** Conegundes LMC, Silva JM, Pinto NCC, Fernandes MF, Scio E. (Brazil)

46. **WOUND HEALING ACTIVITY OF GELS CONTAINING AN EXTRACT OF *CECROPIA PACHYSTACHYA* LEAVES.** Duque APN, Pinto NCC, Mendes RF, Aragão DMO, Castañon MCMN, Scio E. (Brazil)

47. **ANTIOXIDANT ACTIVITY OF *CECROPIA PACHYSTACHYA TRÉCUL* (*URTICACEAE*) IN NORMAL AND DIABETIC RATS.** Fernandes MF, Aragão DMde O, Assis CM, Pires PP, Scio, E. (Brazil)

48. **PREPARATION OF PARVIFOLINE DERIVATIVES AS TUBULIN INHIBITORS.** Silva-García EM, Cerda-García-Rojas CM, del Río Torres RE, Joseph-Nathan P. (Mexico)

49. **GC/MS ANALYSIS AND BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OIL FROM THE DRIED AND FLESH LEAVES OF *ANNONA CACANS* (ANNONACEAE).** Lourenço CC, Marinho JVN, Aranha-Netto L, Siqueira CAT, Serain AF, Mesquita JT, Tempone AG, Salvador MJ. (Brazil)

50. **CHEMICAL COMPOSITION AND ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FROM *PLATYMISCIUM GRACILE BENTH. SAWDUST*.** Cuéllar JE, Martínez J, Gil JH, García CM, Rojano BA, Durango DL. (Colombia)

51. **EVALUATION OF TOTAL BETALAINS OF *STENOCEREUS SPP* AND CHARACTERIZATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY.** Gil Escamilla JO, Hernández-Navarro MD, Hernández López AV, Figueroa-Ibarra LM, Gómez-Oliván LM, Dublán-García O, Islas-Flores H, Hernández-Ortega MM. (Mexico)

52. **ANTIOXIDANT EFFECT OF BETALAINS FROM *STENOCEREUS SPP* AGAINST N-NITROSODIETHYLAMINE IN MICE.** Hernández-López AV, Figueroa-Ibarra LM, Hernández-Navarro MD, Juaregui-Rodriguez B, Gil-Escamilla JO, Gallegos-Ortiz MR, Osorio-Esquivel O, Gómez-Oliván LM, Dublán-García O, Islas-Flores H. (Mexico)

53. ***CANNABIS SATIVA L.*: DEVELOPMENT AND VALIDATION OF A NEW HPLC METHOD WITH UV/DAD AND ESI-MS^n DETECTION FOR THE ANALYSIS OF NON-PSYCHOACTIVE CANNABINOIDS.** Brighenti V, Maran D, Pellati F, Tardugno R, Benvenuti S. (Italy)

54. **BRONCHODILATOR AND ANTITUSIVE EFFECTS OF *BLEPHAROCALIX SALICIFOLIUS* ("ANACAHUITA").** Jimenez-Hernandez J, Consolini AE. (Argentina)

55. **PHENOLIC PROFILE AND CYTOTOXIC PROPERTIES OF POLAR EXTRACTS FROM BASAL LEAVES AND FLOWERS OF *ISATIS TINCTORIA L.*** Taviano MF, Filocamo A, Ragusa S, Dugo P, Cacciola F, Guzman ML, Hsu H-T, Galletti G, Miceli N. (Italy, USA)

56. **ANTI-GLYCANT AND ANTI-DIABETIC ACTIVITIES OF AROMATIC GUANYLHYDRAZONE DERIVATIVES.** Sarmento PA, Silva-Júnior EF, França PHB, Ribeiro EAN, Bastos MLA, Aragão DMO, Fontes ES, Araújo-Júnior JX. (Brazil)

57. **A STUDY OF GUANYLHYDRAZONE DERIVATIVES WITH VASORELAXANT ACTIVITY USING DYNAMIC SIMULATIONS, MOLECULAR DOCKING AND 2D-QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS.** Silva-Júnior EF, Herculano EA, França PHB, Costa CDE, Ribeiro EAN, Aquino TM, Araújo-Júnior JX. (Brazil)

58. ***BACCHARIS TRIMERA (LESS.) DC (ASTERACEAE)* EXTRACTS ARE PROMISING ANTIBACTERIAL AND ANTIOXIDANT AGENTS.** Feres-Netto C, Pedrosa DM, Fernandes LS, Costa YFG, Pinto NCC, Scio E, Del-Vechio-Vieira G, Sousa OV, Alves MS. (Brazil)

59. **MOLECULAR DESCRIPTORS AS PREDICTING CRITERIA IN THE SEARCH OF ACETYLCHOLINESTERASE (AChE) ISOFLAVONE-LIKE INHIBITORS.** Orduz-Diaz LL, Coy-Barrera ED. (Colombia)

60. **IN-SILICO STUDIES ON *PTEROCAPARNS* AS AN ALTERNATIVE IN TREATMENT OF ALZHEIMER'S DISEASE.** Orduz-Diaz LL, Coy-Barrera ED. (Colombia)

61. **NEW MULTITARGET DRUGS DERIVED FROM NATURAL POLYPHENOLS.** Begines P, Roldán J, Oliete A, Plata GB, Padrón JM, López O, Maya I, Fernández-Bolaños JG. (Spain)

62. **EVALUATION OF GEL PRODUCTION AND ANTIRADICALAR ACTIVITY IN SEVERAL ALOE SPECIES.** Grčić N, Capela P, Dias ACP. (Portugal)

63. **ANTILEISHMANIAL ACTIVITY OF *GUAREA GUIDONIA* (*MELIACEAE*).** Torres Suarez, E, Coy-Barrera CA, Delgado-Murcia LG, Coy-Barrera ED. (Colombia)

64. **UFLC-ESI-MS-BASED PROFILING, CYTOTOXICITY AND ANTILEISHMANIAL ACTIVITY OF EXTRACTS OF *AZADIRACTHA INDICA* SEEDS.** Monroy-Velandia D, Torres Suarez E, Coy-Barrera CA, Delgado-Murcia LG, Coy-Barrera ED. (Colombia)

65. **ANTI-INFLAMMATORY ACTIVITY OF EXTRACS FROM AN IN VITRO CULTURE OF *SAMBUCUS NIGRA L.*** Cruz-Cruz DG, Méndez-Sánchez LI, Herrera- Ruiz ML, Capataz Tafur J, Sánchez-Ocampo PM. (Mexico)

66. **ANTI-INFLAMMATORY ACTIVITY OF *SAMBUCUS NIGRA* EXTRACTS FROM CALLUS IN VITRO CULTURE.** Cruz-Cruz DG, Méndez-Sánchez, LI, Herrera- Ruiz ML, Capataz, Tafur J, Sánchez-Ocampo PM. (Mexico)

67. **ACUTE TOXICITY "IN VIVO" OF METHANOLIC EXTRACT OF *RHIPSALIS BACCIFERA.*** Echeverría-Pérez GM, Castro-Torres IG, Castro-Torres VA, Martínez-Vázquez M, Naranjo-Rodríguez EB. (Mexico)

68. **HYDROALCOHOLIC EXTRACT OF *ERYNGIUM CARLINAE* FOR THE TREATMENT OF HYPERCHOLESTEROLEMIA IN MICE.** Castro-Torres IG, De la O-Arciniega M, Naranjo-Rodríguez EB, Martínez-Vázquez M. (Mexico)

69. **SYNTHESIS AND BIOLOGICAL ACTIVITY OF ALKOXYLATED CHALCONE DP7 ON IMPORTANT PLANT PATHOGENS.** Remškar A, Betancur PJF, Trilleras VJE, Valencia JA, Arboleda VJW. (Slovenia, Colombia)

70. ***KALANCHOE BRASILIENSIS CAMB. (CRASSULACEAE)*: AN INTERESTING SOURCE OF BIOACTIVE SUBSTANCES TO TREAT INFECTIOUS DISEASES CAUSED BY *SALMONELLA* STRAINS.** Mayorga OAS, Florencio JR, Feres-Netto C, Pedrosa DM, Costa YFG, Pinto NCC , Scio E, Sousa OV, Alves MS. (Brazil)

71. **IN VITRO ANTIBACTERIAL ACTIVITY OF *HYPOESTES FORSSKAOLII VAHL. ROEM & SCHULT. (ACANTHACEAE)* AGAIST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: PRELIMINARY RESULTS.** D'Ambola M, Nocera FP, Vassallo A, Florio S, Severino L, De Martino L. (Italy)

72. **SEASONAL VARIATION AND CHEMOTYPE IDENTIFICATION IN VOLATILE AND NON-VOLATILE FRACTIONS FROM *SCHINUS MOLLE* LEAVES BY CHROMATOGRAPHIC PROFILING COUPLED WITH CHEMOMETRICS.** Macias YP, Bernal FA, Coy-Barrera E. (Colombia)

73. **PHOTODYNAMIC ANTIMICROBIAL ACTIVITY OF *SYZYGIUM CUMINI* EXTRACTS ON *KLEBSIELLA PNEUMONIAE.*** Quigua ORM, Valle-Molinares RH, Vallejo LWA, Diaz UCE, Arboleda VJW. (Colombia)

74. **IDENTIFICATION OF PHENYLPROPANOID GLYCOSIDES FROM *ALOYSIA POLYSTACHYA (GRISEB. ET MOLDENKE)* BY HPLC-MS ANALYSIS.** Bertelli D, Graziosi R, Plessi M. (Italy)

75. **ESSENTIAL OILS AS POTENTIAL FUMIGANT AGENTS FOR THE CONTROL OF *SITOPHILUS ZEAMAIS.*** Patiño WR, Patiño OJ, Cuca, LE, Delgado WA, Prieto JA. (Colombia)

76. **SYTHESIS OF BIOACTIVE CHROMENES INSPIRED ON THOSE ISOLATED FROM *PIPER CF. CUMANENSE KUNTH (PIPERACEAE).*** Parra AJE, Patiño OJ, Avila MMC, Cuca SLE, Gamba-Sánchez D. (Colombia)

77. **CYTOTOXICITY OF DQ-35 IS MEDIATED BY G2-ARREST AND MICRONUCLEI FORMATION WITHOUT NUCLEAR DAMAGE.** Ocampo, Y, Rivera D, Acuña J, Caro D, Castro J, Olivero J, Motilva V, Müller K, Franco L. (Colombia, Spain, Germany)

78. **SUSCEPTIBILITY OF TWO STAPHYLOCOCCUS AUREUS STRAINS FROM THE CLINICAL ENVIRONMENT TO ANNONA PURPUREA FRACTIONS.** Márquez VRL, Jiménez CAP, Tamara RY, Parejo AMS, Muñoz NAS, De La Rosa TCR. (Colombia)

79. **HISTOCHEMISTRY AND GAS CHROMATOGRAPHIC PROFILE OF THE ETHYL ETHER FRACTION EXTRACTED FROM FRESH SHEETS OF *CNIDOSCOLUS ACONTIFOLIUS* (EUPHORBIACEAE).**Márquez VRL, Martínez PMA, Jiménez CAP, Tamara RY, Parejo AMS, Estrada RDJ, Carpio ZCM, Castillo COJ, De La Rosa TCR. (Colombia)

80. **ESSENTIAL OILS FROM CITRUS SINENSIS, CYMBOPOGON CITRATUS, PINUS SYLVESTRIS AND LIPPIA ORIGANOIDES EXHIBIT LARVICIDE ACTIVITY AGAINST RHIPICEPHALUS SANGUINEUS..** Bravo M, Uzcátegui J, Sanabria M, García L, Rojas L, Gualtieri M. (Venezuela)

81. **SECONDARY METABOLITES FROM CHILEAN PLANTS AND THEIR BIOLOGICAL ACTIVITY.** Alarcón JE, Cespedes CL, Muñoz E, Quiroz S. (Chile)

82. **ETHANOL CRUDE EXTRACT FROM LEAVES OF *SINNINGIA SCHIFFNERI* (GESNERIACEAE) AND THEIR**

FRACTIONS AS NATURAL PHOTOSENSITIZER IN PHOTODYNAMIC CHEMOTHERAPY. Serain AF, Andreazza NL, Stefanello MEA, Salvador MJ. (Brazil)

83. **THE SYNERGISTIC EFFECTS OF ANTIOXIDANTS IN THE CONTROL OF REDOX BALANCE IN CULTURED FIBROBLASTS AND POSSIBLE INFLUENCE ON PATHOLOGIC CALCIFICATION.** Boraldi F, Costa S, Annovi G. (Italy)

84. **FLASH CHROMATOGRAPHY MS-TARGETED ISOLATION OF NATURAL PRODUCTS UNDER NORMAL PHASE CONDITIONS.** Righi D, Azzollini A, Queiroz EF, Wolfender JL. (Switzerland)

PLENARY LECTURES

PL01

PLANTS AND PEOPLE: A LONG HISTORY OF HUMAN-ENVIRONMENT INTERACTION THROUGH ARCHAEOBOTANICAL RESEARCH

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The history of the human species is intrinsically related to that of plants. Their role in nutrition but also the myriad of different purposes (medicines, drinks, dyes, shelter, fodder, fuel, crafts, cosmetics, rituals, building, etc.) for which plants are used demonstrate their central role in societies. In the past, this relationship pervaded every aspect of human life (subsistence, health, clothing, heating…) and thus, the study of archaeological plant remains provides an extraordinary opportunity for exploring human-environment interactions throughout time. Consequently, archaeobotany has become a most valuable tool for approaching the subject by encompassing both the archaeological and scientific records.

My lecture aims at showing the power of archaeobotanical research for exploring plant-human interactions in the past through examples from different research projects in the Mediterranean region. From prehistoric hunter-gatherers to Medieval farmers, across the western Mediterranean, I will survey plant encounters providing evidence of the important role of plants for both prehistoric foragers and agriculturalists.

Although humans have been hunter gatherers for a much longer period than farmers, a significant part of archaeobotanical research has focused on agricultural communities so, the way plant food and resources were used by prehistoric pre-agrarian groups is still largely unknown. In my presentation, I will discuss not only examples from farming communities but also latest research on pre-agrarian plant exploitation. Particular emphasis will also be given to ethnographic approaches as a tool for better understanding the intertwining relationship between plants and people in the past.

PL02

THE IMPACT OF DIET, PRE- AND PRO-BIOTIC STRATEGIES ON GUT MICROBIOTA COMPOSITION AND HEALTH

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A rich balance of human gut microbial species is necessary to sustain healthy metabolism and immune function. Disturbance in the colonic microbial balance can have negative consequences for health resulting in elevated inflammation and infection which are also contributory factors in diabetes, bowel disease and cancer. Changes in the species composition of the human faecal microbiota can be detected within several days of transferring to a new diet. There is a growing awareness that the microbial balance in the colon may become increasingly perturbed with aging and therefore hastens the onset of certain diseases. Diet and age are therefore major factors driving microbial community composition both in the short and long term. Significant progress has been made in defining some of the dominant members of the microbial community in the healthy large intestine and in identifying their roles in metabolism. For example, certain bacterial phylotypes identified from 16S rRNA gene sequences respond to increases in particular dietary non-digestible carbohydrates across many individuals. This is despite considerable inter-individual variation in microbiota composition. Furthermore, controlled dietary intervention studies have shown that only certain specific groups of Firmicutes are stimulated by resistant starch or inulin, while other representatives of this same phylum are stimulated by complex non-starch polysaccharide (NSP) fibres such as wheat bran or pectin. Functional analysis of cultured strains suggests that these Firmicutes bacteria are likely to play a key role in initiating the degradation of these insoluble substrates in the colon. The relationship between dietary intake of fermentable carbohydrates and metabolic outputs of the gut microbiota, including the potentially beneficial short chain fatty acids, propionate and butyrate is of particular interest in terms of human health. In addition wheat bran products have a high content of potentially bioactive phenolic acids, especially ferulic acid, that are largely bound within the plant cell wall. These phenolic acids are released only upon degradation by microorganisms in the large intestine which can also have health consequences. To determine health effects requires an excellent understanding of the bacterial groups involved in substrate breakdown, the distribution of metabolic pathways and cross-feeding interactions involved in product formation and how this is driven by the gut environment. Currently, there is a vital need for better awareness of the impact of diet, prebiotic and probiotic strategies in driving human colonic microbial composition for maintaining healthy gut function and wellbeing across all age groups.

PL03

PLANT PATTERNS FOR ETHNOBOTANICAL FOOD AND NUTRACEUTICAL USES: THE CASE STUDY FROM TRADITIONAL VILLAGES OF BALI (INDONESIA)

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The medicinal properties of plants have increasingly being studied in the last decades due to the importance of extract active principles for nutraceutical applications. Traditional Ethnobotanical Knowledge (TEK) represents one of the most important ways to achieve the goal of increasing and promoting such information. Due to increasing urbanization and technological innovation, fewer and fewer people belonging to Western culture maintain this kind of knowledge whilst still there are villages in less developed areas of the World, where this knowledge is still widely used and handed down from generations. TEK is especially threatened in tropical areas, where single indigenous individuals and entire communities are rapidly changing their culture under the influence of Western models (Brosi et al. 2007; Quinlan and Quinlan 2007; Voeks 2010).

In this study, we analyse ethnobotanical information for the Indonesian island of Bali, as a relevant example in the Asian context conserving its traditions and culture, in order to identify and analyze patterns of plants ethnobotanically used on a local scale. Due to the abundance of native flora, this study should contribute also to increase the knowledge on the application of wild plants in the treatment or prevention of diseases through traditional medicine.

Ethnobotanical data were collected using different interview methods (semi-structured interviews, key informant interviews, individual discussions, and focus group discussions) (Alexiades and Sheldon 1996; Sujarwo et al. 2015). Interviews were carried out in 13 Traditional villages (Bali Aga) involving 50 informants, mainly belonging to male gender, since female were less confident in giving information. In order to assess similarities in plant pattern within villages, we used the Jaccard similarity index. The similarity matrix was then used as reference matrix for cluster analysis with the complete linkage method. Three principal coordinates analyses (PCoA) were run by using the similarity matrix, with species grouped as follow: i) according to groups obtained from cluster analysis, ii) according to the biological form, iii) according to the use human made of each plant.

The 113-recorded species belong to 50 families and 91 genera. The dominant life forms are trees (39.29%), followed by shrubs (21.43%), perennial herbs (19.64%), climbers (18.75%), and woody climbers (0.89%). The most frequently used parts are leaves, fruits, tuberous roots, and seeds. The cluster tree clearly showed a separation between species into 12 groups. Few species were completely separated from the others, while half of the species are grouped inside a pattern. That shows certain homogeneity among plant used in the villages, as confirmed from the three principal coordinates analysis.

Our findings confirmed the central role of TEK as source of information to preserve and to enhance the culture of native people. The wide use of traditional species they made underline the medicinal properties of Bali plants, both for food uses and to extract active principles for nutraceutical applications.

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3. Quinlan, M. and Quinlan R. *Modernization and medicinal plant knowledge in a Caribbean horticultural village*. Medical Anthropology Quarterly 2007, 21:169–192.
4. Sujarwo, W., et al. *Ethnobotanical study of Loloh: Traditional herbal drinks from Bali (Indonesia)*. Journal of Ethnopharmacology 2015, 169:34-48.
5. Voeks, R. A. *Ecotourism and ethnobotanical erosion: A possible rescue effect in Brazil's Chapada Diamantina*. In: Recent development and case studies in ethnobotany, eds. U. P. Albuquerque and N. Hanazaki, 2010, pp. 228–245. Sociedade Brasileira de Etnobiologia e Etnoecologia.

PL04

HELICHRYSUM ITALICUM: WAKING UP THE SLEEPING GIANT OF MEDITERRANEAN HERBAL MEDICINE

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Helichrysum italicum (Roth.) Don. (Asteraceae), an iconic plant of the Mediterranean area, has now become also an icon of luxury because of its use in glamorous perfumes and personal care products [1]. *H. italicum* is, however, also an important medicinal plant, and recent studies have provided the basis for a veritable *Helichrysum* Renaissance, rationalizing the fascinating ethnopharmacology of the plant in the light of molecular investigations on its constituents and their pharmacological targets [2]. Extracts from *H. Italicum* have the potential to be developed as a novel ingredient for medicinal and healthfood products just like its essential oil has been in perfumery and aromatherapy, but awakening this sleeping giant of the Mediterranean herbal medicine will required a multidisciplinary joint effort. One of the major challenges to be addressed is the establishment a reliable supply chain to overcome the plight of spontaneous harvest that is threatening the wild population of the plant in Sardinia and Corsica, where the most valuable chemotype of the plant grows. A second issue that needs to be addressed is the high variability of the phytochemical profile of the plant, where the concentration of heterodimeric pyrones, the major bioactive constituents of the plant, can range from undetectable to almost 1%.

1. Appendino G. et al., *Helichrysum italicum: The sleeping giant of Mediterranean herbal medicine*. Herbalgram 2015; 105: 34-45.
2. Taglialatela-Scafati O. et al. *Antimicrobial phenolics and unusual glycerides from Helichrysum italicum subsp. microphyllum.* J Nat Prod 2013; 76: 346−353

PL05

THE ROLE OF THE FOOD CHEMIST IN THE ENHANCEMENT OF THE FOOD FARMING CHAIN

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The food sector plays a strategic role in both Italian and world economy, as well as in the social development.
The food industry is facing lots of changes compared to the past and in this scenario the physical-chemical and microbiological analysis of raw materials and of final products are essential.
To date the consumer safety is the main requirement, and in this filed the food chemist is one of the main figures suitable for the whole food production chain control, from the origin of raw materials and processing methods , until their proper distribution.
The micro and macro constituent analyses allows us to determinate not only the traceability of the product, but also its health aspects. The monitoring of organic, inorganic and microbiological contamination is the most important tool for food safety determination.
The food chemist figure is important also in the monitoring of row product cultivation and making processes; in fact, it plays a fundamental role in sector operators or entire companies training.
By using innovative and new green technologies, several benefits derive by its work such as:
improvement of production; innovation of production technologies; legal, economic and tax on start -up consulting; quality and safety of the environment and the workplace ;assessment of risks due to the presence of contaminants from the production chain; consulting on the study of effects on human health of food prepared.
The scientific progress allows us to get high performance targets and to reduce the wastes processes by using low environmental impact technologies.

PL06

BIOCATALYSIS: THE GREEN SIDE OF CHEMISTRY

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Sustainability has become one of the key words of our modern society. When applied to chemistry it has been codified in the so-called "green chemistry" principles:1 the invention and the exploitation of chemical processes which do not use at all or limit the use of substances that might be dangerous to the environment and to human health.
And what is more intrinsically green than nature itself, with its products and its catalysts? Nature is an inexhaustible source of bioactive molecules. The structural complexity and the "fragility" of these natural products makes them an ideal training ground for new synthetic strategies. More specifically, biocatalysis, exploiting the peculiar properties of enzymes in terms of selectivity and efficiency under mild reaction conditions, offers interesting solutions.
This presentation will discuss some of the results obtained by our research group in this area, moving from the regio- and stereoselective oxidoreduction of polyhydroxylated steroids,[2] to the regioselective acylation of natural glycosides catalyzed by lipases and proteases in organic solvents.[3]
Some examples will be also related to the synthetic exploitation of laccases, a group of oxidoreductases belonging to the so-called blue-copper oxidases.[4] These enzymes proved to be efficient catalysts for the oxidization of a broad range of molecules, including alkaloids[5-6] and phenols,[7] directly or in the presence of low-molecular-weight redox mediators. Reactive radical intermediates, generated by biocatalyzed oxidation at the expense of molecular oxygen, further undergo self-coupling reactions leading to the formation of C-O and C-C dimers and oligomers, are trapped by reactive cosubstrates in domino reactions, or, in more rare examples, originate hydroxylated products.

1. Anastas P, and Warner JC. *Green Chemistry: Theory and Practise*, Oxford University Press, 2008
2. Monti D, et al., *One-pot multienzymatic synthesis of 12-ketoursodeoxycholic acid: subtle cofactor specificities rule the reaction equilibria of five biocatalysts working in a row*. Adv. Synth. Catal. 2009, 351, 1303–1311 and references therein
3. Riva S. *Enzymatic modification of the sugar moieties of natural glycosides.* J. Mol. Cat. B-Enzymatic 2002, 19-20, 43-54 and references therein
4. Riva S. *Laccases: blue enzymes for green chemistry*. Trends Biotechnol., 2006, 24, 219-226
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6. Sagui F. et al., *Laccase-catalyzed coupling of catharanthine and vindoline: an efficient approach to the bisindole alkaloid anhydrovinblastine* Tetrahedron, 2009, 65, 312–317
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PL07

EFFICIENT ANALYTICAL STRATEGIES FOR AN EARLY IDENTIFICATION OF BIOACTIVE NATURAL PRODUCTS AT THE HERBARIUM SAMPLE SCALE

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With the recent progresses made in metabolite profiling methods and miniaturization of bioassays, a question that arises is: do we still need to perform conventional large scale bioactivity-guided isolation of natural products (NPs)?
High resolution mass spectrometry (HRMS) and data dependent MS/MS analyses provide very valuable information on secondary metabolites for in-depth metabolome annotation studies [1]. The recent development of molecular network (MN) approaches for the mining of such data gives the possibility to establish relationships between metabolites, thus significantly improving the efficiency of dereplication, particularly when combined with high quality chemotaxonomic data [2]. Such types of information can be generated with a few mg of extract only and are readily applicable to herbarium scale samples.
For complete *de novo* identification of new compounds MS-targeted micro-isolation can be performed, and sensitive 1D and 2D microNMR data are acquired with microgram amounts of purified metabolites. For bioactivity determination, many bioassays also fit to this scale.
Using an ideal combination of such methods, it is thus virtually possible to fully identify any bioactive principles in this way.
Integration of other filters to this approach, such as permeation studies on extracts, additionally provide key information on the possible bioavailability of NPs prior to their isolation [3]. Furthermore the link of a given bioactivity result to those previously reported for compounds similar to those identified can be rationalised through *in silico* chemical space approaches.
Ideally, a combination all these state-of-the-art methods should enable the identification of valuable NPs efficiently at the analytical scale. In such a way large scale MS-targeted isolation of such selected NPs only can become a very rational way to conduct investigations.
Various examples will illustrate the power, but also the bottlenecks, of such tools. Discussion on the potential of such approaches and the changes of paradigm that they might induce in natural product research will be made.

1. Wolfender JL, et al. *Current approaches and challenges for the metabolite profiling of complex natural extracts.* J Chromatogr A 2015; 1382: 136-164
2. Allard PM, et al. *Integration of Molecular Networking and In-Silico MS/MS Fragmentation for Natural Products Dereplication.* Anal Chem 2016; 10.1021/acs.analchem.5b04804
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PL08

UNTARGETED METABOLOMICS TO IDENTIFY BIOACTIVE COMPOUNDS AND SYNERGISTS FROM BOTANICAL MEDICINES

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A central challenge in botanical medicine research is distilling complex mixtures down to bioactive compounds. To accomplish this goal, the gold standard approach is bioassay-guided fractionation, in which the mixture is subjected to successive rounds of purification and bioassays until an active compound is identified. Bioassay guided fractionation has historically played a critical role in drug discovery, but is, nonetheless, fraught with challenges. The process is biased towards the most abundant and easily isolatable mixture components, which may not be the most biologically active. Furthermore, if multiple compounds contribute either additively, antagonistically, or synergistically to the observed biological activity of the mixture, activity may be lost upon isolation.

As a complementary strategy to bioassay-guided fractionation, our research group has developed untargeted metabolomics strategies to aid in the identification of bioactive mixture components. These strategies involve profiling botanical mixtures using ultraperformance chromatography coupled to high resolving power mass spectrometry on an Orbitrap mass spectrometer. The resulting chemical data is then integrated with biological assay data using biochemometric data analysis strategies. Several case studies will be presented illustrating how this approach can be applied to identify antimicrobial compounds from fungi and botanical extracts.

Acknowledgments: This work is a collective effort of many outstanding scientists. These include Dr. Nicholas Oberlies, Dr. Huzefa Raja, Dr. Daniel Todd, and Dr. Joshua Kellogg at the University North Carolina Greensboro, Dr. Olav Kvalheim at the University of Bergen in Norway, and Dr. Alexander Horswill at the University of Iowa. Countless undergraduate and graduate students have also made critical contributions. Funding was provided by the National Center for Complementary and Integrative Health, a component of the National Institutes of Health (grant number R01 AT006860).

PL09

EVALUATION OF THE BIOLOGICAL ACTIVITY OF PHYTOCHEMICALS USING *IN VIVO* MODEL ORGANISMS

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Phytochemicals, and among them phenolic compounds, have been related with a variety of beneficial effects on human health. However, the actual mechanisms subjacent to their *in vivo* biological activity are not fully understood. Classically, they have been associated to their antioxidant and radical scavenging abilities. That view has mostly originated from *in vitro* assays performed with the compounds as they are present in the plant, whereas *in vivo* studies that take into account the complex interactions that occur in a complete organism are limited. It is known that most phenolic compounds are little bioavailable and extensively metabolized in the human organism, so that the compounds that may reach the biological targets are different from those present in plant tissues. Currently, it is thought that the mechanisms involved in the biological activity of phenolic compounds are more complex than initially supposed and could involve metabolites interactions with different signaling and transcription factors, and modulation of the activity of enzymes implied in cell defense mechanisms.

Different *ex vivo* and *in vivo* approaches, such as the use of cell cultures or animal (e.g., murine) models can be employed to elucidate the mechanisms involved in the biological effects of phytochemicals. However, animal studies are expensive and have bioethical limitations, and assays in cell lines are not always easy to extrapolate and would require the use of the compounds that can be found at the cellular level, which are not always known and/or commercially available. In this respect, the assays in simple model organisms, such as the nematode *Caenorhabditis elegans*, represent an attractive alternative to perform *in vivo* studies with phytochemicals before their assessment in higher animals and humans.

C. elegans offers several advantages for biochemical and genetic studies. It possesses a short lifespan and allows an easy and inexpensive storage and culturing on Petri dishes or liquid media. It also shows a strong conservation in molecular and cellular pathways in relation to mammals, with a relevant number of human disease genes and disease pathways present in the worm. Furthermore, its robust genetic foundation that includes a completely sequenced genome facilitates the application of techniques such as transformation and RNAi for genetic manipulation. Finally, as an invertebrate, its use does not pose ethical concerns. For these reasons, it is increasingly used as a model to study biological effects of beneficial and toxic substances as well as to identify new pharmacological targets.

These aspects will be reviewed in the present talk, where examples on the application of this *in vivo* model for the assessment of the biological effects and mechanisms of activity of phenolic compounds will be presented.

Acknowledgments. The GIP-USAL is financially supported by the Spanish Government through the grants BFU2012-35228 and AGL2015-64522-C2-2-R.

PL10

ANTIOXIDANT THERAPIES THROUGH SIGNAL TRANSDUCTION AND GENE-REGULATORY PATHWAYS

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Oxidative stress caused by the body imbalance of oxygen and nitrogen reactive species (ROS/RNS) may induce gene-regulatory effects and influence signal transduction pathways. Our previous findings on the effect of a mango bark extract (*Mangifera indica* L.) showed a dose-dependent inhibition of tumor necrosis factor (TNFα) and nuclear factor κB (NfκB) both *in vitro* and *in vivo* indicating that its antioxidant effect was down-regulated by these factors[1]. Other transcriptional factors (JAK 1/2, STAT 3, AKT, MAPK, IKK) and the VEGF have been also inhibited by mangiferin, the major component of the mango extract[2]. It has been described recently that the Antioxidant Responsive Element (ARE) acts as specific enhancer of antioxidant enzymes regulation through the transcriptional factor Nfr2[3]. This factor may exert an autoregulatory effect on mafG and BSV1 transcriptional regulation[4,5]. These efects are underlying the human antioxidant defense pathways and may explain the potential benefits of antioxidant therapies for chronic degenerative diseases.

Acknowledgments:work supported by FONDOCYT Project 2012-2013-2A1-58.

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ORAL COMMUNICATIONS

OR01

MATERIAL AND IMMATERIAL CONSTITUTIVE ELEMENTS OF HEALING SITES - THE MAYANTUYACU CASE STUDY IN THE PERUVIAN AMAZON.

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Since ancient times, healing sites have been identified for peculiar geomorphological assets, hydrological structures and a specific biosphere.
As the place is considered already part of the therapy, the process of healing becomes linked to many different elements. The emergence of modern medicine and the indifferent localization of the sites linked to the planning of care therapies and the undifferentiated urbanization has in fact neglected - apart from a few isolated cases - this aspect of the cultures of health. Western medicine and ethnomedicine are today bridging gaps towards a new approach by exchanging knowledge and experiences.
The research project involves a multidisciplinary team of anthropologists, physicists, chemists, ethnobotanical, architects and medical doctors from different institutions. Its primary objective is the analysis of a specific place and the different processes generating healing. The case study is Mayantuyacu, a healing center of ashaninka medicine located in the Peruvian Amazon. The aim of the process is to identify the combination of geomorphology, hydrology, biosphere, medical plants and traditional mythology, music, landscape and architecture involved in the action of healing and caring.
The research on field, laboratory and academic is articulated in several disciplines and the first results are planned to be published by the University Press of Apus Graph in coedition with the Unesco Chair of the University of Genoa.
The research analyzes the following elements: water, music (icaros), plant teachers, ceremonial architectures (malocas) involved during therapies and healing activities and their impact on different species (plants, animals and humans). The cross reference of the results of the different research field activities generates an independent and objective set of empirical evidence.
1. Architecture: the study of the ethnographic relevance of sacred spaces and their periodic re-construction in ceremonial events constitutes the central event of the transmission of a cosmovision and within the dynamics of a living landscape. A Techno morphology of the site and a digital 3D record of the cosmology of the main maloca is under development.
2. Water: the study of the electrical conductivity measurements on different samples is indicating the different structures of the waters and their effects in the preparation of medicines.
3. Music: the study of the heart mechanical vibrations with laser Doppler vibrometry compares the recorded signals for an individual in different conditions. An analysis of the music uses the decomposition method in time domain, in order to study self-similar structures of the listened music.
4. Ethnomedicine: The study of the different properties of the ashanika plant teachers is presented through clinical and botanical cases, in order to translate the healing process into a scientific methodology.

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OR02

THE ROLE OF FAMILIAR GARDENS IN PRESERVING THE ETHNOBOTANICAL KNOWLEDGE OF A PERUVIAN NATIVE COMMUNITY

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The study was conducted in the native community of Maime (Pasco, Peru), belonging to the Yanesha ethnic group. The main economic activity of the inhabitants is the cultivation of coffee to sell at the near city of Villa Rica. During the years the social and cultural influence of this city has become stronger and stronger and led to a profound transformation of the habit, lifestyle, cosmovision of native people. The home gardens have been much studied in the tropics, where are generally characterized by a wide variety of plants useful for different purposes, so as to resemble the surrounding ecosystem. Home gardens host edible, decorative, medical native species; which traditionally have a subsistence function and are the result of a cultural heritage. In this work, we aimed to test whether home gardens still had an actual role in Yanesha families for the supply of traditional herbal medicinal resources, with special reference to those considered as most important.

A floristic inventory with native guides was carried out across the whole territory of the community covering both the wild vegetation and home gardens, in order to determine the current use of natural health remedies and the way of transmitting traditional skills. We visited 23 family gardens, accompanied by the natives, for evaluating the frequency of medicinal plants, wild or cultivated in the vicinity of the houses. Three samples for each species were collected, with permission, to be dried and identified at the university herbarium UNMSM in Lima. We also evaluated the relationship between the importance of a species for the community (IV), the frequency of use and its frequency in the gardens. This was done by calculating the value of the Spearman correlation coefficient between the variables.

Eighty-four medicinal taxa have been inventoried, including 28 wild and 56 cultivated species. Among the main cultivated plants the most common were *Colocasia esculenta*, which occurred in 55% of visited gardens, *Mangifera indica* (36%), *Musa × paradisiaca* and *Nicotiana tabacum* (both in 32% of cases), *Citrus limon*, *Persea americana*, *Piper umbellatum* and *Solanum quitoense* (27%). The most frequent spontaneous include *Acmella alba*, *Plantago major* and *Verbena litoralis* (46%). Sixteen species with a high IV were preserved in family gardens. Six of these (incl. *Bixa orellana*, *Minthostachys mollis*, *Crassula* sp. and 3 species of the genus *Cyperus*) were exclusively found in home gardens. Particularly, *Cyperus* species, called "epe" , in Yanesha , or "piri piri" , in Spanish, are thought to have different properties, which sometimes fall into the magical sphere (Valadeau, 2010; Luziatelli, 2010). Overall, 25% of medicinal plants found during the floristic inventory were extremely rare in natural habitats. Among them, only 10% was cultivated in at least 3 home gardens, while more than half did not occurred. Apparently home gardens do not play either the ancient subsistence function or a more current role in the protection of the most relevant species for the community. Substitutability of medicinal plants , a lack of awareness of the resource degradation issues and accessibility of alternative remedies, such as modern medicines, are factors having a bearing on this trend.

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OR03

BIODIVERSITY USE: PRODUCTIVE CHAIN AND ETHNOKNOWLEDGE (ITACOATIARA, AMAZONAS-BRASIL)

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The Amazonian biodiversity has several potentialities among which are medicinal plants. These are used in the traditional way, supported in ethnoknowledge which is culturally constructed for generations. This study was conducted in Itacoatiara - AM, where these plants are indicated for the treatment of various diseases. There are no public policies for the improvement and strengthening of productive chains directed to medicinal plants, although the importance of medicinal plants as a social necessity, economic and plant biodiversity use policy is an element of social justice.

For this work has been done fieldwork in Itacoatiara (Amazonas, Brazil) in January 2016 and a bibliographic research, aiming the use of biodiversity for medicinal purposes and the determination of the structures that comprise the production chain.

In Itacoatiara, peasants cultivate medicinal plants in their small farms / yards. These are used for family use and part of the production goes to the county fair. Medicinal plants are part of a production chain, where production starts with the peasant work, which, from its ethnic knowledge relating to medicinal plants, destinate part of their production to market, which start to have exchange value, and in a domestic case, a use value. In the distribution and circulation there is the middleman (*atravessador*), which goes to the producers to buy their production and take it to sell in the county fairs. In the case of Itacoatiara, often the middleman function is exercised by the peasant himself, who takes his production to the fair where he owns a sale place. At the fair of the rural producer of Itacoatiara - AM, it was identified the marketing of cat's claw, which is indicated for inflammation; embauba for the treatment of diabetes, high blood pressure and hemorrhage; grass smelling used to control the heart racing. There are also potions (*garrafadas*) being produced, which are composed by several medicinal plants, for example, jatoba, jucá and sara tudo, when in field work they were packed in two-liter bottles, priced at R$ 10,00 (~ U$ 2.5). In this context the ethnoknowledge is of high importance in the commodity trading process (medicinal plant); there is the diversity of medicinal Amazonian plants a functionality as a key factor to marketing in the fair of rural producer in the municipality of Itacoatiara. The productive chain of medicinal plants strengthens and is strengthened by ethnoknowledge and the needs of its use.

Acknowledgments: To PPBio/CNPq, CT-Agro/CNPq, CAPES (Pro-Amazônia) and CENBAM/CNPq/MCTI for the financial support.

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OR04

BIODIVERSITY OF MELIPONINI BEES IN ECUADOR: AN INTANGIBLE HERITAGE TO PROTECT GENUINE HONEY

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Megabiodiversity results from events of speciation and extinction in natural history. Bees producing honey in cerumen pots belong to the tribe Meliponini with some 400 species in the Neotropics (Camargo and Pedro, 2007). They are known by the ethnic name "stingless bees". A fossil record of the extinct *Cretotrigona prisca*, from the late Cretaceous amber in New Jersey, USA, c.a. 65-96 Ma (Michener and Grimaldi 1988) is the oldest bee presence in our planet. Cultural traditions are a source to know stingless species richness in Argentina (Zalmudio and Hilghert, 2012). The drop of meliponiculture, the reduced management and the loss of colonies due to the increase of disturbed habitat, had more impact to decline *Melipona beecheii* than competition with Africanized honey bees in Mexico (Villanueva et al., 2005). The biodiversity of stingless bees in Ecuador is recorded from diverse contexts. The driver of this work was the entomological origin of pot-honey needed for the proposal of quality standards in the Ecuadorian honey norm (NTE INEN 1572). The paradox of a great biodiversity of Meliponini pot-honey and the presence of fake honeys.

Honeys were collected in stingless bee hives or purchased in the local markets. Stingless bee specimens were prepared for taxonomic identifications by Dr. Pedro SRM. The ethnic names were retrieved as knowledge of ethnospecies besides the uses of bee products and the type of bee management.

The biodiversity of Ecuadorian stingless bees observed during the honey quality research contributed to increase the number of taxa identified in previous studies (Coloma, 1986; Rasmussen, 2004; Camargo and Pedro, 2007) up to 132. The ancestral knowledge session in the Plan Buen Vivir "Sumak Kawsay", has no budget to support meliponiculture in Ecuador. Both pot-honey hunting and stingless bee keeping are individual initiatives. There is no census of stingless bee keepers in Ecuador, and we contributed to that database. One of the artificial honeys uncovered with an authenticity test (Vit, 1998), and characterized by physicochemical indicators (Vit et al., 2016) was detected by two Kichwa female assessors from the Amazon in Pastaza province during a Free-Choice Profile sensory study (Vit and Deliza, 2015). The Project "Route of Living Museums of Meliponini Bees in the World" lodges intangible ancestral knowledge of indigenous and rural meliponicultors to protect the environment where they thrive. It is also an action for preserving their cultural heritage.

Acknowledgements: To Consejo de Desarrollo Científico, Humanístico, Tecnológico y Artístico, Universidad de Los Andes CDCHTA-ULA for continuous support to the Research Group APIBA ADG-FA-97-08. To the Scholarship Prometeo-SENESCYT-UTMACH 2014 – 2015 to P. Vit. To Instituto Ecuatoriano de Normalización INEN to ponder pot-honey. To stingless bee keepers of Ecuador for preserving their ancient art.}

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OR05

POLLEN AS INDICATOR OF HUMAN BEHAVIOUR

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Archaeobotany is an extraordinary tool to investigate and comprehend the ethnobotanical evidence on a long time perspective. Palynology applied to archaeological sciences puts particular emphasis on the pollen produced by plants handled during human activities. In fact, the palynology of archaeological sites (on-site spectra) helps to recognize anthropogenic pollen indicators (API) (Mercuri et al., 2013a), and to investigate the human role in exploiting or changing the plant cover near the site. As pollen is mainly transported into the site by humans, and in general only a minor part arrives by air or water, high percentages or concentration of pollen in a certain context are indicative of transport of high amount of organic matter in the layer, including both collections of plants and faeces containing pollen from eaten plants (Faegri et al., 1989; Mercuri, 2008a).
Pollen samples have been collected from archaeological layers of more than a hundred sites selected from prehistorical and historical sites located in Italy and in the central Sahara (Mercuri, 2008b; Mercuri et al., 2015). The samples were prepared using tetra-Na-pyrophosphate, HCl 10%, acetolysis, separation with Na-metatungstate hydrate, HF 40% and ethanol (Florenzano et al., 2012). Pollen slides were mounted on glycerol jelly. *Lycopodium* tablets were added for calculation of concentrations (expressed as pollen per gram - p/g). Pollen was identified at 1000x magnifications, with the help of atlases and the reference collection of the laboratory of Modena.
Pollen analyses focused on selected anthropogenic pollen indicators (cultivated plants, weeds, ruderal flora) that were recurrent in spectra from archaeological sites of the Mediterranean area (Mercuri et al., 2013a,b).
The majority of pollen from archaeological sites is a clear marker of human environments shaped by food production (e.g. cereals, legumes) and plant exploitation by people and domestic animals (developing chicory or plantain vegetation). Similarities with current uses of plants in popular medicine and traditions are easily found by observing the human/nature relationships over a long time scale. Plant accumulation/transport in archaeological sites is evident from high amounts of pollen (and macroremains) that may represent a selection of particular species for food (e.g. *Triticum*), medicine (*Artemisia*) fodder (grasses), fuel (oaks, and other trees), textiles (hemp), construction or other uses. Moreover, the constant recovery of species with high nutritional values, such as cereals, testifies the food history and the agrarian vocation of the modern-day territories. Pollen data show unambiguous evidence of past human handling of useful species which commonly were multipurpose plants (e.g. *Typha* and *Cannabis*) through the selection and transport of species, confirming deep knowledge on the 'green world' by ancient societies/human groups.

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OR06

A PRELIMINARY STUDY OF METHODOLOGIES FOR AN INVESTIGATION ABOUT ICHTHYOLOGY AND GASTRONOMY IN CLASSICAL SOURCES

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This work proposes an interdisciplinary approach to ichthyology and fishing in the Mediterranean sea area in the Greek-Roman world. The main purpose is to answer to one basic question and to other questions strictly related to the former: which fish species widely well-known in the ancient times are still appreciated, investigated and cooked in modern times? Which differences or analogies could we notice? Which fish species, instead, we may say, fell in obsolescence? Moreover, which methodologies should be used in similar cases? The denomination of fish species (or plant species), indeed, changes through the centuries, making the exact identification of each rather difficult. The classical literature is full of scientific treatises and references about sea products, cooking, medicine, pharmacology and didactic poetry, in the form of complete works or, more often, of fragments. The works of Hippocrates the physician, Aristotle's *History of animals*, the *Parts of animals* and *Generation of animals*, Theophrastus of Eresus, Aristophanes of Byzantium, the Ovid's treatise on fishing, Pliny the Elder, Apicius, Oppian's *Halieutica*, Claudius Aelianus the naturalist, Pollux and several more are the primary sources of this investigation. Furthermore, the Greek comedy abounds in references to food and, in particular, to fish. The ancient comedy is a realistic picture of life in Greece and Rome. For example, Antiphanes (fr. 130 K-A): "So, we have the stewed Sting-ray (*Dasyatis pastinaca*), the back of the Ray (*Raja brachyura*), the Mullet (*Mugil cephalus*), the Sea Bass (*Dicentrarchus labrax*), the Snapper (*Lutjanus campechanus*), the stomach of the Fishing Frog, the head of the Conger (*Conger conger*), the hip of the Tunny (*Thunnus thynnus*), the Red Mullet (*Mullus barbatus*), the neck of Glaucus (*Glaucus atlanticus*)". Lots of information about sea products may be deduced by the above mentioned Oppian. For example, (Hal., 1.98): "Now fishes differ in breed and habit and in their path in the sea, and not all fishes have like range. For some keep by the low shores, feeding on sand and whatever things grow in the sand; to wit, the Sea-horse (*Hippocampus* sp.), the swift Cuckoo-fish (*Synodontis multipunctatus*), the yellow Erythinus (*Strombus erythrinus*), the Citharus and the Red Mullet (*Mullus barbatus*) and the feeble Melanurus (*Halichoeres melanurus*), the shoals of the Trachurus (*Trachurus mediterraneus*) and the Sole (*Solea solea*) and the Platyurus (*Thamnocephalus platyurus*), the weak Ribbon-fish (*Desmodema polystictum*) and the Mormyrus (*Mormyrus caschive*) of varied hue and the Mackerel (*Scomber scombrus*) and the Carp (*Cyprinus carpio*) and all that love the shores" It would be interesting to understand which species exactly the Citharus belongs to. In the Athenaeus' *Deipnosophists* the Citharus is called 'acharnus' and it is mentioned too in Callia's Ciclops: "A roasted harp-fish, and a ray, and the head of a well-fed tunny". LSJ[1] describes it as: "a kind of flatfish, sacred to Apollo". Plinius the Elder with derogative words says: "it's the worst type of rhombus". The Citharus is probably a species of flatfish, belonging to the *Pleuronectiformes*.

The aim of the work was to compare modern and ancient knowledge about fish species. The sources were analyzed to better understand the technical lexicon of ichthyology and fishing, the philological aspects and the ancient gastronomy.

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OR07

THE PALEONUTRITION FROM 500 BC TO 500 AD: CASE STUDIES BETWEEN THE ADDA AND TICINO RIVER (NORTHERN ITALY)

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In the last decades, remains of archaeological vegetal foods have been given increasing attention by the domain scientific literature. These consist of finds with recognizable morphology, or adhering to vessels, or fragments of biogenic material. As the analysis of this paleobotanical material through optical or SEM microscopy is high time consuming, new finds are often only reported with no additional micromorphological or chemical documentation.

The University of Insubria and the Laboratory of Archaeobiology of the Como Civic Museums have just launched a new research initiative on the microscopical analysis of archaeological remains of charred food. Preliminary results have already been published on the "bread" of the Bronze Age in the Garda Lake area, northern Italy (Castelletti et al., 2015; Castelletti et al., *in press*).

The analysis focuses on micromorphology and assesses the degree of conservation of structures by optical microscopical inspection (in particular reflected light microscopy), scanning electron microscope (SEM), combined with chemical survey by electron microprobe (EDAX), and IR spectroscopy.

This work concerns charred food remains of proto-historic age, classical and early Middle Ages discovered in several archaeological sites in the area between the Adda and the Ticino river, northern Italy. The remains are related to the Golasecca Culture, Roman Imperial Age and the early stages of the Middle Ages, and date back to a period ranging from 500 BC and 500 AD, approximately. The finds derive from funeral pyres or fires of buildings. Remains of phytoliths, and other anatomical structures related, for instance, to the caryopses of cereals and to the seeds of legumes, are still recognizable. Traces of fungal attacks, mainly in the form of hyphae and spores, are also observable. These data make it possible to identify the ingredients, the procedures for the preparation of food, the techniques of raw materials processing and the nutritional value of recognizable components.

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ORo8

NOT ONLY FOR BEAUTY, THREE RITUAL PLANTS IN CLASSICAL TIMES

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The plants have always been important in human history, not only because they provide food, medicines, fuel and materials, but also for their social uses, including religious ones, as the funeral rites. In Mediterranean the traditions linked to plants can have very ancient roots, which however are codified from the Archaic Greece to the Roman World.
During a PhD research the archaeobotanical remains (seeds/fruits and charcoals), recovered in graves and funerary structures (1st – 5th cent. AD, Roman Age) of Modena (Emilia Romagna, Northern Italy), have been investigated and are still under study. Up to now, about 35 contexts from 3 different necropolis were analyzed. (Rinaldi *et al.*, in press; Riso *et al.*, 2016). In the laboratory, moreover, we are studying the plant iconography of hundreds tombstones (30 graves, end 5th – 3rd cent. BC) from Paestum (Ferrari *et al.*, *in litteris*). These studies also permit a comparison of the archeobotanical data and that from the Greek-Roman written sources regarding the role of plants in the classical societies (e.g. Baumann, 1993; Bernhardt, 2008; Caneva, 2010; Caneva and Bohuny, 2003; Castoldi, 2014; Ducourthial, 2003; Forster, 1936; Frazer, 2012; Giesecke, 2014; Raven, 2000; Repici, 2000; Sena Chiesa and Pontrandolfo, 2015).
In Modena graves have so far been identified around 50 carpological taxa, in accordance with the results obtained from a review of about 300 Roman cremations of Northern Italy (Rottoli and Castiglioni, 2011). In the investigation of the Paestum's tombstones, we have identified 10 plant species with certainty. We noticed that three woody plants are present both in the pictures of Paestum and, with their "fruits", among Roman funerary offerings in Modena and they also appear in many classical sources: olive (*Olea europaea*), date palm (*Phoenix dactylifera*) and grapevine (*Vitis vinifera*), are plants that represent a common thread between Greek and Roman world, for their beauty and the symbolic meanings ascribed to them. These plants, among the oldest fruit crops known in the Mediterranean area, have properties which made them essential for nutraceutic and medicine fields, from ancient times until today. Olives, date palm and grapevine are, among the vegetables, the perfect example of form related to the usefulness and symbolic aspects connected with the uses in everyday life.

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OR09

PERSICARIA LAPATHIFOLIA (*POLYGONUM LAPATHIFOLIUM*, PALE SMARTWEED) SEEDS AS FOOD DURING THE IRON AGE SOUTH OF THE ALPS? NEW DATA FROM THE SITE OF PADOVA-S.EUFEMIA (NE-ITALY)

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It has been demonstrated that wild herb species were intentionally gathered for human consumption as an addition to diet North of the Alps during the Iron Age; in particular, high quantities of *Persicaria lapathifolia* (pale smartweed) often occurred in German and Denmark Iron Age bog bodies, cadavers of sacrificed or executed people [1]. In this study we will discuss whether pale smartweed and other Polygonaceae were used as food during the Iron Age also South of the Alps. We also discuss the hypothesis that the use of pale smartweed as food arrived in N-Italy from the Carpathians area (SE-Europe).

We present new palaeocarpological data from the site of S.Eufemia, located in the city of Padova (NE-Italy), mainly used during the Iron Age between VIII and V cent. BC for ritual purposes [2]. We examined the macrofossil records of coeval sites in N-Italy and in SE-Europe to verify the occurrence of pale smartweed. We finally checked its use as food during the XIX and XX centuries in Europe. Pale smartweed is one of the three most represented carpological *taxa* in the site 3,4 (ca.14% of the total), particularly in wood charcoal layers found in wide pits (VII-VI cent. BC). These structures were excavated to set on large ritual fires and for the deposition of human and animal remains ceremonially sacrificed. In coeval N-Italy sites this species is well represented (> 10%) only in a house in Bressanone (NE-Italy) [5]. Pale smartweed is a therophyte which have a paleotemperate distribution ranging, in the Old World, from N-Africa to the whole Europe as far as Siberia and sub-Siberian Asia. In NE-Italy it is widely present in areas, even very large, along water courses on silty-sandy substrates rich in nutrients. In the Carpathians area (e.g. Bulgaria) [6]; Popova 2009) this species was used as a parallel source of food between V and III cent. BC. A large and pure assembly of seeds of this *taxon*, clearly purposefully collected, dating back to the XII cent. was recovered in Germany [1]. Pale smartweed leaves and seeds represented a supplementary food source forced by poverty, famines and wars during the XIX cent.[7] and are also nowadays eaten raw or cooked (e.g. in Polish) [8,9]. Some Poligonaceae are food plants in certain countries in Asia [10]: rootstocks are consumed raw or roasted and used to obtain starch in Mongolia, China [11] and Siberia [7]. *Persicaria maculosa* is nowadays one of the 56 wild herbs constituting a dish typical in NE-Italy [11]. We can conclude that pale smartweed was probably used as food during the Iron Age also South of the Alps, even if this practice seems restricted to NE-Italy: probably it came from the Carpathians area and didn't diffuse during that period towards NW-Italy, where it was considered as supplementary food only later during the XIX cent. [7]. The use of Poligonaceae was forced by poverty and famines, as attested for Italy and NE-Europe during the XIX cent., and also by adverse climate conditions, nowadays encountered in certain Asiatic regions. In NE-Italy the use of Poligonaceae has remained until now in the food tradition. It is not to be excluded a symbolic value of pale smartweed, since it was involved in ritual celebration in S.Eufemia and was part of the last meals of bog bodies.

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OR10

WAYUU TRADITIONAL MEDICINE AND THE USE OF MEDICINAL PLANTS IN THE CONTROL AND PREVENTION OF HYPERGLYCEMIA

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The Wayuu Indians, who live in the department of La Guajira (Colombia), have traditionally used dry forest plants for medicinal purposes to prevent, cure or control the various diseases that afflict them. Diabetes has become one of the diseases that affecting millions of people worldwide, of which the Wayuu are no exception. The aim of the study was to obtain information from practicants of Wayuu traditional medicine (*Chaman, Outsüü*) about the role played by some medicinal plants in the control and prevention of hyperglycemia.
It was realized a quantitative research, through interviews with shamans settled in three (3) municipalities in the department of La Guajira (Maicao, Uribia and Manaure) where there is the largest indigenous population. The information obtained was compared with the existing literature on a scientific level.
The outcomes showed that the Wayuu using 16 plants for the treatment of hyperglycemia, overhanging by its effective *Bauhinia glabra* (bejuco cadena), *Curatella americana* (peralejo), *Prosopis juliflora* (trupillo), *Eugenia jambolana* (uvita morá), *Parquinsonia aculeata* (sauce guajiro), *Cissus sicyoides* (uvita e' culebra), *Jacquinia pungens* (manca mulo). The most commonly used methods were cooking (50%) and the infusion (25%); the parts of the plants most frequently used were the whole plant (37.5%), stems, leaves and bark (18.75% each) and roots (12.5%). Currently, the plants reported are subject of experimental research and according to the scientific literature there are significant advances in the control and prevention of hyperglycemia worldwide.

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OR11

ETHNOPHARMACOLOGICAL STUDY OF SOME PLANTS TRADITIONALLY USED AS ANTIDIABETIC IN THE COLOMBIAN PACIFIC REGION

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Diabetes mellitus type 2 (DM2) is a metabolic and multifactorial disease which nowadays is suffered by more than 382 million people[1]. One of the most important complications in the treatment of diabetes is that it can manifest with obesity, presented in about 60 to 90% of patients with DM2[2]. The intrinsic relationship between the two diseases can be accelerated by oxidative stress that is generated from the non-oxidative metabolism of lipids and carbohydrates [3]. This metabolic profile in diabetic patients requires an integrated treatment of the disease. The worldwide increase in the mortality of this disease has promoted multiple researches for new and better therapeutic alternatives [4]. Thus, the wide variety of unexplored natural substances offers an alternative treatment of this disease. The knowledge developed by the traditional medicine in El Choco offers valuable information about a variety of plants used effectively as treatment against diabetes and/or associated symptoms. However, the ancient practices of the afro communities in the Colombian Pacific behind the preparation of these natural medicines have not been enough studied experimentally. The objective of this research is to provide a scientific support to the traditional use of some medicine plants recognized as natural antidiabetics in the Colombian Pacific.

The studied species were selected via ethnobotanical study, taking into account those species with using value greater than 60%. From dried and powdered material of *Artocarpus altilis*, *Bauhinia picta*, *Momordica balsamina*, *Neurolaena lobata* and *Vismia macrophylla*, hydroalcoholic extracts were obtained by maceration. These extracts were characterized chemically by a preliminar phytochemical analysis on TLC and by quantification of total phenols[5]. They also were submitted to spectrophotometric and autographic assays to identify substances capable to inhibit the enzymes α-glycosidase and pancreatic lipase [6]; finally decoloration proofs of the free radical DPPH• [7].

The extracts of the five species were characterized by the presence of phenolic compounds, steroids and/or triterpenoids, being the most representative the phenolic compounds, with estimated values between 5.04±0.78 to 34.00±1.11 mgEAG/g of sample, with greater presence in the extracts of *V. macrophylla*. The hydroalcoholic extracts from the leaves and bark of *V. macrophylla* possess the greater capacity to inhibit the enzymatic activity of the α-glucosidase with IC_{50} values between 18 and 104 times lower than the IC_{50} values of the acarbose (678±2.90 µg/mL), respectively. The leaves extract of *N. lobata* caused the greatest inhibition of pancreatic lipase with an IC_{50} = 17.00±0.87 µg/mL, showing a slightly lower activity than orlistat (4.44±1.07 µg/mL). Besides, in the autographic assay with pancreatic lipase, around 37 inhibitory zones were identified between the six extracts, related to the presence of flavonoids, saponines and steroids, especially in *N. lobata*, *B. picta* y *M. balsamina*. All the extracts, except the *M. balsamina*, show an excellent behavior as inhibitors of the free radical DPPH• with values IC_{50} between 10.63±1.08 and 152.90±0.67 µg/mL. By relating the total phenolic content of the six extracts with the results of the three activiades evaluated, it was possible to establish that the three biological activities have a correlation above 75% with the total phenolic content, indicating that these substances are largely responsible for the effects of extracts on enzymes and DPPH•. In conclusion, the species *V. macrophylla* and *N. lobata* are the most promising as raw material for the search of new integral treatments for diabetes type 2.

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OR12

ALKALOIDS FROM *HIPPEASTRUM CANASTRENSE* J. DUTILH & R. S. OLIVEIRA (AMARYLLIDACEAE)

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Amaryllidaceae is a well-known family of monocotyledonous which are distributed widely in Brazilian territory, being found in the biomes Amazon, Cerrado, Caatinga, Pampa, Pantanal and Atlantic Forest. This plant family is characterized by the presence of an exclusive group of isoquinoline alkaloids, which have been isolated from plants of all the genus of this family.[1] Several biological activities have already been reported for these alkaloids including acetylcholinesterase (AChE) inhibition, antimalarial, anticancer, antiviral, among others.[1]

The aim of this work was to carry out a chemical investigation of the alkaloids present in the Brazilian species *H. canastrense*. Seventeen compounds were purified including typical Amaryllidaceae alkaloids and some are new homolycorine-type derivatives.

The species *Hippeastrum canastrense* J. Dutilh R. S. Oliveira were collected and divided in fresh bulbs (900 g) and aerial parts (720 g), crushed separately and extracted with methanol. The combined macerate was evaporated to dryness and the crude extract was acidified to pH 2 with 2% H_2SO_4, the neutral material removed using Et_2O (3 x 200 ml) and EtOAc (3 x 200 ml). The aqueous phase was then basified up to pH 11 with NH_4OH (25%, v/v) and extracted with *n*-Hex (10 x 200 ml), followed by EtOAc (10 x 200 ml) and finally EtOAc:MeOH (3:1, v/v, 3 x 200 ml). These enriched-alkaloidal fractions were submitted to extensive chromatographic techniques for alkaloid purification and were also tested against mouse hepatoma cells (H1C1C7) and the fraction EtOAc from aerial parts along with the alkaloid candimine were tested against human breast adenocarcinoma cell line (MCF-7).

The Amaryllidaceae alkaloids sanguinine, hippeastrine, candimine, 7-methoxy-*O*-methyllycorenine, homolycorine, albomaculine, 2α-methoxyhomolycorine, 2α,7-dimethoxyhomolycorine, 2α-hydorxyhomolycorine, 2α-hydroxyalbomaculine, 8-*O*-demethylhomolycorine, lycorine and norpluviine were isolated and identified in *H. canastrense*. Furthermore, the complete characterization of four new homolycorine-type alkaloids is currently under study.

Concerning the cytotoxic assay, a percentage range of cell-death between 70% to 80% were observed for the extract EtOAc from bulbs and aerial parts and for the extract *n*-Hex from bulbs. The range of concentration of all extracts varied between 4-500 μg.ml^{-1}. The fraction EtOAc from aerial parts displayed the best cytotoxic activity against hepatoma cell line (cell-death percentage of 73% in 4 μg.ml^{-1} of extract) and was also tested for the human breast adenocarcinoma cell line (MCF-7), along with candimine. The IC_{50} 0.58 and 28.7 μg.ml^{-1} were observed for EtOAc extract and candimine, respectively. In this attempt, the extract showed a remarkable cytotoxic potential for these tumor cell line.

In summary, the indigenous Amaryllidaceae *H. canastrense* showed to be able to synthesize a great number of homolycorine-type derivatives, this feature is typically observed in the *Hippeastrum* genus. The alkaloid-rich fractions from *H. canastrense* displayed an important cytotoxic activity against mouse hepatoma and human breast adenocarcinoma cell lines. In this attempt, cytotoxic assays for the all isolated compounds are still in progress.

Acknowledgements: the authors thank CAPES, FAPES and CNPq

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OR13

PHYTOCHEMICAL INVESTIGATION OF ETHANOLIC EXTRACTS FROM *KIGELIA AFRICANA* (LAM.) BENTH. FRUITS

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Kigelia africana (Lam.) Benth. is a tree endemic to the African continent (tropical zone). All the plant parts, including leaves, fruits, stem bark, and roots, are widely used in the local medicine. The plant is commonly known as "sausage tree", because of the singular shape of the big fruits. The latter constitute the most used drug obtained from the tree, and are considered a valid remedy to treat several ailments. Beyond conventional uses, such as the antimicrobial, the fruits are also employed in religious rituals to promote fertility and prosperity.

Powdered fruits of *K. africana* collected in Mali (Africa) were subjected to solvent (ethanol) extraction. The extract underwent a preliminary TLC screening with UV spectroscopy detection. Successively, the extract was further purified through SPE cartridges, in order to be analyzed by HPLC-PDA and HPLC-ESI-MS.

TLC analysis evidenced the presence in the ethanolic extract of polyphenols, iridoids, and phytosterols. A deeper investigation carried out through LC techniques with both PDA and mass spectrometric detection, allowed to identify and quantify six phenolic acids, three iridoids, and two unassigned components, presumably being two extra iridoids.

Acknowledgements: Authors wish to thank the University of Messina through the "Research and Mobility" project.

OR14

NOVEL SECONDARY METABOLITES EXTRACTED FROM THE ECUADORIAN PLANT *MACROCARPAEA LENAE* J.R. GRANT

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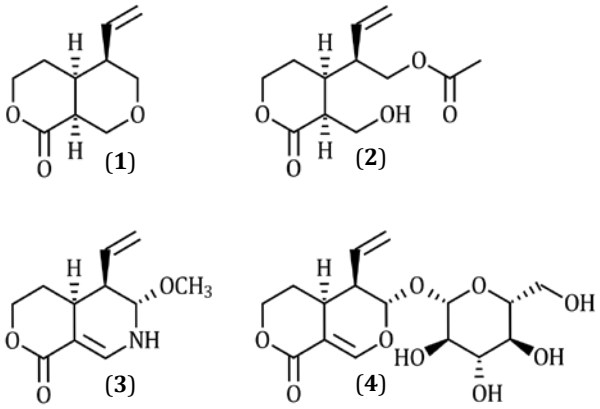
Macrocarpaea lenae J.R. Grant is a plant belonging to the family Gentianaceae, growing in Colombia, Ecuador and Peru between 1000-3500 m a.s.l., mainly on the eastern Andean slopes. The genus *Macrocarpaea* counts 75 recognized species, more than 30 of which are present in Ecuador. The species *Macrocarpaea lenae* J.R. Grant was classified for the first time in 2003 [1], after being collected in the Parque Nacional Podocarpus, in the Zamora-Chinchipe province; however the samples employed in this studies were recollected near the town of Saraguro, in the province of Loja. In fact this plant is employed in the traditional medicine of the Saraguro people with the name of *Tabaco de Cerro*, were it is applied to the treatment of cold and of some supernatural diseases.

This is the first chemical study on this species; however the genus *Macrocarpaea* counts on one publication [2], about the extraction of xanthones from *Macrocarpaea glabra*.

Dry leaves were exhaustively extracted four times with ethyl acetate and methanol. The methanol extract was then subjected to acidic extraction with 2% sulfuric acid, followed by alkalinization to pH 11 and repeated extraction with chloroform, affording the alkaloid fraction of the plant. The extracts were subjected to chromatographic fractionation, both on silica gel and C-18 reversed phase, until the obtainment of pure metabolites.



In a previous edition of this congress (Marsala, 2014), some spectroscopic evidence about the presence of the alkaloid *gentianine* was shown. After that, four secondary metabolites were isolated as almost pure compounds. Two of these molecules resulted to be unknown in scientific literature (**1-2**), while the other two are known as *hirsutanine A* (**3**) and *sweroside* (**4**). The structure of the new molecules was elucidated with spectroscopic techniques, through ^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC and NOESY NMR experiments and by mass spectrometry. In this moment, some intent to detect a biological activity is in progress.

Acknowledgements: Part of this study was conducted under the financial support of the PROMETEO PROJECT to GG, patronized by Secretaría de Educación Superior, Ciencia, Tecnología e Innovaciòn of Ecuador in 2014-2015.

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OR15

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *HELIANTHEMUM SESSILIFLORUM*

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Helianthemum is a genus of plants including around 110 species, belongs to the Cistaceae family also known as rock rose. This genus is growing in America, Europe and Northern Africa. However, the Mediterranean region is considered its center of diversity (Mabberly, 1997). *Helianthemum sessiliflorum* Pers. is one of species of this genus that showed previously anti-inflammatory and analgesic activities. In addition, the aerial parts of this plant are recommended in folk medicine in case of cutaneous lesion. The phytochemical investigation of the AcOEt and *n*-BuOH extracts of the aerial parts of *H. sessiliflorum* resulted in the characterization of one new lignan named 1-*O*-acetyl prinsepiol **1**, together with twenty seven known bioactive compounds **2-28** (Benabdelaziz et al., 2015). Furthermore, the antioxidant and antibacterial activities of different extracts of *H. sessiliflorum* were determined.

The structures of all the isolated compounds **1-28** were determined by spectral methods including 1D (^{1}H and ^{13}C NMR) and 2D NMR (COSY, HSQC, TOCSY, HMBC and NOESY), HR-ESI-MS, UV, IR, values of optical rotation and chemical correlations with known compounds that have been described in the literature. Dried aerial parts (1Kg) of the plant material were macerated with 70% EtOH (3 × 10 L) at room temperature. The EtOH extract was concentrated then diluted with H_2O and partitioned successively with cyclohexane (3 × 150 mL), AcOEt (3 × 150 mL) and *n*-buthanol (3 × 150 mL). The AcOEt extract (3.2 g) was fractionated on VLC (SiO_2) with the solvent system cyclohexane/AcOEt (100:0 to 0:100) then AcOEt/MeOH (100:0 to 0:100) to give four fractions (F1-F4). The purification of these fractions afforded twenty secondary metabolites **1-20**. The *n*-butanol extract (8 g) was chromatographed on a polyamide column eluted with H_2O/MeOH (100:0 to 0:100) to give five main fractions (F1-F5). The purification of the obtained fractions gave eight polyphenolics **21-28**.

Repeated column chromatography over silica gel (SiO_2), reversed-phase (RP-18), polyamide SC-6 and sephadex LH-20, and HPLC (RP-18) of the AcOEt extract afforded twenty compounds, the new furofuran lignan; 1-*O*-acetyl prinsepiol **1**, along with nineteen known ones named 1α-hydroxypinoresinol 2, (+)-cycloolivil 3, (−)-pinellic acid 4, benzoic acid 5, *p*-hydroxybenzoic acid 6, protocatechuic acid 7, vanillic acid 8, gallic acid 9, (−)-epicatechin 10, (−)-catechin 11, (−)-epigallocatechin 12, (−)-gallocatechin 13, astragalin 14, tiliroside 15, quercetrin 16, isoquercetrin 17, myricitrin 18, β-sitosterol 19 and daucosterol 20. The chemical investigation of *n*-butanol extract using also several chromatographic methods, afforded eight known polyphenolic compounds called isolaricireinol 9'-*O*-β-D-glucopyranoside 21, nicotiflorin 22, kaempferol 3-*O*-vicianoside 23, rutin 24, neoisorutin 25, vicenin-2 26 and two auronols as enantiomeric pairs; hovetrichoside C 27 and 28. The antioxidant activity was performed by DPPH radical scavenging method which indicated that ethyl acetate extract had the best antioxidant potential (IC_{50} = 32.75 ± 2.07 µg/ml). The significant linear correlation was done between the values of the total phenolic/flavonoid contents and antioxidant activity of plant extracts. The antibacterial activity of different extracts was assessed against some bacterial strains. The ethyl acetate and *n*-butanol extracts showed moderate activity. This could be explaining the traditional use of this plant against skin infections.

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PROANTHOCYANIDIN A2 FROM *DIPLOTROPIS PURPUREA* LEAVES

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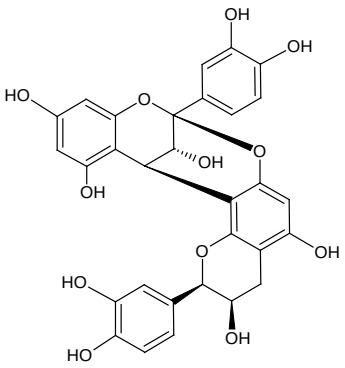
Diplotropis purpurea var *leptophylla* (Kleinhoonte) Amshoff, which is known as sucupira, sucupira-preta and others (Stasi and Hiruma-Lima 2002) has been chosen as part of our bioprospection research line. It belongs to Fabaceae family and Papilionoideae sub-family. Chemical study on this species has been described as flavonoids, triterpene and steroids isolated from trunk barks (Braz-Filho *et al.* 1973). In order to identify other compounds, this work describes the phytochemical study of methanolic extract from leaves of *Diplotropis purpurea*.

Leaves of *Diplotropis purpurea* were collected in June 2012 at the Reserva Particular do Patrimônio Natural (RPPN) in Presidente Figueiredo, AM. The plant material was extracted with hexane, methanol and water by using ultrasound bath for 20 min for each solvent repeated 3 times. The methanolic extract (4 g) was partitioned to obtain 3 phases: CH_2Cl_2, EtOAc and MeOH/H_2O. The EtOAc phase was submitted to open chromatographic column on Florisil using the solvents CH_2Cl_2, EtOAc and MeOH as gradient elution system. 32 fractions were obtained and analyzed by TLC. Combined fractions 15-17 (21 mg) were separated by preparative TLC using CH_2Cl_2/MeOH 8:2 as elution system yielding compound 1 (16.6 mg).



The phytochemical work yielded one biflavonoid as the major compound of the fraction 15-17. The fraction was analyzed by TLC, MS and NMR. The fraction showed a red spot when revealed with anisaldehyde sulfuric reagent. The HR-ESI-MS spectrum revealed the molecular ion at *m/z* 577 $[M+H]^+$, indicating $C_{30}H_{24}O_{12}$ as the molecular formulae. The ^{1}H-NMR spectra analysis showed signals at δ_H 4.07 (*d*, *J* = 3.27, H-3) and δ_H 4.41 (*d*, *J* = 3.27, H-4) belonging to the C-ring of flavanol. HMBC spectra correlations at δ_H 4.41 with δ_C 107.21 and 152.42 confirmed a doubly linked interflavanyl bonds of D ring of the second flavanol moiety described in the literature (Nam et al., 2015). Other signals at 4.93 (*s*), 4.24 (*d*, *J* = 3.73 Hz), 2.76 (*dd*, *J* = 17.0 and 5.2 Hz), 2.90 (*dd*, *J* = 17.0 and 2.2 Hz) allowed the identification of F ring.

All these data in comparison with the literature allowed the biflavonoid identification as proanthocyanidin A2. As far as we know, this is the first report of this biflavonoid in the genus *Diplotropis*. Additional studies will be performed to evaluate the antibacterial activity.

Acknowledgments: CT-Agro/CNPq, Pro-Amazônia/CAPES, Programa Ciência sem Fronteiras/CNPq, PPBio/CNPq, INCT/CENBAM/CNPq, FAPEAM.

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CYCLOARTANE GLYCOSIDES FROM *ASTRAGALUS GOMBO*

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The genus *Astragalus* is represented in Algeria by about 40 species, including *Astragalus gombo* Coss. & Dur. This endemic perennial plant grows in sandy arid and desert pastures of Algeria (Quezel and Santa, 1963). Several *Astragalus* species are used in traditional medicine as antiperspirants, diuretics, tonics, in treatment of nephritis, diabetes, leukemia and uterine cancer (Avunduk et al., 2008). Previous phytochemical studies on *Astragalus* saponins have shown the importance of cycloartane-type triterpene glycosides as major compounds (Barbić et al., 2010). They are known for their interesting biological properties, including immunostimulating, antiviral, anti-inflammatory and cytotoxic activities. The chemical investigation of the *n*-butanol extract of the aerial parts of *Astragalus gombo* resulted in the isolation of six new cycloartane-type triterpene glycosides **1**–**6**, in addition to another known cycloartane triterpene glycoside **7** (Maamria et al., 2015).

The structures of the isolated compounds have been determined on the basis of 1D and 2D homo- and heteronuclear NMR and mass spectrometry, as well as by comparison with reported literature data. Part of the air-dried and powdered plant material of *A. gombo* (aerial parts; 1 Kg) was macerated two times (10 l x 2, each 48 h) with EtOH-H_2O (70:30) at room temperature. The EtOH extract was concentrated and partitioned with petroleum ether, EtOAc and *n*-butanol (each solvent, 500 mL x 3). The *n*-BuOH extract (14 g) was separated over a VLC chromatography (vacuum liquid chromatography) using reverse-phase material. Elution was performed with H_2O-MeOH (100:0 to 0:100) to give fourteen fractions (F1-F14). Fraction F10 (679 mg) was subjected to silica gel column chromatography with the solvent system CH_2Cl_2-MeOH (100:0 to 60:40) to give 8 sub-fractions (F10-1 to F10-8). The sub-fraction F10-1 (166 mg) was chromatographed by semi-preparative HPLC using an elution H_2O-acetonitrile (50:50, 50:50, 0:100, 0:100) as mobile phase to yield compounds **3** (Rt= 9.521 min, 80.7 mg) and **4** (Rt= 12.346 min, 32.4 mg). Sub-fraction F10-3 (95 mg) was purified by semi-preparative HPLC using a gradient of H_2O-acetonitrile (75:25, 44:56, 0:100) to yield compounds **1** (Rt= 5.851 min, 2 mg), **2** (Rt= 6.678 min, 2.5 mg), **5** (Rt= 7.743 min, 2.9 mg) and **6** (Rt= 8.728 min, 5.9 mg). Fraction F11 (300 mg) was subjected to CC on Sephadex LH-20 in $CHCl_3$ giving 5 sub-fractions (F11-1 to F11-5). Preparative TLC of sub-fraction F11-2 (50 mg), developed with $CHCl_3$-MeOH (90:10), afforded compound **7** (15 mg).

Column chromatography over silica gel (SiO_2), preparative TLC, sephadex LH-20 and HPLC (RP-18) afforded seven cycloartane glycosides **1-7**, from the *n*-BuOH extract of the aerial parts of *A. gombo*. This result is in agreement with previous studies performed on *Astragalus* species. The phytochemical investigation of *A. gombo* confirms the occurrence of cycloartane triterpene glycosides in the *Astragalus* genus. In fact, this chemical study allowed the isolation and characterization of seven cycloartane glycosides of which six compounds **1-6** called gombosides A-F are described for the first time. The known compound **7** was identified as tomentoside II.

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NATURAL AND SEMISYNTHETIC COMPOUNDS FROM ARGENTINIAN *POLYGONUM ACUMINATUM* AGAINST HUMAN PATHOGENIC YEASTS

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In the last years, fungi have become the major cause of human infections, especially among immunocompromised hosts, having an enormous impact on morbidity and mortality. Although there are many available drugs for the treatment of systemic and superficial mycoses, they are not completely effective for their eradication. In addition, they all possess a certain degree of toxicity and quickly develop resistance due to inappropriate and abusive use. Therefore, there is an urgent need for the development of new antifungal chemical structures alternatives to the existing ones.[1] In this context, exploring natural and semisynthetic products, turn out to be of great importance. Previously, we reported antifungal activities of polygodial isolated from *Polygonum acuminatum* Kunth and some molecules derivatives from this natural product, against a panel of human pathogenic fungi.[2] In this work, we expanded the list of yeasts using clinical isolates of *Candida* and *Cryptococcus* strains. *P. acuminatum* was collected during the flowering season (March 2014) in Santa Fe province (Argentina) and deposited at the Herbarium FCA-UNL (Arturo Ragonese) with identifying data SF # MD97. Natural products polygodial (**1**) and *p*-methoxycinamoylpolygodial (**2**), as well as semisynthetic compounds confertifolin (**3**), drimenal (**4**) and isodrimenol (**5**) were obtained according to previous reports[2] and tested against clinically isolated fungi from Centro de Referencia en Micología (CEREMIC) and Malbrán Institute. They included 9 strains of *Candida* spp. (5 of *C. albicans* and 4 of *C.* non-*albicans*) and 6 strains of *Cryptococcus neoformans*. Inocula of yeasts suspensions were obtained according to reported procedures and adjusted to 1 x 10^5 Colony Forming Units (CFU)/mL.[3] MIC_{50} was defined as the lowest concentration of each compound that showed 50% growth reduction respect to the control and was determined using VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). Amphotericin B was used as positive control and tests were performed by duplicate.

Table 1. MIC_{50} (µg(mL) mean concentrations of compounds (1-5) inducing 50% growth reduction compared to control.
CCC= Centro de Referencia en Micologia (Rosario, Argentina), IM=Malbran Institute (Buenos Aires, Argentina), I= inactive >250µg/mL.

		MIC_{50} (µg/mL)					MIC_{100}			MIC_{50} (µg/mL)					MIC_{100}
Strain	*V. specimen*	**1**	**2**	**3**	**4**	**5**	Anf	Strain	*V. specimen*	**1**	**2**	**3**	**4**	**5**	Anf
C. albicans	CCC 125	7.8	62.5	125	31.2	31.2	0.49	*C. parapsilosis*	CCC 124	15.6	62.5	I	62.5	125	0.98
C. albicans	CCC 126	7.8	62.5	250	31.2	62.5	1.9	*C. neoformans*	IM 983040	1.9	7.8	250	31.2	15.6	1.9
C. albicans	CCC 127	3.9	250	I	125	250	0.49	*C. neoformans*	IM 972724	3.9	3.9	250	15.6	15.6	7.8
C. albicans	CCC 128	15.6	250	I	125	125	0.49	*C. neoformans*	IM 042074	1.9	15.6	62.5	15.6	15.6	3.9
C. albicans	CCC 130	7.8	125	125	62.5	250	0.49	*C. neoformans*	IM 00319	3.9	3.9	31.2	15.6	15.6	3.9
C. kefyr	CCC 123	7.8	1.9	125	62.5	125	0.49	*C. neoformans*	IM 972751	3.9	3.9	125	15.6	15.6	7.8
C. lusitaneae	CCC 131	7.8	125	I	62.5	I	0.98	*C. neoformans*	IM 031631	3.9	7.8	62.5	15.6	15.6	7.8
C. krusei	CCC 117	3.9	125	I	125	I	0.49								

Results are shown in Table 1. All *Candida* and *Cryptocuccus* strains were more sensitive to natural products polygodial (**1**) ($MICs_{50}$ between 1.9 and 15.6 µg/mL) and *p*-methoxycinamoylpolygodial (**2**) ($MICs_{50}$ between 3.9 and 250 µg/mL) than to semisynthetic derivatives confertifolin (**3**), drimenal (**4**) and isodrimenol (**5**). Among these, compound **4** showed higher activity than **3** and **5**, since it inhibited the growth of all evaluated strains with $MICs_{50}$ between 15.6 and 125 µg/mL. Compounds **3** and **5** were active against all *Cryptococcus* strains evaluated ($MICs_{50}$ between 15.6 and 250 µg/mL) but less active against *Candida* strains and in some cases they resulted inactive. With these results we conclude that compounds isolated from the natural source *P. acuminatum* are highly more powerfull as antifungal than semisynthetic ones.

Acknowledgments: CONICET for MD doctoral fellowship and PIP 112201501005 24CO, UNR project BIO411

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COMPARATIVE PHYTOCHEMICAL PROFILES OF *LIPPIA ORIGANOIDES* GROWN WILD AND *IN VITRO*

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Lippia origanoides (Verbenaceae) is commonly known as "sálva-de-marajó", generally used due to its medicinal properties (carminative, repellent and for the treatment of infectious diseases, genitourinary and gastrointestinal disorders) and food (condiment) [1]. It occurs in Northern South America, mainly in Brazil, Colombia and Venezuela [2]. *In vitro* culture techniques are an alternative to sustainable and large-scale production of plants for medicinal use. The main goal of this study was to evaluate the effects of different plant growth regulators on *L. origanoides in vitro* development and to achieve the best chromatographic profile in order to enable a good separation of the chemical constituents of this ethnomedicinal plant.

Aerial parts of *L. origanoides* collected from Amazon region (Manaus- Brazil) were extracted by percolation using commercial ethanol to obtain the hydroalcoholic crude extract (LONM). A mother-plant kept in a greenhouse located at Rio de Janeiro Mountain Region was used as donor to *in vitro* culture establishment and an hydroalcoholic crude extract was also prepared from it (LONP). In this study cultures of LONP maintained *in vitro* in basic medium [3] without growth regulators (MS0) or supplemented with 2.5 µM of naphthalene acetic acid (NAA) or 0.5 µM of jasmonic acid (JA) or 100 µM salicylic acid (SA) were prepared. After 60d, plantlets were evaluated for number of shoots and nodal segments, shoot length, rooted plants (%), callus development and fresh/dry biomass. The data were submitted to ANOVA and the mean values were compared by Tukey's test. Crude hydroalcoholic extracts for the treated plants were prepared by maceration and the results were compared to LONM and LONP. High-Speed Countercurrent Chromatography (HSCCC) and High Performance Liquid Chromatography (HPLC) coupled with ultraviolet (UV) were the techniques used for the comparison among the extracts. HSCCC results were monitored by Thin Layer Chromatography (TLC) and HPLC-UV results were compared with a profile of 6 flavonoid standards analysed in the same chromatographic conditions.

Hormonal treatment of *Lippia origanoides* by tissue culture influenced qualitatively and quantitatively the chemical profile when compared to natural plant (LONP). The highest number of shoots/plant was obtained with MS + 2.5µM NAA. A general solvent system using hexane-ethanol-water (4:3:1, v/v) was employed to achieve a good separation by polarity of the constituents of the extracts by HSCCC. The chemical profiling showed an increase of the number of steroids and terpenes for the *in vitro* cultures. MS0; MS + 0.5µm JA and MS + 100µM SA treatments showed a yellow fluorescence on the TLC plate that was indicative of the presence of flavonoids. Through analysis by HPLC-UV using isocratic mobile phase composed of methanol-acetone-acetic acid-phosphoric acid-water (200:100:10:10:200, v/v), the presence of naringenin was verified in wild plants, MS + 0.5µm JA and MS + 100µM SA.

Acknowledgments: Oliveira, D. R. for supplying the plant material.

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PROFILE CHARACTERIZATION OF VOLATILE COMPOUNDS ON WILD AND "IN VITRO" PLANTS OF *CLINOPODIUM ODORUM* (LAMIACEAE) USING HS-SPME/GC-MS ANALYSIS

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Clinopodion Odorum is used as a flavouring agent in food, and has gained considerable importance in the traditional medicine by the local inhabitants as an ant catarrhal, antispasmodic, stringent, carminative, diuretic, laxative, stomachic, anti-oxidant, antiacid, stimulant, soporific, vermifuge, menstrual suppressor, or in the treatment of flatulence, colic, altitude sicknesses, headaches and stomach-aches, as well as an anti-spasmodic and to help in parturition. As its production occur entirely from wild collection, this species is subject to a high extraction pressure resulting from its use as an herbal component of yerba mate composite (an Argentine traditional infusion), folk medicine and in the preparation of liquor companies.

The aim of this work was to choose among the in vitro plants of *C. odorum* which grew in the best media condition and development a procedure for the standardization of an analytical methodology using HS-SPME/GC-MS for the analysis and characterization of volatile organic compounds. Additionally, the results were interpreted using statistical methodology by applying principal component analysis (PCA) and conglomerate analysis (CA) to establish similarities and differences with the wild plant.

To increase stock populations for multiplication rate assessment, clonally plants (plants originally derived from seed) were initially propagated on different media for 4–5 months. Multiplication experiments were carried out using nodal segments with axillaries buds of in vitro germinated seedling aged on different culture media. The identification of volatile components was performed using a HS-SPME/GC-MS. A variability pattern found is repeated with each of the volatile compounds, for this reason it was necessary to consider and perform a principal component (PCA) and a hierarchical cluster (CA) analysis.

Considering the composition of each volatile compound had been showed similarities and differences, so the results have a wide variability. (-) Menthone is the predominant monoterpene produced in the most of plant media studied either control or with the addition of growth regulators. Pulegone showed a higher sensitivity to the nutrient composition media, it biosynthesis is favored on low salt concentrations media. This component is accumulated in higher concentration on half-strength salt media (MS½ and B5½).

The biosynthesis and emission of volatile compounds on in vitro C. odorum plants is a developmentally regulated process which is finely modulated by medium nutrient composition and growth regulators.

Acknowledgments: to FONCYT (National Argentine Scientific Founds)for supporting this research: Start up: PICT-2013-0458-:Argentine.

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CAPSICUM ANNUM SEED AS SOURCE OF FOOD: PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION

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In Mexico, chilli (*Capsicum annum*) seed has been consumed since pre-Hispanic times. Currently, the dry seed is ground and used for preparing diverse dishes. Production of chilli (in Mexico) is about 2.2 million tons per year (SAGARPA, 2015) of which 40% is commercialized as dry chilli. In dry form, it contains 15-20% of seed that represents some thousand tons of food. The seed has been extensively studied from an agronomic point of view (as seed for sowing) (Moreno *et al*., 2011; Reyes *et al*., 2006), but its physicochemical (or nutritious) characteristics are scarcely known (Carrillo *et al*., 2009). The aim of this research was to study the physicochemical and microbiological characteristics of the seed of four varieties of *Capsicum annum* in order to know its value as food and to promote its consumption.

The seeds of four varieties of dry *Capsicum annum* were studied: "mulato", "ancho" "pasilla" and "guajillo". The constituents of the chilli were quantified: pericarp, peduncle, membranes and seeds. Chemical composition was determined: moisture (atmospheric oven), protein (by Kjeldhal), fat (by solvent extraction), ashes (by calcination) fiber (by acid and alkaline extraction), minerals (by atomic absorption), fatty acid profile (by gas chromatography). Molds, yeasts and mesophilic microorganisms were also determined. The study was carried out in triplicate.

Results are given as average for all four types of chilli/seeds, with exception for percentage of seeds and microflora. The major constituent of the analyzed chilies was the pericarp 70.81%, the peduncle represents 5.74% and the membranes 2.57%. Seed content was 21.24%, 18.74%, 18.17% and 14.16% for "pasilla", "mulato", "guajillo" and "ancho" chilies, respectively. The number of seeds (inside one chilli) was 294 and the weight of a seed was 8.7 mg. The chemical composition of seeds was as follows: moisture 7.37%, protein 19%, fat 16.14%, fiber 37.8% and ashes 3.5%. The seeds contain the following minerals (ppm): Na (20), K (263), Mg (30), Ca (1.2), Zn (0.7), Fe (4.9). Among heavy metals, the lead was present at 8 ppm. Population of mesophilic bacteria was 10^4 CFU/g, 10^3 CFU/g, 10^2 CFU/g and 0 CFU/g for "guajillo", "ancho" "mulato" and "pasilla", respectively. The presence of molds or yeasts in seeds was no detected.

With respect to other three, the "guajillo" seeds are smaller, clearer and brighter, and the chilli contains the lowest number of seeds. There were significant differences in chemical composition as well as in minerals content among varieties of seeds. Lead identified in seeds could come from irrigation water. Even though the seeds are enclosed in the pericarp, they contain mesophilic microorganisms.

The study revealed that the chilli seeds analyzed have a balanced chemical composition from a nutritious point of view: a high content of protein, fat and fiber.

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OR22

THE PEPTIDE PROFILE OF PARMIGIANO REGGIANO CHEESE: EVOLUTION THROUGH THE GASTROINTESTINAL TRANSIT AND UTILIZATION BY BIFIDOBACTERIA AND LACTOBACILLI

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Parmigiano Reggiano cheese (PR) is a raw-milk, hard cooked, long-ripened cheese of high quality and nutritional value, produced in a restricted area in northern Italy. Long ripening times allow for extensive proteolysis of milk proteins by the rennet and the enzymes of starter and non-starter bacteria [1]. Proteolysis contributes to digestibility and flavor, reduce allergenicity, and yields potentially bioactive peptides (e.g. antihypertensive or immunomodulatory) [2]. This study aimed to identify the fate of PR peptides during gastrointestinal transit and to evaluate if they could modulate the gut microbiota, supporting the growth of beneficial bacteria such as bifidobacteria and lactobacilli.

Differently aged PR samples (16, 24, and 36 months) were subjected to *in vitro* digestion, simulating oral, gastric, and duodenal transit. The digests were given to 27 strains of *Bifidobacterium* and 30 of *Lactobacillus* as the sole nitrogen source to evaluate their ability to utilize the PR peptides. Cultures of human gut microbiota were carried out to evaluate if PR digests could affect the abundance of bifidobacteria and lactobacilli among intestinal bacteria. The peptides in PR samples, in the digests, and in cultural broths were characterized by HPLC/ESI-MS/MS and quantified by UPLC/ESI/MS. Peptide utilization by bacteria was analyzed by principal component analysis (PCA)

Before digestion, a common peptide profile was detected in all the ripened PR samples, regardless of their ripening time. A total of 63 peptides were identified, with a length 2 to 103 residues and a molecular weight spread over a wide range. The digestion caused the disappearance of the serum proteins and most of the original peptides, while 71 new peptides were found, with an increase of shorter ones. The peptides of PR digests can be utilized as nitrogen source for the growth of pure cultures of *Bifidobacterium* and *Lactobacillus*, belonging to species which are natural colonizers of the human hindgut and/or which could find application as probiotics. All the strains grew in PR-based media, in many cases with high biomass yield. Bifidobacteria generally consumed a greater amount of peptides than lactobacilli, in terms of both the mean peptide consumption and the number of peptides consumed. PCA revealed strong strain specificity in the ability to utilize each single peptide, but did not reveal any grouping based on the main characteristic of the peptides (e.g. the length or the abundance of hydrophobic, polar, and positively/negatively charged aminoacids). In microbiota cultures, the PR digest promoted the growth of bifidobacteria, even though rapid and effective consumption of peptides by proteolytic colonic bacteria made impossible any relationship between growth of bifidobacteria and peptide preferences. These results confirm the positive role of PR on growth of beneficial bacteria and potential modulation of the intestinal microbiota, as a further mechanism of health promotion. The increase of bifidobacteria is universally regarded as a health promoting effect, albeit the complexity of the community inhabiting the colon that can take advantage of PR peptides.

Acknowledgement: The study was supported by Fondazione Cassa di Risparmio di Modena, grant no. SIME2013.0627

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OR23

EVALUATION OF THE ANTIOXIDANT CAPACITY AND CONTENT OF POLYPHENOLS OBTAINED FROM TEA (CAMELLIA SINENSIS) OF FOUR BRANDS SOLD IN COLOMBIA BY EXTRACTION AT ROOM TEMPERATURE

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Herbal medicines have long been used to treat chronic diseases such as cancer, neurodegeneration and diabetes, usually in the form of herbal teas, also called tisanes [1]. Green tea is catching more interest than other beverage due to its healty benefical effects and has become the most consumed beverage all over the word, after water [2], moreover containing catechins wich have anti-oxidative, anti-carcinogenic, anti-microbial, anti-viral, anti-nflammatory, and anti-diabetic properties [3]. The consumption of green tea in Colombia is a recent trend andthe market is continuously growing, then the most common commercially available types of green tea weretested in this study; Oriental, Lipton, Hindú and Jaibel. The main objective of this study was to determine andcompare the amount of polyphenols present in tea samples considering extraction in water at room temperature and stirring every 30 seconds for 5 minutes. The antioxidant capacity was determined by the 1,1- diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay and the oxygen radical absorbance.

Capacity (ORAC) assay. Concentrations ranging from 22.36 ± 0.98 to 41.29 ± 0.86 mg Trolox equivalents / g dry sample for DPPH assay and 22.95 ± 1.31 to 46.25 ± 2.05 mg Trolox equivalent / g dry simple were determined for ORAC assay. It was also found that the amount of total phenols in tea samples ranging from

2.53 ± 0.25 to 14.63 ± 0.53 mg gallic acid equivalent (GAE) / g dry sample and total flavonoid content concentration was obtained from 2.67 ± 0.20 to 7.08 ± 0.38 mg catechin equivalent / g dry simple. The antioxidant activity and total flavonoid content were highly correlated for both DPPH (r^2= 0.9911) as ORAC (r^2 = 0.9968). Tea extracts from the Oriental brand had the highest polyphenol content showing greater biological activity, contrary to Jaibel tea brand which recorded the lowest concentrations in all analyzes.

To date, there appear to be no thorough studies on how antioxidant activity of teas may be affected by hot or cold water steeping and how this may be related to their polyphenol content. The results obtained contribute to gaining further knowledge on how the potential health benefits of this popular beverage may be maximized by the different methods of preparation.

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OR24

CHEMICAL CHARACTERIZATION OF JATOBÁ: OIL, FRUIT AND FLOUR

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Brazilian tropical fruits occurred in Cerrado typical biome, that originally, covered about 2,039.386 km^2, just under 24% of the Brazilian territory. Because high incidence of endemic life forms, has placed the biome among the world's "hot spots" (ALHO, 2005). The native fruits trees occupy a prominent place in Cerrado ecosystem. *Hymenaea coubaril* L. var. *stilbocarpa* (Hayne) Lee et Lang. (Caesalpinaceae), or Jatobá tree produces fruits which contain a flour pulp which is extracted and consumed by native population for diet helps, to reduce the risk of gastrointestinal and coronary illnesses (LORENZI & MATOS, 2008). The aim of this word was study the biometry, characterized fresh and dry mass, polyphenols contents of fruits, identify majoritarian content of bark oil, and mineral characteristically of flour fruits.

The fruits were collected in Brazil, transported and measured *in natura* (integers, bark and seed out, flour). The biometry parameters was does to digital pachymetry and data measured in mm. The fruits were measured (mg and mm) and segregated in bark, seeds and flour. Oil was obtained for hydrodistillation by European Pharmacopeia 8th (2014) where 100 g de bark were extracted for water distillation in Clevenger apparatus for 4h. The oil obtained was reunited and perceptual yield was estimated. The CG analyses was executed Shimadzu GCMS-QP2010 system, and the volume injection was 0.4 µL. The software used was GCMS solution (Shimadzu). Polyphenols analyzed to HPLC, compered con 25 standards and expressed in ppm. The mineral analyzed was do for IPC-MS and results expressed in mgkg^{-1}

The median of length, width and weight of fruits, bark, seeds, flour were 114.8613 ± 5.9089 mm to 41.0563 ± 2.0701 mm, 51.4589 ± 4.1760 g in fruits; where bark weight was 35.3274 ± 2.5618 g, in flour with seeds and only flour were 16.0731 ± 2.1006 g and 6.5886 ± 0.7437g, and seeds 8.9898 ± 3.0260 g by fruit. From to 100 g of Jatobá bark were obtained for hydrodistillation in Clevenger apparatus about 2.0976 ± 0.5260 % of essential oil. The majoritarian compounds of jatobá oil were Germacreno D, Alpha-copaene Cadineno-delta, Muurolene Gamma, Biciclogermacrene, Cadineno-gamma. The polyphenols identify were tirosole (522 ppm) and t-cinnamic acid (230 ppm). The nutritional composition of flour were Na 4.954, Mg 604.636, K 1507.245, Ca 917.821, Mn 147.098, Fe 45.579, Cu 28.516 mg Kg^{-1} (ppm), Cr 0.287, Ni 0.551, As 0.071, Se 0.380, Cd 0.125, Pb 0.181 ppm. The importance of this study was to obtain quality biometric parameter settings (organography) depending on the fruit maturation stage (mature fruits), to obtain essential oil and flour. In general, green fruit has a higher oil content as a function of the defense mechanisms against herbivores. Germacreno D (19.56%), the chemical marker, is potential anti-microbial, bactericide and antioxidant, justifying the ethnomedicinal use. The antioxidant activity in flour is present, and in function of polyphenols with characteristics act by antioxidant in cellular membranes. The nutritional composition suggest the use of this flour in preparations of functional foods, as breads, cakes or foods rich in calcium us adjuvant alimentary.

Acknowledgements: To CNPq, FAPEMIG and UNIME.

OR25

POLYPHENOLS, MICROBIOTA, AND BIFIDOBACTERIA: RECENT INSIGHTS

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Information from metagenome analysis suggest that considerable differences in gut microbiota composition occur among subjects, where this diversity is responsible of different metabolic activities. Consistently, studies dealing with bioavailability of phytochemical metabolites demonstrated that different transformations are largely attributable to the diverse bacterial populations.

Objects of this study were chlorogenic acid (C-QA) and secoisolariciresinol diglucoside (SDG), a major phenolic acid and one of the most common lignans in the western diet, respectively. C-QA exerts several health effects, due to the antioxidant properties. The dietary intake of lignans is recognized to exert several positive effects, due to metabolites produced by bacterial transformations occurring in the colon. The resident microbiota releases the aglycone secoisolariciresinol (SECO) and transforms it into the enterolignans enterodiol (ED) and enterolactone (EL) that present improved bioavailability and potent biological activity, especially as phytoestrogens.

Interindividual diversity in C-QA and SDG metabolism were explored. C-QA biotransformation were performed with 9 different human colonic microbiota, through resting cells experiments. The diverse bacterial communities were able to hydrogenate, dehydroxylate C-QA, or break the quinic acid moiety also before hydrolyzing its ester bond. All the transformation pathways converged on 3-(3-hydroxyphenyl)-propanoic acid, the final metabolite in most of the samples, with some novel compoud identified for the first time in the transformation carried out with the microbiota of a sole volunteer. To evaluate whether the fate of C-QA may be modified by the presence of a potentially probiotic strain able to hydrolyze C-QA to CA, continuous microbiota cultures were supplemented with *B. animalis* subsp. *lactis* WC0432, a strain able to hydrolyze C-QA into CA. The transformation pathways of C-QA in presence of *B. animalis* subsp. *lactis* WC0432 did not change, even though the kinetics were diverse.

Transformations carried out by colonic microbiota on SDG were performed to identify and quantify the metabolites produced *in vitro* by the human gut microbiota of five healthy subjects, to evaluate *in vitro* the efficacy of a potential probiotic strain of *B. pseudocatenulatum* able to hydrolyze SDG, and to identify *in vivo* the lignan metabolites detected *in vitro* and their possible conjugates through the analysis of urine and fecal samples of the same volunteers after ingestion of flaxseed. The two glucose moieties of SDG were successively removed to yield SMG, and then SECO. Deglycosylation was not the limiting step of the biotransformation pathway, since the microbiota of all the subjects was efficient in releasing SECO. Consistently, the utilization of the probiotic strain potentially increasing the release of the aglycone did not improve the bioavailability of enterolignans. SECO underwent successive dehydroxylations and demethylations yielding ED (4–18% conversion) and EL (0.2–6%) after 24 h. Novel intermediates related to SECO, matairesinol and anhydrosecoisolariciresinol, were identified in fecal cultures. These metabolites were also found after flaxseed consumption in feces and urine in their native form and/or modified by phase II human enzymes (glucuronide, sulfate and sulfoglucuronide conjugates).

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CHARACTERIZATION OF FOS AND INULINS BY HPAEC-PED ANALYSES

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The analysis of carbohydrates in food has extreme nutritional importance because they are a primary source of energy and have healthy beneficial effects resulting mainly from dietary fibers and other unavailable carbohydrates resistant to digestion.
High-Performance Anion-Exchange Chromatography (HPAEC) coupled with pulsed electrochemical detection (PED) is a powerful analytical tool in carbohydrate separation due its ability to separate all classes of alditols, aminosugars, mono, oligo and polisaccharides, according to structural features such as size, composition, anomericity and linkage isomerism. Moreover, this technique has been a major impact on the analysis of oligo- and polysaccharides. The compatibility of electrochemical detection with gradient elution coupled with the high selectivity of the anion-exchange stationary phases allows mixtures of simple sugars, as well as oligo- and polysaccharides such as frurcooligosaccharides (FOS) and inulin to be separated with high resolution in a single run. FOS and inulin are effective prebiotics , which beneficially affect the host by selectively stimulating the growth and/or activity of the microbial groups. They occur naturally in many foods of vegetable origin, such as onions, Jerusalem artichokes, asparagus, leeks, garlic and chicory.
Carbohydrate analyses were performed with a Dionex apparatus consisted of a GP50 low-pressure quaternary gradient pump equipped with an autosampler AS 50 and a pulsed electrochemical detector (ED 50) consisting of an amperometric flow through cell and two electrodes: a silver-silver chloride reference electrode and a gold working electode. The ED 50 detector delivered to the electrochemical cell the following potential waveform: E_1= 0,1 V (t_1=0.00-0.40s), integrating from 0.20 and 0.40s, E_2=-2.0 V (t_2= 0.41-0.42s); E_3= 0.6V (t_3=0.43s); E_4=-0.1 V (t_4=0.44-0.50s).
Elutions of FOS and inulin at different degree of polymerization were performed at room temperature using Dionex Carbopac columns and suitable elution gradient programs.
MALDI-MS measurements were performed using a MALDI-LR TOF mass spectrometer (Micromass, Manchester, UK) operating in the positive linear ion mode. Ions formed by a pulsed UV laser lamp (λ =337 nm), were accelerated at 15 keV. Laser strength was varied from sample to sample to obtain the best signal. For all sample different matrices were tested.
FOS and inulin having different degree of polymerization were characterized by HPAEC-PED methods specially optimized and validated. The assignment of chromatographic peaks was based on the generally accepted assumptions that the retention time of a homologous series of carbohydrates increased as the DP increased. In the investigated short chain FOS products, a study on the detector response in relationship with the degree of polymerization was also carried out. To verify the chain lengh distribution of the analyzed FOS and inulion by HPAEC-PED, the same products were analyzed by MALDI-TOF MS. The proposed HPAEC-PED methods allow the determination of FOS and inuln which are usually employed by food industry as functional ingredients either for their prebiotic properties or as a fat replacer giving a fat-like mouth feel and texture.
FOS and inulin at different degree of polymerization can be charactirize by optimized HPAEC-PED methos. The proposed methods method may also contribute to clarify the real importance, the prebiotic effectiveness and the biological role of these carbohydrates.

OR27

MACRONUTRIENT COMPOSITION OF GIANT WATER BUG (*LETHOSERUS SP.*) EDIBLE INSECT

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Macronutrient malnutrition is characterized as a disease of poverty in the midst of plenty. It means that while macronutrients rich sources are readily available they are not consume or not intake on adequate amounts by vulnerable groups through a lack of awareness. In Mexico and Thailand there are a large reproduction of an under-utilized edible insect of the Belestomidae family, giant water bug, *Lethoserus americanus,* from Mexico and *Lethoserus indicus* from Thailand, both inhabitants of fresh water rivers and streams that can be easily collected either in the airborne when attracted to bright lights or with nets from the water. The aim of this study was assess macronutrient composition of Giant Water bug from Mexico and from Thailand to investigate if there is a difference in chemical composition of insects from two different geographical locations where insects are consumed.
Insect samples were obtain in summer time 2014, at the Chang Mai night market in Thailand and conventional sampling collected at Xochimilco canals of Mexico City, and macronutrients assess as proteins, lipids, minerals, fiber and soluble carbohydrates of adults in dry basis, was provided according AOAC, 1995 techniques.
Data obtained for insects from Mexico 1), and Thailand 2) were: 1) proteins 60.12%, 2) 53.11%; lipids 1) 5.72%, 2) 8.15%; minerals 1) 5.46%, 2) 6.75%; fiber 10.95%, 2) 12.23%; soluble carbohydrates 1)17.75%, 2) 19.74%. The macronutrient composition of giant water bug, show a difference in macronutrients, due to the environment flora and fauna of the water conditions, where they acquire the nutrients necessary for growth and reproduction. Proteins are higher in insects from Mexico, but the rest of macronutrients are lower. Giant water bugs are available all year round and are a good source of proteins necessary for the human health.

OR28

PROFILING OF MAJOR AND TRACE ELEMENTS OF THE MEDITERRANEAN SEPIA INK BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

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Cephalopods, such as cuttlefish, contain very few non-edible portions and constitute a valid alternative to the over exploited fish and lean meat sources, due to a typical high protein/fat *ratio,* which makes them highly valuable for human diet. Although they represent a minor fishery source in Europe, specific environmental factors of the Mediterranean area encourage their biodiversity and abundance (Keller et al., 2016), thus contributing positively to the expansion of fisheries and trading of cephalopods and derived products. Many studies already focused on the nutritional composition of cuttlefish's edible parts coming from the Mediterranean area (Özyurt et al., 2006; Villanueva et al., 2004), but no study has been still performed on the assessment of the nutritional value of the Mediterranean cuttlefish ink, increasingly appreciated in the Mediterranean culinary culture. The aim of the present work was to investigate the nutritional composition of the *Sepia officinalis* ink in terms of major (Na, Mg, K, Ca and P) and trace (Cu, Zn, Cr, Fe, Mo, Co, V, Ni, Mn, As, Cd, Pb and Hg) elements, by means of inductively coupled plasma mass-spectrometry (ICP-MS).

Black inks of *Sepia officinalis* specimens sampled in six different sampling sites, located along the north- and south- eastern Sicilian coasts, were considered. Each ink sample was pre-treated by a microwave digestion system and the major and trace element profile was subsequently characterized through a fully validated ICP-MS method. A Principal Component Analysis (PCA) was also conducted to reduce data dimensionality and cluster major and trace metal contents depending on the considered variables (*e.g.* sampling sites). Furthermore, data were statistically interpreted by chemometrics and a benefit/risk analysis was conducted to assess the nutrient/contaminant intake derived from the consumption of *Sepia* ink.

Sepia ink revealed to be both a reservoir of "healthy" elements, and a sink of potential toxic metals.

Mg (6246.4±452.1 μg g^{-1}) and Na (6426±531.7 μg g^{-1}) showed to be the most abundant major elements. Among trace elements, Fe and Cu were found in considerable levels (35.95±5.53 μg g^{-1} and 11.09±1.28 μg g^{-1}, respectively), while trascurable amounts of Cr, Zn and Mo were revealed in all the samples (<0.60 μg g^{-1}). Heavy metals such as As, Cd, Pb and Hg showed to be related to the environmental conditions of the sampling area. Indeed, ink samples from Capo Milazzo showed the highest levels of all considered elements (Pb 1.90±0.24 μg g^{-1}, Hg 0.90±0.08 μg g^{-1}, As 0.90±0.04 μg g^{-1} and Cd 0.09±0.006 μg g^{-1}), due to the strong pollution of such industrialized area. The benefit/risk analysis was performed considering a typical portion of Sepia ink (~3g). It highlighted a good contribution of major (K, Mg and Na) and essential trace metals (Cu and Fe) to the established RDAs. Although particularly high levels were found in the ink samples from Capo Milazzo, the assumption of potentially toxic elements *via* Sepia ink did not represent a risk for human consumers.

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OR29

EFFECTS OF JASMONIC ACID AND INDOLE-3-ACETIC ACID IN THE VOLATILE PROFILE OF *ANEMIA TOMENTOSA IN VITRO* PLANTS, AN AROMATIC FERN

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Ferns are the second largest group of plants. However, research related to their *in vitro* development aimed at the production of individuals for the ornamental plants market, leaving the chemical research beside. The essential oil from wild plants of *Anemia tomentosa* var. *anthriscifolia* has been studied and showed the presence of triquinane-type sesquiterpenes in wild plants and *in vitro* plants (1) (2), which are responsible for its woody scent.

In this work, we tried to optimize the production of the trichinane sesquiterpenes in *in vitro* cultures of *A. tomentosa* by adding different concentrations of the plant growth regulators jasmonic acid (0,1; 1; 10 µmol) and indole-3-acetic acid (2.8; 5.7; 11.4 µmol) to the MS medium culture. Plant's leafs (± 5g) were subjected to SDE extraction (Simultaneous Distillation and Extraction). The samples were analyzed by GC/FID and GC/MS in Agilent 6890N and Agilent 5973N systems, both with HP-5MS fused silica capillary columns Hydrogen was used as carrier gas for GC/FID and helium for GC/MS. The percentage composition was obtained by normalization from FID. Volatile compounds were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (3) Cultures were also analyzed for their fresh biomass production (FB), dry biomass (DB) and number of fully expanded leaves.

The major components found in SDE extracts were the monoterpenes trans-pinocarveol (24.23%), pinocarvone (19.73%) and α-pinene (17.04%). The major averages in fresh and dry biomass production were observed in treatments with jasmonic acid (FB: 0.8g; 0.4g; 0.25g DB: 0.78g; 0.35g; 0.24g respectively to treatments 0.1;1;10 µmol), and the lower averages were found in the treatment with indole-3-acetic acid (FB:0.30g; 0.17g; 0.12g DB: 0.22g; 0.12g; 0.08g). Also, the number of fully expanded leaves was greater in the treatments with jasmonic acid (12, 10, 8 respectively to treatments 0.1;1;10 µmol). The chemical profile of the volatile components presented a predominance of monoterpenes over sesquiterpenes for the *in vitro* plants (both treatments) similarly to previous work without the use of growth regulators (2). Besides an increase in the biomass production, jasmonic acid increased the diversity of volatiles, whereas indole-3-acetic acid appears to suppress this diversity.

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OR30

BIOCONVERSION OF ALKALINE HYDROLYSATES OBTAINED FROM AGROINDUSTRIAL WASTE TO PRODUCE FLAVORING AGENTS

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Agro-industrial waste is an important supply of useful renewable resources in the production of raw industrial materials; however, the potential use has been little explored. Ferulic acid (FA), from agro-industrial waste, constitutes a viable source of antioxidants and precursors of flavoring agents that are of interest in the food, cosmetic and pharmaceutical industries. This product can be used as a substrate for the biological synthesis of "natural additives", offering multiple economic and environmental advantages such as low costs, high availability of starting materials and efficient use of undervalued waste that causes environmental problems. A biotechnological process was evaluated to obtain natural flavoring agents through the microbial conversion of FA (which was obtained through base hydrolysis of lignin rich agricultural byproducts) using phytopathogenic fungi, such as *Botryodiplodia theobromae* and *Colletotrichum acutatum.*
The FA content in the alkaline hydrolyzate, obtained from agro-industrial waste, such as white and yellow corn bran, corncobs, rice husks, bagasse, banana pseudostems, coconut husks and coffee pulp, was quantified.
Three liquid media (Czapeck-Dox, Sabouraud and hydrolyzed) were formulated to evaluate the mycelial growth of the fungi *B. theobromae* and *C. acutatum*. The media were adjusted with agar (2%) and the corncobs were hydrolyzated to a final concentration of 1000 or 1400 mg FA / mL. The radial growth of the fungi was measured and the inhibition percentage was calculated. The biotransformations of FA and the hydrolyzation were performed in a Czapeck-Dox medium in 250 mL Erlenmeyer flasks (stirred shaker). The progress of the biotransformation was followed with the chromatographic techniques coupled to a mass spectrometry (GC-MS).
After performing alkaline hydrolysis under standard conditions, the highest FA content (1385 mg/100g) was obtained from the corncobs. This hydrolyzate was used as the source of AF in the biotransformation process with the fungi. The compounds 4-ethylguaiacol, 4-vinylguaiacol, vanillin, homovanillic alcohol and acetovanillin were identified as biotransformation process metabolites from FA by the fungi *C. acutatum* and *B. theobromae*. Taking into account the metabolites found in the biotransformation processes, a metabolic pathway for the biotransformation of FA by *C. acutatum* and *B. theobromae* was proposed.
For both fungi, it is observed that the hydrolyzate substantially limited the growth of microorganisms, with a maximum inhibition of ~ 90% (C. acutatum) and ~ 88% (B. theobromae). Possibly, the hydrolyzate contained hydroxycinnamic and benzoic acids, which inhibit the growth of microorganisms, and lacked the nutrients needed for the development of the fungi.
To carry out the biotransformation process using the hydrolyzate as a substrate, it was necessary to supplement the culture medium with nutrients; the Czapeck-Dox medium was appropriate for obtaining flavoring agents such as 4-ethylguaiacol, 4-vinylguaiacol and vanillin, among others.

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TO THE RESCUE OF COFFEE FRUIT (*COFFEA ARÁBICA*) AS A FUNCTIONAL FOOD

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The food industry releases large amounts of byproducts, which are a source of serious pollution and environmental problems, posing a threat to the environment by contaminating water and soil. Coffee is one of the most traded commodities in the world, but generates a lot of waste products during processing from the coffee fruit to the drink [1]. Peels, pulp and skin comprise almost 45% of the coffee fruit, and are the main products of food industry. Despite recent findings demonstrating high levels of antioxidant compounds and other phytonutrients, little progress has been made in its use as an additive or as a food *per se* [2]. Aim of this work was to obtain a functional food, by developing a snack made out of coffee pulp (*Coffea arábica*) dehydrated with a solar dehydrator, roasted and finally covered with chocolate. For this purposes, two chocolate substitutes were used, the SILKO SUMMER AND CEBES LSX-80 which were provided by the Company Aarhus Karlshamn Mexico, S.A de C.V. and were compared with a trademark Moctezuma® Chocolate.

Pulp *Coffea arábica* cherry was used already in the mature state, and crops were located in areas surrounding the city of Morelia, Michoacán. Coffee cherry pulp was washed and separated manually for subsequent dehydration and analyses. After solar drying, the pulp was dried at 100 ^{o}C for 10 min in order to obtain a crispy texture. Dehydrated and roasted pulp underwent total polyphenols quantitation by Folin Ciocalteu methodology. The roasted pulp was then covered with chocolate substitutes: SILKO SUMMER AND CEBES LSX-80 and results compared with those obtained using a cover of the trademark Moctezuma® Chocolate. Sensory studies were conducted using testing panels for choosing the best coverage. Finally, the finished product underwent additional analyses to quantify major components and to determine the energy content of this new snack.

After solar dehydration, the moisture content in the pulp was 12.72%. Results of proximate analysis showed that pulp was characerized by moisture (13.72%), ash (6.16%), total fat (8.85%), protein (3.85%), dietary fiber (29.26%) and total carbohydrates (38.16%). High methoxyl pectin was 1.35% in hulls and 4.58% in pulp. The caffeine content in the pulp was 0.42%. The total polyphenol resulted in a concentration of 2.09 mg/L. Sensory results concerning the cover (substitutes vs commercial chocolate) indicate a greater acceptance of the formulation using the commercial chocolate. Proximate analysis of the finished product revealed carbohydrates (32.0%), fiber (29.9%), proteins (3.8%) and lipids (22.8%). Finally, a snack portion of 100g will provide 378.3 Kcal.

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OR32

METABOLITE FINGERPRINTING OF *PUNICA GRANATUM* L. (POMEGRANATE) POLYPHENOLS BY MEANS OF HPLC-UV/DAD, ESI-MS AND MS^2

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Punica granatum L., commonly known as pomegranate, is an ancient fruit widely consumed all over the world as fresh fruit or juice. In addition, it is extensively used in therapeutic formulas, cosmetics and food seasonings. The increasing interest in its health-promoting properties is fully justified by the most recent findings, according to which the fruit can be a useful agent for treating a wide range of human disorders and diseases.

Pomegranate juice is used in cosmetics and food for its well-known antioxidant activity. Other properties are documented for pomegranate mesocarp and exocarp extracts, such as anti-inflammatory, antimicrobial and anticancer; these extracts are also able to exert a cardiovascular protective role. The wide range of health-promoting biological activities described above are related to the synergistic presence of a unique and complex phytochemical composition that encompasses anthocyanins, phenolic acids and hydrolyzable tannins.

However, despite the large number of studies on pomegranate chemical composition, there is no validated analytical method that includes the simultaneous determination of all pomegranate constituents. In the light of all the above, the development and validation of a new highly efficient analytical method for the complete metabolite fingerprinting of all pomegranate polyphenols by means HPLC-UV/DAD, ESI-MS and MS^2 was performed in this study for the first time.

While pomegranate juice was directly analysed after simple centrifugation, different extraction techniques, including maceration, heat reflux extraction, ultrasound-assisted extraction and microwave-assisted extraction, were compared in order to obtain a high yield of the target analytes from pomegranate peels. Dynamic maceration with a mixture of water and ethanol 80:20 (v/v) with 0.1% hydrochloric acid as the extraction solvent provided the best result in terms of recovery of pomegranate secondary metabolites.

The quali- and quantitative analysis of pomegranate secondary metabolites was performed by HPLC-UV/DAD, HPLC-ESI-MS and MS^2 analyses. The application of the fused-core column technology allowed us to obtain an improvement of the HPLC performance in comparison with that of conventional particulate stationary phases, thus enabling a good separation of all constituents in a shorter time and with low solvent usage.

The analytical method was completely validated to show compliance with ICH guidelines and then it was successfully applied to the chemical characterization of commercial and experimental pomegranate cultivars, thus demonstrating to be an efficient tool for the fingerprinting of this plant material. The quantitative data collected were submitted to principal component analysis (PCA), in order to highlight the possible presence of pomegranate varieties with high nutraceutical value. From the statistical analysis, four experimental varieties showed a remarkable content of bioactive compounds in the peels, while commercial varieties still represent the best source of healthy juice.

The ability to fully analyze different parts of pomegranate cultivars conform to the ICH guidelines could be useful in the chemical analysis of nutraceutical and food products containing this plant extract. This research showed also the great potential that pomegranate peels have in relation to hydrolysable tannins and could turn the current waste stream of pomegranate peels into a new source of healthy animal food or herbal products.

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TYPIFICATION OF AVOCADO FRUIT (*PERSEA AMERICANA* MILL.) CULTIVATED IN COLOMBIA BY MULTIVARIATE STATISTICAL ANALYSIS WITH A VIEW TOWARD INDUSTRIALIZATION

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In Colombia avocado production is growing and stands it as the third world producer after Mexico and Dominican Republic (FAO Statistical; 2013); commercial crops of avocados *var. guatemalensis*, *var. americana* (known as 'criollos') and hybrids between them are distributed throughout Colombian territory (Perea M. 2010), therefore avocado fruits with different phenotypic characteristics such as shape, size, epicarp color as well as taste and texture can be found. In this study seventeen avocado samples cultivated in various regions of Colombia were included mainly for further information on this fruit. Moreover, we wonder if they present variation on fat content and fatty acid profile to select the best ones for food industry and to industrial oil extraction purposes.

The fruits were weighed and epicarp, mesocarp and endocarp were separated from each other. Mesocarp was vacuum drying and fat content was determinated by Soxhlet extraction. Fatty extracts were derivatized with the complex BF_3/MeOH 14% (AOCS; 1973) and the fatty acid methyl esters were analyzed by GC/MS. Data obtained were processed using multivariate statistical analysis.

Avocado fruits included in this study have weights between 60.1 and 606.8 g. Epicarp proportion is varying from 6.3 to 18.6%; mesocarp proportion from 42.8 to 80.5% and endocarp from 7.8 to 39.2%. Crude fat content is between 5.4 and 28.4% w.b. which correspond to a calorific value from 72.9 to 274.0 kcal/100 g fresh pulp. Cultivars *Guatemalteco*, *Hass* and *Fuerte* had the highest fat content, expressed as percent of fresh pulp (mean ± standard deviation): 27.9% ± 0.5, 25.5% ± 0.1 and 24.8 ± 0.1, respectively. This feature show them as cultivars interesting for industrial oil extraction purposes. Monounsaturated fatty acids- MUFA's- were the major constituents in avocado oil regardless of cultivar (56.8- 83.3%); oleic acid was the principal MUFA with values between 36.6 and 73.2%, followed by palmitoleic acid (4.2- 15.0%). Palmitic (13.2- 32.8%) and stearic (0.5- 1.2%) acids were the saturated fatty acids found in avocado oil. Polyunsaturated fatty acids- PUFA's- as linoleic acid (7.7-16.5%) and linolenic acid (0.4-1.6%) were found. Avocados *var. guatemalensis* had the major concentration of MUFA's (78.3-82.8%) and minor content of saturated fatty acids (13.8-17.5%). Avocados *var. americana* had higher proportions of saturated fatty acids (28.5- 33.7%) and less content of MUFA's (56.8-59.7%). A principal component analysis including the morphological variables and fatty acid profile for each cultivar, shows that the seventeen samples can be typified into four groups. The first group is composed of cultivars with characteristics of avocados *var. guatemalensis.* A second group is integrated by cultivars with features of avocados *var. americana.* The third cluster is located between the above two groups and contains hybrid cultivars of avocado. Cultivar grown in Santa Bárbara- Santander forms the last cluster and presents a particular aniseed smell that is characteristic of avocados *var. drymifolia.*

In conclusion, there is a great variability between different samples included in this study based on their phenotypic features and content of metabolites, which may be result from genetic diversity; therefore four clusters that obey the three avocado races and hybrids of these were formed. This statistical model allows approaches on genetic diversity of cultivars which their genotype are not known; nevertheless, this information must be confirmed by analysis of genetic material.

Acknowledgments: The authors acknowledge the financial support provided by Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas (Contract No. 0459-2013, RIFRUTBIO) and the Department of Chemistry at Universidad Nacional de Colombia- Sede Bogotá.

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VALIDATION OF ANALYTICAL METHOD BY HPLC-DAD FOR IDENTIFICATION AND QUANTIFICATION OF CAFFEOYLQUINIC ACID DERIVATIVES IN *CYNARA SCOLYMUS L.* TABLETS AND CAPSULES

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Cynara scolymus L. leaves, popularly known as artichoke, have cholagogue and choleretics properties and are used for the treatment of digestive disorders. Its effects are attributed to caffeoylquinic acid derivatives. This study aimed at validating the analytical method for determination of caffeoylquinic acid derivatives in artichoke tablets and capsules by HPLC-DAD.
It was used a C18 column (250 x 4.6 mm, 5 um) with temperature maintained at 40 °C, flow rate 1.2 mL/min, injection volume of 25 µL and detection at 330 nm. The mobile phase was composed by a gradient of phosphoric acid 0.5% and phosphoric acid 0.5% in acetonitrile. Samples were prepared at the concentration of 4 mg/mL in a mixture of methanol and water (3:7), using not less than 20 tablets and accurately weighing the quantity equivalent to the labeled amount of artichoke extract. The method was adapted from European Pharmacopoeia - 7th edition to the finished product and inclusion of markers cryptochlorogenic, neochlorogenic, isochlorogenic acids and cynarin. Method selectivity evaluation was carried out comparing three samples: one without hydrolysis induction, one with acidic hydrolysis, and the other with basic hydrolysis. For precision analysis, six samples at 100% concentration were analyzed in the same day. Linearity of standard compounds was performed by three analytic curves with six concentrations for each compound. From the linearity curve it was possible to calculate the quantification and detection limits. The accuracy evaluation was carried out through the standard addition method at concentrations of 80, 100 and 120% with three replicates. Finally, 3 commercial products of artichoke tablets or capsules were evaluated to determine the caffeoylquinic acid derivatives contents.
The method proved to be selective, precise and accurate. Furthermore, the standard compounds showed linearity in the concentration range from 1 to 50 µg/mL. Detection and quantification limits of standards compounds ranged 0.20 to 1.05 and 0.68 to 3.48 µg/mL, respectively. Commercial products A, B and C had different labeled amount of artichoke extract (200, 335 and 300 mg, respectively) and different concentrations of standard compounds per mg of extract. Brand B was the only one that presented cynarin on its profile and got significantly higher concentrations ($p < 0.005$ at one-way ANOVA followed by tukey) of neochlorogenic, chlorogenic and isochlorogenic A and C acids in relation to other commercial products. Moreover, brand B had the highest total content of caffeoylquinic acid derivatives (26.73 µg/mg) followed by C (9.32 µg/mg) and A (8.47 µg/mg). Such differences may bring a significant impact on therapeutic outcome. In conclusion, the method was suitable for the identification and quantification of caffeoylquinic acid derivatives in tablets and capsules, which could be used in quality control, ensuring the safety and efficacy of herbal medicine.
Acknowledgement: FAPDF, CAPES, CNPq, FINATEC and DPP - University of Brasilia.

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ESSENTIAL OILS: PHYTOCHEMICAL COMPOSITION, CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY

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Essential oils (EOs) are very complex mixtures composed of natural volatile constituents. They are produced by aromatic plant specialized glands as defensive secondary metabolites [1, 2]. EOs are well known from the Middle Ages and can be obtained in most cases by steam or hydro-distillation [1, 2]. Nowadays, the use of these phytocomplexes is increasing both in ethnomedicine and in companies involved in the pharmaceutical, agronomic, food and cosmetic fields. Due to the EO complexity, deep qualitative and quantitative investigations are necessary to explore their composition, to guarantee their genuineness and to allow a rational investigation about their potential biological activities. In the light of all the above, this research project was focused on the development of highly efficient methods to study the phytochemical composition of several EOs.

All the EOs were analysed both on Agilent gaschromatograph coupled with mass spectrometer (GC-MS) and Agilent gaschromatograph coupled with flame ionization detector (GC-FID), for qualitative and semi-quantitative purposes, respectively. Compounds were separated on an Agilent Technologies HP-5 cross-linked 5% diphenyl-95% dimethyl polysiloxane (30 m x 0.32 mm i.d., 0.25 mm film thickness) capillary column. The elution was obtained under programmed-temperature. Helium was used as the carrier gas. Compounds were identified by comparison of their linear retention indices (LRI) relative to C_8-C_{40} *n*-alkanes obtained on the HP-5 column with those provided in the literature[3]. Furthermore, the identification of the several constituents has been carried out by comparison of their mass spectra with those recorded in the National Institute of Standards and Technology (NIST version 2.0d, 2005) and, when it was necessary, the identification was carried out by co-injection of available reference compounds. Relative percentage amount of individual components was expressed as the percent peak area relative to the total composition of the essential oil obtained by the GC-FID analysis. Semi-quantitative data were acquired as the mean of triplicate analyses for each sample.

The study was focused on EOs obtained from plants cultivated in Italy or in other countries, with particular attention to *Lavandula angustifolia* P. Miller, *Lavandula x intermedia* Emeric ex Loisel., *Thymus vulgaris* L. and *Cedrelopsis grevei* H. Baillon.

The chemical characterization developed in this work had a key role in classification, selection and explanation of the possible biological activity of the each EO, which was evaluated in collaboration with other institutions, on cell lines including A549, Caco-2, Hep-2 and WKD. Moreover, EOs were tested on several microorganisms such as, *Candida albicans, Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus mutans*.

The study of EOs composition and the biological properties highlighted interesting correlations between the analytes and the bioactivity. As demonstrated by the results obtained, the development and application of analytical methods to study the EOs composition is highly recommended to ensure their genuineness and therapeutic efficacy. Finally, the achieved results might be a valuable contribution in the selection of effective phytocomplexes and molecules to fight microbial infections, as highlighted by WHO.

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DETERMINATION OF SQUALENE IN E.V.O.O. BY DIFFERENT ANALYTICAL TECHNIQUES

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Squalene (SQ) is an isoprenoid compound with 30 carbon atoms, containing six double bonds and it is present mainly in the oil of cod liver. For the first time it was isolated from the oil liver shark, but is widely distributed in nature in both the vegetable and animal tissues (Tsujimoto, 1916). In humans, about 60% of SQ is absorbed from food and main source is olive oil, particularly extra virgin olive oil (E.V.O.O.) which contains about 0.2-0.7% (Smitth, 2000). Many studies report different beneficial properties of SQ such as: a) antioxidant effect; b) protective effect against skin photo-oxidation, c) ability to boost the immune system, d) aptitude to adjust the amount of carrier proteins of LDL fat (Kelly, 1999; Nicolaides et al., 1968).

One of the first studies related to the SQ determination involved a colorimetric method (Rothblat et al., 1962); generally SQ in foods, oils and fats was determined by titrimetric (AOAC, 1999) or chromatographic procedures (HPLC, GC, hypenated chromatographic techniques) (Popa et al, 2015). The purpose of this study was to compare three different analytical techniques (UPLC-DAD, GC-MS and NMR) for the SQ quantification in EVOOs.

A fast UPLC-DAD method was used for SQ determination in 33 biological EVOO samples. Analytical sample treatment included the clean-up purification by using Discovery SPE DSC-Si Silice Tubes. The SQ analysis was carried out using an *Acquity UPLC® Waters BEH C18* column of 1.7 μm (2.1 x 50 mm).

The GC-MS analysis were carried out on samples previously subjected to chromatographic purification on column packed with glass wool and deactivated silica. The mass spectrometer was used in full scan and in SIM mode for the identification of the characteristic fragments.

^{1}H NMR analysis required the simple dilution of 125 μl of sample in 475 μl di $CDCl_3$. Standard ^{1}H spectra were run at 500 MHz with 70° pulses repeated for 64 transients, 2 sec as acquisition, and 2 sec of relaxation time (about 10 minutes of experimental time).

The UPLC method has proved useful and versatile, the sample preparation was found to be relatively fast (about 30 min) and the chromatographic run took only about 3 min.

The GC method showed a good sensitivity and reproducibility, however this technique provided for long sample preparation time and long time of analysis.

The NMR, on the other hand, showed that in about 15 min, including sampling, integration and quantification, turns out to have a good precision and accuracy.

Statistic analysis of the data showed that all EVOOs are grouped by country of origin and that the Sicilians have more SQ than the samples of other countries.

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OR37

CHEMICAL STUDIES ON *PSIDIUM FRIEDRICHSTHALIANUM* NIED. FRUIT FLAVOUR AND THEIR PRECURSORS

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The Myrtaceae family comprises woody trees and shrubs containing a variety of oils which release characteristic odours. Centres of diversity for the family are the west tropics (mainly South America), Australia, and tropical Asia. There are four genera of interest which produce edible fruit: *Psidium*, *Eugenia*, *Syzygium,* and *Feijoa*. Among them, "guayaba agria" or sour guava (*Psidium friedrichsthalianum* Nied.) grows naturally from southern Mexico to northern South America. The fruit is consumed fresh (pulp) or it is used to prepared juices and sorbets, and exhibits a pleasant and intense aroma. Until now, there is only one study that reports the volatile composition of this fruit [1], however no any sensory analysis was performed before to identify the odour-active volatiles.

The odour-active volatiles of whole fruits were isolated by Solvent Assisted Flavour Evaporation (SAFE) of dichloromethane extract. GC-O and GCMS analyses allow identifying all volatile organic compounds relevant to the flavour of this fruit, by comparison with standards. A thiol-rich fraction was obtained by affinity chromatography on mercurated agarose gel [2]. The glycosidic-rich fraction was isolated by selective adsorption on Amberlite XAD-4 glass column and methanol elution [3]. Glycosidically bound volatiles were released by enzymatic hydrolysis with a β-glucosidase (emulsin, Sigma-Aldrich) and analysed also by GCMS. Based on the presence of sulphur volatile compounds, the cysteine-S-conjugates were isolated by using a Dowex 50WX8 resin and eluting with ammonia [4]. The identity of glucosides and cysteine-S-conjugates were elucidated from their analyses by HPLC-ESI/MS.

GC-O and GC-MS analyses of *P. friedrichsthalianum* fruit SAFE extract allow identifying ethyl butanoate, (Z)-3-hexenal, and ethyl hexanoate as key aroma compounds of this fruit. Other relevant odorants to the overall aroma were the sulphur compounds: dimethyl disulfide, 2-methyl 1,3-dithiolane, methional, 3-sulfanylhexyl acetate, 3-sulfanyl-1-hexanol, and bis(methylthio)methane, as well as β-dodecalactone. Sulphur compounds were reported here for first time as odour-active volatiles in this fruit and based on sensory experiments, they could be considered the responsible for the characteristic flavour of this fruit. Hexyl β-D-glucopyranoside was identified as aroma precursor after HPLC-ESI/MS analyses of the glycosidic mixture and GCMS analyses of enzymatically released volatiles with a glucosidase. The role of hexyl-β-D-glucopyranoside and 3-(1-hexanol)-*L*-cysteine as precursors of hexanol, and 3-sulfanyl hexanol, respectively, was demonstrated by enzymatic hydrolysis of corresponding precursor mixtures. In conclusion, the relevance of aliphatic esters, C_6-compounds, and sulphur compounds as odour-active volatiles of *P. friedrichsthalianum* fruit was demonstrated in this study through the correlation of analytical data and sensory analyses.

Acknowledgments: Authors greatly appreciate the financial support provided by Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, contract No. 0459 – 2013, Red Nacional para la Bioprospección de Frutas Tropicales-RIFRUTBIO.

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FLAVONOIDS AS A SCAFFOLD FOR THE DEVELOPMENT OF ANTITRYPANOSOMATIDIC AGENTS

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Parasites of the family of Trypanosomatidae are agents of serious human diseases, including African sleeping sickness, Chagas disease and Leishmaniasis. Since drugs currently in use have limitations in terms of safety, efficacy and duration of treatment, there is an urgent requirement for new effective drugs[1]. The folate pathway is a successful target for the treatment of bacterial infections and some parasitic diseases (e.g. malaria). However, the classical inhibitors of dihydrofolate reductase (DHFR) are ineffective against Leishmania and Trypanosoma because Pteridin Reductase 1 (PTR1), a trypanosomatidic enzyme, overlaps DHFR activity[2-3]. Therefore, PTR1 is a promising target for the development of improved therapies.

Computational studies were performed to screen a library of 98 natural compounds from plants of different origins. We combined target-based screening on pteridine reductase 1 with a phenotypic screening on Trypanosoma brucei, for hit identification. Flavonoids turned out to be an interesting class to be explored as PTR1 inhibitors. Even though flavonoids have been widely explored and often pleiotropic properties can be observed leading to promiscuous inhibition, they represent an interesting starting point for drug discovery in trypanosomatidic infections. Aiming to explore the flavonol scaffold, 16 compounds were synthesized and biologically evaluated. Four X-ray crystallographic structures were solved and, together with docking analysis, allowed SAR elucidation. Twelve compounds showed EC50 values against T. brucei below 10 μM. A wide panel of in vitro toxicity properties were evaluated to guide compounds selection. Compound **2** (3,6-dihydroxy-2-(3-hydroxyphenyl)-4H-chromen-4-one) was selected for pharmacokinetic studies. Encapsulation of compound **2** in PLGA or cyclodextrins resulted in lower toxicity when compared to the free compound. Combination studies with methotrexate revealed that compound **13** (3-hydroxy-6-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one) has the highest synergistic effect and a dose reduction index at the EC50 value of 14.6. Our results suggest that, using an appropriate chemical and delivery strategy, flavonoids may be promising leads for drug development against trypanosomatidic diseases.

Acknowledgments: This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 603240 (NMTrypI - New Medicine for Trypanosomatidic Infections).

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SYNTHESIS AND EVALUATION LEISHMANICIDAL OF ANALOGUES *ENT*-BEYER-15-EN-18-OL AND ISOESTEVIOL.

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Leishmaniasis (or leishmaniasis) is a set of zoonotic and anthroponotic diseases caused by protozoa of the genus Leishmania. It occurs mainly in three forms: leishmaniasis visceral (LV), cutaneous (LC) and mucocutaneous (LM). It is estimated that each year about 1.3 million infected people, being deadly to near 25,000 [1].

In the search for new compounds against the aforementioned parasite, two isomers at C-4 of *ent*-Beyer-15-en-18-ol Figure 1) isolated from the plant *Baccharis tola* [2] showed high activity, *in vitro*, against amastigotes of Leishmania panamensis (LC), with 80% inhibition at 4.6 ug/ml.

These compounds are present in very low percentages in the plant, with the disadvantage of not being available in Colombia.

Stevioside and rebaudiosides are widely used as non-caloric sweeteners [3], which are ideal for obtaining *ent*-Beyer-15-en-18-ol in important quantities (Fig. 1), allowing tests to animal scale and facilitates the generation of good amount of synthetic derivatives [4,5].

Figure 1. Synthesis of *ent*-Beyer-15-en-18-ol from stevioside.

Various derivatives showed increased activity than the starting compound. especially those who retained the hydroxyl at C-1

Acknowledgments: Rodriguez, G. was sponsored by Colciencias-COLOMBIA (project No 111,565,843,059, contract 618-2014)

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OR40

GANGLIOSIDES N-ACETYL AND N-GLICOLYL GM3. STRATEGY TO SUPPORT THE VSSP PLATFORM IN VACCINES AGAINST CANCER.

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For several years when the production of the gangliosides began in the CQB starting from erythrocytes, it was revealed that the yields obtained by this procedure were very low to be able to satisfy the demands required in medical practice due to the low concentration of gangliosides in erythrocytes. A strategy was reported for the production of the gangliosides N-acetyl and N-glicolyl GM_3 in order to guarantee the VSSP platform in the development of vaccines against cancer to be used in clinical trials. Furthermore, the socioeconomic importance that these vaccines have for the country demands high capacity to produce these gangliosides. Although it was possible to scale up to 50L the extraction process starting from mammalian erythrocytes, the obtained average yield of N-AcGM_3 and N-GcGM_3 would not be able to satisfy the possible demand, especially if the technical capabilities of the infrastructures and the delays that may occur during the process of productive extractive-purification have to be taken into consideration. Additionally, big international regulatory restrictions exist on the use of components of animal origin, thus having a strong impact on medicinal drug policy making. The different steps starting from the extraction procedure to the chemical synthesis were described as well as the characterization physical-chemical characterization and the chemical-pharmacieutical evaluation allowing to establish quality specifications. Moreover, a physico-chemical comparability of natural vs synthetic compounds produced, the limitations that the proposed process may have to become a scalable procedure, the scientific state of the art and the technological solutions were also investigated. In 2009 the procedure of the laboratory scale up was proposed and the development of the N-AcGM3 was prioritized in 2010 due to the expectations that in Europe vaccines containing these components were going to be registered. The production of the synthetic N-AcGM3 began in 2011 and subsequently in 2012 a process was developed able to triplicate produt yield. Since then, dozens of lots of the synthetic N-AcGM3 were produced allowing to perform comparability studies and to carry out pharmaceutical developments, biological evaluations and clinical evaluations.

OR41

EFFECT OF SECONDARY METABOLITES OF CANELO BARK FRACTIONS (*DRIMYS WINTERI FORST*) ON THE VIABILITY OF *HELICOBACTER PYLORI*.

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In Chile the seroprevalence of *Helicobacter pylori* is bigger than 75% in children under 5 years and reaches 50% in lower socioeconomic class and 15% in the middle class. (Figueroa et al., 1993) This high infection at infantile level, perhaps explains why this country has one of the highest rate of gastric cancer in the world. (FONTEC No. 94-0356, August, 1996.) H. pylori eradication requires the use of at least two antibiotics, bismuth or associated to an antisecretor, a treatment that is massively used in almost all patients, but showing discomfort when swallowing, liver damage and other effects (Medical Journal of Chile, 2000) .For this reason the effects of a natural extract of Canelo were investigated *(Drimys winteri Forst*) on the viability of the pathogen *in vitro.*

An extract of 183 grs. Was prepared of dried and ground bark of Canelo and one liter of 96% ethanol. It rested 48 hours, obtaining 3.34 grs. Of a foamy material and semi cristallized in a rotary evaporator. Soluble fractions were then removed in a silica gel chromatography column, with mixtures of increasing polarity hexane etilo. Chromatography was made in a thin layer of resulting 150 fractions, which were revealed with iodine and analyzed with ultraviolet light. Some initial, intermediate and final fractions for biological testing and analysis by NMR protons. For selected biological tests, *Helicobacter pylori* was cultured in agar TSA supplemented with horse serum, supplement Dent and Vitox, both laboratory used Oxoid Limited. 6.51×10^9 bac/ml in special conditions of pressure, temperature and humidity, in a growth chamber for 48 hours and subsequently measured and tabulated results. It was used 10 µl of compounds dissolved in DMSO, different concentration on planting.

The fractions of a higher biological activity were those intermediate fractions whose NMR spectrum contained a predominance of signals corresponding to aliphatic chains as odoriferous substances such as Polygodial, Isotadeonal and structures derived from Drimano. Other intermediate fractions showed the presence of flavonoids, with clear signs of similar spectrum Safrole and presence of α- glycoside. All these fractions concentrated in 80 µg/ ml in vitro biological tests showed halos of inhibition of bacterial growth from 2.2 cm. The final fractions gave as result aliphatic chains compatible with Sesquiterpenes probably derivativated from Drimano without double bonds and without oxygen functions, which in the biological tests in concentration of 80 µg / ml. presented great and immeasurable growth inhibition bacterial hales. It could be concluded that all intermediate and final fractions obtained containing flavonoids and Drimanos, had bactericidal effect on the pathogen in vitro, showing halos of inhibition of bacterial growth, higher than those obtained with Gentamicin used as a bactericide control. It can be concluded that Canelo bark contains Flavonoids and Drimanos that in vitro bactericidal, act as phytodrug on Helicobacter pylori, microorganism that is responsible of duodenal ulcers and gastric cancer in humans.

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OR42

ESTERS OF FATTY ACIDS AS ALTERNATIVES OR ADJUVANTS OF ANTIBIOTICS IN ANIMAL FEED

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The widespread use of antibiotics in the animal feeding industry is causing great concern for the increasing occurrence of antibiotic resistance. Natural substances such as essential oils, organic acids and short chain fatty acids may constitute an alternative to antibiotics, since they can exert antimicrobial and antioxidant activities (Desbois and Smith, 2010). In this study, the antimicrobial activity of a commercial blend of short chain fatty acids and their esters (BL), was evaluated.

BL composition was investigated by GC-MS. Its antimicrobial activity against pathogenic strains of *Escherichia coli*, *Salmonella enterica* subsp. *enterica* Typhimurium, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus uberis* was tested by Kirby-Bauer method (Fekete et al., 1994) and was compared with 4 antibiotics commonly added to animal feeds: doxycycline hyclate (DH), lincomycin hydrochloride (LH), chlortetracycline with sulfadimethoxine (CS), and amoxicillin with colistin sulphate (ACS). Furthermore, the synergic effect of BL and ACS in different amounts was evaluated by Kirby-Bauer test.

The composition of BL, analized by GC-MS, was a mixture of mono-glycerides of butyric acid (C4), lauric acid (C12); caprylic acid (C8) and capric acid (C10). The activity of BL was globally comparable with the one of antibiotics, with the only exception of ACS, that showed a higher inhibitory efficacy. Different mixtures of BL and ACS showed higher antimicrobial activity than ACS itself, suggesting a synergy between traditional antibiotics and different antimicrobial compounds.

The utilization of BL or similar formulations could constitute a valid alternative to antibiotics and address a double goal of prevention and therapy: its administration in animal feed could reduce the possibility of bacterial infection in livestock overcoming the concerns about the development of antibiotic resistance, and a synergic mix with appropriate antibiotics used in therapy could enhance the effectiveness of the treatment.

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OR43

EFFECT OF A MIXTURE OF ALLERGENS AS ORAL IMMUNOTHERAPY ON A MURINE MODEL OF ALLERGEN INDUCED ASTHMA

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Worldwide 235 million people suffer from asthma and a constant increase in the global prevalence, morbidity, mortality, and economic burden associated with asthma is being observed [1,2]. Asthma is clinically characterized by bronchial hyperreactivity and symptoms of airway obstruction [3]. The global burden of allergic diseases demands effective disease prevention strategies and in recent years, promising studies have emerged regarding oral immunotherapy (OIT). A novel formulation obtained from a mixture of four allergens: *Candida albicans, Staphylococcus aureus, Staphylococcus epidermis* and *Dermatophagoides pteronyssinus* at fixed concentration has been developed [4]. In order to explore its efficacy the aim of the present work was to evaluate the effect of the oral consumption of this allergen mixture on MDA and protein carbonyl content, and histology changes on lung tissue in a murine model of allergen induced asthma.

BALB/c female mice (7-8 weeks old) were sensitized) intraperitoneally on day 0 and 7 with 20 µg of ovalbumin emulsified in 0.2 ml of sterile phosphate buffered saline containing 2 mg of aluminum hydroxide. On days 14, 16, 18, 20 and 22 after initial sensitization, mice were air challenged by exposure for 20 min to nebulized 1% dust mite (*Dermatophagoides pteronyssinus*) or 0.1% Californian Pepper tree pollen (*Schinus molle*) delivered via a nebulizer. The allergen mixture was administered at 1mg/kg dose from day 23 to 28. Another air challenge was done from day 29 to 37 [5]. On day 38 animals were divided in two groups: One group was euthanized with xylazine /ketamine mixture an lung was extracted for MDA formation and protein carbonyl content. The other group was anesthetized with xylazine and ketamine (50 and 10 mg/kg, respectively) and transcardial perfusion fixation was performed for histopathology analysis.

Allergen mixture treated mice showed a decrease in MDA formation and protein carbonyl content compared with the untreated mice. Histopathology indicate a protective effect of allergen mixture in inflammation and mucus hyper-secretion due to goblet cell hyperplasia. Our study shows significant protective efficacy of allergen mixture against oxidative stress and inflammation during asthma and provides evidence that use of the allergen mixture can help in better management of inflammatory lung disorders.

Acknowledgments: This work was supported by the Instituto Politécnico Nacional, COFAA and CONACyT .

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OR44

HYPOGLYCEMIC ACTIVITY OF *IN VITRO* PLANTS OF *AZADIRACHTA INDICA*

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Azadirachta indica is multipurpose plant that has been used in traditional medicine, because of its high content of active principles mainly tetranortriterpenoids, which are known for their therapeutic potential were screened for pancreatic α-amylase inhibition, a known anti-diabetic target (Ponnusamy *et al.*, 2015). In México its used to treat diabetes *mellitus* (DM), the second cause of death according to the National Institute of Statistics and Geography 2014. The aim of the present work was to assess for their hypoglycemic activity extracts of different polarity from *in vitro* and wild plant of *A. indica*. Biological activity are related to the chemical profile of the extracts.

For evaluate the hypoglycemic activity of *in vitro* and wild plant's extracts (hexane, ethyl acetate, methanol and aqueous) of *A. indica,* we used normoglycemic and diabetic (glucose levels >120mg/dl) CD-1 mice. The DM type II was induced intraperitoneally with STZ (40 mg/kg) after intraperitoneal injection of nicotinamide (68 mg/kg) (Masiello *et al*., 1998). Extracts from *A. indica* and glybenclamide were administered at dose of 200 mg/kg and 600 µg/kg, respectively (Akinola *et al*., 2010) administration was orally every 24 hours for seven days. The extracts were further subjected to phytochemical studies (Chaovanalikit & Wrolstad, 2004; Dewanto *et al*., 2002; Capataz *et al.,* 2007; Dai *et al* 1999)

The oral administration of *A. indica* extracts at dose of 200 mg/kg for seven days produced a hypoglycemic effect. Hexane extract of *in vitro* plant was which showed greater decrease in blood glucose (44.19%), decreasing by 20.72% more than glybenclamide while aqueous and methanol extract from wild plant were exerted the highest effect (33.6 % and 26.8%). The chemical profile by TLC of hexane extract of *in vitro* plant showed the presence of two diferents bands (maybe salannin, nimbidin) in comparison to TLC of the other *in vitro* extract; though its total secondary metabolites content less compared with the other extracts. Extracts *in vitro* plant *A. indica* were showed greater decrease in blood glucose that wild plant extracts. *In vitro* propagated plants of *A. indica* are an alternative source of hypoglycemic compound to wild growing plants. This activity were associates with its traditional use for diabetes treatment.

Acknowledgments: Financial support from CONACyT (grant 18395). Méndez-Sánchez is indebted to CONACyT for fellowship awarded (N° 22133).

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OR45

BIOLOGICAL ASSESSMENT OF *PISTACIA LENTISCUS* EXTRACTS: PHYTOPHARMACOLOGICAL PROPERTIES

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Pistacia lentiscus is an evergreen shrub belonging to the *Anacardiaceae* family, and particularly abundant in the Mediterranean area, including the Iberian Peninsula, Greece, North Africa and the Near East. Both, the aerial parts and the resin of this shrub have been widely used in ethnomedicine for the treatment of cough, eczema, stomach-ache, icterus, diabetes, or respiratory diseases, among others [1,2]. Numerous studies indicate, for instance, that the resin exhibits important antioxidant, anti-inflammatory, hypolipidemic and anticancer activities [3].

In this communication we present the obtention of aqueous and organic extracts from *Pistacia lentiscus* leaves *via* a lixiviation process and their biological assessment. In particular, their antioxidant profile, their capacity for inhibiting glycosidases and acetylcholinesterase, as well as the *in vitro* antiproliferative activity against a series of human solid tumors have been analyzed.

The aqueous extract turned out to be a potent inhibitor of α-glucosidase, and acetylcholinesterase, so it could be useful in the treatment of type 2 diabetes and Alzheimer's disease, respectively.

Moreover, both, the aqueous and organic extracts exhibited a good antiproliferative activity against the tested cell lines.

Acknowledgments: We thank the Dirección General de Investigación (CTQ2011-28417-C02-01, CEI10/00018), Junta de Andalucía (FQM134, P11-CVI-7427), Instituto de Salud Carlos III (PI11/00840), FEDER funds, 7thI EU Framework Programme (FP7-REGPOT-2012-CT2012-31637-IMBRAIN) for the financial support.

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DO YOU REALLY THINK ANTIOXIDANTS ARE EFFECTIVES AND SAFE?

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In the last decade a great nutritional revolution has been carried out in the world, not only by efforts to reduce caloric food but also by the added advantage to prevent or relief several diseases, through ingestion of secondary metabolites. One of the most important groups of this class of compounds are the Antioxidants (Fang et al, 2002). Cardiovascular sickness in addition to neurodegenerative, gastrointestinal, immunologic diseases and of course sexual difficulties and aging are susceptible to be treated with these compounds (Peluso et al., 2015). On the other hands, several commercial antioxidants are considered as safe and display structural analogies to the experimental and natural antioxidants, although people think that these substances are carcinogenic and their consumption has already been banned (Carocho et al., 2013).

A concise search of Antioxidant Effects in the last five years was conducted in a great diversity of scientific journals, including those of Chemistry, Biology, Biochemistry, Pharmacy, Nutrition and Medicine. Later on, data obtained and experiments were organized in several groups (see below)

Results and Experimental sections of each paper were compared accordingly to principles of biology, chemistry and biochemistry; several major problems were detected, as follows (Baur et al., 2006, Lewandowska et al., 2013; Schaich et al., 2015):

- A low bioavailability with a fast hepatic metabolism
- An unknown and specific target
- Absence of a rational therapeutic scheme
- The lack of a confident and reliable mechanism of action
- A lot of chemical assays, not ever extrapolated
- Ignorance of chemical reaction course with a radical, a metal, an anion or a cation

Recently more evidences have indicated an additional risk from antioxidants including cancer, since many molecules originally classified as antioxidants possess a high prooxidant capability (Nimse et al., 2015, Perera et al., 2011) Then the inappropriate use of antioxidant could be dangerous for human health, since several biochemical reactions involve a radical mechanism, e.g. prostanoid biosynthesis, oxygen transport and erection mechanism, among others. Finally and after more than 20 years of antioxidant expectative, at the end of the conclusion section there are frequent additional explanations about doubts of the results and the need for more studies as well as precise *in vivo* investigations to clarify the real effect to prevent or cure some human diseases.

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CYTOTOXIC ALKENYLPHENOLS FROM *PIPER ERIOPODON* (PIPERACAE)

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According to the World Health Organization, cancer disease is one of the leading causes of death worldwide and in 2030, the number of people with cancer will reach 26.4 millions in the whole world. Natural products have been the most representative sources of small molecules for cancer therapy and provided enormous contributions to new drug discovery and mechanisms of action. Moreover, many developing countries are still using traditional medicines due to their low cost and limited access to pharmaceutical drugs. The ethnobotanical knowledge represents threfore the basis for the discovery of new active principles. In fact, the interest on simple phenolic structures has been renewed because of the antioxidant, anti-inflammatory, anti-estrogenic, anti-mutagenic and anti-carcinogenic effects of these compounds both *in vitro* and *in vivo*. In Colombia many species are traditionally used by local healers and it is necessary to link chemical compositions to biological effects. Phytochemical reviews on the genus *Piper* have shown that it contains high amount of phenolic compounds such as flavonoids, lignans and simple phenols that we have recently demonstrated to exert high cytotoxic activity against cancer cells. The present study aims to investigate the cytotoxic activity of two new compounds isolated from the leaves of *P. eriopodon*, against PC3 (prostate) and A549 (lung) cancer cell lines, by the colorimetric assay of MTT (methyl tetrazolium tiazole). Their structures were elucidated on the basis of spectroscopic analysis of 1D and 2D RMN.

The investigated compounds were obtained using conventional chromatographic techniques and NMR spectra were measured on Bruker Avance spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The human cell lines A549 (lung carcinoma) and PC3 (prostate carcinoma) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with inactivated fetal bovine serum (FBS, 10%), penicillin (100µg/ml) and streptomycin (100 µg/ml) in a humidified atmosphere at 37 °C in 5% CO_2. The solvent used in the colorimetric assay was DMSO (0.2%). Cells were harvested, counted and plated ($1x10^4$ cell/well) in a 96 well microtiter plate. After 24 h, cells were exposed to various concentrations of the two compounds (100, 50, 10, 5, 1, 0.1 and 0.5 µg/mL). Plates were incubated for 48 h at 37 °C in humidified air with 5% CO_2. After the indicated incubation times, 10 µL of MTT solution was added to each well (0.45 mg/mL final concentration) and incubated for 4 h. Finally the medium was removed and 100 µL of saline lysis buffer was added. Cellular viability was determined by the MTT reduction assay and the optical density was measured at 595 nm.

The two new alkenylphenols exhibited considerable antiproliferative activity against A549 and PC3 human cancer cell lines with IC_{50} values between 2.5 and 30.0 µg/mL. The values of the IC_{50} of these compounds are comparable to those of reported to embelin, a quinone that bound selectivity to XIAP protein, inducing apoptosis in cancer cells.

Acknowledgments: Authors acknowledge the financial support provided by the Administrative Department of Science, Technology and Innovation from Colombia and the Department of Chemistry at Universidad Nacional de Colombia- Sede Bogotá.

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CHEMICAL CHARACTERIZATION AND CYTOTOXIC EFFECT OF LEAF ESSENCIAL OIL FROM *LIPPIA GRACILIS* AND *LIPPIA SIDOIDES*

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Essential oils are complex mixture of different compounds that confers to them a wide range of biological properties. Several studies have shown in vitro and in vivo antitumor activity of many essential oils obtained from plants and this activity has been related to the presence of monoterpenes in their composition (Ferraz et al., 2013; Nikolic et al., 2014). Lung cancer is the deadliest type of cancer for both men and women, and the number of deaths each year is increasing. The available therapies are not completely efficient and they show serious side effects. So, development of new therapies is necessary in order to overcome these barriers. In this scenario, the essential oils from plants of *Lippia* species can be feature as a potential source of therapeutic agents for the development of alternative drugs against lung cancer. Thus, we have performed the chemical characterization of essential oil from different chemotypes of *Lippia sidoides* (Cham.) and *Lippia gracilis* (Schauer). Moreover, their cytotoxic effect on lung carcinoma cells A549 was assessed.

The essential oil from leaf of both species was extracted by hydrodistillation using Clevenger equipment (Ehlert et al., 2006). Chemical composition analysis was performed in a gas chromatograph coupled to a mass spectrometer (GC–MS) and quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (FID). The cytotoxic effect of increasing concentrations of essential oils (from 20 to 160 µg/mL) was evaluated by MTT colorimetric assay. To evaluate alterations in morphology, after treatment, cells grown over circular coverslips were, fixed with methanol, stained with hematoxylin–eosin and examined by light microscopy.

GC-MS analysis has shown that both essential oil from *L. gracilis* chemotype 110 (LGEO110) and from *L. sidoides* chemotype 104 (LSEO104) have carvacrol as the main compound. On the other hand, thymol was the most abundant monoterpene found in the composition of *L. gracilis* essential oil chemotype 102 (LGEO102) and in *L. sidoides* essential oil chemotype 106 (LSEO106). Even though all chemotypes evaluated have shown cytotoxic effect on A459 cells, the effect of those rich in thymol, was more pronounced than that rich in carvacrol. The CC50s obtained for LGEO106 and LSEO102 were, respectively, 72.42 and 97.93 µg/mL. For LGEO110 and LSEO104, the CC50s were 158.60 and 150.70 µg/mL, respectively. In fact, it was demonstrated by Melo et al. (2014) that essential oils rich in thymol and the thymol isolated are more effective compared to carvacrol. Morphological changes, including cytoplasm and chromatin condensation and nuclear fragmentation were observed in all chemotypes evaluated in this work. Once more, the effect of thymol-rich essential oils was strongest than carvacrol-rich ones. About 95% of A459 cell treated with 75.0 µg/mL of LGEO106 have shown some morphological change whereas the proportion of changes induced by the same concentration of LGEO110 did not differ from the negative control. The same profile of action was observed between LSEO102 and LSEO104. Thus, we can conclude that the differences observed in the cytotoxic effect of essential oils from different chemotypes of *L. gracilis* and *L. sidoides* might be due, at least in part, to the presence of thymol or carvacrol in their composition. Finally, the morphological changes detected suggest that the cytotoxic activity can be due to essential oil ability in triggering cell death processes by apoptosis.

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COMPARATIVE ASSESSMENT OF THE APOPTOTIC POTENTIAL OF CUBAN AND BRAZILIAN PROPOLIS IN HUMAN LARYNGEAL EPIDERMOID CARCINOMA CELLS

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Propolis has been used in folk medicine for centuries because of its beneficial effects including anticancer properties. Due to significant differences in the chemical composition of samples originating from different geographic and climatic zones, the evaluation of antitumor effects of each type of propolis is crucial. The aim of this study was to compare the cytotoxic mechanism of Cuban red propolis (CP) and Brazilian green propolis (BP) on human laryngeal carcinoma (Hep-2), based on their apoptotic potential.

The apoptotic efficiency of both samples of propolis was confirmed using a series of assays, including cell viability, leakage of lactate dehydrogenase, mitochondrial membrane potential, fluorescence staining and the expression of apoptosis-related genes (p53, caspase-3, bax, bcl-2, bcl-xL, and p21).

Cell viability and cytotoxic assays suggested a dose-dependent toxic effect of CP and BP extracts with possible association of intracellular reactive oxygen species production. Apoptotic potencial was confirmed by decrease of mitochondrial membrane potential and fluorescence staining, revealing that both samples induced cellular apoptosis via activation of p53, caspase-3, bax, p21 signaling, and downregulation of bcl-2 and bcl-xL. Particularly, CP extract showed a higher apoptotic potential than BP extract. Our findings open perspectives to study the main components of each propolis, which may lead to the development of a suitable drug for the treatment of laryngeal cancer

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MASTICADIENONIC AND 3-HYDROXY MASTICADIENONIC ACIDS INHIBIT PROLIFERATION PROSTATE CANCER CELLS *IN VIVO* AND *IN VITRO* BY INDUCING APOPTOSIS.

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Previous studies indicate the importance of triterpenoids as anticancer agents *in vivo*, although their cytotoxicity to human cancer lines is small. Therefore, one form to evaluate some triterpenes as anticancer agents is to assess *in vivo* their anti-tumor effect (Yadav, et al., 2010). Studies by our group showed that masticadienonic (AMD) and 3α hydroxyl masticadienonic (3αAMD) acids, triterpenes isolated from *A. adstrigens*, are the responsible for the anti-inflammatory and cytotoxic activities of this species (Oviedo, et al.,2004). Taking into account above we decided to evaluate *in vivo* the antitumor properties of these triterpenes using nude mice. The cell cycle arrest and induction of apoptosis by AMD y 3αAMD *in vitro* also were determined.

Xenografts were induced by inoculation to 60 nu/nu male mice a suspension of PC-3 cells. Three weeks later the mice were randomly distributed into ten experimental groups with n = 6 each. Two groups were administered with vehicle, the following two with cisplatin 4 mg/kg and the other with AMD and 3αAMD (60, 125, 250 mg/kg) respectively. At the end of the xenograft experiment, the tumors were dissected and fixed in 10% formaldehyde, dehydrated and embedded in paraffin to determined PCNA and Ki67 expression by immunohistochemistry assay. Apoptotic cells were detected in tumor tissues using detection kit Promega® kit (G7131) *in situ*. To determine a possible mechanism of AMD and 3αAMD tumor action, determination of inhibition of proliferation by crystal violet, cell cycle arrest and induction of apoptosis *in vitro* were performed. The expression of p-Akt was determined by western blot using the supernatant of lysed PC-3 cells previously treated with AMD and 3αAMD.

The antitumor activity of AMD and 3AMD was demonstrated by the reduction of tumor growth similar to ci s-platinum (CP) induced by inoculation of PC-3 cells in nude mice. The Ki67 protein is associated with cell proliferation and has important clinical implications in cancer. Ki67 analysis of tissues showed that the groups treated with AMD, 3AMD and CP have fewer proliferating cells and intensity of Ki67 compared with the control group. PCNA protein facilitates and controls DNA replication. Detecting PCNA in tumor tissues showed greatest number of positive cells and intensity brand in the control group compared to the groups treated with AMD and 3α-AMD. Our results showed that AMD and 3AMD induce apoptosis in both tumor tissues and PC-3 cell as well as they arrested the PC-3 cycle cell. Also our results showed that AMD and 3AMD inhibit the activation of Akt in PC-3 cells.

The lethal dose was determined in CD-1 mice resulting 400 mg / kg for AMD and 250 mg / kg for the 3α-AMD.

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THE USEFULNESS OF OREGANO'S PHYTOCOMPLEX CRUDE EXTRACT: NOVEL ANTI-PROLIFERATIVE EFFECTS IN ADRENOCORTICAL TUMOR CELLS

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Oregano (*Origanum vulgare* L.) is a common aromatic plant broadly used in the Mediterranean and Asian Regions. Since ancient times, it has been consumed in the diet as flavor enhancer and used in folk medicine as curative herb treating respiratory diseases, painful menstruation, rheumatoid arthritis, dyspepsia, etc. Recently its role as an anticancer phytocomplex has been suggested in human preclinical models, although a crude extract of oregano has been never evaluated into adrenocortical tumor cell models. This study analyzed the anti-proliferative effects of crude extract of oregano into 2 tumor cell lines, SW13 and H295R.

Main components of oregano were studied by GC/MS analysis. Different procedures evaluated the effects of oregano, testing its properties on cell viability, survival and morphology. Moreover cell death processes and cell cycle were investigated as well as main intracellular pathways modulation.

Our findings demonstrated that the crude extract of oregano may decrease cell viability, vitality and survival of SW13 and H295R cells. In addition, oregano induced cell death through necrotic process and altered cell cycle, producing a decrease in G0/G1 phase and an increase in subG0/G1 phase. Western blot revealed an impact on MAPK and PI3K/Akt pathways, with specific reactivity decrease, affecting p-Erk1/2 and p-Akt. This work underlines the anti-proliferative activity of oregano as a crude extract in tumor cell models (SW13 and H295R cells), but more data are needed to substantiate its potential use in the treatment of adrenocortical neoplasia or as an integrative diet phytocomplex.

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ANTITUMOR EFFECT OF AQUEOUS EXTRACT OF *JATROPHA DIOICA SESSÉ EX CERV* LYMPHOMA L5178Y IN CD_1 / C MICE.

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Cancer is a group of diseases with high incidence nationally and worldwide [1]. In 2008, the mortality rate in Mexico was in the third place. Most of the time, treatments are very expensive and sometimes toxic and inefficient, moreover it is necessary to search for more antitumor options. Natural alternatives, including medicinal plants, may represent an option for the treatment of these pathologic condition. One example is the plant called "Sangre de Drago" (*Jatropha*), that is used as an antitumoral. The aim of this study was to evaluate the antitumor effect of aqueous extract of *Jatropha dioica* in a model of lymphoma L5178Y, in Balb/c mouse.

Jatropa doica was collected in Tezontepec de Aldama, Hidalgo, Mexico. The aqueous extract was prepared as an infusion from the root of the dried plant that was processed and triturated according to the Pharmacopoeia Herbolaria Mexicana [2] (2001). The decoction was tested in three different doses (0.92 mg/Kg, 2.40mg/kg and 4.8 mg/kg) equivalent to one, three and six cups per 70 kg of body weight. For antitumor test, animals were divided into five groups of seven male Balb/c mice 30 g and subjected to an adaptation period of seven days. Into each mouse the tumor L5178Y lymphoma was inoculated intraperitoneally. Survival time and tumor growth (weight) in mice were evaluated using the statistical test analysis of variance (ANOVA).

Results show that medium and high doses of the aqueous extract of *J. dioica* inhibited tumor L5178Y growth in mice balb/c and mice survived more days compared with the control and the vincristine group of animals. The antitumor effect can attributed to the presence of terpenes and cyclic peptides and to the presence of a protein called *curcin* that has been tested in vitro showing effects on the activity of N-glycosidase[3]. Similarly, an antitumor effect was also observed in a model of P-388 lymphocytic leukemia using a lactam *Jatropha macrorhiza* [4]. The group treated with *J. dioica* appeared healthier than the vincristine group.

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HERBAL DRUG INTERACTIONS AND REMEDIES: A CHALLENGING PROBLEM IN THERAPY

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Although herbal medicines are by far the largest component of non-orthodox remedies they do not have exclusive claims; it must be made clear that there are various types of other alternative treatments ranging from preparations of animal origin, to minerals, vitamins and amino acids. It must be appreciated that the quality control over most herbal remedies, which are not officially registered as medicines, is often poor and more likely to be non-existent, and that most herbal preparations are not standardized for potency in biological test systems. As a consequence their potency may vary considerably from sample to sample. Furthermore they may be contaminated or adulterated with pesticides, toxic metals, botanicals, animal substances and/or orthodox drugs which may lead to additional and unexpected adverse drug reactions and interactions.

We reviewed various Researches and review articles for this work and the findings are includes in this article. Generally too little is known about the consequences of such combinations although the clinical reports of interactions that infrequently appear in the medical and pharmaceutical press suggest that many more interactions may be occurring that are not realized as such and are not reported in the literature.

Reducing the risk of drug interactions is a challenge that embraces a number of considerations. The following are guidelines to reduce and manage drug interactions.

Factors such as age, the nature of the patient's medical problem (e.g., impaired renal function), dietary habits, smoking, and problems such as alcoholism influence the effect of certain drugs and should be considered during the initial patient interview.

Acknowledgments: Authors are thankful to Mr.Ramashankar Singh (Chancellor, ITM University), Dr. D. S. Chauhan (Managing Director, ITM University), Prof. (Dr.) Vandana Kushwah (Vice-Chancellor, ITM University) and Dr.Richa Kothari (Head, School of Pharmacy ITM University) for providing financial support and necessary facilities for this work. We are also thankful to all supportive staff of School of Pharmacy, ITM University who were directly or indirectly involved in this work.

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ECHINACEA ANGUSTIFOLIA DC. EXTRACT QUALI-QUANTITATIVE ANALYSIS AND FIBROBLAST CELL GROWTH EVALUATION

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Echinacea angustifolia DC., native from North America, is one of the three *Echinacea* species that are widely used as herbal remedy, mainly for their immunostimulant properties. Chemical analysis of plants in the genus Echinacea has identified several groups of medically important components including polysaccharides, flavonoids, caffeic acid derivatives, essential oils, polyacetylenes, alkylamides (1).

The aim of this study was the quali-quantitative analysis of secondary metabolites in extracts obtained by different extractive techniques, the evaluation of polysaccharides content and *in vitro* cicatrizing activity on fibroblast cell lines.

The extraction of bioactive compounds from dried roots of *E. angustifolia* was performed by different techniques as maceration, ultrasound, microwave and enzymatic-assisted extraction. Temperature and pH were monitored during extraction procedures (2 hours). Quali-quantitative analysis was performed by HPLC-DAD system (2), whereas the soluble polysaccharides content was evaluated by phenol-sulphuric acid method (3). The stimulation of fibroblast cell growth was carried out by wound healing assay.

Solvent extraction consists of liberating plant compounds into a recipient solvent. The choice of the solvent type depends on the solubility of the solute in the solvent and the combination with an extraction process could ensure the maximum yield.

Extracts of *E. angustifolia* roots showed similar qualitative phytochemical profile reporting the presence of caftaric acid, chlorogenic acid, caffeic acid, ferulic acid, chicoric acid, echinacoside, verbascoside, 3,4 and 3,5 di-*O*-caffeoylquinic acid. On the other hand extracts reported significant quantitative differences and the enzymatic-assisted extraction was found to be more efficient to recover high amount of secondary metabolites than other extraction techniques in aqueous solvent. Compounds as 3,4 and 3,5 di-*O*-caffeoylquinic acid were found in traces in almost all extracts (4). Echinacoside, instead, was the main phenolic component in this species. Polysaccharides in Echinacea root were quantified and the extracts also showed quantitative differences, also evident when stimulation of fibroblast cell by *in vitro* assay was performed.

Roots of *Echinacea angustifolia* are an important natural source for chemical-pharmaceutical and food supplement field and enzymatic extraction could be useful to increase the secondary metabolite yield.

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TURNERA DIFFUSA (DAMIANA) AS SOURCE OF HEPATOPROTECTIVE AND HYPOGLUCEMIC COMPOUNDS

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Liver diseases and diabetes are serious health problems worldwide. Mexico is a country with a great biodiversity of plants and a historical tradition of using them. *Turnera diffusa* (damiana) is a shrub native from México with different traditionally uses[1]. Previously we reported the hepatoprotective and hypoglycemic effect of a methanolic extract obtained from this plant[2], as well as its great antioxidant potential[3]. Therefore, based on a bioassay-guided fractionation of a methanolic extract obtained from the aerial part of *T. diffusa*, we present in the present contribution the isolation and identification of the compounds responsible for the pharmacological action, as well as the efforts towards the elaboration of a phytomedicine with this plant.

The extracts were obtained by maceration with MeOH at room temperature and where further purified by solid phase extraction, silica vacuum liquid chromatography, reverse-phase and countercurrent chromatography, to obtain the compounds responsible for the pharmacological action. Purity was determined by HPLC and tlc. Structures were established by NMR, MS and ion trap HPLC/MS. Cytotoxicity and hepatoprotection were assessed in an in vitro model using HepG2 cell line and in an ex vivo model by means of precision-cut hepatic tissue slices. Silibinine was used as positive control. Hypoglycemic activity was evaluated in vivo with normoglycemic CD1 mices. Insulin was used as positive control.

The fractionation procedure yielded a C-glycoside of luteoline which exhibited a four times greater hepatoprotective effect than the widely used hepatoprotective agent silibinin against carbon tetrachloride damage in an *in vitro* model using HepG2 cells. The activity was confirmed using an *ex vivo* model with precision-cut hepatic tissue slices. From another fraction a terpene was isolated and identified, which resulted responsible for the hypoglycemic action of this plant. All these results validate the traditionally uses of the plant, and open insight to further investigations Although these findings are promising, the recuperation of the compounds is low and costs associated for purifications are high; therefore we are trying now to obtain an standardized extract of this plant that could fulfill the requirements of a phytomedicine proposal.

Acknowlegment: Funding SEP-CONACYT 2012 grant No. 180977 and PRODEP: funding 103-5/15/14156

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OR56

ROSSOPURO™: A RED YEAST RICE EXTRACT FOR THE MANAGEMENT OF HYPERCHOLESTEROLEMIA

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Red yeast rice extract is obtained following rice fermentation by *Monascus purpureus*. It has been used for centuries in traditional Chinese medicine as remedy of different diseases related to circulation and digestion. Evidence exists about its efficacy in the control of blood cholesterol levels. In fact, the main product of fermentation is monacolin K, which inhibits the synthesis of cholesterol by inhibition of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, the key enzyme of cholesterol synthesis in human body (1,2). The aim of the current study was to evaluate the efficacy of ROSSOPURO™, our red yeast rice extract rich in active compounds, in improving lipid profile in an animal model of diet-induced hyperlipidemia.

C57BL/6J mice were used for experimental procedures. Animals were divided into 4 groups according to treatment (14 animals/group). 3 groups were fed daily with a high-fat diet for 4 consecutive weeks. After this period, serum lipids were analyzed (total cholesterol and triglycerides) and the animals, maintained with high-fat diet, were treated by gavage for further 4 weeks according to the following scheme: group 1: administered with ROSSOPURO™; group 2: treated with traditional red yeast rice (reference item); group 3: treated with vehicle. Conversely, the negative control group (group 4) were fed with a rodent standard diet and received the vehicle by gavage.

At both the pre-dose (week 4) and the end of the treatment, blood samples were collected from the mandibular vein of all the animals under a light anesthesia, after a fasting period. The blood samples were divided into different test-tubes without any anticoagulant and analyzed by the means of the automatic analyzer VitroVet (SCIL), in order to assess serum lipid profile (total cholesterol, HDL, LDL, triglycerides), glucose, lipase, lactate dehydrogenase, creatine kinase, CRP. The atherogenic index (AI) were calculated by HDL-cholesterol/total cholesterol and LDL-cholesterol/HDL-cholesterol. During all the in vivo study, the animals were monitored for clinical signs and mortality, body weight, food and water consumptions.

Results of the current study showed that ROSSOPURO™ improved lipid profile of animals better than the reference item. These results suggest that our technological approach lead to a bio-enhanced ingredient of dietary supplements and candidate it as a valid alternative or complementary therapy to conventional treatment for the hypercholesterolemia management, and thus have a role in cardiovascular prevention strategies.

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OR57

EFFECTS OF PERALEJO (*CURATELLA AMERICANA* L.) IN THE EXPULSION OF KIDNEY STONES, USING TRADITIONAL METHODS

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At the experimental level, peralejo bark (*Curatella americana* L.) has been used to demonstrate their antihyperglycemic activity in mice, but there are no scientific reports of its use in dissolving of kidney stones. At the level of wayuu pharmacopoeia a single case of use in such activity is reported. The aim of the research was demonstrate through traditional methods the effectiveness of the bark in the expulsion of kidney stones.
It was used bark branches with blackish gray color, 5 to 8 cm wide, washed with clean water and dried 2 days indirect sunlight. The bark was crumbled manually and chips obtained were placed in a glass bottle containing potable alcohol 60° at a rate of 330 g of bark per 1 liter of alcohol, this was kept in a dark place, and stirred for 1 minute daily by 14 days. Then, it proceeded to filter through a clean cloth and the filtered liquid was placed in 20 ml plastic bottle. Because of difficulties in achieving alcohol, this was replaced by artisanal rum of sugar cane (chirrinche) with grades similar to potable alcohol. Through the Health Center and community in general were selected approximately 50 patients with problems of clinical diagnosis of stones in ureters, urethra and kidney chalices level. Clinic patients were also included for treatment after consultation with the urologist. Each patient was given a bottle with the plant dye, prescribing a dose of 20 drops in a tablespoon in the morning and before bedtime until finish 20 ml of the bottle contents.
The outcomes demonstrate the effectiveness of peralejo bark at all levels of renal structure, noting that small stones are dissolved at 5 days of treatment, and patients report urination with sandstone, while patients with larger stones delayed even 2 months from the start of treatment and then expel them. All patients reported, almost immediately, pain decrease caused in the lumbar part by the presence of kidney stones, and after treatment scans show the absence of them.

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OR58

NRF2-MEDIATED ANTIOXIDANT ACTIVITY OF *T.ROSEA* (BERTOL) DC AND *T CHRYSANTHA* (JACQ) G. NICHOLSON EXTRACTS.

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Plants from the genus *Tabebuia* have been used in traditional medicine as anti-inflammatory, anti-carcinogenic and anti-microbial agents, in rural areas of South America. Also, several ethnobotanical and ethnopharmacological studies have shown the therapeutic potential of these plants for the treatment of several human diseases. These studies suggest a potential use of plant biodiversity in order to find new molecules with potential importance in pharmacology and therapeutics (Jiménez-González., et al. 2013). However, most of the studies have been done on *Tabebuia impetiginosa* (Franco Ospina., et al. 2013). The aim of this study was to evaluate *in vitro* the antioxidant activity of the extracts from the inner bark of *Tabebuia rosea* and *Tabebuia chrysantha* mediated by the nuclear factor E2-related factor 2 (Nrf2), using cell lines HEK-293 (Human kidney), HepG2 (human hepatocellular carcinoma) and HeLa (human cervical adenocarcinoma).

The antioxidant activity of extracts (n-hexane, chloroform, ethyl acetate, n-butanol and aqueous) obtained from the inner bark of *T rosea* and *T. chrysantha* was evaluated using the ORAC technique. The effect of extracts on the viability of HEK-293, HepG2 and HeLa cells was determined using the MTT method. To evaluate the protective effect of extracts to the H_2O_2-induced oxidative stress we did use the MTT method. The effect of extracts on the translocation of Nrf2 to the nucleus was evaluated with the Nrf2 transcription factor assay kit (Cayman Chemical Co), according to the manufacturer's instructions. The induction of the antioxidant response genes (*NQO1*, *HO-1* and *MT1A*) was evaluated using real-time PCR with the TaqMan® RNA-to-C_T ^{TM} 1-step kit (Applied Byosistems).

The ethyl acetate extract obtained from the inner bark from both *T. rosea* and *T. chrysantha* displayed the highest ORAC activity (12523 and 6325 μmoles Eq Trolox/g extract, respectively). The evaluation of the expression of antioxidant response genes such as *NQO1* and *HO-1* showed that these extracts have the ability to induce the expression of both genes in HeLa and HEK-293 cell lines, after 6 and 12 hours of exposure to the extracts, whereas in the HepG2 cell line only the *NQO1* gene expression was induced. The extracts also had the ability to activate and translocate Nrf2 to the nucleus after 4 hours of exposure of HepG2 and HeLa cells to the extracts. These results suggest that the ethyl acetate extracts from the inner bark of *T. chrysantha* and *T. rosea* have an important antioxidant activity and may be used as a new source of natural antioxidants

Acknowledgments to: Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contrato RC-0572-2012-Bio-Red-Co-CENIVAM, Vicerrectoría de Investigaciones Innovación y Extensión Universidad Tecnológica de Pereira and Fundación Universitaria Autónoma de las Américas for financial support.

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ANTIOXIDANT ACTIVITY AND APOPTOSIS INDUCTION ASSESSED BY FLOW CYTOMETRY OF A *BACTRIS GUINEENSIS* POLYPHENOL EXTRACT

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Bactris guineensis (Aracaceae), also known in Costa Rica as güiscoyol or uvita, corozo in Honduras, Panamá, Colombia and Venezuela, is a wild palm that grows in the plains. Its fruit has a high polyphenol content (824.4± 77.5 mg GAE/100 g), compared with another fruits like blueberry and plums.

The antioxidant activity of *Bactris guineensis* was reported in literature with the methods DPPH and ORAC (Osorio *et al.* 2011 and Rojano *et al.* 2012), however it is important to correlate it with another assays closer to *in vivo* systems, like the effects on cell culture.

Full ripe fruits were collected in the province of Guanacaste, Costa Rica. The fruit was extracted with acetone/water and then subjected to polyphenol purification using an Amberlite XAD-7 column (150 mm x 20 mm). The column was washed with water, the phenolic compounds were eluted with methanol:water (80:20), concentrate under vacuum (40°C) and freeze dried. The extract was analyzed by UPLC-MS.

Antioxidant activity was assessed by using the DCFDA fluorescent dye. The intracellular ROS production was induced on the Vero cell line with tert-butyl-hydroperoxide (TBHP) in presence and absence of the polyphenol extract. The fluorescence positive cells were determined by flow cytometry and the analysis performed by CellQuest software.

In order to analyze if the observed cytotoxic activity of the polyphenol extract on the colon carcinoma cell line SW 620 (IC_{50}: 16.6□1.3□g/mL) was because of an activation of apoptotic mechanisms, apoptotic cells were quantified using Annexin V-FITC/PI assay and analyzed by flow cytometry.

The polyphenols identified by UPLC-MS in the extract were cyanidin 3-rutinoside, procyanidin B1 and procyanidin 3-rutinoside. The antioxidant effects of the polyphenols inside of cells, using the DCFDA assay, shows that the extract reduced the induced intracellular ROS in a dose-dependent manner, with an IC_{50}: 151.7 □g/mL. Also the antioxidant activity was determined by DPPH (IC_{50}: 3.3 ± 0.2 µg/mL) and ORAC (979.1 µmol TE/g extract).

The cytotoxic effect observed over the SW 620 cell line seems to be happen by apoptosis. We observed 50.3% of cells in early apoptosis and a 17.7% of cells in late apoptosis, after the polyphenol extract treatment. However, further investigation will be needed in order to explain the apoptotic mechanisms involved in its activity.

In conclusion, with its polyphenol content, high antioxidant activity and its cytotoxic effect on colon carcinoma cells, *Bactris guineensis* seems to be a fruit with a high potential as functional food.

Acknowledgments: financial support by Vicerrectoría de Investigación, Universidad de Costa Rica (Project No. 735-B2-659)

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OR60

IN VITRO ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF COLOMBIAN PLANTS AS POTENTIAL NEUROPROTECTIVE AGENTS

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Neurodegenerative diseases comprise a complex and heterogeneous group of central nervous system (CNS) disorders, characterized by the progressive and irreversible loss of neuronal cell populations from specific regions of the brain. The prototypical neurodegenerative disorders include Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). A common feature of these diseases is the extensive evidence of oxidative stress, which might be responsible for the dysfunction or death of neuronal cells that contributes to disease pathogenesis (Barnham et al, 2004). AD is the most prevalent neurodegenerative disorder worldwide and is the major cause of dementia associated with aging. It is a multifactorial disorder with a complex pathogenesis. The large number of biochemical pathways are distinguish by abnormal accumulations of extracellular (β-amyloid plaques) and intracellular (tau) proteins, mitochondrial abnormalities and neuroinflammatory processes; triggering a progressive degeneration of memory and cognitive function. Although the causes and mechanisms of neuronal death in AD are unknown some factors to play an important role, such as the cholinergic hypothesis and oxidative stress. The inhibition of AChE is an important target strategy not only for AD, also for senile dementia, ataxia and PD. Actually, cholinesterase inhibitors are the only approved drugs for treating of AD as Galantamina and Rivastigmina; however, these only improve cognitive ability and have considerable side effects. Consequently, the development for new therapeutically agents more effective and safety is required. In recent years, the research have focused on the search for multitarget agents like a promising option (Rosini et al, 2008). In this work was performed an *in vitro* screening for antioxidant and acetylcholinesterase inhibitory activity of Colombian Lauraceae, Piperaceae, Rutaceae and Myristicaceae species.

The preliminary activity was determinate using an *in vitro* TLC-bioautographic assay using silica gel as stationary phase and two different mobile phases, 92 ethanolic extracts of 49 Colombian species were evaluated. The antioxidant potential was assessed by DPPH• scavenging activity by TLC-bioautographic, the active extracts were evaluated the total antioxidant capacity and the IC_{50} were determined and expressed as VCEAC (mg/L) in comparison with ascorbic acid (Deng et al, 2011). AChE inhibitory activity was carried out by bioautographic assay described previously (Marston et al., 2002). The enzymatic activity of extracts was measured using an adaptation of the colorimetric Ellman method (Ingkaninan et al., 2003).

In the preliminary TLC assay were evaluated 18 Myristicaeae, 32 Lauraceae, 30 Piperaceae and 12 Rutaceae extracts. In DPPH• assay 38 extracts (300 µg) of the total tested showed compounds with high capacity of radical scavenging, the marked active extracts are mainly of the families Lauraceae and Myristicaceae. In most cases these compounds has medium to high polarities, however some Piperaceae extracts exhibit nonpolar compounds with high radical scavenging capacity. In the AChE assay, 15 extracts (300 µg) of 7 species (Lauraceas and Rutaceas) showed compounds with high inhibitory activity. The species of greatest activity against AChE belong to the *genus Zanthoxylum* and *Ocotea* and they are characterized by the presence of alkaloids; these compounds are widely recognized to be AChE inhibitors (Mukherjee et al, 2007). By comparison, to the antioxidant and anticholinesterase activities, it was possible to identify least nine extracts (5 species) which have activity in the both assays, therefore they can be considered promising for rational isolation of potential multitarget therapeutic agents in the treatment of neurodegenerative diseases.

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ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY OF *HAEMATOXYLOM BRASILETTO* KARST

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Traditional medicine includes the use of medicinal plants that have been used for thousands of years by rural and indigenous communities. Many plants contain antioxidant compounds which protect cells from the adverse effects of free radicals and oxidative stress, a cause of cancer (3). *Haematoxylum brasiletto* woody plant, is used in traditional medicine in the state of Guerrero, México for the treatment of: kidney infections, kidney stones and anti-inflammatory agent. (1,2)

In this study it was evaluate the antioxidant activity of extracts and fractions in different concentrations by DPPH method 0.1 mM. The powder of heartwood of *H. brasiletto* (1 kg) was extracted with 96% EtOH for three times to give crude extract (50 g), the extract was then suspended in a mixture of water and MetOH 3:2 and partitioned with hexane, CH_2Cl_2 and EtOAc successively to give fractions HBM-2A (500 mg), HBM-2B (2 g) and HBM-2C (10 g). It was found that the ethanolic extract (IC_{50} = 152,□g/mL ± 0.47) and the organic fraction HBM-2A (IC_{50} = 80,14□g/mL ± 0.25), HBM -2B (IC_{50} = 40,17 □g/mL ± 0.15) and HBM -2C (IC_{50}= 40,05□g/mL ± 0.25) showed an antioxidant capacity significantly higher among extracts and fractions analyzed (p<0.001, □□□0.05). The Trolox was taken as a positive control for antioxidant activity with a high percentage of inhibition of (IC_{50} = 43,9 □g/mL ± 0.41) compared with the results obtained for extracts and fractions of *H. brasiletto*.

Additionally, we evaluated the antiproliferative activity of those fractions by MTT assay on RAW 264.7 and A549 cancer cell lines, using L-929 as a not cancerous cell line. HBM-2B showed a significant inhibitory effect on A549 (22.57±0.86 µg/mL), and RAW 264.7 (23.87 ± 0.84 µg/mL) cells. This fraction exhibited activity against L-929 not cancerous cell (30.89 ± 0.84 µg/mL). It can be concluded that extracts of *Haematoxylum brasiletto*, are important sources of antioxidants with anti-proliferative potential.

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ANTIOXIDANT, HYPOGLYCEMIC AND ANTIMICROBIAL ACTIVITY OF TWO ALGERIAN TANNIN SORGHUMS CRUD EXTRACTS CORRELATED WITH POLYPHENOLS AND TANNIN CONTENTS

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High levels of phenolic acids, procyanidins and anthocyanins have been reported in sorghums. Polyphenols including tannins have been shown to have antioxydant and free radical scavering effects.
Antioxidant activity of 70% acetone extracts of two Algerian tannin sorghum seeds, brown *tannin sorghum* (BTS) and red tannin sorghum (RTS), was examined in relation to its contents in total polyphenols and tannins. Total phenols contents estimated by Folin-Ciocalteu method and tannins contents estimated y HCl- Vanillin method. antioxidant activity. investigated by Free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH*) scavering essay. Hypoglycemic activity of methanol and acetone extracts of red tanin sorghum was examined on rates submitted to alloxane treatment and the antibacterial activity was tested using disc diffusion method.
Total phenol varied between 35.20 ± 2 in BTS extract and 10.34 ± 3mg g^{-1} in RTS extract s. while Tannins contents were between 61.36± 1.3 mg g^{-1} and 25.34 ± 5 mg g^{-1}. The highest radical scavenging effect was observed in BTS with IC_{50} = 5 µg ml^{-1} crud extract. The potency of radical scavenging effect of this sorghum extract was about 5 times greater than synthetic antioxidant Butylated Hydroxy-Toluene (BHT) and two times more than Butylated Hydroxy-Anisole (BHA). In addition, the RTS showed an IC_{50} value of 15 µg ml^{-1} which still more effective than BHT.
Glycemia values of rates treated with acetone extract were significantly lower than those of positive control and those of rates treated with methanol extract. This, probably due to the difference in tannins composition between the two genotypes.
Sorghum extracts showed a high inhibitory effect on *S. epedermidis* and *S. aureus* growth while a lower effect was observed on *E.coli* growth.
The results obtained in the present study indicate that polyphenol and tannin content correlated strongly with their biological activities and that tannin sorghum seeds are a potential source of natural bioactive compounds.

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POSTERS

PP1_01

NUTRACEUTIC EFFECT OF CUETLAS *ARSENURA ARMIDA C* EDIBLE INSECT LOCAL FOOD AT IXCAQUIXTLA, MEXICO

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Entomophagy, insect consumption is a cultural tradition in Mexico since prehispanic time, practice that provides balance nutrients to several ethnic groups in Mexico. Selected species from insects play an important role at festivities and common insects are source of the requested nutrients for a daily diet of population. Ixcaquixtla, semirural village located South East Puebla, State at an arid zone with a low food production has several species of edible insects that local people intake but not as a regular basis, since they have not information regarding the nutraceutic functions these small animals can provide to human health. Nutraceutical term is defined as food or food components that provide health or medical benefits, including prevention or treatment of decease in humans. Cuetlas common name of the larvae stage of a butterfly is one of the insects consumed by local people of Ixcaquixtla. The aim of this research is to chemical analyze macronutrient composition and investigate the nutraceutic benefits in health and inform to local people of them.
Convention sampling was take place at August 2014, near by the village and raw dry insects were analyzed proteins, lipids, minerals, fiber and soluble carbohydrates in dry basis according AOAC (19995) techniques.
Data obtained was: proteins 56:93%; lipids 14.76%; minerals 10.07%; fiber 2.13%; soluble carbohydrates 16.11%. Cuetlas content a good amount of proteins, essential macromolecules for human life, essential fatty acids important sources of fuel for brain cells and in particular for hearth and skeletal muscle, minerals, not determined individually, for metabolism processes, fiber, and soluble carbohydrates for energy.
Cuetlas edible insects for Ixcaquixtla community provide nutraceutic nutrients that benefit and improve local people health.

PP1_02

ARGENTINIAN MEDICINAL PLANTS AGAINST POSTHARVEST PHYTOPATHOGENICS FUNGI ISOLATED FROM ORANGES, STRAWBERRIES AND PEACHES

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Continuous and excessive use of synthetics compounds in order to prevent fungal infection in postharvested fruits has caused dangerous consequences not only to the environment but also to human health. In this context, exploring natural sources based on native plant extracts or compounds isolated from them, turns out to be of great economic and social importance.[1] In this work, three Argentinean native species with previously reported human antifungal properties (*Solidago chilensis* Meyen[2], *Polygonum acuminatum* Kunth[3,4] and *Drimys winterii* Forst[5]), were evaluated against three pathogenic fungi (*Penicilium digitatum*, *Botrytis cinerea* and *Monilinia fructicola*) which strongly affect oranges, strawberries and peaches respectively. *S. chilensis*, *P. acuminatum* and *D. winterii* were collected during the flowering season (March 2014) in Santa Fe province (Argentina) and deposited at the Herbarium FCA-UNL (Arturo Ragonese) with identifying data SF # MD16, MD97 and MD108 respectively. Air-dried aerial parts of each species (100 g) were powdered and successively macerated (3×24 h each) with petroleum ether (H), ethyl acetate (EtOAc) and methanol (MeOH) with mechanical stirring to obtain the corresponding extracts, after filtration and evaporation.

For the antifungal evaluation, standardized strains from National Institute of Agricultural Investigation (INIA) Salto (Uruguay) and National Institute of Agricultural Technology (INTA) San Pedro (Argentina) were used. *P. digitatum* INIA-S22 (*P.d.*), *B. cinerea* INIA-S26 (*B.c.*) and *M. fructicola* INTA-SP345 (*M.f.*) strains were grown on Potato-Dextrose-Agar (PDA) using Petri dishes for 48 h up to 6 days at 15-18 °C (as needed for each fungi growth). Inocula of spore suspensions were obtained according to reported procedures (CLSI)[6] and adjusted to 1 x10^4 Colony Forming Units (CFU)/mL.[1] Minimum Inhibitory Concentrations (MICs) of each plant extract were determined by using broth microdilution techniques according to the guidelines of the CLSI for filamentous fungi (M 38 A2).[6] Imazalil and carbendazim were used as positive controls. Endpoints were defined as the lowest concentration of extract resulting in total inhibition (MIC_{100}) of visual growth compared to the growth in the control wells containing no antifungal.

Table 1: Minimum Inhibitory Concentrations (MICs) (µg/mL) of each plant extract evaluated. I= inactive (MIC > 1000 µg/mL). *P.d: Penicillium digitatum, B.c: Botrytis cinerea, M.f: Monilinia fructicola*

Plant Material	Extract	MICs (µg/mL) *P.d*	*B.c*	*M.f*
S. chilensis (aereal parts)	H	I	1000	125
	AcOEt	I	1000	125
	MeOH	250	500	1000
P. acuminatum (aereal parts)	H	250	250	31.2
	AcOEt	500	500	31.2
	MeOH	I	I	I
D. winterii (bark)	H	500	500	125
	AcOEt	1000	1000	125
	MeOH	I	I	I
Imazalil		7.8	0.9	0.12
Carbendazim		15.6	7.8	0.49

Results are shown in Table 1. Regarding *S. chilensis*, MeOH extract was the most active one, with MICs between 250-1000 µg/mL and showing the highest potency against *P.d.*, H and EtOAc extracts were more active against *M.f.* (MICs= 125 µg/mL) but hardly active against *B.c.* (MICs= 1000 µg/mL). *P. acuminatum* H and EtOAc extracts were moderately active against *P.d.* and *B.c.* (MICs between 250-500 µg/mL) but showed important inhibition for *M.f.* (MICs= 31.2 µg/mL), while MeOH extract resulted inactive. *D. winterii* H and EtOAc extracts were hardly active against *P.d.* and *B.c.* (MICs between 500-1000 µg/mL) but they exhibited activity against *M.f.* (MICs= 125 µg/mL), MeOH extract also turned out inactive.

Acknowledgement: CONICET for MD doctoral fellowship and PIP 11220150100524CO, UNR project BIO411.

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PP1_03

CACAO CO-PRODUCTS: AN ALTERNATIVE TO OBESITY CONTROL

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Overweight and obesity have been highly increasing in the last years, becoming a serious public health problem; among the strategies to control the problem are functional foods therefore different strategies are being sought to control this epidemic, including functional foods. Cacao is rich in flavonoids mainly (-)-epicatechin and fiber, which have been showed therapeutic effects thus, the use of cacao co-products represents an attractive alternative as an adjuvant for handling overweight and obesity. Therefore the aim of the present work was to evaluate the effect of cacao co-products on body weight, lipid profile, blood pressure and hepatic enzymes in a rodent model with obesity induced by diet [1,2].

In this study, Wistar male rats were fed with an obesogenic diet (21% of fat) and 20% fructose solution as consumption water during 5 weeks. After 5 weeks of feeding with the high fat diet, rats were distributed into 4 groups: Control, I, II and III (obesogenic diet plus 141.54, 23.55 and 11.751mg of equivalents of Gallic acid respectively obtained from cacao co-products). After 5 weeks of treatment blood samples were obtained via retro-orbital punctures; blood was allowed to clot for 30 min, centrifuged at 3500 r.p.m. for 15 min, and then the serum was collected and analyzed. Glucose and triglycerides were determined using commercially available colorimetric kits (Randox S.A.s, Mexico). Systolic blood pressure was measured by a non-invasive tail-cuff method before starting the dietary induction period, at week 5 and at study completion [3].

The group that presented an improvement in the cardiometabolic profile was the one that received a total dose of 23.55 mg of cacao co-products phenolic content showing a significant decreased ($p<0.05$) in cholesterol (36%), triglycerides (63%), c-LDL (37%) concentrations, and also in the relation TG/c-HDL (64%) compared to the data obtained in the obesogenic group. No significant changes ($p >0.05$) were observed in the c-HDL concentration. Blood pressure decreased significantly ($p<0.05$) in a 27% and also the hepatic enzymes decreased their concentration ($p<0.05$). The results suggest that phenols of cacao co-products improve the lipid profile of obese rats indicating that the use of co-products as raw material for the production of functional foods can be used as an adjuvant in the management of overweight and obesity.

Acknowledgments: This work was supported by the Instituto Politécnico Nacional and CONACyT .

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PP1_04

BOTRICIDE ACTIVITY OF DRIMENOL AND DERIVATIVES

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Botrytis cinerea, also known as "gray mould fungus", causes serious pre- and post-harvest diseases in at least 200 plant species, including agronomically important crops and harvested commodities. Nevertheless, the magnitude of the fungicidal treatments against this fungus has induced the appearance of resistant isolates, necessitating the identification and development of new molecules.[1]

The "canelo" is a medicinal tree native form Chile that has been traditionally used by the Mapuche people, phytochemical studies have demonstrated that this plant contains mainly sesquiterpenes of the drimane-type and flavonoids, being the majority drimenol and polygodial. Several studies have reported biological activity both essentials oil of "canelo" and their metabolites.[2,3]

Table 1. Effect of drimenol and by products on in vitro mycelial growth of *B. cinerea* an estimation of median effective doses (ED50) was based on colony diameter measurements after 4 days of incubation.

Compound	Ec $_{50}$ (ppm)
Drimenol	80
Drimenona	92,1
Ac-epox-drimenol	314,2
Bc 1000	32

Fungitoxicity of drimenol and their derivatives and the commercial fungicide BC1000 was assessed using the radial growth test on malt-yeast extract agar. The compounds except the BC-1000, were dissolved in dichloromethane at different final concentrations (20, 40, 80, 160 ppm). The medium with or without Drimenol, their derivatives and BC1000 was poured into 6 cm diameter Petri dishes. After evaporation of the solvent, the Petri dishes were inoculated with 0.5 cm agar discs with thin mycelium of *B. cinerea*. Cultures were incubated in the dark at 22 °C during several days. Mycelial growth diameters were measured daily. Results were also expressed as EC50 (the concentration that reduced mycelial growth by 50%). Each experiment was done at least in triplicate.

In this work the effect of drimenol and its derivatives, Ac-Epox-drimenol (1) and Drimenone (2), on *B. cinerea* have been studied. Drimenol, a sesquiterpene of drimane, was obtained from D. winteri's bark according to standar procedure.[4] Table 1 showed the effect on *Botrytis cinerea* mycelial growth at different concentrations.

Results showed what derivatives (1) and (2) were able to inhibit the hyphal growth at the same concentrations used for drimenol. Nevertheless, the inhibition effect of (1) was lower than drimenol and (2). However, all compounds tested in this work showed a much lower antifungal activity than the commercial fungicide, Bc1000 (Table 1).

Acknowledgments: The authors thanks to Fondecyt for Grants 1130742 and 11140194.

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PP1_05

A NEW IRIDOID AND OTHER CONSTITUENTS FROM *PTEROCEPHALUS NESTORIANUS* NAB.

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The Kurdistan Region of Iraq has a centennial tradition in the use of medicinal plants; however, the majority of them are still uninvestigated from a phytochemically and pharmacologically point of view. Therefore, recently, we decided to start an ambitious project on the isolation and characterization of main bioactive compounds from Kurdish medicinal plants. Within this program, we report, for the first time, the main constituents isolated from *Pterocephalus nestorianus* Nab. (Dipsacaceae), known in Kurdistan with the name of Lawa. This plant grows in sunny, dry, rock crevices, mostly in Western Asia (Iran, Turkey), and it is used in Kurdistan for treating oral diseases and inflammation. *Pterocephalus* species are, indeed, widely used as ornamental plants and in several folkloric medicines all around the world.

Dry flowers and roots of *P. nestorianus* (100 g) were separately exhaustively extracted by maceration at room temperature in solvents of increasing polarity: hexane, EtOAc, MeOH, MeOH-H_2O, 70:30. Subsequently, chlorophylls were removed from the MeOH extracts by SPE technique. The chlorophyll free extracts were then separately fractionated by repartition between solvents and repetitive MPLC on successive C-18 reversed phase columns. The structures of isolated compounds were established by 2D NMR spectroscopic methods and, in case of known compounds, by comparison with literature.

The major bioactive isolated products were kaempferol glycosides and three iridoids: loganic acid (**1**), loganin (**2**) and the new dimer, secologanyl ester (**3**). These iridoids show a characteristic high anti-inflammatory activity, which thus corroborate the traditional uses of the plant.

Acknowledgments: This research has been financed by the University of Salahaddin-Erbil/Iraq and the University of Pavia-Italy, in the framework of the split-side program for the support to the PhD studies of Fuad O. Abdullah.

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PP1_06

BOTRICIDE ACTIVITY OF EUGENOL AND DERIVATIVES

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Eugenol is a major component of essential oil isolated from the Eugenia caryoplyllata, which has been widely used as an herbal drug. It is a remarkably versatile molecule incorporated as a functional ingredient in numerous products and has found application in it's a vast range of pharmacological activities has been well-researched and includes antimicrobial, anti-oxidant and anticancer activities, amongst others.[1,2]

Botrytis cinerea, also known as "gray mould fungus", cause serious pre- and post-harvest diseases in at least 200 plant species, including agronomically important crops and harvested commodities, such as grapevine, tomato, strawberry, cucumber, bulb flower, cut flower and ornamental plants (Jarvis, 1980).[3]

Fungitoxicity of eugenol and their derivatives and the commercial fungicide BC1000 was assessed using the radial growth test on malt-yeast extract agar. The compounds except the BC-1000, were dissolved in dichloromethane at different final concentrations (20, 40, 80, 160 ppm). The medium with or without eugenol, their derivatives and BC1000 was poured into 6 cm diameter Petri dishes. After evaporation of the solvent, the Petri dishes were inoculated with 0.5 cm agar discs with thin mycelium of *B. cinerea*. Cultures were incubated in the dark at 22 °C during several days. Mycelial growth diameters were measured daily.

In this work the effect of eugenol, eugenol acetate, isoeugenol, isoeugenol acetate and 4-allyl-6-nitro-2-methoxyphenol (nitroeugenol), on *B. cinerea* have been studied. Figure 1 showed the effect on *Botrytis cinerea* mycelial growth at different concentrations.

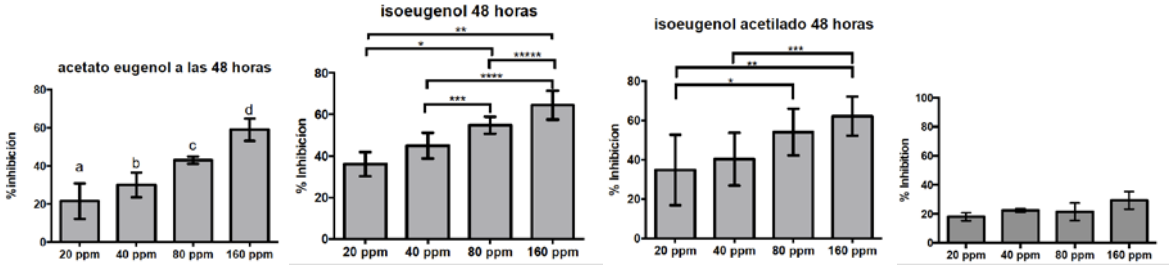

Figure 1. Effect of eugenol and derivatives on mycelial growth of B. cinerea in solid media. Percentage of growth inhibition was determined after 48 hours of incubation. Every bar represents the average of at least three independent experiments ± standard deviation.

Results showed what eugenol acetate, isoeugenol and isoeugenol acetate were able to inhibit the hyphal growth with values of EC50 of 112.77, 56.55 and 67.84 ppm respectively. However, all compounds tested in this work showed a lower antifungal activity than the commercial fungicide, Bc1000.

Acknowledgement: The authors thanks to Fondecyt for Grants 1130742 and 11140194.

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PP1_07

THE LONG HISTORY OF PASTORALISM IN SOUTHERN ITALY AS SHOWN BY ARCHAEOPALYNOLOGY

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Pastoralism is one of the main economic activities of the people living in Southern Italy. Animal breeding and transhumance (seasonal migration of livestock and herders between mountains and valleys or coasts) have a long tradition in this territory and certainly played an important role in shaping the landscape. Thanks to the long-time scale archaeobotanical research, it is possible to find the roots of this traditional practice in regions where vegetation and landforms have been significantly modified by sheep tracks and pastures.

The study of pasture indicators of biological origin from archaeological sites of southern Italy is particularly useful in discriminating land uses and pastoral/breeding activities, allowing the identification of pastoral sites and activities when they are virtually impossible to identify in the archaeological record alone (Florenzano 2015).

This research has been carried out on samples from the main archaeological sites of Basilicata region located in a transect from the Apennines (1 site) to the coast along the Bradano river (7 sites). The 8 sites have been studied in collaboration with the University of Basilicata and the University of Texas at Austin (Florenzano 2013).

A set of 121 pollen samples were taken from archaeological layers – small trenches, rooms or floors of houses, and spot samples. According to the chronology of these archaeological sites, the temporal transect spans about 20 centuries - from the Archaic to Medieval periods.

In general, the local evidence of human-induced environments is marked by high values of Anthropogenic Pollen Indicators (API), plant taxa directly associated with human activities, such as crop plants, floras of arable weeds, and ruderal floras (Mercuri et al. 2013). In pollen diagrams, the clearest signal for pastoralism is given by the abundance of plants reflecting animal breeding and grazing areas, such as daisy-family (Cichorieae and Asteroideae; Florenzano et al. 2015). The high presence of other pollen taxa may be indicative though indirect evidence of pasturelands: e.g. plantain-*Plantago* is common in trampled areas, and nettle-*Urtica* in nitrophilous environments. In addition to these pollen pasture indicators, non-pollen palynomorphs-NPPs can be used to assess the presence of past fauna, in particular herbivores. Ascospores of dung-related fungi *Sordaria*, *Sporormiella*, *Podospora* and *Cercophora* are the most reliable indicators associated with local grazing activities (van Geel et al. 2003). Data point to an open landscape dominated by pastures and cereal fields. Important evidence of pastoral farming rises from the joint record of pollen grazing indicators and spores of coprophilous fungi. This dataset highlight the pressure of pastoralism in the past and support the idea of the importance of the ancient pasture farming as a major agent of landscape transformation in this Mediterranean region.

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PP1_08

MULTIDISCIPLINARY INVESTIGATION ON EARLY-MID HOLOCENE WILD CEREALS FOUND AT TAKARKORI (CENTRAL SAHARA)

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Plant macroremains from rock shelters of central Sahara give information about the environmental conditions during the Holocene, and the adaptive strategies of human groups living in the area. Takarkori was excavated by the Italian-Libyan Archaeological Mission in the Acacus and Messak (directed by S. di Lernia, Sapienza University of Rome) and its chronology ranges from *ca.* 10,200 to *ca*. 4,600 cal yr BP (Cremaschi et al. 2014).

For the morphometrical analysis, fertile spikelets of *Panicum laetum*, *Echinochloa colona* and *Sorghum bicolor* subsp. *verticilliflorum* were selected as representative of different cultural contest. 1,600 desiccated specimens, found as 18 "seed accumulations" in the site, were measured using image analysis techniques and data were elaborated by means of Principal Component Analysis (PCA). For the molecular analysis, aDNA was extracted from the spikelets testing different protocols and then was studied by means of the DNA barcoding approach, using four chloroplast markers (rbcL, matK, trnH-psbA, trnL). A neighbour joining clustering was performed on the combined dataset of the barcode sequences using the statistical package PAST – PAleontological Statistics. Because of the present-day importance of wild cereals in the Saharan and sub-Saharan regions, ethnobotanical information were investigated from literature.

The archaeobotanical record shows homogeneous typology and uniform size of the spikelets. Moreover, some relations are founded in the comparison with the measures of the relevant modern species. This could be associated with the action of collection of those cereals by the human groups who lived at Takarkori (Fornaciari et al. submitted). Bioinformatic analysis of the aDNA sequences allows to inspect the phylogenetic relationships between the archaeobotanical records and the modern species of African wild cereals (Fornaciari et al. submitted). Next Generation Sequence (NGS) analysis is in progress, and will allow a deeper study of the domestication level, the origin and developing of minor cereals in Africa, eventually helping the archaeologists in understanding changes in cultural trajectories. The specimens found at Takarkori, selected by type, are evidence of a deep knowledge of the plants distributed in the region and of the presence of a small group of wild cereals collected continuously in the area for a very long time. Grains of this wild species are still nowadays essential in the human diet in Africa and are harvested by nomadic people for food, fodder and many other purposes (Mercuri 2008).

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PP1_09

BIOACCESSSIBILITY AND BIOACTIVITY OF POLYPHENOLS EXTRACTED FROM SIX CHERRY CULTIVARS

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There is a possible association between consumption of fruit and vegetables and reduced risk of cancer, particularly cancer of the digestive tract. This anti-cancer activity has been attributed in part to polyphenols present in foods [1]. Cherries in particular are a rich source of polyphenols, especially anthocyanins and hydroxycinnamic acids [2].
Cherries from six different cultivars (Durone della Marca, Celeste, Durone Nero I, Bigarreau, Lapins and Moretta) were *in vitro* digested with a procedure that mimicked the physiochemical conditions of the gastro-intestinal tract [3]. After digestion, polyphenol-rich extracts were obtained by preparative chromatography on C_{18} column. Polyphenols were also extracted from un-digested cherries with methanol-water-formic acid solution (70:28:2 v/v) and chromatographed on C_{18} column. Total polyphenols and anthocyanins concentration were determined by Folin-Ciocalteau assay and pH differential method, respectively [3]. Polyphenols were identified using liquid chromatography mass spectrometry. The digested and un-digested preparations were assessed for their cytotoxic and anti-proliferative activities using two human colon adenocarcinoma cell lines (Caco-2 and SW480) and IC_{50} values of anti-proliferative activity were determined.
The total polyphenol concentration in the un-digested samples varied from 70.2mg/100g of cherry in the cultivar Durone della Marca to 280.6mg/100g of cherries in the cultivar Durone Nero I. The total anthocyanin content varied from 0.12mg/100g of cherry in the cultivar Durone della Marca to about 17mg/100g of cherry in the two darker cultivars (Lapins and Moretta). *In vitro* digestion resulted in a decrease in both total polyphenols and anthocyanins. Total polyphenols decrease ranged from 56 to 80%, whereas total anthocyanin decrease was more than 90% in all the tested cultivars. Mass spectrometry showed that the gastro-intestinal digestion decreased the content of hydroxycinnamic acids and anthocyanins. Other classes of polyphenols are more stable to digestion. Breakdown products of polyphenols are also present. Digested and un-digested polyphenol-rich extracts showed no cytotoxic activity. Un-digested polyphenol-rich extract from the cultivar Bigarreau showed the highest anti-proliferative effect (IC_{50} of 7.1 ± 1.1 µg polyphenols/mL) on the SW480 cells. The *in vitro* digestion increased the anti-proliferative activity of cherry extracts. Polyphenol-rich extracts from digested Durone della Marca and Celeste were the most active with IC_{50} values of 1.7 ± 0.8 and 3.5 ± 1.2 µg polyphenols/mL, respectively. All of the tested extracts showed no anti-proliferative activity on Caco-2 cells.
Our results showed that cherry polyphenols were effective anti-proliferative agents on the more undifferentiated colon adenocarcinoma cells SW480 and that their digestion increased the effect. The increase may be due to the formation of polyphenol metabolites. A great difference was observed between the cultivars used. Further research is required to identify the compounds responsible for the observed anti-proliferative effect.

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PP1_10

INFLUENCE OF THE GELATO BASE FORMULATION ON THE SURVIVAL OF *LACTOBACILLUS CASEI* STRAINS DURING GELATO PRODUCTION AND STUDY OF THEIR IMPACT ON THE RHEOLOGICAL PROFILE

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Present research is focused on the production of Italian artisanal gelato enriched with probiotics. It has been used two potentially probiotic strains deposited in UNIMORE Microbial Culture Collection (UMCC) isolated from Parmigiano Reggiano : *Lactobacillus casei* PRA 041 and *Lactobacillus casei* 225 PRA. The study was carried out evaluating different formulations of gelato bases and proceeding with direct inoculation of the strain into the gelato mix, or, instead, by inoculating the cells in milk, effecting fermentation and adding the fermented milk into gelato mix. We studied the influence of formulations different for fat contents and with or without a prebiotic fiber (inulin) on the survival of the strains and the changes in the rheological properties of gelato.

Lactobacillus casei Pra 041 and *Lactobacillus casei* Pra 205 stock cultures were kept at -80°C in cryogenic vials containing MRS Broth (Oxoid Ltd, Basingstoke, Hampshire,England) and glycerol. It was prepared a biomass. The cells are been separated by centrifugation at 5000 g for 10' at 4°C. Two bases for traditional gelato have formulated able to be prepared either using the hot process, either the cold process. The bases do not contain in the formulation the powdered fat. One of the bases, called BASE A was to include a 10 % of a prebiotic fiber .The prebiotic fiber inulin Orafti GR (Beneo- Orafti Aandorenstrat 1 , B - 3300 Tienen Belgium) has been used. The second base, called BASE B, however, has not to include any fiber . Both bases (Base A and Base B) were added with pasteurized cream to obtain mixture to 2.7% , 4% and 6 % fat. A first series of tests was carried out adding to each formulation the sweet inoculum (19.2%). The second series of tests was carried out adding the acid inoculum (19.2%). With these tests, it has been tried to find the formula that guaranteed greater protection during freezing phase. This formula was then tested with both the strains (*L.casei* PRA 041 and *L.casei* PRA 205) and also using both the cold process, and the hot process. Gelato has been performed by Carpigiani Compacta gelato machine and stored into a ventilated showcase ISA equipped with a system of autodefrost and set at a temperature of -14 ° C (T air) that corresponded to a T of the gelato in pan of -11 / -12 ° C

Contrary to what reported in literature, the fat content or the presence of inulin , does not substantially influence the survival of the microbial strains to the freezing phase. To obtain a good survival of the strains inoculated need to have a formulation respectful of the correct parameters of balancing and a good stabilization. The speed, then, with which the gelato machine is able to freeze the mixture, is the other crucial aspect. During freezing phase there is a reduction of the initial charge equal to 1log, it is essential starting from a lactic initial charge> 10^8ufc/g, to obtain a charge in gelato of at least 10^7ufc/g. This value is in accordance with the recommendations as daily intake/g for probiotics. In order to evaluate any changes to the structure of the gelato, it has been used, instead of the spreadability of gelato, parameter very subjective, its hardness. It is used for this purpose a dynamometer on which was installed the penetrometer probe. Through the analysis of the data some differences were found in structure between the standard gelato and the gelato added of bacteria, mainly when it has been added an acid inoculum.

PP1_11

HERBAL REMEDIES IN THE NORTH OF ALGERIA: POTENTIAL ADVERSE INTERACTIONS WITH ANTICANCER AGENTS

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Interest in the use of herbal products has grown in the world. Recent estimates suggest an overall prevalence for herbal preparation use of 13% to 63% among cancer patients. With the narrow therapeutic range associated with most anticancer drugs, there is an increasing need for understanding possible adverse drug interactions in medical oncology.

In this presentation, a literature overview is provided of known or suspected interactions of the most used herbs in Oran, a North West town in Algeria, with conventional allopathic therapies for cancer.

Herbs with the potential to significantly modulate the activity of drug-metabolizing enzymes (notably cytochrome P450 isozymes) and/or the drug transporter P-glycoprotein include garlic (*Allium sativum*), peach leaves (Prunus persica), Fenugreek (*Trigonella foenum graecum*), saltbush (Atriplex halimus), Barberry (Berberis vulgaris), and many other plants. All of these products participate in potential interactions with anticancer drugs.

It is suggested that health care professionals and consumers should be aware of the potential for adverse interactions with these herbs, question their patients on their use of them, especially among patients whose disease is not responding to treatments as expected, and urge patients to avoid herbs that could confound their cancer care.

PP1_12

PORTULACA OLERACEA L. IN THE ERA OF GLOBALISATION: A SPECIES OF GREAT NUTRACEUTICAL VALUE

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Portulaca oleracea L. is a common ruderal, synanthropic, cosmopolitan taxon, highly polymorphic, typical of warm sites (Danin and Raus, 2012; Danin et al., 2014; Danin et al., submitted). In Italy its status as a native species is doubtful (Pignatti, 1982). It is well known since the antiquity for its medicinal and nutrient qualities (Bosi et al., 2009): all parts of the plant have therapeutic properties (Gastaldo, 1987). It has been used for a long time as an analgesic, anti-inflammatory, antipyretic, diuretic, emollient, lenitive and anaphrodisiac. Many of such properties have been recently confirmed; furthermore, *P. oleracea* is very rich in omega-3 polyunsaturated fatty acids (Ezekwe et al., 1999), so that its use is recommended to contrast the excess of fatty acids assumed by fast foods (Picchi and Pieroni, 2005) and its seeds are good to counteract diabetes mellitus (El-Sayed, 2011).

In this work we tested the content of fatty acids in seeds from Roman period to present, as a comparison to the content of fatty acids of the aerial parts of the plant: 12 fresh plant and seeds samples, collected in Summer 2015, 19 seeds samples coming from Italian herbaria, dating from 1920 to 2011, and 20 seeds samples coming from archaeological excavations, dated from 1st to 15th cent. AD. Total lipids were extracted according to a modified method of Folch (1957) and methyl esters were analyzed by gas chromatography (GC)-FID.

We found a high content of polyunsaturated fatty acids in fresh plants and seeds. Moreover, a quite similar profile with comparable saturated, monounsaturated and polyunsaturated fatty acids was observed in both seeds and aerial parts of the plant, even if significant differences were measured in singular fatty acid percentages. Furthermore, comparing seeds samples from different historical periods, we determined an evident decrease in polyunsaturated fatty acids depending on increasing time, from ~69% of fresh seeds up to ~11% in archaeological samples with the consequent increase in saturated fatty acids. This is probably due to a degradation process, caused by atmospheric oxygen exposition and unsaturated fatty acids oxidation during time. The applied reported methodology would be useful for the determination of fatty acids content and profile also in ancient samples of seeds and parts of plants.

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PP1_13

ANTIOXIDANT ACTIVITY AND APOPTOSIS INDUCTION ASSESSED BY FLOW CYTOMETRY OF A *BACTRIS GUINEENSIS* POLYPHENOL EXTRACT

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Bactris guineensis (Aracaceae), also known in Costa Rica as güiscoyol or uvita, corozo in Honduras, Panamá, Colombia and Venezuela, is a wild palm that grows in the plains. Its fruit has a high polyphenol content (824.4± 77.5 mg GAE/100 g), compared with another fruits like blueberry and plums.

The antioxidant activity of *Bactris guineensis* was reported in literature with the methods DPPH and ORAC (Osorio *et al.* 2011 and Rojano *et al.* 2012), however it is important to correlate it with another assays closer to *in vivo* systems, like the effects on cell culture.

Full ripe fruits were collected in the province of Guanacaste, Costa Rica. The fruit was extracted with acetone/water and then subjected to polyphenol purification using an Amberlite XAD-7 column (150 mm x 20 mm). The column was washed with water, the phenolic compounds were eluted with methanol:water (80:20), concentrate under vacuum (40°C) and freeze dried. The extract was analyzed by UPLC-MS.

Antioxidant activity was assessed by using the DCFDA fluorescent dye. The intracellular ROS production was induced on the Vero cell line with tert-butyl-hydroperoxide (TBHP) in presence and absence of the polyphenol extract. The fluorescence positive cells were determined by flow cytometry and the analysis performed by CellQuest software.

In order to analyze if the observed cytotoxic activity of the polyphenol extract on the colon carcinoma cell line SW 620 (IC_{50}: 16.6□1.3□g/mL) was because of an activation of apoptotic mechanisms, apoptotic cells were quantified using Annexin V-FITC/PI assay and analyzed by flow cytometry.

The polyphenols identified by UPLC-MS in the extract were cyanidin 3-rutinoside, procyanidin B1 and procyanidin 3-rutinoside. The antioxidant effects of the polyphenols inside of cells, using the DCFDA assay, shows that the extract reduced the induced intracellular ROS in a dose-dependent manner, with an IC_{50}: 151.7 □g/mL. Also the antioxidant activity was determined by DPPH (IC_{50}: 3.3 ± 0.2 µg/mL) and ORAC (979.1 µmol TE/g extract).

The cytotoxic effect observed over the SW 620 cell line seems to be happen by apoptosis. We observed 50.3% of cells in early apoptosis and a 17.7% of cells in late apoptosis, after the polyphenol extract treatment. However, further investigation will be needed in order to explain the apoptotic mechanisms involved in its activity.

In conclusion, with its polyphenol content, high antioxidant activity and its cytotoxic effect on colon carcinoma cells, *Bactris guineensis* seems to be a fruit with a high potential as functional food.

Acknowledgement: financial support by Vicerrectoría de Investigación, Universidad de Costa Rica (Project No. 735-B2-659)

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PP1_14

ASSESSMENT OF THE HEAVY METAL CONTENT IN AROMATIC SPICES BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

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Aim of the study was to determine the content of Cd, Hg, As and Pb in spices commonly available in the Italian market, since such heavy metals pose a major health concern and are considered among the most hazardous chemicals (WHO, 2007). Results were compared with the maximum permissible limits set by the national Legislative Decree (L.D.) n.107 implementing the Council Directive 88/388/EEC, and by international organizations, such as Food and Agriculture Organization (FAO) and World Health Organization (WHO). The risk derived from the intake of heavy metals *via* the assumption of spices was assessed, considering the Tolerable Weekly Intake (TWI) and the Provisional Tolerable Weekly intake (PTWI) respectively for Cd and Hg, and the 95% lower confidence limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) for As and Pb.

Seven samples of 3 types of spices produced in different countries (namely, cinnamon from Indonesia, Madagascar and Vietnam; curcuma from India and Sri Lanka; ginger from India and Japan) were considered. Each sample was pre-treated by a microwave digestion system; then, the heavy metal content was determined through a validated ICP-MS method. One-way analysis of variance (ANOVA) and post-hoc Tukey's honest significant difference test were applied to check for significant differences ($p<0,05$) among the content of each heavy metal in the samples.

All the tested spices revealed to contain heavy metals and to be a potential contamination route for human consumers. Cd was detected in all the samples: its lowest and highest content were respectively measured in the Indian ginger (0,023±0,011) and in the Sri Lankan curcuma (0,288±0,004 mg Kg^{-1}). Cd showed to accumulate below the limit set by the LD n.107 (1 mg Kg^{-1}) and FAO/WHO (0,3 mg Kg^{-1}). Considering Hg, the curcuma from Sri Lanka showed the highest Hg content, representing the 28% of the total toxicants investigated in such spice. Nevertheless, all the samples met the national and international safety limit of 1 mg Kg^{-1}. The cinnamon from Madagascar and the Japanese ginger were characterized by the lowest and highest As content (0,006 ±0,003 and 0,697± 0,003 mg Kg^{-1}, respectively). However, As levels were within the safety limit set by the LD n.107 (3 mg Kg^{-1}) and FAO/WHO (1 mg Kg^{-1}). Pb made for the 65% of the total investigated metals: the lowest and the highest Pb content were respectively found in the Indian curcuma and in the cinnamon from Vietnam (0,171±0,005 and 2,224±0,708 mg Kg^{-1}, respectively). Pb content was found to be below the permissible national and international limit (10 mg Kg^{-1}) in all the spices. Assessing the heavy metal intake through the assumption of spices, it resulted that in the cinnamon from Vietnam, the contribution of Cd and Pb respectively exceeded the TWI (151%) and $BMDL_{01}$ (431%); while in the Japanese ginger, the contribution of As ($BMDL_{01}$= 663-24,8%) and Pb ($BMDL_{01}$= 219%) were well above the safety limits. Finally, the Sri Lankan curcuma contributed most to the intake of hazardous elements, since each metal investigated significantly exceeded the safety limits set by EFSA and JEFCA.

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PP1_15

ANTI-INFLAMMATORY EFFECT AND PHYTOCHEMICAL SCREENING OF *FICUS PERTUSA*

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Ficus pertusa (*Moraceae*) grows in regions as Loreto, Madre de Dios and Pasco (Perú) [1]; it has been traditionally used to treat to rheumatism and women's ailments [2,3]. In studies chemical previous have reported the presence of flavonoids, alkaloids, tannins, saponins and anthraquinones [4]. The main objetive was to contribute to the phytochemical study of the stem bark of this specie and assess their anti-inflammatory effect.

Plant material was collected in Loreto and identified in the NHM (UNMSM) collection under number 3796. The phytochemical screening was in crude ethanolic extract of dry stem bark. The anti-inflammatory activity was evaluated on carrageenan-induced paw edema in rats [5] and experimental groups of rats were treated with vehicle, diclofenac gel 1%, ethanol extracts gel (EEG) 0.5%,1% and 10%.

The phytochemical screening revealed presence of phenolics compounds, quinones, flavonoids, condensated tannins, terpenes, lactones α,β-unsaturated and alkaloids. Flavonoids and stilbenes can be considered markers quimiotaxonomicos of the Moraceae [6]. Researches shows terpenes, terpenoids, lactones and alkaloids in the genus *Ficus* [7-10]. For the anti-inflammatory test, the EEG 10% used in our study inhibited the edema induced by carrageenan at 15% as compared diclofenac 1% gel in 2 hours.

Our investigation revealed anti-inflammatory effect, *in vitro* of our gel extract ethanolic.

Acknowledgments: This work has been granted by UIGV

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PP1_16

VISCERAL ADIPOSE TISSUE REDUCTION THROUGH CACAO CO-PRODUCTS CONSUMPTION

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The consumption of diets with high levels of fat and carbohydrates has an impact on the weight leading to develop overweight, obesity, cardiovascular diseases and dyslipidemia among others[1]; the use of functional foods rich in polyphenols is an alternative for the treatment of such conditions. Therefore the objective of this study was to evaluate the effect of cacao co-products on the weight of visceral adipose tissue in a rodent model with obesity induced by diet [2,3].
In this study, Wistar male rats were fed with an obesogenic diet (21% of fat) and 20% fructose solution as consumption water during 5 weeks. After 5 weeks of feeding with the high fat diet, rats were distributed into 5 groups: Control, Obesogenic, I, II and III (obesogenic diet plus 141.54, 23.55 and 11.751mg of equivalents of Gallic acid respectively obtained from cacao co-products). After 5 weeks of treatment rats were sacrificed and the visceral adipose tissue was weighted.
The group that consumed the obesogenic diet had visceral adipose tissue accumulation which was reflected in its weight; the increment was 8 fold compared with normal diet group. The treated groups reduce the visceral adipose tissue in 12 % and 20% in groups II and III, while group I did not show significant difference ($p > 0.05$) with obesogenic group. The results suggest that the decrease in visceral adipose tissue could be attributed to phenols presents in cacao co-products making them a good material for the production of functional foods that can be used as an adjuvant in the management of overweight and obesity.

Acknowledgments: This work was supported by the Instituto Politécnico Nacional and CONACyT..

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PP1_17

ANTIMALARIAL ACTIVITY OF XANTHONES ISOLATED FROM GARCINIA MANGOSTANA

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Malaria is a parasitic endemic disease that is widely distributed worldwide and affects a large economically disadvantaged population living in rural areas of tropical and subtropical countries.[1,2] Conventional treatments include compounds such as aminoquinolines, antifolates, Atovaquone® a hydroxy-naphthoquinone, and artemisinin derivatives. The use of these compounds in affected populations presents disadvantages related to their relatively high cost and the development of resistant parasites.[3,4] Therefore, there is an urgent need to discover and develop new and better drugs. Unfortunately, the drug discovery and development processes are not only expensive but also highly time-consuming and complicated. One of the most important bottlenecks in the discovery and development of new drugs from natural products is the availability of sufficient plant material to obtain pure active molecules for more advanced evaluation.

Based on the previously reported in vitro antiplasmodial activity of several xanthones from Garcinia mangostana, two xanthones, α-mangostin and δ-mangostin, were isolated from mangosteen husk, and the *in vitro* antiplasmodial and cytotoxic effects were determined. α-Mangostin was more active against the resistant Plasmodium falciparum chloroquine-resistant (FCR3) strain (IC_{50}= 0.2± 0.01 μM) than δ-mangostin (IC_{50} = 121.2 ± 1.0μM). Furthermore, the therapeutic response according to the administration route was evaluated in a Plasmodium berghei malarial murine model. The greatest therapeutic response was obtained with intraperitoneal administration; these xanthones reduced parasitemia by approximately 80% with a daily dose of 100mg/kg administered twice a day for 7 days of treatment

Acknowledgements: This work was sponsored by Colciencias-COLOMBIA (grant 111571249866), CIDEPRO (CIIEs, Universidad de Antioquia)

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PP1_18

UNCOVERING ARTIFICIAL HONEYS FROM ECUADORIAN MARKETS

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Honeys are imitated using less expensive sweeteners to earn more profits on this sugary bee product. Economically motivated adulteration (EMA) is the term used for fraudulent and intentional additions or substitutions of substances to increase economic earnings (Easter Strayer et al., 2014). Honey standards are the official reference for authenticity testing but besides specialized techniques, these analysis are not always available in developing countries. The suitability of a simple authenticity test (Vit, 1995; Vit, 1998) is compared here with physico-chemical honey quality standards to assess the presence of artificial honeys in the Ecuadorian market.

Artificial honeys well identified by sensory observations were collected in 12 Ecuadorian provinces. The ash, free acidity, qualitative diastase activity, qualitative hydroxymethylfurfural (HMF), moisture, reducing sugars, and apparent sucrose contents were measured following COVENIN 2136-84 (1984a) methods. The 21 artificial honeys and one genuine honey from a Pichincha province apiary were tested for authenticity after 1:1 honey:distilled water, and vigorous shaking with 1:1 honey dilution:diethyl ether (Vit, 1998).

Venezuelan (COVENIN 2191-84) and Ecuadorian (NTE INEN 1572) standards from National Honey Norms were compared with the physico-chemical composition of Ecuadorian fake honeys. Qualitative diastase activity and HMF of the Ecuadorian fake honeys analyzed did not satisfy the honey standards, as in a study with 500 commercial honeys from Venezuela (Vit et al., 1994). Heated and old genuine honeys also may have low diastase activity and increased HMF as defects. Ash and moisture are not a problem for manufactured honeys that tend to be more viscous and with lower moisture than genuine tropical honeys; therefore these two parameters are not handy to pursue detection of fake honeys. The remaining group of three honey quality factors discriminated only few fake honeys. Higher free acidity (up to 102.0 meq/kg), higher apparent sucrose (up to 23.89 g/100g) and lower reducing sugars (51.80 g/100g) than the maximum 40 meq/kg, maximum 5 g/100g, minimum 65 g/100g contents respectively permitted in the honey standards (COVENIN, 1984b; NTE INEN, 1988) were found. The test based on the number of phases of honey dilutions shaked with diethyl ether, was reliable to uncover all the 21 fake honeys from Ecuadorian markets.

Acknowledgments: To Consejo de Desarrollo Científico, Humanístico y Tecnológico, Universidad de Los Andes CDCHT-ULA for continuous support to the Research Group APIBA ADG-FA-97-08. To the Scholarship Prometeo-SENESCYT-UTMACH june 2014 – june 2015 to P. Vit. To Instituto Ecuatoriano de Normalización INEN to extend invitations to join the Technical Committee to review the NTE INEN 1572 Honey Norm.

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PP1_19

ANTI-INFLAMMATORY SCREENING OF PLANT EXTRACTS USED IN FOLK MEDICINE OF COLOMBIAN CARIBBEAN COAST

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Responding to the need to find new, more effective and safe therapeutic alternatives to treat the clinical conditions associated with inflammatory processes and attempting to exploit the ethnobotanical richness of the Colombian Caribbean Coast, we evaluated the anti-inflammatory potential of seven total ethanolic extracts of botanical species widely used in the folk medicine of this region.

The total extracts of: *Ambrosia cumanensis, Bauhinia guianensis, Capparis odoratissima, Cecropia peltata, Hyptis capitata, Mammea Americana* and *Murraya exótica;* were obtained by maceration with ethanol. Presence of different metabolites was determined qualitatively, while flavonoids and phenolic compounds were determined quantitatively. The anti-inflammatory potential of the extracts were assessed determining their activity on the production of nitric oxide (NO•), in RAW 264.7 macrophages cell line, quantified spectrophotometrically by the accumulation of nitrite (NO_2^-), using the Griess reaction[(1)]. Additionally, NO• scavenging effect was determined, using sodium nitroprusside as a radical donor. The extracts were tested at non-toxic concentrations and below 100 μg/mL in all cases, which were previously determined evaluating cell viability through the MTT reduction colorimetric assay. Ultimately, we evaluated possible antioxidant effects of the extracts, using the free radical scavenging DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and ABTS$^{+\bullet}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) spectrophotometric methods[(2, 3)].

The total ethanolic extracts of *Ambrosia cumanensis*, *Cecropia peltata, Hyptis capitata, Mammea americana* and *Murraya exótica* showed a potent inhibitory activity of the NO• production (70-100%), without significant scavenging effect of the NO• free radical. These extracts did not show significant scavenging effects of DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ free radicals, at the same concentrations used in tests with cell cultures, suggesting that its potential anti-inflammatory activity is unrelated to neutralization of oxidative stress associated with inflammatory processes. These five plant species should continue to be studied, in order to isolate and identify compounds responsible for the activity, which could become a starting point for the discovery of new anti-inflammatory agents.

Acknowledgments: This study was supported by the University of Cartagena (Grant 054-2013). Jenny Castro is deeply grateful to Colciencias and the University of Cartagena for her PhD fellowship through the National Program for Doctoral Formation (Grant 647-2014).

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PP1_20

BIOGUIDED FRACTIONATION OF *Trichilia hirta* SEEDS EXTRACT WITH CYTOTOXIC ACTIVITY AGAINST A BREAST CANCER CELL LINE

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Caribbean Colombian flora has long been used to treat diseases, *Trichilia hirta* is a species conventionally used by patients to treatment the cancer(1). In this work, a bioguided fractionation of the ethanolic extract of *T. hirta* seeds was performed based on its cytotoxic activity on the MDA-MB-231 breast cancer cell line, as well as the cancer cell selectivity of the extracts and its active fractions on 3T3-L1 fibroblasts.

Seeds of *T. hirta* were washed, dried at room temperature, ground to a powder and extracted with ethanol until exhaustion. The dry extract was fractionated using chromatographic techniques under a bio-guide scheme based on the cytotoxic activity against MDA-MB-231 cells, employing the methyl-tetrazolium bromide colorimetric method (MTT)(2). The extract and fractions were classified into three categories as: active (LC_{50}≤20 µg/ml), moderately active (20<LC_{50}<100 µg/ml) and inactive (IC_{50}≥100 µg/ml)(3). In order to determine the selectivity index (SI), we evaluated the cytotoxic effect of the extract and fractions against 3T3-L1 fibroblasts as normal cells and calculated it as a ratio of $LC_{50Fibroblast}/LC_{50CancerCell}$.

Chromatographic separation of *T. hirta* total extract (19,9 g), led to obtain four major fractions identified as TH-F1 (12,46 g - 62,6%), TH-F2 (2,55 g - 10%), TH-F3 (0,75 g - 3,7%) and TH-F4 (1,02 g - 5,1%), which were evaluated for their cytotoxic activity. The total extract was active on MDA-MB-231 cells (LC_{50}=7.71 µg/mL - SI=1,86). This biological activity was kept on three fractions, TH-F1 (LC_{50}=15.35 µg/mL), TH-F2 (LC_{50}=14.13 µg/mL), TH-F3 (LC_{50}=58.75 µg/mL). Interestingly, the cytotoxicity was higher in fractions eluted with dichloromethane, indicating that the main active compounds have low polarity. This study provides an important basis to further research directed to the isolation and identification of active constituents potentially useful to cancer treatment, using Colombian Caribbean plants as a valuable source.

Acknowledgements: This study was supported by Colciencias and the University of Cartagena (Grant 110756933390). Daneiva Caro is deeply grateful to Colciencias and the University of Cartagena for her PhD fellowship through the National Program for Doctoral Formation (Grant 647-2014).

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PP1_21

CAPTURING THE DIVERSITY OF FUNGAL POPULATION IN HUMAN COLONIC MICROBIOTA THROUGH CULTURE-DEPENDENT AND INDEPENDENT APPROACHES

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A small fraction of gut microbiota is represented by fungi, that remain relatively unexplored. ones have never been deeply investigated. This study wanted to investigate the inter-individual and intra-individual differences of fungal population in 3 fecal samples from 8 healthy subjects, within the time-span of a year. The composition of the fungal population, in terms of concentration and taxonomic assignment, was explored with both culture dependent and independent methods at the start of the study, after 3 months, and after a year. Strategies of interaction with the host of the isolates were investigated determining the capability to adhere and to form biofilms, and the induction of Human β-defensin 2 secretion by Caco-2 cell lines (HBD-2).

Isolation of fungi from fecal samples was carried out on PDA supplemented with antibiotics. The isolates were clusterized into different biotypes through RAPD-PCR, then amplification and sequencing of rDNA genes and ITS spacer lead to taxonomic identification. Further characterization of the *C. albicans* strains was done through SSCP and MLP. Each single biotype was tested for biofilm production and cellular adhesion. HBD-2 levels were determined in Caco-2 epithelial cells stimulated with fungal isolates. Composition of the fungal microbiota was monitored in periodically collected fecal samples by 454-pyrosequencing of the internal transcribed spacer regions of 1 and 2 rRNA genes.

Cultivable fungi were quantified at three time points in the feces of 8 healthy volunteers. The counts ranged from 1.4×10^2 to 4.5×10^5 cfu/g, and only for 5 out of 24 samples the charges were under the limit of detection. A wide quantitative intra-voluntary variability over the time was detected. The majority of the biotypes belonged to the genera *Candida*, and in particular to the species *C. albicans* and *C. zeylanoides*, and to *Galactomyces*. All but one *C. albicans* strains were able to produce large amounts of biofilm after 48 h of incubation in complete medium, whereas both the non-*albicans Candida* (NAC) strains and all but one of the other fungi did not produce biofilms. All *C. albicans* strains adhered very efficiently to the epithelial cells. Appreciable, but lower levels of adhesion were also observed with the NAC strains, but not for the other fungi. The intestinal Caco-2 epithelial cells released high levels of HBD-2 when challenged with *C. albicans* isolates, whereas the concentration of HBD-2 was markedly lower when Caco-2 cells were stimulated with NAC strains and other fungi. Metagenome analysis of fungal population succeeded only for 13 out of 24 fecal samples. 95 diverse OTUs were identified in the 13 samples, resulting in detection of 59 genera and 36 additional lineages not classified at genus level. On average, 24 OTUs/sample were detected. The sample presenting the widest biodiversity hosted fungi ascribed to 45 diverse OTUs, the least diverse, 8. *Ascomycota* dominated the fungal population (89.2% of total reads), with only 6.8 and 4.0% of *Zygomycota* and *Basidiomycota*, respectively. Most of the reads belonged to the genera *Penicillium* and *Aspergillus*. Large differences in fungal population determined by cultural methods and metagenome were detected. However, isolation of fungi was necessary to study *in vitro* the interaction of the isolates with the host.

PP1_22

SUBDOMINANT BUT CONSTANT *LACTOBACILLUS* POPULATION IN THE HUMAN GUT MICROBIOTA AS REVEALED BY WHOLE GENOME SEQUENCING

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The genus *Lactobacillus* includes 217 species that colonize plants, foods, sewage, and the gastrointestinal tract (GIT) of humans and animals. In the human GIT, *Lactobacillus* population is made up by both resident and transient members, these latter being ingested with fermented or spoiled food and beverages, and with probiotics. This study longitudinally surveyed the *Lactobacillus* species and strains in the GIT of a healthy subject over a period of almost 2 years, through mining the metagenomic whole genome sequence (WGS).

Metagenomic whole genome shotgun sequences were analyzed with LMAT (Livermore Metagenomics Analysis Toolkit) software package (Ames et al., 2013) (version 1.2.4, with Feb 2015 release of database LMAT-Grand). The "Grand" database, release Feb'15 was used. We converted raw LMAT output into a list of species and strains of interest, filtering them by the Average Read Score (values > 0).. *Lactobacillus* species and strains were selected from the filtered data to do the subsequent calculations about persistence. The number of bacteria per gram of faeces belonging to each species and strain was extrapolated from the respective relative amount (%), assuming that the three fecal samples have the same concentration of microorganisms (1×10^{12} cells g^{-1}). The data were expressed as Log(cells g^{-1}).

In three time-points (0, 670 and 700 days), 58 different species were identified, 16 of them being retrieved for the first time in human feces. *L. rhamnosus*, *L. ruminis*, *L. delbrueckii*, *L. plantarum*, *L. casei*, and *L. acidophilus* were the most represented, being estimated at the magnitudes of 6-8 Log(cells/g). Other species were detected at the magnitude of 4-5 Log(cells/g). Many of them have never been associated to human feces (e.g. *L. composti*, *L. farciminis*, *L. farraginis*, *L. harbinensis*, *L. namurensis*, *L. parabrevis*, *L. sanfranciscensis*, *L. shenzhenensis*, *L. suebicus*, *L. versmoldensis*, *L. zeae*), thus modifying the vision of *Lactobacillus* ecology. WGS also enabled the identification of 86 *Lactobacillus* strains, belonging to 52 species. 43 strains likely occupied the GIT as true residents, being recurrently found over a time-span of 23 months with limited quantitative fluctuations. As a whole, a stable community of lactobacilli was disclosed, with wide and understudied biodiversity. A major challenge is still determining the specific GIT district where this plethora of lactobacilli replicates and grows, in order to discriminate if they are indigenous resident of the colon, or whether they reach it shedding from upstream sites that they colonize. This can make the difference in terms of microbe-immune system relationship.

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PP1_23

PRELIMINARY STUDY OF PESTICIDE RESIDUES IN PETITGRAIN DISTILLATED FRACTIONS

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Citrus petitgrain essential oils are obtained by steam distillation of the leaves, buds and small branches of the different citrus species and are used in soap-manufacturing, perfumery and cosmetics. Among these, bitter orange petitgrain is the most appreciated and is produced mainly in the Mediterranean countries and Paraguay (Dugo G et al., 2002). To guarantee genuineness and quality of products it is necessary to evaluate some organic contaminants, like organophosphorus and organochlorine pesticides, even if a maximum limit of residues has been established by legislation only for citrus fruits. Most scientific studies have focused on the determination of these organic contaminants residues in citrus essential oils, but not in petitgrain ones (Di Bella et al., 2010; Dugo G.mo et al., 2010). In this study, citrus petitgrain essential oils and their distillated fractions collected at predetermined time intervals during a full cycle of distillation were analyzed to try to evaluate the contamination.

Organophosphorus and organochlorine pesticides were investigated respectively by Dani Master, HRGC-FPD, and TSQ Quantum XLS Thermo Scientific, HRGC-MS/MS, in lemon petitgrain oils samples 3 from conventional agriculture and 3 from biological agriculture and 3 bitter orange oils samples. Furthermore, during the steam distillation cycle of each sample, four fractions were collected every 25 minute each. All samples, produced in Messina (Sicily, Italy) in the season 2014/2015, and all fractions were added to internal standard solution and directly analyzed, without any clean-up procedure.

Residues of organochlorine pesticides were always < LOQ both in petitgrain oils, as they are and in their fractions. Among the organophosphorus, methyl (mean value of 0.026 mg/L) and ethyl chlorpyriphos (1.920 mg/L) and methyl pirimiphos (0.027 mg/L) were found in 100% of bitter orange petitgrain oils. During the distillation cycle the pesticides content increased significantly in the third and fourth fractions: in fact it was observed an average increase of 50% and 100 % respectively.

In lemon samples obtained from conventional and biological agriculture ethyl chlorpyriphos was the only determined pesticide with a mean concentration of 0.990 and 2.320 mg/L respectively. In their fractions it was found the same trend as observed for bitter orange samples.

Since the final fractions showed a higher concentration of pesticides compared to the initial ones, it could be assumed to reduce the extraction process, which had a yield very low percentage.

Obviously these data will be implemented with the study of the volatile fraction of each distillated fractions.

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PP1_24

PRODUCTION OF SINGLE CELL PROTEIN BY *SACCHAROMYCES CEREVISIAE* FROM FOOD WASTE

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Currently one of major problems in the world is waste, particularly food waste, both in the developing and the developed countries (Huang et al. 2015). Large-scale retail distribution wastes especially fruit and vegetables waste contain considerable amounts of fermentable and non fermentable sugars that could be utilized to produce value-added products by using microorganisms such as single cell protein (SCP). Food waste have been used as animal feed (Theodoros et.al., 2013), for oxidative and hydrolytic enzyme production (Zilly, et.al 2012), cellulases production (Oberoiet. al 2010), enzymatic extract production (Silvestre et al 2012), and as growth media for starter cultures such as kefir for bread making and alcoholic and dairy fermentations. The aim of this work was to study the promotional effect of whole discarded food, deriving from the large-scale retail distribution, on SCP production by *Saccharomyces cerevisiae* yeast using substrates consisting of fruit and vegetable mixtures through a batch fermentation processes (Plessas, et al. 2008).

Expired food waste, previously collected from large-scale retail distribution in the period between January and July 2015, were used. Expired vegetables such as tomato, lettuce, potato, aubergine, pumpkin, pepper and cauliflower together with expired fruit such as apple, strawberry, pinapple and pear were characterized and used as growth media. Dry matter content, ash content and protein concentration were determined according to the Association of Official Analytical Chemists method (AOAC, 1984). *S. cerevisie* ATCC4126 was used.

A 15 l total volume automatic Biostat E reactor (B. Braun Melsungen AG, Germany), run in *batch* mode and equipped with the common controls was used: pH: 5.05 without any further correction; mixing: 300; air flow: 4 l/min; air sterilization: filtration through 0.2 mm Sartofluor filter and pressure: 200 mbar. At the end of the batch cultivation substrate was tested for total cell count (CFU/mL), biomass concentration (g/L) and protein content (%).

The percentage of protein in the SCP from an initial value of 15.3% reaches a final value of 39.8%.

The supernatant total nitrogen values remain almost constant during the entire fermentation process. The supernatant dry matter passes from a 18.9 g/100ml value at time 0, to 12.7g/100ml after four days of fermentation, and then present an increasing trend up to the end of the fermentation process, this behavior is probably due to the decrease of the sugar.

The great amount of food waste daily produced by the large-scale retail distribution makes their elimination necessaries. Unprocessed food waste could be widely used for the formulation of add value products.

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PP1_25

DEVELOPMENT OF A NEW MULTIRESIDE METHOD TO ANALYZE 67 CONTAMINANTS IN FOOD WASTE BY GC-MS/MS

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The contaminants found in food can be derived from the environment, from the cultivation and or production processes. Their presence may impair the food quality and also involve an health risk. Due to the chemical stability of these substances, they can contaminate also vegetables and fruits, limiting the possibility of reusing their waste such matrices as substrates for compost or animal feed. The aim of this work was to set a new analytical method to analyze simultaneously 67 contaminants belonging to different classes (PCBs, PAH, organochloride, organophosphorus and other types of pesticides) based on QuEChERS technique and to analyze 70 samples of food waste.

31 samples of fruit waste (pears, strawberries, peaches, bananas, pineapples and mango) and 39 samples of vegetable waste (stripped and white zucchini, fennels, eggplants, tomatoes, lettuces, sweet peppers, cauliflowers and cucumber) were analyzed. The two tested extraction methods consists in a liquid-liquid extraction with hexane and ethyl acetate and two typologies of QuEChERS, one composed of $MgSO_4$ and NaCl and the second composed of $MgSO_4$ and CH_3COONa. The choice of the best preparation method was done based on the recovery values obtained. In particular, the first allow to obtain recoveries in the range of 21,44% - 97,76% instead with the second the recoveries were between 78,84% and 134,11%. These data permit to choose the second method. Thus all the samples were analyzed by GC-MS/MS (GC/QqQ tandem mass spectrometry, Thermo Scientific) with SRM modality selecting two MS/MS transitions for each compound, the first to quantify and the second for the confirmation.

The method validation allowed detection at levels ranged from 0,2µg/L and 10 µg/L, instead LOQs were between 0,6 and 30 µg/L and R^2 was always up to 0,9806. Analysis of vegetables and fruits showed that only the 24,28% (17 samples of which 13 fruits and 4 vegetables) of the waste samples had residues up to the specific LODs. All the residues, disciplinated by the EC Regulation n°396/2005 and subsequent amendments and additions, were under the specific MRLs. These results consent to adfirm that this kind of food waste can be used in safety to obtain new products with high added value.

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PP1_26

MOLECULAR CHARACTERIZATION OF HYBRID STRAINS OF *PLEUROTUS* BY ISSR

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Mushrooms of *Pleurotus* genus are commercially cultivated in worldwide by their nutritional and medicinal properties (Huerta *et al*., 2010). The germplasm obtaining from native strains is very important for genetic improvement of edible mushroom, allowing the production of hybrids with attractive attributes. In recent years, many researches have been reported for hybrid production through neohaplonts mating, previously recovered by chemical dedikaryotization (Valencia and Leal, 2002, Guadarrama, et al., 2014) and is convenient to realize the genetic analysis of both monokariotic components and hybrid strains produced. The use of genetic markers inter simple sequence repeats (ISSR) for determination of polymorphisms in basidiomycetes has been a method with high reproducibility and highly informative (Wang et al 2012; Raina et al 2001). The objective of present study was the genetic characterization of parental strains, reconstituted strains, neohaplonts and hybrids strains of *Pleurotus* by ISSR markers.

Two wild strains of the genus *Pleurotus* collected in Huajuapan de Leon, Oaxaca, Mexico were used, also four hybrid strains and two strains reconstituted, all generated by compatible pairings of neohaplonts previously produced were used (Guadarrama, et al., 2014). For gDNA extraction of each strain was used the ChargeSwitch gDNA Plant Kit (Invitrogen, USA) and for identification of genetics profiles six ISSR markers were used: UBC807 (AGAGAGAGAGAGAGAGT), UBC811 (GAGAGAGAGAGAGAGAC), ISR02 (CAGCAGCAGCAGCAG), ISR11 (CACCACCACGC), ISR12 (GAGGAGGAGGC), ISR15 (GCAGCAGCACT). PCR reaction for ISSR were in 25 µL reaction mixture containing 30 ng template DNA, 2 mM oligonucleotide, 23 µL PCR SuperMix (Invitrogen). The ISSR amplification condition was: 5 min initial denaturation at 94 °C; 40 cycles consisting of 1 min denaturation at 94 °C, 1 min primer annealing ranges from 53.2 to 63.8°C and 3 min extension at 72°C and a final extension for 10 min at 72 °C. Amplified ISSR fragments were separated on 1.5% agarose.

84 fragments reproducible with 96% of polymorphism were detected. The six oligonucleotides amplified fragments in the 14 strains studied, with the number of amplified fragments ranging from eleven (ISSR11) to seventeen (ISSR12) and ranging in weight to 200-2200 bp. Mallick and Sikdar (2014) uses these oligonucleotides in strains of *Pleurotus florida* and *Lentinula edodes* and get 13 bands for oligonucleotide ISSR11 300-3000 bp, oligonucleotide ISSR12 and get 12 bands from 300 to 2500 bp. With the analysis using markers ISSR patterns reproducible banding were obtained, thus achieving characterize strains of *Pleurotus*, parental, reconstituted, neohaplontes and hybrid allowing to detect genetic variation between strains of *Pleurotus* hybrid regarding parental which they arose.

Acknowledgnents: Financial Support: IPN-SIP Project: 20160240, ICyTDF 200/2012 (PICSO12-096).

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PP1_27

SERJANIC ACID OBTAINED FROM *CECROPIA TELENITIDA* REGULATES BLOOD LIPID LEVELS AND REDUCES THE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES

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Cecropia telenitida is a species of tropical tree located in the Andean region of Venezuela, Colombia, Peru and Ecuador (Frannco-Roselli, P. et al., 1997). In this area, *C. telenitida* is used as a traditional medicine for the treatment of inflammatory diseases and diabetes mellitus type 2. (Schinella G et al., 2008; Aragao DM., et al 2010). Serjanic acid is one of the most abundant pentacyclic triterpenes present in the roots of *C. telenitida* (Montoya Peláez, et al 2013). In a preliminary work we have shown this molecule has hypoglycemic activity and reduces the genetic expression of pro-inflammatory cytokines in adipose tissue of a mouse model of diet-induced obesity and insulin resistance (Balcazar N. et al., 2014). In this study we evaluated the effect of serjanic acid in plasma lipid and adipokine levels using the same mouse model. We also analyzed the genetic expression of pro-inflammatory cytokines in a human macrophage cell line model after serjanic acid treatment. Our objective is to evaluate the effect of serjanic acid in the molecular mechanisms involved in the development of systemic insulin resistance.

An ethyl acetate extract of air-dried roots of *C. telenitida* was partitioned successively with hexane and ethanol. The ethyl acetate was concentrated, diluted in methanol and purified by Sephadex LH-20. The fractions containing triterpenes were pooled and used for analytical and biological assays. Conventional isolations as preparative HPLC were used to purify serjanic acid.

We evaluated the effect of serjanic acid on blood lipid and adipokine levels in C57BL/6J mice fed with a high-fat (HF) diet. After 10 serjanic acid oral doses, animals were sacrificed. Blood samples were taken to evaluate total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, leptin, adiponectin and resistin levels.

Differentiated human monocytic THP-1 cell line, activated with lipopolysaccharide (LPS) and INF- γ, was used to evaluate acid serjanic anti-inflammatory action. Gene expression levels of TNF-α, IL-6, IL-1β and MCP-1 were detected by real-time RT-PCR.

Data showed no change in triglycerides and LDL cholesterol levels. However, serjanic acid administration reduced total cholesterol in mice fed with a HF diet. In this group, HDL cholesterol blood levels were increased after the administration of serjanic acid. The administration of this molecule also reduced significantly plasma leptin levels and we observed a slightly increase of adiponectin and resistin levels. We corroborated that serjanic acid reduces mRNA expression of pro-inflammatory cytokines MCP-1, IL-1β, IL-6 and TNF-α in human activated macrophages. *Conclusions:* This study demonstrates that the administration of serjanic acid could modulate blood lipid and adipokine levels in an obese and insulin resistance mouse model. These results also confirm the anti-inflammatory action of serjanic acid in a human cell model.

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PP1_28

PRELIMINARY STUDY ON THE PHENOLYC PROFILE OF BOLETUS AEREUS

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Boletus aereus and related species are among the most common and popular edible mushroom due to their particular sensory quality. The chemical composition, as well as the phenolic composition, of edible mushrooms was evaluated by many authors, but a very low reference is available on *B. aereus*.

In the present work, it is given a method to investigate 24 phenolic compounds in *B. aereus* by UHPLC system.

Several samples of *Boletus aereus* were collected in November 2015 at the woods of Peloritani Mountains, Messina, Italy. After collection half of those were maintained at -20°C and the other half were dried on filter paper at room temperature for ten days and then in oven at 45°c for 20min, to simulate an industrial process of preservation.

A known amount of fresh mushrooms was extracted with a mixture of methanol/water 80:20 in ultrasonic system and then analyzed with a Wathers Acquity UHPLC system, equipped with UV detector at 280 nm.

All the tests for the method validation were carried out: recovery values is about 80-90% obtained by spiked sample before extraction; the calibration curves of 24 standard of phenolic compound were carried out in the linear range of 0,025 – 0,2 µg, with LOD = 0,00043 µg and LOQ = 0,0014 µg for Hydroxytyrosol.

Then another amount of the dried mushroom was analyzed by the same procedure.

Hydroxytyrosol and t-Cinnamic acid and were identified and quantified in all fresh samples of *Boletus aereus* reaching up a mean concentration of 101 mg/kg and 0,94 mg/kg respectively. Whereas in the dried sample only Hydroxytytrosol was found, having a mean concentration of 299 mg/kg.

No other compound among those investigated was detected.

Protocatechuic acid, p-Hydroxybenzoic acid and p-Cumaric acid, were detected in previous study on edible mushrooms, but they have not made a comparison between fresh and dried matter, furthermore there are no references about Hydroxytyrosol and t-Cinnamic acid in *Boletus spp*.

Currently, the data obtained in the present study seem to be very interesting, especially if compared to the preservation methods.

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PP1_29

ETHNOBOTANICAL INVESTIGATION IN THE ITALIAN CENTRAL ALPS: HEALTH BENEFITS FROM EDIBLE PLANT SPECIES

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In popular tradition, plant resources are often employed multi-contextually, for example, both as food and medicine (Pieroni et al., 2002). Considering this dual feature, we decide to carried out an ethnobotanical survey in Valtellina, a valley of the Lombardy region, bordering Switzerland, where the traditional use of the plants is still alive.

More than 400 people were interviewed from nine municipalities using semi-structured questionnaires. Data were analysed by quantitative parameters such as ethnobotanicity (EI) and ethnophytonomic (EPI) indices, factor informant consensus (FIC). Abandoned and current uses were compared. Each reported species were exsiccated and the samples deposited at the Herbarium of the Brera Botanical Garden, Milan State University. Their scientific names were identified based on International Plant Name Index (IPNI).

Inhabitants quoted 56 out of 220 collected plant species as medicinal foods belonging to 26 families (Asteraceae, Rosaceae, Gentianaceae, Brassicaceae and Lamiaceae, mainly). Forty-seven species were wild, twelve cultivated and one purchased. About 16% was reported for its exclusive use in the considered category. The local people still resorted to 94.6% of their known uses. The plant parts most commonly used were leaves and fruits (20.6% respectively), flowers (19.1%), aerial parts (14.7%) and roots (11.8%). Almost all plants were used in the preparation of herbal tea, soups, salads, omelets and liqueurs.

The preference ranking identified *Achillea moschata* Wulfen at first place, followed by *Taraxacum officinale* F.H.Wigg., *Artemisia genipi* Stechm., *Sambucus nigra* L. and *Chenopodium bonus-henricus* L..

The study participants considered the medicinal cuisine an integral part of healthy practices, with beneficial effects mainly on the digestive (46.4%), respiratory (10.9%), nutritional (10.9%) and genitourinary (9.4%) disorders.

Our data testify that some functional foods are rooted in the traditional culture of the study area.

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PP1_30

EFFECT OF COMBINED ADMINISTRATION OF *ARTHROSPIRA* (SPIRULINA) *MAXIMA* AND WASTE OF *COCOS NUCIFERA L.* ON LIPID PROFILE IN MALE MICE FED WITH HYPERCHOLESTEROLEMIC DIET

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Metabolic Syndrome (SM), is a serious disease with many risk factors such as insulin resistance, dyslipidemia, decrease of high density lipoproteins (HDL), abdominal obesity and hypertension; which is associated with the increased in development of metabolic and cardiovascular diseases. For counteract the SM they have been included functional foods in diet that may contents antioxidant as polyphenols, dietary fiber, and polyunsaturated fatty acid Ω 3 y 6; one of these foods is a unicellular cyanobacterium *Arthrospira* (Spirulina) *maxima*, has been demonstrated to have antiatherogenic activity, suggesting benefits effects on hyperglycemia and hypercholesterolemia. Other functional food with high beneficial potential is coconut (*Cocos nucifera L.*), which have pointed antioxidants, antiviral, antifungal, antidiabetic, immune, hepatoprotective, cardioprotective, antibacterial effects. Therefore, the objective of the present study was to evaluate the effect of combined administration of *Arthrospira* (Spirulina) *maxima* and waste of *Cocos nucifera L.* on lipid profile in male mice fed a hypercholesterolemic diet.

Males ICR mice (28-30 g body weight) were acclimated for 7 days, with 12 hours light/dark periods, temperatures 22 °C, with water and food *ad libitum;* the animals were randomly assigned in 8 groups (n=10), 1) normal diet (DN), 2) hypercholesterolemic diet (DH), 3) normal diet + spirulina (DN + S), 4) normal diet + coconut waste (DN + C), 5) normal diet + spirulina combination coconut waste (DN + SC), 6) hypercholesterolemic diet + spirulina (DH + S), 7) hypercholesterolemic diet + coco waste (DH + C), 8) hypercholesterolemic diet + spirulina combination coco waste (DH + SC); the doses administered were of 400 mg/kg of Spirulina (S) and 350 mg/kg of coconut waste (C) and the treatments were administrated daily and intragastrically for 6 days. At the end of study, a blood sample was obtained, then by centrifugation getting serum, mice were sacrificed following the recommendations of the NOM-ZOO-062. From the serum obtained were determined total cholesterol, triglycerides, HDL-C and glucose by Wienner Lab Selectra with RANDOX Kits, LDL-C was calculated using the Friedewald formula.

Results shown a significant decrease in triglycerides concentration by combining spirulina and coconut waste comparing to the DH group, this is a positive effect because suggesting an improving on transportations of triglycerides with lipoproteins due to antioxidant capacity that have Spirulina to capturing free radicals and dietary fiber from Coconut waste, fermentable in the large intestine and produces short chain fatty acids. As for the effects on lipid profile in total cholesterol concentration, HDL-C, LDL-C there was no significant difference with any treatments. In conclusion, in this present study for 6 days was observe a positive therapeutic effect to decrease the triglycerides concentration in mouse serum, probably would have more effect making a new study changing doses and treatment time.

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PP1_31

BIOLOGICAL ACTIVITIES OF *LAVANDULA ANTINEAE* MAIRE. ENDEMIC SPECIES IN ALGERIA

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Lavandula antineae Maire (Lamiaceae) is an endemic medicinal plant of Algeria which is traditionally used for the treatment of Chills, Bruises, oedema and rheumatism (1). However, no antioxidant or antimicrobial activities of its extracts have been previously published. The objective of this study is to evaluate antioxidant and antimicrobial activity of *Lavandula antineae*.

The hydromethanolic crude extract and its fractions (chloroform, diethyl ether, ethyl acetate and n-butanol) were investigated for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH),reducing power and β-carotene–linoleic acid tests. Total phenolic contents and flavonoid contents were also measured. Antimicrobial activity was examined against 10 microbial strains by disc diffusion and agar dilution methods.

Total phenolic and flavonoid contents of the extracts varied between 12.49-262.35 mg GAE/g extract and 1.35-4.03mg QE/g extract, respectively. The highest radical scavenging activity was detected in ethyl acetate and diethyl ether fractions. Ethyl acetate fraction had also the highest reducing capacity. The most effective fractions on lipid peroxidation are diethyl ether and ethyl acetate fractions. In case of antimicrobial screening, crude extract and its fractions showed moderate antimicrobial activity against tested microorganisms. Among the tested samples, the crude hydromethanolic extract have been the most active.

The findings of the present study suggest that hydromethanolic extract and its fractions of *Lavandula antineae* possess antioxidant and antimicrobial properties and hence can be a potential natural source in health and medicine.

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PP1_32

PRIMARY SCREENING OF BIOLOGICAL ACTIVITIES IN EXTRACTS OBTAINED FROM AROMATIC AND MEDICINAL PLANTS GROWN IN COLOMBIA

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Herbal therapy could be beneficial in attenuating severity of human diseases. Colombia has a rich herbal repertoire and the Vademecum on Medicinal Plants prescribes medicines for a variety of diseases, but the number of plants studied is limited.

The current work was undertaken to identify plant species from Colombia whose extract (EXT) could be selected as primary source for discovering natural medicines to alleviate symptoms of cancer, dengue, and infectious caused by Methicillin-*Resistant Staphylococcus aureus* (MRSA).

Supercritical fluid (CO_2) extraction was carried out to obtain plant EXTs, which were collected at 40 (fraction 1) and/or 80 (fraction 2) bar. Fifty samples from 27 plant species were profiled for cytotoxicity and antiproliferative activities using MTT screening, in six human and animal cell types derived from targets of xenobiotic toxicity (liver, kidney, skin), and from cancerous organs (liver, cervix, breast). Hierarchical clustering of cytotoxic profiles was performed using CC_{50} values, and the sum of individuals weighted hierarchy values (WH) was adopted as si milarity indicator. Inhibitory effect on dengue virus (DENV) was evaluated by using both area fraction output method (with ImageJ freeware) and virus-antigen reduction test. A modified microdilution method was used for testing inhibition on the MRSA growth.

Some EXTs were non-cytotoxic (n = 12; CC_{50} > 200 µg/mL; ∑iWH > 7.0) or cytotoxic (n = 14; CC_{50} < 200 µg/mL; ∑iWH < 3.2) to all cell types, whereas others exhibited cell type–specific cytotoxicity (n = 24; CC_{50}: 500 – 77.8 200 µg/mL; ∑iWH: 6.3 – 3.3). Fractions of EXTs from the same plant frequently showed different patterns of cytotoxicity. EXTs from *Bursera tomentosa* and *Eriope crassipes* seem to be active (IC_{50} < 50 µg/mL; selectivity index > 6.4) against cells (HeLa) from cancer cervix, though none of EXTs presented relevant activity on other cancerous cell. Five EXTs reduced (66.8 – 31.4%) cellular death caused by infection at least of one DENV-serotype, and different pattern of antiviral activity respect to fraction of EXT was observed. *Hyptis suaveolens* fraction 1, but not fraction 2, was the only EXT active against all four DENV serotypes (IC_{50}: 16.6 – 78.7 µg/ml; SI > 5.4). Relevant activity against MRSA (MIC: 0.029 - 0.059 mg/mL) was found in EXTs from *Piper subflavum var. Espejuelanum*, *Piper eriopodon,* and *Hyptis suaveolens*.

Analysis of biological activities of products derived from plants is an option for identifying starting samples for discovering of herbal medicines. This study resulted in the identification of EXTs from Colombian plants with promising activities against cervix cancer, DENV and MRSA. Protective efficacy studies in an animal model are required to confirm activities.

Acknowledgnents: This research was carried out thanks to financial support from the Colombian Institute of Science and Technology (Colciencias), Grant RC-245-2011 (Patrimonio Autónomo del Fondo Nacional de Financiamiento para la Ciencia, Tecnología e Innovación, Francisco José de Caldas).

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PP1_33

GREEN SYNTHESIS OF NEW LIGANDS STARTING FROM CHIRAL *p*-HALOGENATED AMINES

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This research was carried out with a new and more efficient method of synthesis, achieving the principles of Green Chemistry. This new technique reduces the use of dangerous chemicals in the design and development of the synthesis. On the other hand, imines are versatile ligands, which are synthesized from the condensation of a carboxylic compound and an amine. These compounds and their metallic complexes are very important as catalysts in different biological systems, polymers, colorants and in the medical and pharmaceutical fields.

We report the synthesis of three new chiral imines derived from 2-thiophencarboxaldehyde and optically active primary aromatic amines bearing halogen atoms in *para*- position: (*R*)-(+)-1-(4-fluorophenyl)ethylamine, (*S*)-(-)-1-(4-chlorophenyl)ethylamine and (*S*)-(-)-1-(4-bromophenyl)ethylamine, by using a technique from Green Chemistry: "Solvent free reactions", which gave excellent yields of the imines. In Solvent free reactions, the processes occur in many cases in a more efficient and selective way than in reactions in solution, since in the solid state the molecules in the crystal had arranged in a regular and compact way. Solvent free reactions have also many advantages: low pollution, low cost and easy work-up.

The reaction of 2-thiophenecarboxaldehyde and (*R*)-(+)-1-(4-fluorophenyl)ethylamine in a 1/1 ratio leads to the corresponding imine (*R*)-(-)-[1-(4-fluorophenyl)-*N*-(tiophene-2-yl)methylidene]ethylamine, as a white solid. Mp = 69-70°C, yield = 97%, $[\alpha]_D^{25^\circ}$= - 153.7° (c = 1, $CHCl_3$). In the same way, the reaction of 2-thiophenecarboxaldehyde and (*S*)-(-)-1-(4-chlorophenyl)ethylamine gives (*S*)-(+)-[1-(4-chlorophenyl)-*N*-(thiophene-2-yl)methylidene]ethylamine as a white solid. Mp = 41-42°C, yield = 98%, $[\alpha]_D^{25^\circ}$ = +174.3° (c = 1, $CHCl_3$). Finally, the reaction of 2-thiophenecarboxaldehyde with (*S*)-(-)-1-(4-bromophenyl)ethylamine produces (*S*)-(+)-[1-(4-bromofenil)-*N*-(tiofen-2-il)metilideno]etilamina as a white solid also. Mp = 58- 59°C, yield = 95%, $[\alpha]_D^{25^\circ}$ = +198.7° (c =1, $CHCl_3$).

The products were fully characterized by FT-IR, NMR ^{1}H and ^{13}C, EI-MS and the structures of the new ligands was confirmed by X–ray diffraction studies.

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PP1_34

GREEN SYNTHESIS OF A NEW CHIRAL LIGAND HALOGENATED AND ITS Pd(II) COMPLEX

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Nowadays, many groups worldwide carry out numerous researches about the origin and treatment of carcinogenic diseases, where the design and synthesis of new drugs are constantly proved with the goal of eliminating or controlling such diseases. This report is about sulfur-containing molecules in their structure to form a chiral ligand with both the C=N group and a Sulfur atom. These features have special interest in the formation of metallic complexes since metals can also coordinate with the Sulfur atom. As antecedent, our research group has already synthesized Pd(II) complexes with anticancer activity. Due to the importance of these complexes in the medical field, one of our main objectives is the synthesis of metallic complexes using chiral ligands.

We report the synthesis of a new chiral ligand derived from 2-(methylthio)-benzaldehyde and the optically active primary aromatic amine: (S)-(-)-1-(4-chlorophenyl)ethylamine, using a technique from Green Chemistry: "Solvent free reactions", which gave excellent yields of the imine and the Pd(II) complex. In Solvent free reactions, the processes occur in many cases in a more efficient and selective way than in reactions in solution, since in the solid state the molecules in the crystal are arranged in a regular and compact way. Solvent free reactions have also many advantages: low pollution, low cost and easy work-up.

The products were characterized by using spectroscopic techniques: FT-IR, NMR H and C, EI-MS and the structure of the Pd(II) complex was fully confirmed by X-ray diffraction. The crystalline structure of the complex showed that the Palladium atom coordinates with the Sulfur atom and the Nitrogen atom of the imine group, as expected.

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PP1_35

ANTIPROLIFERATIVE ACTIVITY ON HUMAN PROSTATE CELLS OF ESSENTIAL OILS OF THREE LEBANESE *SALVIA* SPECIES

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Prostate cancer is the leading cause of death from cancer in older men and the most commonly diagnosed cancer in men overall. Many patients are diagnosed when the cancer has already advanced to metastasis. Most patients with advanced disease respond initially to androgen-ablative therapies, but frequently in this stage, prostate cancer growth and development become independent of androgen and renders androgen ablation therapy ineffective [1]. At present available treatments for advanced, androgen-independent prostate cancer are only marginally effective [1]. In the Lebanese folk medicine, *Salvia* species are used by many people in various villages and towns for the therapeutic value of their essential oils and water extracts [2]. In this study we report the potential anticancer effect in androgen-insensitive (DU-145) and androgen-sensitive (LNCaP) human prostate cancer cells of essential oils from three *Salvia* species well known in Lebanon as component in several multiherb products used for the treatment of cancer and other diseases*: S. aurea* L., *S. judaica* Boiss and *S. viscosa* Jacq.

Aerial parts from *Salvia aurea S. judaica* and *S. viscosa* were collected at the full flowering stage from plants wild growing at El Kfour, Lebanon, in August 2012. The air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h using n-hexane as a solvent according to the standard procedure described in European Pharmacopoeia (2008). Analytical gas chromatography was carried out as previously reported [2]. The biological activity of the essential oils, was investigated against human prostate cancer cells, testing several biochemical parameters [2, 3], such as cell vitality (MTT assay), cell membrane integrity (lactate dehydrogenase release) and caspase-3 activity. In addition, the expression of Bcl-2 and Bax proteins was evaluated.

Results revealed *that all* the *test* essential oils were able to inhibit, after 72 h of treatment, the growth of cancer cells. Our data also demonstrate that these natural products induce apoptotic cell death that could be related to an overall action of the sesquiterpene compounds, particularly caryophyllene oxide, found in comparable concentration in all three samples. In conclusion, the experimental evidences presented here, could provide a further *in vitro* scientific support for the use of these species in traditional herbal preparations known in Lebanon and surrounding countries for the treatment of cancer. In addition, the selectivity of the essential oils *S. aurea*, *S. judaica* and *S. viscosa* in targeting cancer cells, sparing normal cells, suggests that these natural products for their active components could be candidate for further analysis with the aim to reduce the toxic side effects of chemotherapeutics in prostate cancer patients.

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PP1_36

ANTIMICROBIAL PROPERTIES OF GARLIC (*ALLIUM SATIVUM* L.) AND ONION (*ALLIUM CEPA* L.) EXTRACT ON COMMON CARP (*CYPRINUS CARPIO*) RESTRUCTURED MEAT DURING STORAGE IN 4 °C

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Aquaculture is an activity that has been the basis for numerous investigations that have been reflected in important technological advances (Montaño et al., 2006). Common carp (*Cyprinus carpio*) is a species that has great adaptability and reproductive capacity; additionally it is a source of high quality protein. However, the high water content, the proper bacterial flora of fishery products and high enzyme activity, cause it to be very susceptible to deterioration (Medina et al., 2008) in order to counteract these damages have been used antimicrobials natural origin such as garlic and onions, they contain phenolic compounds and sulfur which exert an antioxidant and antimicrobial effect. The aim of this study was to evaluate the effect of an aqueous extract of garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) on microbial characteristics during the shelf life of restructured meat of common carp (*Cyprinus carpio*).

A mixture of aqueous extracts of garlic and onion was conducted in order to assess the mixture with greater amount of phenolic compounds and antimicrobial activity. For the determination of total phenolic compounds (TFC), was according to López et al. (2014). For antimicrobial activity, microbial challenge was performed with microorganisms of importance in public health such as *E coli, S. aureus and P. aeruginosa* (NOM-113-SSA1-1994). A restructured meat was designed based on common carp at which the mixture of extracts of garlic and onion was applied and its antimicrobial effect was evaluated for 21 days under refrigeration (4°C) also carried out an acceptance test with 200 judges untrained. All studies were conducted in triplicate, analyzed by ANOVA and Tukey means difference ($p < 0.05$).

It was found that a 50:50 mixture of the aqueous extracts of garlic and onion had the best TFC content ($p < 0.05$) and antimicrobial effect ($p < 0.05$) inhibiting growth of *S. aureus, E. coli, and P. aeruginosa*. The aqueous extract was applied to the restructured meat observed that after cooking, 17% of the TFC is retained with respect to the amount obtained in the initial mixture was also in 42% of the antioxidant activity. No microbial growth was observed during the 21 days of storage, not so for the control that had growth at 5 days of storage. Acceptance testing resulted in significantly final product acceptance by consumers. These results might suggest that the use of carp meat in combination with TFC garlic and onion are an alternative consumption of this species of economic importance, being a product sensorially accepted also can be a method useful conservation and low cost.

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PP1_37

MEDICINAL PLANTS OF COLOMBIA WITH POTENTIAL FOR TREATMENT INFECTIOUS DISEASES

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Infectious diseases have generated problems associated with morbidity, aftermath and mortality, in humans and in animal species, affecting public health and livestock production. Currently, about 25% of deaths worldwide are directly related to infectious disease, occurring mainly in the region of the Americas with 44%. Additionally, it has been reported that 57.8% of the events of emerging diseases are of bacterial origin, being mostly of new pathogens (40%) and antimicrobial resistant microorganisms (31%). In this sense, the use of plants for treatment of common infectious diseases has been known for many years and therefore plant species have been used as a source of drugs for modern medicine, either by providing pure compounds or as prototypes modified to synthesis of new drugs. To work with natural products based on traditional knowledge, it has been proposed as an effective method for the identification of medicinal plants, extracts and bioactive principles, reducing empiricism and improving the chances of success in drug development. 2,404 medicinal species for Colombia, where 1,656 are native to the Neotropics and only 12.5% have references that show their traditional therapeutic use are known; allowing establish that the country has little research on the subject and has not been rated this botanical heritage as a health alternative.

Therefore, the objective of this work was to increase the phytochemical knowledge of some medicinal plants of the Colombian biodiversity. Eleven plants were selected with the greatest factor consensus for the treatment of infectious diseases among herbalists subsequently extracts of these plants were subjected to evaluation of antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* the method of HT-spoti. During the study it found that 80% of the plants showed antimicrobial activity against at least one of the selected bacteria, *Kohleria spicata* being the only plant that present activity against four microorganisms. In addition, this species with *Arnica montana*, *Equisetum giganteum* and *Levisticum officinale* were those with the greatest potential to possess antimicrobial MIC values below 4 mg/mL. This project is presented as the first contribution to chemical and biological knowledge of some medicinal species of Colombian diversity, contributing to the assessment of the botanical heritage as a resource for the development of new innovative therapeutic agents.

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PP1_38

INHIBITORY CAPACITY OF AQUEUS OREGANO EXTRACT (*ORIGANUM VULGARE* L.) ON MICROORGANISMS(*S. AUREUS, E. COLI AND SALMONELLA TYPHIMURIUM*) AND IT´S EFFECT ON THE SHELF LIFE OF A GEL-TYPE MADE OF PROTEIN FROM GIANT SQUID (*DOSIDICUS GIGAS*).

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The use of natural extracts in food applications and especially in highly perishable products is based on certain characteristics such as availability, functional attributes, antimicrobial properties, color contribution and improvement of flavor. Studies have shown, it can be used as additives, because of his antioxidants and antimicrobial effects. Organic extracts from oregano (*Origanum vulgare* L.) had shown antimicrobial effects against *E. coli, S. aureus* and *Salmonella typhimurium*, the principal components of this extracts are non-polar and has interactions with the cell wall. The information from aqueus extracts from this source recently had grown and it´s been proved on different microorganisms and different products, but not specifically giant squid products. Our goal is the use of aqueus extracts form oregano in the production of a gel-type product made of protein extracted from giant squid (*Dosidicus gigas*) as an alternative to extend the shelf life of this product.

Extraction of the components from oregano was made using a buffer of phosphates (100mM pH 6.0, with NaCl 0.6M) as the extraction medium, using a ratio 1:10 (oregano: solution) and 30 minutes of extraction in continuous agitation. The antimicrobial effect against microorganisms was determined by the Bauer-Kirby method. Added concentration of extract to the gel-type was determined by flavor and color contribution, with a maximum of 5% (v/w). Gel-type products were evaluated in three characteristics: sensory attributes (flavor and color), structure attributes (hardness, elasticity and continuous aspect) and microbiology attributes (total coliforms by MPN method).

The oregano extract obtained shown antimicrobial characteristics against *S. aureus* ATCC 25922 (17 mm) and *E. coli* ATCC 25923 (12 mm), but not against *Salmonella typhimurium (from clinical source)*. Four concentrations were evaluated, 0%, 1%, 3% and 5%; as the concentration was higher, the sensorial, structural and microbiological characteristics were maintained, as shown in table. 1

Concentration (v/w)	0%	1%	3%	5%
Days of storage	7	9	13	17

Table. 1- Percentage of extract added to the gel-type product and the days of storage that characteristics were maintained

Microbiological analysis until the 20th day didn´t shown growth of microorganisms that use lactose and can produce CO_2, determined in the first step of the determination of total coliforms by MPN method. It can be due to the aseptic elaboration process and the manufacturing steps.

The increase in days of storage and maintenance of characteristics can be attributed to the growth inhibition of microorganisms related to detrimental effects, and the interactions between extract components and proteins of the gel-type.

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PP1_39

ERYSIMUM CRASSIPES AS NEGLECTED MEDICINAL PLANT: STORAGE AT EARLY BRONZE AGE KÜLLÜOBA/TURKEY

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At present, in modern medicine, numerous species of *Erysimum* are well known as containing chemical compounds in form of the steroid glycosides used against cardiac diseases, and essential oils with expectorant, laxative and diuretic effects (Bauer et al. 1960, Maslennikova et al. 1967, Umarova et al. 1977, Makarevich et al. 1993, Lei et al. 2000, Jawla et al. 2010). Essential oils like dillapiole are detected recently in other medicinal *Erysimum* species as anti-inflammatory compounds (Akhgar and Roknabadi 2015). As a part of Iranio-Turanian vegetation, species of genus *Erysimum, E. crassipes* is widely distributed in Anatolia and in the Near East (Davis 1965-1988). Despite the extended use of numerous *Erysimum* species for medicinal purposes, ethno-medicinal use of *E. crassipes* is mentioned solely from the Highlands of Northern Jordan (Oran and El-Aisawi 2014). Archaeologically, at an Early Bronze Age site in Turkey, *Erysimum crassipes* found in a small pot with ca. 2,5 million seed counts, constitutes the first recorded find in the World (Cizer 2015). Essential oil and glycoside profile of modern *E. crassipes* seeds can be obtained using different chromatographic and spectrometric methods (Es-Safi et al. 2005, March et al. 2006). The seeds are collected by a plant taxonomist from well documented accession in Turkey. Identification and definition chemical compounds of *Erysimum crassipes* and their effects on human body may enlighten the possible medicinal use of *Erysimum crassipes*. Such kind of study will not only be crucially important for the plant's archaeological meaning, but also for the modern biochemistry and medicine.

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PP1_40

LEVELS OF HEAVY METALS IN OCTOPUS (*OCTOPUS VULGARIS*) FROM SOUTH TYRRHENIAN: PRELIMINARY RESULTS

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The common octopus (*Octopus vulgaris*) is a voracious predator normally distributed on rocky, sandy and muddy bottoms. It is characterized by fast growth rates and a short lifespan and known for its ability to accumulate high levels of elements essential to metabolic functions and nonessential elements. In general, feeding is considered to be the primary pathway for trace element bioaccumulation in cephalopods and second seawater. Although *O. vulgaris* is benthic, living in direct contact with the substratum, which represents another possible pathway for trace element accumulation. The objectives of the present study were to evaluate Cd, Pb and Hg levels in the muscle of octopus (*Octopus vulgaris*) collected from the southern Tyrrhenian Sea in Italy and to assess the health risk related to human consumption.

Samples (n.12) of *Octopus vulgaris* were caught along the coast of the southern Tyrrhenian Sea between September and December 2015. Organisms were stored in individual plastic bags and immediately frozen onboard in order to minimize mobilization of metals between organs/tissues. Samples were kept at -25°C for a maximum of 2 weeks before dissection. In the laboratory, arm and mantle was dissected excluding the skin. Weight, mantle length and sex were determined for each individual and was subsequently homogenized by means of a laboratory mixer for metal analysis. Aliquots of each sample were digested in ultrapure 65% HNO_3 and H_2O_2 in a microwave digestion system. Pb, Cd and Hg concentrations were determined by atomic absorption spectrometer (GF-AAS).

Cd and Hg concentrations were found very low in all samples of octopus muscles (Cd: 0.011±0.018 mg/kg w.w.; Hg: 0.017±0.040 mg/kg w.w.) and were below the legal limit for human consumption. The concentration of Pb was generally high in samples of muscle (0.851±0.662 mg/kg w.w.) and all tested samples were above the maximum concentration level (Reg CE 1881/2006) excluding this product to human consumption.

To establish possible human health implications related to consumption of octopus, the Pb estimate weekly intakes (ISMEA, 2010) were subsequently compared with the provisional tolerable weekly intake (PTWI) of 25 µg/kg of body weight (EFSA, 2010).

Considering the level of Pb, the consumption of octopus muscle may increase Pb intake, but it would not contribute significantly to the PTWI. In contrast, may reach high EWI values in the heavy consumer of octopus, when the other main contributors to dietary Pb intake were included in the exposure assessment.

The preliminary results obtained in the current study showed the presence of Pb in all samples analyzed, underlying its presence in the environment. Monitoring studies on heavy metals in *Octopus vulgaris* and other species of the marine food chain in a greater number will provide more detailed information of the human exposure to Pb in this area.

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PP1_41

PHYSICOCHEMICAL EVALUATION OF THE OLEORESIN FROM *COPAIFERA PAUPERA* COLLECTED SEASONALLY IN ACRE, BRAZIL

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The copaiba oleoresin has been increasingly commercialized worldwide as a valuable non-timber forest product from the rain forests. Efforts have been driven toward assessing chemical standards to establish the quality and aggregate value to this as raw material. In Acre State, Brazil, the oleoresin collectors categorize the copaiba trees as white, yellow, red and black types, according to the bark color, regardless the botanical species. Our goal was to assess the terpene composition, refractive index, density and acidic index variations in the oleoresin, according to a seasonal collection of *Copaifera paupera* Herzog, which embraces all the types above.

Oleoresins collected from forty-eight GPS-marked individuals of *C. paupera* in the Embrapa Experimental Field area, at Rio Branco-Porto Velho road, Acre State, Brazil. Samples and color type were duly registered. Samples (15-50 ml) were centrifuged (2100 rcf, 30 min, 10 °C) to eliminate debris. Densities were measured in a pre-calibrated Mettler digital densimeter. Refractive indices (RI) were determined using an Abbe refractometer. Acidic indices (AI) were obtained according by Pharmacopeia titration method. Chemical compositionss were assessed by GC-MS using a HP 6890N chromatograph equipped with a HP-5 MS column (30 m×0.32 mm×0.25 µm); helium flow 0.5 ml/min; 1:20 split ratio mode; oven temperature from 70°C (held 5 minutes) to 290 °C at 4°C/min (held 10 minutes); ion source at 250 °C; EI 70 eV. Sample aliquots (1 µL from 5 mg/ml CH_2Cl_2 solution) were previously methylated with diazomethane. Constituents were identified by comparing mass spectra stored in Wiley 59943B data basis and retention index.

RI varied from 1.45-1.54 and density from 0.956 a 1.056 g/ml (statistically non-significant, $p>0.05$) for all the oleoresin samples. Acidic index ranged from 30-100 mg KOH/g. All samples were constituted by mixtures of sesquiterpene and diterpene acids. Most frequent compounds, β-caryophyllene (20% avg, maximum produced in April) appeared in 77% and copalic acid (3% avg) in 84% overall samples. Caryophyllane/humulane-type sesquiterpenes were predominant in the red-, black- and yellow-type copaiba, whilst elemane/cadinane-type were predominant in the white copaiba oleoresin, as corroborated by Permanova statistical analysis. These two tendencies reflects competitive biosynthetic routes in the plant. Danielic acid was found abundant and exclusively in the white-type copaiba. This latter oleoresin is the least preferred by buyers, a fact that may be related to lower β-caryophyllene contents. Values obtained for RI, density and AI were tentatively related to the chemical composition and compared with those obtained to edible fats oils. Some patterns to detect adulteration in oleoresins from *C. paupera* destined to commercialization were preliminarily inferred.

Acknowledgments: Finanacial support: Embrapa Acre (Macroprogram 3)

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PP1_42

ACETIC ACID BACTERIA AND CELLULOSE PRODUCTION: STRAIN SELECTION AND POLYMER CHARACTERIZATION

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Bacterial cellulose (BC) is an insoluble, extracellular polysaccharide that is produced by different bacteria species. It is an attractive biomaterial having potential use in many fields as natural polymer or in composite materials. Among BC producing acetic acid bacteria (AAB), *Komagataeibacter xylinus* is the representative species of cellulose synthesis.

In this study 50 AAB isolated from Kombucha tea, a beverage obtained by the fermentation of tea through a culture of yeasts and AAB, were screened for their ability to produce BC. Candidate strains were pre-selected on the basis of qualitative traits such as rapid growth on basic culture media containing glucose and ability to produce BC as a layer or deposit in testing tubes. Quantitative assays to select the best candidate for optimized BC production in different culture conditions and culture media (standard GY medium and the optimized medium (ET)) were conducted by microscale tests.

Process scalability and optimization of BC production were tested in batch using the standard GY medium and the optimized one (ET). Then, the selected BC membrane was characterized from a microstructural and thermo-mechanical point of view.

Selected strains showed high variability in BC production. Strain K2G30 achieved the highest BC yield in all tested conditions and therefore it was fully characterized and identified. Differences in BC yield due to carbon sources were stated. The pool of strains collected and typed are a microbial resource to study mechanisms of cellulose synthesis and to develop tailored cellulose processes; they also represent a starting point for a further optimization in order to increase the cellulose yield.

The SEM observation of the BC membrane produced by K2G30 highlighted a high degree of porosity due to the ultrafine 3D network of BC nanofibres. Moreover, it did not show any difference between the two sides of the membrane, which presented an average thickness of about 20 µm. The XRD analysis estimated a degree of crystallinity (Segal equation) of about 80%. The water absorption rate (WAR) tests proved the excellent ability of the polymer to absorb water, since the BC produced by K2G30 achieved a WAR of about 400% in 48 hours.

The BC membrane produced by K2G30, on account of its microstructure, promising thermo-mechanical behavior and high water-absorption ability, is an ideal candidate for the production of biomedical devices, especially for skin repair, and food additives.

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PP1_43

AMARANTH GRAIN (*AMARANTHUS HYPOCHONDRIACUS*) AS SUSTAINABLE ALTERNATIVE PROTEIN SOURCES TO MEAT

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Amaranth grain, native wild plant from Latin America was cultivated by the Aztecs since ancient times and consumed as part of their daily diet and also used in religious ceremonies. Amaranth corps were banned by Spaniards upon their conquest of Mexico, however the plant has continued to grow as weed since that time, and it´s genetic base has been maintained. Nowadays several species of *Amaranthus caudatus, Amaranthus crudents* and *Amaranthus hypocondriacus* are still cultivated in some states of Mexico. Currently the main protein source for most Mexican diets is meat, which due to it´s cost of production, meat is not available to all social groups. Therefore other sustainable sources of protein such as Amaranth plant should be investigated. The aim of this study is to evaluate nutritional value of macronutrients that can be use as sustainable alternative protein sources focus on partial replacement of meat in a healthy balanced diets.
Macronutrients of *Amaranthus hypocondriacus* grains were assessed in dry basis, according to AOAC 1995 methods. Samples were obtained early summer 2015 at Mexico State. The samples were dried at 60°C for 24 h and milled to a fine powder to analyze moisture, proteins, lipids, minerals, fibre and soluble carbohydrates. Proteins were calculated from the nitrogen content by the Kjeldahl method using the conversion factor 6.25. Lipids were determined by an extraction process with petroleum ether at 120°C for 6 h using a Goldfish apparatus, minerals were analyzed after incinerating the sample at 600°C in a muffle furance for 6h.Crude Fibre by acid hydrolysis, followed by alkaline hydrolysis and soluble carbohydrates by [100-(protein+lipids+minerals+crude fibre)].
Data obtained were: moisture 3.8%; proteins 14.80%; lipids 6.5%; minerals 2.22%; crude fibre 3.15%; soluble carbohydrates 73.33%. Data obtained might vary due to biotic and abiotic environment conditions. Consumption of Amaranthus grain is not in a regular diet due to the lack of information about the benefits that they provide to health. However, Amaranthus plant offer an alternative source of protein to meat, additionally grains contain rich sources of fatty acids and minerals recognized for their potential health benefits.

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PP1_44

EVALUATION OF THE HYPOLIPEMIANT AND HYPOGLUCEMIANT EFFECT OF A BASE POWDER TO ELABORATE FUNCTIONAL FOOD OBTAINED FROM TOMATO PEEL (*PHYSALIS IXOCARPA*) IN MICE

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Dyslipidemia is characterized by abnormalities in blood lipid levels, such as cholesterol, triglycerides, HDL high density lipoproteins and low density lipoproteins LDL. Having no control in this condition may occur diabetes, which is the increased blood glucose, which results in the development of various complications, considering one of the leading causes of mortality in Mexico; so the search for alternative treatments to control these conditions is necessary. In this research the hypoglycemic and lipid-lowering activity of a base powder was evaluated to develop functional food derived from tomato peel; by the presence of substances in its peel, as acilsacarosas, it is credited with lipid-lowering and hypoglycemic effect.

Male ICR mice were used (25-35 g body weight) and were acclimated for 7 days, with 12 hours light/dark periods, temperatures 22 °C, with water and food *ad libitum;* the animals were randomly assigned in 6 groups (n=6), 1) normal diet (N), 2) hypercholesterolemic diet (H), 3) normal diet + tomato peel 500mg/kg (N+CT), 4) hypercholesterolemic diet + tomato peel 125mg/kg (H+CT), 5) hypercholesterolemic diet + tomato peel 250mg/kg (H+CT), 6) hypercholesterolemic diet + tomato peel 500mg/kg (H+CT), the treatments were administrated daily and intragastrically for 7 days. . At the end of the study, a blood sample was obtained, and then by centrifugation serum was obtained, then mice were sacrificed following the recommendations of the NOM-ZOO-062. From the serum obtained were determined serum glucose and lipidic profile, by using vitaLab Selectra II, LDL-C was calculated using the Friedewald formula.

The results obtained, shown in relation to the glucose concentration a significant difference between the group H+CT 500 mg / kg compared to the H group; in relation to cholesterol, there was a significant difference between the group H+CT 250mg / kg compared to the H group; in relation to triglycerides there was a significant difference between H+CT 500 mg / kg group compared to the H group; in relation to LDL-C there was a significant difference between group H+CT 500mg / kg compared to the H group. It is concluded that the powder obtained from the tomato peel has a hypoglycemic and lipid lowering activity, with the dose of 500 mg /kg the most effective, suggesting that such activity is because the tomato peel inhibits the absorption of cholesterol in the body.

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PP1_45

BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *ARTOCARPUS HETEROPHYLLUS LAM*

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Artocarpus heterophyllus is an exotic fruit native of Asia, commonly known as jak, which has been used in traditional medical as a treatment for diabetes mellitus, anti-inflammatory, anthelmintic, antibacterial and hypoglycemic. In Mexico it has been cultivated and commercialize for many years ago, as juice, jam or fresh fruit. However, in last years it has been object of studies because its biological properties. The aim of the present study was to quantified the amount of bioactive compounds such as total phenolic and total flavonoid contents of leaves, exocarp, mesocarp, and seeds, as well to evaluated its radical scavenger capacity by DPPH assay.

Fruit and leaves were obtained of Jack Fruit Quiñones Company located in Nayarit, Mexico. The fruit was identified by the Herbarium of the UNAM´s Botanical garden in Mexico City. A voucher specimen that identifies the plant as *Artocarpus heterophyllus* has been deposited at the Department of Pharmacy (FS9157). The extracts of leaves, shell, and seeds were performed following the method Asongalem et al. (2004) with a few modification. The pulp was macerated and soaked in 80% EtOH (Umesh et al, 2010). Hydro alcoholic extract of leaves, shell, pulp and seed were analyzed with the phytochemical preliminary evaluation. The content of total flavonoid content was performed following the procedure of Osorio et al. (2011) and the total phenolic compounds by the methodology of Akrout et al. (2011).

The antioxidant activity was measured by the DPPH assay. Finally, the acute toxicity of each of the hydro alcoholic extracts was measured by the lethal dose 50 (DL50) method in CD1 strain mice (OECD 423, 2001).

Preliminary phytochemical assay revealed that *A. heterophyllus* contained tannins, glycosides, flavonoids and phenolic compounds. The total phenolic content of the leaves was 75.44 ± 0.14 mg galic acid eq. / 100 g d.b., shell have 72.24 ± 0.33 mg galic acid eq. / 100 g d.b, pulp 8.79 ± 0.09 mg galic acid eq. / 100 g d.b. and seeds have 11.04 ± 0.16 mg galic acid eq. / 100 g d.b

The total flavonoid content of the leaves was 38.88 ± 0.66 mg catechin eq./100 g, d.b., shell have 27.57 ± 0.10 mg catechin eq./100 g, d.b, pulp 6.10 ± 0.36 mg catechin eq./100 g, d.b and seeds have 7.50 ± 0.14 mg eq. catechin/100 g. The antioxidant activity of the hydro alcoholic extract presented the higher inhibition percentage of DPHH radical (50% of inhibition).

The hydro alcoholic extracts did not cause mortality, morbidity symptoms, or deleterious effects. LD_{50} value was greater than 2000 mg/kg, which indicates that it is not toxic, so it can be used for later biological studies.

The present study showed that the hydroalcoholic extract have a strong antioxidant activity.

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PP1_46

ANTIOXIDANT ACTIVITY OF PORIFERANS FROM THE COLOMBIAN CARIBBEAN ROCKY COAST BY DPPH AND ABTS METHOD

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Marine invertebrates, especially the species belonging to the Phylum Porífera represents a rich source of new biological and chemical compounds of great interest. Many of these bioactive molecules have been considered to have great potential, such as antibacterial, anticancer, anti-inflammatory and antioxidants, among other potential biological activities. The main goal of this study was to evaluate the antioxidant activity of aqueous extracts from the Colombian Caribbean Poriferans: *Suberites aurantiacus*, *Desmapsamma anchorata* and *Ircinia campana*, using the in vitro models DPPH and ABTS; and the results were compared against the results obtained with the antioxidant commercial, hydroquinone. In the ABTS assay, the aqueous extracts and fractions containing precipitated proteins exhibited high inhibitory activity of radical cation showing more than 77% of inhibition in comparison with the hydroquinone, while the free radicals captation by DPPH in the *I. campana* was 146,73% when compared to the positive control (91,01%). The results suggest that the marine sponges of the rocky coast of the Colombian Caribbean are promising sources of antioxidants and others molecules with useful potential to be used in biotechnological processes.

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PP1_47

SESQUITERPENES AND MONOTERPENES FROM *AMMOIDES ATLANTICA* (COSS. ET DUR.) WOLF

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The genus *Ammoides* (Apiaceae) tribe of Ammineae includes two species in Algeria, one of which is endemic: *Ammoides atlantica* (Coss. et Dur.) Wolf; the other one, *Ammoides pusilla* (Brot.) Breistr, is widespread in the Mediterranean region. These species are used in folk medicine as antibacterial, anti-diarrhoea, anti-fever, antiinfluenza and to treat vitiligo in addition of their use as condiments (Bellakhdar J, 1997).

Ammoides atlantica is biennal or perennial plant with thick stem fitted with a rosette of basal leaves. Slightly branched stems. Leaves Umbels 3-6 rays. Fruit 2-2.5 mm, it is found in lawns mountains above 1000 m (Quezel and Santa., 1962).

In Algerian traditional medicine, the arial parts of *Ammoides atlantica* are reported to have a wide range of biological activities such as antibacterial, antioxidant, anthidiarrheic and diabetic activies (Ababsa et al., 2013; Laouer et al., 2003).

Ammoides atlantica arial parts were subjected to extraction with solvents polarity increased, *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1) and MeOH. The study of the extracts was carried out using different chromatographic techniques such as Silica gel, SephadexLH-20, MPLC, and rp-HPLC. Part of chloroform extract (4.5 g) was subjected to column chromatography using silica gel and eluting with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$ (between 1% and 100%). Fractions of 50 ml were collected and analyzed by TLC.

Preliminary study of $CHCl_3$ extract led to isolation of some pure compounds whose structures were elucidated by 1D-and 2D-NMR Spectroscopy (^1H, ^{13}C, 13C DEPT, DQF-COSY, HSQC, HMBC, ROESY) and confirmed by mass spectrometry.

Seven compounds from $CHCl_3$ extract were isolated: one monoterpene chrysanthenone, two glicosides monoterpenes as l-borneol *O*-β-$_D$-glucopyranoside (Asano et al., 1993) and (-)-cis-chrysanthenol-β-$_D$-glucopyranoside (Miyakado et al., 1974), three guaianolides as 9α-Acetoxyartecanin, 3α-Chloro-9α-acetoxy-4β,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide and apressin (Trifunovic´ et al., 2006) and a new bisabolene sesquiterpene derivative 1,4-dihydroxybisabol-2,11-diene-10-one.

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PP1_48

PLANT CELL CULTURE AS EXPERIMENTAL MODEL APPLIED TO THE BIOSYNTHESIS PATHWAY STUDY OF COUMARIN AND CHLOROGENIC ACID IN *MIKANIA GLOMERATA* (ASTERACEAE)

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Mikania glomerata, popularly known as guaco, are commonly used as treatment for respiratory system diseases due to their bronchodilator effect, activity assigned to coumarin as active compound. However, it has been observed an accumulation of chlorogenic acid and low levels or even absence of coumarin in *M. glomerata,* raising the question whether the coumarin could be used as chemical marker in the quality control of the medicines which contains *M. glomerata*. Therefore, there is a need to investigate the biosynthetic pathway of these secondary metabolites in this species. Plant cell cultures are very promising for the production of secondary metabolites *in vitro*, since they allow a rapid cell proliferation. Moreover, they allow studies of regulation of a particular secondary metabolite under controlled conditions and are easy to manipulate. This research aim was to investigate the use of plant cell and tissue cultures as experimental models applied to the study of biosynthetic pathways of coumarin and chlorogenic acid in this specie.

The explants obtained from leaves of *M. glomerata* were collected from plants kept in the greenhouse of the Department of Plant Biology of the Unicamp Biology Institute. Then they were washed and sterilized to prevent the growth of fungi and bacteria yhay might be associated with the plant. The calli were maintained on MS medium[1] supplemented with 30.0 $g.L^{-1}$ sucrose. To determine the optimal concentrations of the hormones and ensure the best growing conditions, several tests were held with explants in media with different proportions of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzilaminopurina (BAP). Cell suspensions were established by inoculating cells from friable calli into liquid MS medium with the same supplements; however it was observed that increasing the sucrose concentration of the medium to 50.0 $g.L^{-1}$ promotes the growth of these cells. Analysis for identification and quantification of the phenylpropanoid biosynthetic pathway products were done by UPLC-MS, using methodology described by Melo[2].

Media containing 4.52 mM of 2,4-D and 26 uM BAP were established as the best conditions for callus induction for this species since the calli development was fast and there was little root formation. *M. Glomerata* cell cultures have demonstrated viable as a biological model for application in studies of the biosynthetic pathway of coumarin and chlorogenic acid. Preliminary results from tests in which chlorogenic acid was inoculated in *M. glomerata* cell suspensions suggests that there is a positive metabolism modulation, which diverts the biosynthesis pathway to coumarin production. On the other hand, these tests showed that chlorogenic acid content in the cells was negatively correlated with an increasing chlorogenic acid concentration added to the suspensions. Despite the positive results, further investigations are necessary to confirm the potential of the use of plant cell and tissue cultures of *M. glomerata* as experimental models applied to the study of biosynthetic pathways of coumarin and chlorogenic acid in this species.

Acknowledgments: CAPES, CNPq, FAEPEX-Unicamp and FAPESP.

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PP1_49

BIOACTIVITY AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM THE LEAVES OF *ANNONA PARVIFLORA* (A. ST.-HIL.) H. RAINER (ANNONACEAE)

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Annonaceae family are widely distributed around the world and is the largest family in Magnoliales with approximately 135 genera e 2500 species, that is concentrated in the tropics with few species found in temperate regions with trees, shrubs, and woody climbers (Siqueira *et al*., 2015). Many species are valuable for their large pulpy fruits, some are useful for their timber, and others are prized as ornamentals. The genus *Annona* is one of 33 genus of Annonaceae occurring in the Brazil with about of 250 species is described in the country. Among the main chemical groups of metabolites present in this taxon, we mention: alkaloids, acetogenins, volatile oil, flavonoids, among others (Siqueira *et al*, 2011). The *Annona parviflora* species is known as "pinha-brava" and so far have not been described scientific studies of this species, so that encouraged us to study the essential oil. This species as part of a research project focusing on phytochemical investigation of Brazilian medicinal plants searching for new bioactivity of natural products, in this study, we report the chemical analysis of the essential oil from the leaves of *Annona parviflora* by GC/MS and evaluation of their antimicrobial activity.

The leaves of *A. parviflora*, were collected in the Agronomic Institute of Campinas (IAC), the coordinates (S 23° 06' 89") and (W 46° 56' 12"), Jundíai, São Paulo State, Brazil. The botanical identification performed by Prof. Dr. Jorge Yoshio Tamashiro (IB-UNICAMP), and a voucher specimen of the material was deposited in the herbarium of IB-UNICAMP (register number UEC-84100). The essential oil was obtained by hydrodistillation from fresh leaves of *A. parviflora* and analyzed by gas capillary chromatography (GC/MS). Antimicrobial and antileishmanial activities were evaluated *in vitro* according to Siqueira *et al*, (2015).

This is the first report on the analysis of the volatile constituents from the flesh leaves of *A. parviflora* and evaluation of their antibacterial and antileishmanial activity. Fifty-six compounds were identified in the essential oil, representing 94.74% the total oil. The essential oils were characterized mainly due to increased presence of sesqueterpenes (60.07%), monoterpenes (34.67) and other constituents (4.81%), and the main compounds identified were bicyclogermacrene (12.56%), germacrene D (10.32%), β-linalool (8.92%), (E)-caryophyllene (8.74%) and α-pinene (7.43%). This result showed to be in accordance with the classes of volatile constituents found in the Annona genus and in the Annonaceae family (Siqueira et al., 2007). The essential oil from the leaves of *A. parviflora* showed significantly *in vitro* bioactivity against microorganisms (bacteria and protozoa). Antimicrobial activity of the oil was verified against *Escherichia coli* ATCC-10799, *Enterobacter aerogenes* and *Staphylococcus aureus* ATCC-14458 with minimum inhibitory concentration between 1.0 and 0.5 mg/mL. Moreover, the oil also showed promising antileishmanial activity against *L.* (L.) *chagasi* with IC_{50} of 44.2 µg/mL. The significant biological activities presented by the essential oils suggest that this specie is a rich source of biologically active compounds. However, further investigations are needed to confirm its therapeutic potential.

Acknowledgments: CAPES, CNPq, FAEPEX-Unicamp and FAPESP.

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PP1_50

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SEVERAL AGRO-INDUSTRIAL WASTES FROM COFFEE AND COCOA PRODUCTION

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Biodiversity greatly contributes in the pursuit of human welfare offering new alternative resources for bioprospecting and searching of new molecules. Plants are one of these resources, since they are considered as natural laboratories, where a large number of chemical compounds are biosynthesized, especially from secondary metabolism. Compounds present in cocoa and coffee play an important role in the quality of derived foods. Further, these compounds exhibit a wide range of biological effects making them very useful in the prevention of coronary diseases and neurodegenerative disorders [1]. Exploring plants as a potential source of antimicrobial properties and antioxidants could add additional value to agro-products derived from cocoa and coffee, thus reducing effects generated by pollution. The corresponding biological material of coffee was collected in the Experimental Station CENICAFE-Naranjal (04°59'North, 75°39'West) and cocoa samples were provided by the Farm Luker (05°04'North, 75°41'West) at department of Caldas, Colombia. Bioassays were conducted in order to determine the antimicrobial and antioxidant activity from coffee and cocoa extracts**.** Bioassays of antibacterial activity were carried out against bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70503), *Staphylococcus aureus* (ATCC 25923) and Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300).

Antimicrobial activity. Results show promising antimicrobial activity from acetone extracts of coffee, against all bacteria that were tested. *K. pneumoniae* (9%) was the bacteria that showed less susceptibility to the extracts, while *S. aureus* did show the highest percentage of inhibition (52%). Methanolic extracts from cocoa did not show inhibitory activity against *K. pneumoniae* whereas for *E. coli* strain, showed lower percentages of inhibition. In general, the extracts have a higher antimicrobial activity against gram-positive bacteria when compared with gram-negative bacteria, because gram-negative bacteria have an outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through the lipopolysaccharide that cover it. *S. aureus* and methicillin-resistant *S. aureus* strains were susceptible to extracts from cocoa pod and fermented roasted cocoa bean showing an inhibition ranging between 29.58 and 33.08%. The activity of these extracts on bacteria is probably due to the possibility they can generate complexes with extracellular proteins and soluble proteins.

Antioxidant capacity, DPPH method. Values with greater biological activity were presented in coffee leaves extracted with acetone and methanol solvent respectively (393.94 ± 23.13 mg Trolox / g sample and 271.34 ± 11.61 mg Trolox / g sample). These results agree with those results obtained by Dudonné et al. 2009, which state that in the case of green coffee values are higher for methanol extracts (60% of DPPH), even though the reviewed study used aqueous extraction (41% of DPPH). The antioxidant capacity of samples of green cacao and roasted cacao differ between them, as well as between fermented and unfermented cocoa.

*Antioxidant capacity, ORAC method***.** Results prove that performing methanolic extractions; green unfermented cocoa and green coffee shows the highest values of antioxidant capacity measured as ORAC with 1694.323 and 698.794 mmol/g sample respectively, while the mucilage of coffee had the lowest content with 9,955µmol/g sample. The higher activity values were found in coffee leaves and coffee pulp (530.368 and 348.368 mmol/g sample respectively), when samples were extracted using acetone, while samples of cocoa husks and coffee mucilage presented the lowest activities (5,775 and 9,070µmol/g sample respectively). It has been shown that coffee and cocoa beans and products made from them contain a variety of antioxidants such as soluble phenolic compounds and insoluble phenolic polymers [2].

In recent years, bacterial resistance to drugs and development of cardiovascular diseases increased significantly worldwide, so search and discovery of new antimicrobial and antioxidant plant compounds represent a biotechnological alternative and an economically interesting field to improve sectors such as medicine, agro and cosmetics.

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PP1_51

EVALUATION OF ANTIOXIDANT PROPERTIES AND ACUTE TOXICITY OF CYANOBACTERIA PIGMENTS AND METHANOLIC EXTRACTS

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Cyanobacteria are an interesting and innovative source of various biomolecules and some compounds have anti-inflammatory and antioxidants properties. These microorganisms synthesize and accumulate colored proteins (pigments) known as phycobiliproteins (phycoerythrin, phycocyanin and allophycocyanin), participating as an antenna pigments in the photosynthetic apparatus. Different studies have shown the antioxidant capacity of phycoerythrin and phycocyanin (Romay *et al*., 2003). The antioxidant phycocyanin pigment is related to its ability to sequester hydroxyl ions that promote the process of lipo-peroxidation (Romay *et al*., 2001). Consequently, cyanobacteria from the Atacama Desert are an innovative source of natural antioxidants with potential protective role against oxidative stress. The goal of this work is to study the antioxidant properties and toxicity of the pigments of cyanobacteria for subsequent application in the food, pharmaceuticals and cosmetics industry.

Cyanobacteria cells in the exponential growth phase were suspended in 75% methanol. The cells were disrupted by ultrasound, the suspension was centrifuged and the supernatant (75% methanol extract) was recovered by and filtered. The pigments phycoerythrin and phycocyanin were purified from cyanobacteria strains from Atacama Desert by precipitation with ammonium sulfate saturation (0-35% and 35-60%) respectively. The phycobiliproteins were dialized and freeze-dried and used in antioxidant assays. The methanol extract and freezed-died pigments were used in ABTS antioxidant assay based on protocol described by Re *et al*. (1999). The values obtained were expressed as µmoles Trolox equivalent per gram fresh mass or milligram of pigment. Acute toxicity assay was performed using age-synchronized *C. elegans* N2 (wild-type). Nematodes plus added pigments (0.5-0.005 mg/mL) were incubated at 20°C for 24 h. Later, nematodes were counted and classified as active (alive) or inactive (dead).

The ABTS assay done with methanol extracts from cyanobacteria strains LLA-10, CAQ-15 and LLC-10, showed values ranging from 1.95±0.38 to 7.17±0.61 µmoles TE/g fresh mass. The assays with pigments phycocyanin (LLA-FC) and phycoerythrin (CAQ-FE) rendered values ranging from 1.98±0.45 to 3.12±0.56 µmoles TE/mg pigment. These preliminary results suggest that both pigments and methanol extracts from cyanobacteria have antioxidant power that compares to fruits as mulberry, pineapple or passion-fruit (Kuskoski *et al*., 2005). However, it is necessary to determinate the antioxidant power with additional antioxidants methods. We also have observed that the pigments used here showed no toxic effects on *C. elegans.* Finally, this is the first study on the antioxidant activity of purified pigmented proteins and methanol extracts from Atacama cyanobacteria. These results may contribute to future biotechnological applications on the pharmaceutical, cosmetic industries and on functional food developments.

Acknowledgements: CONICYT-Chile (CeBiB FB-0001) and Universidad de Antofagasta (SI-5305; CODEI-5364).

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PP1_52

ADVANCES IN KNOWLEDGE OF CHILIOTRICHUM DIFFUSUM (ASTERACEAE), A NATIVE SPECIES FROM PATAGONIA USED IN TRADITIONAL MEDICINE OF THE ONAS

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Chiliotrichum diffusum is a shrub that inhabits the southwest of Patagonia Argentina, popularly known as "mata negra" or "kóor" (in Shelknam or Ona´s language). The Ona traditionally used flowers for medicinal purposes to "clarify the view" and aerial parts were used in rituals (tattoos), to improve memory, treat headaches, cramps and varices [1, 2]. In previous studies we showed that, as with other Asteraceae, C. diffusum biosynthesized flavonoids and phenolic acids related to analgesic, anti-inflammatory [3] and vasodilator activities, and its traditional uses. In this work we present advances in the study of biological activity and phytochemical characterization.

The aerial parts and flowers Chiliotrichum diffusum (G. Forst.) Kuntze (Asteraceae) were collected in summer of 2003 and 2006, in the village of 28 de Noviembre (Santa Cruz, Argentina). These were dried at room temperature, protected from light, ground and sieved. The aerial parts were exhaustively extracted with ethanol 96° at room temperature; then the extract was fractionated with solvents of increasing polarity. Extraction of flowers was performed with water by decoction [3, 4]. The extracts were analyzed by RP-HPLC-DAD. Cytotoxicity was assessed with the Artemia salina test (BST) and inhibition of root elongation of wheat test. Antiproliferative activity on tumor cell line SH-SY5Y (ATCC® CRL-2266™) was measured using the resazurin method, which is based on the ability of viable cells to reduce this molecule to form a fluorescent product with an excitation wavelength at 544 nm and an emission at 590 nm; fibroblast was used as line control. Antioxidant activity was measured by inhibition test of DPPH (1,1-diphenyl radical-picrylhydrazyl-2).

Ethanolic extract fractions were analyzed at concentrations of 10, 100 and 500 µg/ml, showing a cytotoxicity with a LC_{50} < 40 µg/ml against Artemia salina test. Decocto from flowers showed inhibition of root elongation of wheat of 56 and 69 % (dilutions 0.05 and 0.5 % respectively) with a IC_{50} of 58.1 µg/ml. Decocto also showed antiproliferative activity on the cell line SH-SY5Y (IC_{50} 0.62 ± 0.1 mg/ml) and on fibroblast (IC_{50} 1.35 ± 0.84 mg/ml, dose since 1.25 mg/ml). Consequently, the selectivity index of the antiproliferative activity was 2.1 ± 1.6.

In both extracts, flavonoids and phenolic acids were the major metabolites, standing out in the ethanol, vitexin, kaempferol-O-glc, apigenin-7-O-glc and quercetin-3-O-glc (isoquercitrin). Decocto showed caffeic and chlorogenic acid, quercetin-3-O-gal (hyperoside), isoquercitrin, quercetin-3-O-rham (quercitrin), quercetin and kaempferol-3-O-rham (afzelin). These metabolites have been described as antioxidants, anti-tumor, anti-inflammatory.

In the studies of potential anti-tumor substances, extracts which have a LC_{50} or IC_{50} < 200 µg/ml in BST or root inhibition assay are of interest. In this study, the results obtained with the decocto of flowers were important, and related to the phytochemical profile. These results and the strong antioxidant activity, supporting the traditional uses of this species.

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PP1_53

ESI-MS AND UHPLC-MS ANALYSIS OF RESIDUES OF GREEN PROPOLIS AND EVALUATION OF THEIR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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Propolis is a bee product produced by *Apis mellifera*, mainly composed of plant resins. It presents complex chemical composition and its main bioactive components are flavonoids and a wide variety of phenolic compounds. Propolis is widely used in Brazil for its therapeutical activities, including antitumour, antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory as well as antimicrobial effects. This study aimed to verify the pharmacological potential of a draff of green propolis and to identify and quantify certain ions markers, characteristic of green propolis, certainly present in draff of propolis. For this reason, we proceeded to evaluate the antioxidant capacity, antimicrobial activity, and the chemical profile by ESI-MS, as well as to quantify the concentrations of the ion markers in the residue of green propolis by UHPLC-MS, compared to the propolis control. In this study, we evaluated the composition of the residues of subsequent extractions of raw propolis compared to that of the first extraction (EEP) in order to determine the appropriate number of extractions that could be used. Three samples of green propolis, two of them from small apiaries and another from a larger apiary (all with similar composition) were analyzed. All three samples were collected in the state of Minas Gerais in the Southeastern region of Brazil, where propolis is predominantly derived from *Baccharis dracunculifolia* plant. The chemical composition of the residues and the crude propolis was evaluated by direct insertion electrospray ionization mass spectrometry in the negative ion mode (ESI(-)-MS) using a TQD Acquity mass spectrometer. A comparison of the ESI(-)-MS fingerprints showed that bioactive substances still remain in the residues after one or two extractions. The chromatographic were performed on a UPLC Acquity chromatographer coupled with a TQD Acquity mass spectrometer with an ESI source. A C_{18} BEH Waters Acquity column (2.1mm x 50 mm x 1.7 µm particle size) was used. Solvent A was mili-Q purified water with 0.1% formic acid and solvent B was acetonitrile. The flow rate was 0.2 mL/min and 5 µL of samples were injected; with a linear gradient starting at 5% acetonitrile and increasing to up 100% methanol in nine minutes, held until 12 minutes and then returning to the initial conditions. The compounds were identified by comparison of their ESI-MS/MS fragmentation spectra with literature data (Sawaya et al., 2011). The antioxidant activity was assessed by DPPH and ORAC assays and the antimicrobial activity was studied as well in accordance to Salvador et al., (2011) and Salvador et al, (2002), respectively. To our knowledge this is the first report on the chemical composition and biological activity of the residues of green propolis extracts. Artepellin C was found to be present in green propolis extract (EEP) and also in the residues, however it was present in lower concentrations. Nevertheless, another important substance, dicaffeoylquinic acid, was only found in the propolis extract and in the first two residues. In the DPPH and ORAC assays a sequential loss of antioxidant activity was observed. The antimicrobial activity of EEP and extract of the first two residues was investigated in vitro against gram-positive and gram-negative bacteria and EEP and the first extract presented inhibition values of (1 mg ml^{-1}) or less. We conclude that two sequential extractions of raw green propolis are appropriate to avoid the loss of important chemical components in the residue. Further investigations are necessary to confirm the potential of these natural products and their constituents as useful agents for biotecnological applications. Propolis residue obtained after the third extraction shows no biological activity, however it might be useful for other purposes other than for its antimicrobial and antioxidant activity and further studies of this material would be appropriate.

Acknowledgments: to CAPES, CNPq, FAPESP and FAEPEX-Unicamp for the financial support. .A.C.H.F. Sawaya would like to thank Mr Antonio Carlos President - Apis Flora - for the supply of propolis. Cunha would like thank to Mr Edilson A Emidio (PROVMEL) for the supply of propolis from his apiary.

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PP1_54

DEVELOPMENT AND CHARACTERIZATION OF ACTIVE ALGINATE-BASED EDIBLE FILMS FOR FOOD PACKAGING APPLICATIONS

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In recent years, the demand for safe, high quality, freshness and extend shelf life of food have led to the development of innovative approaches in food packaging named "active packaging" which can be classified into to main kinds:

i- Non migratory active packaging acting without intenctional migration

ii- Active releasing packaging allowing a controlled migration of compounds in the atmosphere surrounding the food or on food surface in contact with packaging.

Recently antimicrobial packaging systems obtained by a hybrid organic-inorganic coating with antimicrobial properties were prepared in our laboratory and the release behavior in water, other food simulants, as well as selected food was evaluated (1, 2).

In this presentation results regarding the development and characterization of alginate-besed edible "active" films will be presented.

The proposed active films represent an innovative material characterized by being biodegradable, and known to be suitable for edible food packaging. Their also show good mechanical properties and high resistance, and has been successfully employed for active food packaging containing natural antioxidant agents. Results will be presented and discussed.

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PP1_55

ANTIMICROBIAL ACTIVITY OF TWO NEW TETRAHYDROQUINOLINES

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One of the most important pathologies contributing to increase morbidity, disability and mortality worldwide are infectious diseases caused by bacteria [1]. In addition, the excessive and inadequate use of antibiotics has led to the emergence of pathogens resistant to drugs currently available, as in the case of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* that have developed different resistance mechanisms [2]. Currently, in the search for new molecules with antibacterial activity two approaches are followed in order to design alternatives for the treatment of infections caused by resistant bacteria: (*I*) search for new molecules obtained from natural sources and (*II*) diversification of molecules by synthesis from different natural sources, with known biological activity. Tetrahydroquinolines (THQ) have ubiquitous distribution among natural products and medicinal agents. For this reason, they have been lsrgely studied and represent an important basis for synthetic chemicals [3]. In this work, two newly synthesized THQ-based (CM725 and CM728) were obtained by using the imine Diels-Alder methodology (DA) and tandem reaction Aza-Michael-Ida dihydropyrrole; afterwards, their antibacterial activity was evaluated on the following strains: Methicillin resistant *Staphylococcus aureus* (SAMr) ATCC 43300, *Klebsiella pneumoniae* ATCC 1705, *Staphylococcus aureus* wild type ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 27853. Minimal Inhibitory Concentration (MIC) was calculated following the recommendations of the CLSI-2012 for microdilution (IC50) by using the Graphpad Prism 5.0 statistical package. A serial dilution set of each molecule was prepared using DMSO as solvent (75, 35, 20, 10 and 5µg/mL). The final volume was 100µL and the final concentration of DMSO in the assay did not exceed 1%. Each treatment was carried out by triplicate and the cultures were incubating at 37°C and the growth was monitoring at OD600 until reaching the stationary phase. Gentamicin (20µg/mL) was used as positive control. The inhibition percentage was calculated from equation used by Valle-Molinares et al. [4].

Fig 1. CM728 Molecule Structure

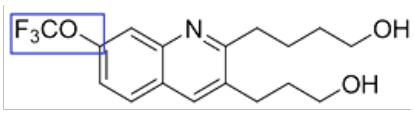
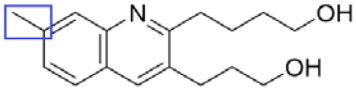


Fig 2. CM725 Molecule Structure



Results show that compound CM728had the lowest MIC (0,06917µg / mL) for *Enterococcus faecalis,* whereas the highest value was 37,01µg / mL for *S. aureus*. However, compound CM725 shows broad spectrum with MIC values of 7,417 µg / mL and 14,84 µg / mL to *Klebsiella pneumoniae* and *Enterococcus faecalis,* respectively. The difference between the activity of these molecules could be attributed to the type of substituents present in the quinoline ring. Other studies have previously reported that aliphatic substituents contribute to enhance the biological activity [5]. In comparison with the antibiotic used as positive control, it is important to point out that these molecules showed better inhibition percentages on the bacteria tested, particularly against resistant bacteria (SAMr). Likewise, MIC values obtained for these molecules are lower than MIC of positive control (20µg / mL). In conclusion, the screening of natural and synthetic molecules represents an interesting strategy for developing new tools to control infections caused by resistant bacteria. The diversity of quinoline ring allows to increase the potential of these molecules by using substituents as an important characteristic to design new drugs.

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PP1_56

PHENOLIC PRODUCTION AND ANTIRADICALAR ACTIVITIES OF IN VIVO PLANTS AND IN VITRO CULTURES OF SEVERAL HYPTIS SPECIES COLLECTED IN CERRADO AREA (GOIÁS, BRAZIL)

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Hyptis is a Lamiaceae genus, consisting of various medicinal and aromatic species widely distributed in South America, particularly in Brazil. Among these species, *H. suaveolens* (SH), *H. marrubioides* (HM) and *H. pectinata* (HP) are widely used in folk medicine in the region of Goiás (Midwest, Brazil). They are used as infusions or ethanol/aqueous extracts for treating diseases such as nasopharyngitis, sinus congestion, skin disorders, headache, stomach problems, fever, dental pain, bacterial and fungal infections, cancer, etc. This practice becomes a major concern in the scientific community because little is known about the reliability and safety of use of these plants. In order to better understand and validate some of the traditional uses of these plants biomass of these plants were studied concerning phenolic composition and antiradicalar activities.

HS, HM and HP plants were collected *in vivo* in the region of Rio Verde (Gioás Brasil). Additionaly, *in vitro* cultures of these species were established. Different hormone combinations of auxin 2,4-D/NAA and cytokinin BAP/Kin were used for *in vitro* cultures. The best combinations were selected for *calli* and/or shoot cultures production. Both biomass of *in vivo* plants and *in vitro* cultures were taken and lyophilized. The dried biomass was ground and aliquots were taken for preparation of ethanolic extracts (80%). These extracts were filtered and subsequently analyzed for phenolic content (HPLC analysis) and antiradical activity (DPPH method).

The *in vivo* plants produced mainly quercetin (Q) and luteolin (L) derivatives, and rosmarinic acid (RA) derivatives. The phenolic profiles of *in vitro* cultures were similar to the *in vivo* counterparts, but with differences in composition; *in vitro* cultures produced more compounds of RA type. The extracts of the three species shown good antiradicalar potential, with the best EC50 obtained for HP (57 µg / ml). *In vitro* extracts of cultures showed similar anti-radical properties; no significant differences were observed for EC50s of *in vivo* and *in vitro* biomass. Phenolic compounds may be responsible for the antiradicalar activities of these species and therefore their positive effect against several pathological processes.

PP1_57

APOPTOTIC EFFECT OF CARDENOLIDE GLYCOSIDES FROM *ASCLEPIAS SUBULATA*

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Aim of the study was to determine the cell death pathways that the cardenolide glycosides with antiproliferative activity found in the methanol extract of *A. subulata* are able to activate.

The effect of cardenolide glycosides isolated of *A. subulata* on induction of apoptosis in cancer cells was evaluated through the measuring of several key events of apoptosis. A549 cells were treated for 12 h with doses of 3.0, 0.2, 3.0 and 1.0 µM of 12, 16-dihydroxicalotropin, calotropin, corotoxigenin 3-O-glucopyranoside and desglucouzarin, respectively. Apoptotic and necrotic cell levels were measured by double staining with annexin V-FITC/PI. Mitochondrial membrane depolarization was examined through JC-1 staining. Apoptosis cell death and the apoptosis pathways activated by cardenolide glycosides isolated of A. subulata were further characterized by the measurement of caspase-3, caspase-8 and caspase-9 activity.

Apoptotic assays showed that the four cardenolide glycosides isolated of *A. subulata* induced apoptosis in A549 cells, which was evidencing by phosphatidylserine externalization in 18.2, 17.0, 23.9 and 22.0% for 12, 16-dihydroxicalotropin, calotropin, corotoxigenin 3-O-glucopyranoside and desglucouzarin, respectively, compared with 4.6% of control cells. Cell death was also associated with a decrease in mitochondrial membrane potential, which was more than 75% in the treated cultures respect to control. The activation of caspase-3 was observed in all cardenolide glycosides-treated cancer cells indicating the caspase-dependent apoptosis of A549 cells. Extrinsic and intrinsic apoptosis pathways were activated by cardenolide glycosides treatment at the doses tested.

In this study we have found that cardenolide glycosides, 12, 16-dihydroxicalotropin, calotropin, corotoxigenin 3-O-glucopyranoside and desglucouzarin, isolated from *A. subulata* induced the cell death trough caspase-dependent apoptosis, which was activated, preferably, by extrinsic pathway.

PP1_58

ANTIPSORIATIC ACTIVITY OF LATIN AMERICAN NATIVE PLANTS

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Preliminary studies performed by our group have shown the marked anti-inflammatory potential of the Latin American native plants *Pereskia aculeata*, *Xylopia sericea* and *Vernonia condensata*, traditionally used to treat inflammatory disorders (Lorenzi. 2009; Da Silva et al., 2011; Pinto et al., 2015). Psoriasis is an important chronic inflammatory and autoimmune-mediated dermatoses which affects 1-3% of the world Caucasian population. So, the present work aimed to investigate the *in vivo* antipsoriatic activity of those species.

The antipsoriatic activity was evaluated by the mouse tail test (Bosman et al., 1992). Different topical pharmaceutical formulations (20 mg) containing 12% of hexane fraction of *P. aculeata* leaves (HF), 5% of methanol extract of *X. sericea* fruits (XE), 5% of ethyl acetate fraction of *V. condensata* leaves (EF), vehicle or Psorex® were topically applied daily for five days a week during two weeks on the tail of Swiss mice (n=5). The naive group did not receive any treatment (vehicle control). Next, the animals were euthanized and the tails were submitted to histopathological analysis using hematoxylin and eosin staining. The slides were histometrically analyzed using the software Image Pro Plus 6.0® as follows: (1) the total length of the scale region; (2) the length of the granular layer of the scale region; (3) the orthokeratosis degree, which was calculated by the ratio of (2)/(1); (4) the relative activity of each treatment calculated by the equation $RA = (OK_T - OK_N) \times 100/(100 - OK_N)$, where OK_T means the orthokeratosis degree of the treated group and OK_N means the orthokeratosis degree of the naive group. ANOVA followed by the Newman-Keuls test was used for statistical analysis (COBEA – Protocol nº 028/2014).

The mouse tail test is widely applied as it is easily accomplished, provides reproducible results and presents high correlation with the activity of antipsoriatic drugs currently used for human skin. In this test, the orthokeratosis degree indicates the therapeutic potential of a tested drug. The naive group and the topical pharmaceutical formulations containing vehicle, HF, XE, EF or Psorex® showed, respectively, 49.62 ± 3.1, 52.25 ± 4.5, 78.14 ± 3.1***, 72.16 ± 2,8***, 62.75 ± 3.1* and 86.83 ± 2.5*** of orthokeratosis degree (* $p < 0,05$; ***$p < 0.001$ *vs* naive). The relative activities were 0 (naive), 5.79 (vehicle), 56.88 (HF), 44.47 (XE), 26.29 (EF) and 74.06 (Psorex®). Our findings strongly suggested these plants present compounds endowed with antipsoriatic potential.

Acknowledgements: This work was supported by the grants from FAPEMIG, CAPES and CNPq.

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PP1_59

ETNOPHARMACOLOGICAL INVESTIGATION OF PLANTS USED BY THE RIVERINE POPULATION ON THE MICROREGION OF NORTH ARAGUAIA, MATO GROSSO, BRAZIL.

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The microregion of North Araguaia in Mato Grosso comprises 14 municipalities covered under the Amazon and the Brazilian Savannah, bathed mainly by the Araguaia and Xingu rivers, and holds an enormous ethnocultural diversity. To survey, identify and record medicinal plant species used by riverine populations that inhabit the microregion of North Araguaia and select native plants for further chemical and pharmacological studies through consultation in specializes literature.

This was a cross-sectional ethnopharmacological study by non-probabilistic sampling (n=60), employing the snowball method for the selection of riverine dwellers locally known as specialists in the use of medicinal plants throughout the 14 municipalities of the microregion. The data was obtained on the period between July and November in 2013 through interviews utilizing a semi-structured form. The relative importance (RI) and the informant consensus factor (ICF) were determined, and comparison between taxonomic groups and biological systems was carried out by principal component analysis (PCA) and Chi-square (X^2). The research was only initiated after compliance with all ethical aspects regarding the informants and federal organizations.

Informants cited 309 plant species belonging to 85 botanical families, of which 73% were native. The most cited species were *Copaifera langsdorffii* Desf. (133) *Lafoensia pacari* A. St.-Hil. (131) and *Cecropia pachystachya* Trécul. (126), and the plants reported in this research were indicated for 18 of 22 disease classifications in ICD-10, with higher numbers on infectious and parasitic diseases (A00-B99, Chapter I), showing 16,6% of usage citations. Among the plants cited for this disease category (A00-B99), *Chenopodium ambrosioides* L. was the species with highest usage citations (50). The RI values ranged from 0.1 to 1.9, with highest values to *C. langsdorffii, C. pachystachya* and *L. pacari* (1.9; 1.8 and 1.7, respectively). As for ICF, the highest values were related to A00-B99 (0.79), J00-J99 (0.79) and K00-K93 (0.78), and the most cited native plants indicated for the treatment of these disease categories were *Bidens pilosa* L., *Vernonia ferruginea* Less and *L. pacari,* respectively. PCAs showed that plants in the Fabaceae family were the most used for the most recurrent categories in the study (A00-B99, N00-N99 and K00-K93). We also verified that the biological systems R00-R99, K00-K93, S00-T98, A00-B99, N00-N99, M00-M99, J00-J99 and F00-F99 show strong correlation to one another and thus tend to be treated by the same plants. Chi-square analysis suggested that the disease categories were in some way different from one another regarding the species of the families used in their treatment. Out of the 9 investigated native plants, *C. langsdorffii* and *Brosimum gaudichaudii* stood out, seeing as their phytochemical and pharmacological properties have been studied, they have also been sold as pharmaceutical products and have a patent deposit.

Local riverine specialists of the microregion of North Araguaia make use of a broad variety of medicinal plants in health self-care, predominantly those used in the treatment of A00-B99, and some of these plants already have their therapeutic potential scientifically validated. However, there are other whose pharmacological effect and safety have not yet been properly investigated. It was also observed that the informants do not use plants at random, but following well established criteria for the selection of the species used for medicinal purposes. Thus, the present study, in addition to serving as a basis for future studies of chemical, pharmacological and agricultural bioprospecting, may also contribute to the development of management, conservation and sustainable use of medicinal flora of the microregion.

Acknowledgments: Financial support from CAPES/Pró-Amazônia, CNPq/Bionorte.

PP1_60

CHARACTERIZATION AND EVALUATION OF SAPONINS FROM *YUCCA BACCATA* AGAINST *GIARDIA INTESTINALIS IN VITRO*

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Human giardiasis is a global public health problem, including Mexico. It has been estimated that about 200 million people are infected with giardiasis annually. Its prevalence in Sonora is high (68%), although there is a national deworming campaign since 1993. Treatments of choice are expensive and may be accompanied by side effects, especially in children. In this context, research in natural products chemistry is a viable option for bioactive compounds with antiparasitic alternative. Recently, has been reported that extracts of native plant *Yucca schidigera* from southwestern United States has anti-Giardia activity. On the other hand, Sonoran Desert has an endemic flora researched. *Yucca baccata* is distributed in the Sonoran Desert and present similar *Yucca schidigera* activity. Previous studies in our laboratory have indicated that *Y. baccata* butanolic extracts are active against *G. intestinalis* in infected gerbils. The aim of this work was to isolate and characterize *Y. baccata* stem saponins and determine their anti-giardia *in vitro* activity.

The results of proton magnetic resonance (NMR_1H) of butanolic extracts obtained from 2.0 kg *Y. baccata* stem (EEYB) flour, indicate the presence of saponins and activity against *Giardia intestinalis*. The EEYB was subjected to column chromatography using silica gel 60, 230-400 mesh and a AcOEt-MeOH-H_2O mobile phase, isolating a saponin spiroestan steroidal type (F8), which was characterized by NMR $_1H$, $_{13}C$, and two dimensions HSQC experiments, HMBC.

On the other hand, an inoculum of 10^5 trophozoites of *G. intestinalis* GS/M-83-H7 in TYI-S-33 growth medium, was used. To this, he added different concentrations of saponin (F8) from (0.1-50 µg / mL). It past 24 hours exposure viability was observed using light microscopy.

Analysis by HR-ESI-MS showed an ion with m / z 739.4232 for C39H63O13. Saponin F8 showed an IC50 of 3.125 ug / mL against G. intestinalis ($P<0.001$), while the IC50 of Metronidazole was 1.5 ug / mL. In conclusion, the anti-giardia F8 saponin activity suggests potential as a dewormer. It is important to continue studying the anti-giardia F8 saponin of *Y. baccata* to elucidate its mechanism of action and toxicity.

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PP1_61

CHARACTERIZATION OF ANTHOCYANINS IN BILBERRY (*VACCINIUM MYRTILLUS* L.) FOOD DERIVATIVES

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Vaccinium myrtillus L. is a spontaneous plant of mountain areas of Northern and Central Europe, widely diffused in the Italian Alps and Apennines. The fruits of this species, commonly named "bilberry", have a large commercial importance in the food (as fresh fruits, jam, juice, liquor), dietary (as dried bilberry fruits or extract) and pharmaceutical industries (as standardized extract in tablet or capsules) .

The bilberry can be considered as one of the few medicinal plants, if not the only, which is not convenient to cultivate; for this reason it is important and urgent to dedicate attention to the protection and enhancement of this small fruit and the ethnobotany, nutraceutical and gastronomic heritage connected to its application.

Extensive studies on bilberries have been conducted over several years by researchers of the University of Modena and Reggio Emilia and the recognition of the virtues health-accredited by these studies and the importance of protecting and enhancing *V.myrtillus* have suggested more recently, within the Chamber of Commerce of Modena, to introduce this "small fruit" in the "Tradition and flavors of Modena" basket with the aim to promote the quality of the Apennine bilberry in the province of Modena, until confined to the provinces of Tuscany.

The phytotherapeutic quality of *V.myrtillus* is substantially attributable to the high content in active principles and to the chemical specificity which characterizes them. Among these surely anthocyanins are the components with health-protecting attributes such as antioxidant properties, which result in capillaroprotective activities, anti-edema, anti-inflammatory and anti-aging.

The common uses of this precious treasure plant range from use in traditional cuisine to the industrial exploitation for pharmaceutical purposes. Fresh is an excellent functional food that, in addition to well-known organoleptic properties, has beneficial virtues. As concentrated juice is a "nutraceutical product" or "food supplement", and for pharmaceutical industry is a real Phytomedicine, "Bilberry dry extract", titrated and standardized in anthocyanins, described in the major Pharmacopoeia, comes in supplements and in various drugs used in ophthalmology for the treatment of retinal diseases and in the treatment of other disorders of the microcirculation.

With the aim to describe the phytochemical profile of *Vaccinium myrtillus* we have carried out many studies and this present research describes the chemical characteristics of many samples of juice, jam and liquor prepared from local company to control the quality and the genuineness of these products. These derivatives, with excellent and typical flavor, are subjected to preparation processes which can affect the phytochemical and biological profile of bilberry.

23 samples of jam, 14 of juice and 14 of liquor "Mirtillino" were analyzed with the European Pharmacopeia method for the determination of the anthocyanins content and, to describe the different anthocyanins in these preparations, an HPLC-UV method according to a validated analytical technique was applied. In parallel, a group of expert tasters evaluated the organoleptic characteristics of the samples and the results of chemical and tasting tests have led to a ranking of the "*Best product award*" for both content anthocyanins and for taste.

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PP1_62

POLIACETILENS EXTRACTION IN *DAUCUS CAROTA*

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The term "polyacetylenes" describes a whole range of natural products that are not polymers, in fact many precursors and many metabolites contain only a single acetylenic bond. The polyacetylenes in the plant kingdom are secondary metabolites; they are therefore metabolics products but does not perform essential vital functions to the plant itself instead as the primary metabolites. Although they are not essential, they still play an important role in the growth and development of the plant defense system. Chemically the polyacetylenes are characterized by long chains of carbon atoms and by the presence of some triple C-C bonds, this makes the molecules particularly reactive. The polyacetylenes, in fact, are unstable and sensitive compounds towards the chemical and enzymatic oxidative degradation.
Carrots are considered the main food source of polyacetylenes . In carrots major polyacetylenes are three and belong to the family of aliphatic C17 polyacetylenes of falcarinol. The structure is shown in figure 1.

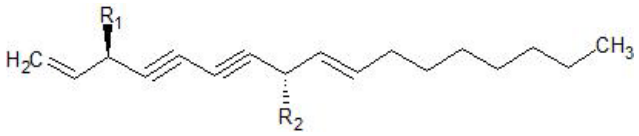

Figure 1: Structure of the C17 aliphatic polyacetylenes falcarinol family.

The attention of the scientific world has centered on falcarinol because it is a bioactive molecule that has been shown to have a pronounced cytotoxic activity against various tumor cells.
The extraction step is represented by a solid - liquid extraction using dicloromentano as an extraction solvent. In order to estimate the concentration of falcarinol in oily matrices extracted from carrots it was necessary to use falcarinol standard for the construction of a calibration line. The falcarinol standard has been found at the Shechem GmbH company (Sirius Fine Chemicals , Fahrenheitstraβe, 1, Bremen, Germany). The product, of an amount equal to 20m , it appeared as a liquid oil is stored in an amber vial. 1H - NMR spectra showed the product is identified and specified a≥95 % purity. For the identification phase of the extracts we used the UPLC / ESI-MS system: mass spectrometry coupled to an ultra high performance chromatographic system. This precise technique, accurate and sensitive offers high selectivity over other HPLC techniques.
The technique used could be applied for the determination of polyacetylenes also at other plants. The ESI (electrospray ionisation source), used in positive mode connected to the mass spectrometer showed the presence of clusters in the gas phase of falcarinol. The trend is to form adducts with the solvent used in the elution step (especially with acetonitrile).

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PP1_63

NUTRACEUTICAL PROPERTIES OF PRUNUS DULCIS EXTRACTS OBTAINED BY SUPERCRITICAL FLUID EXTRACTION

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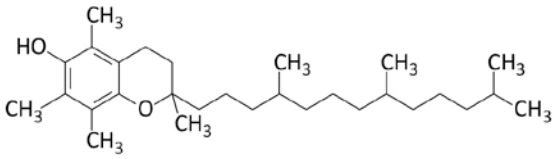
The high nutritional value of the almond seeds stems mainly from their high lipid content, which constitutes an important source of calories, but does not contribute to the formation of cholesterol in humans, because of their high level of unsaturated fatty acids, mainly fatty acids monounsaturated (MUFA), which are inversely correlated with serum cholesterol levels. Furthermore, the almond oil has a high content of tocopherols that has high functional properties and nutraceutical. The α-tocopherol (Fig.1) is chemically (2R)-3,4-Dihydro-2,5,5,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-2H-1-benzopyran-6-ol, a derivative of the hydroxy chromane, formed in turn by a condensed benzene nucleus with a pyran nucleus, and a carrier isoacetilic chain in position 2.



From the same vegetable matrix, subjected to different extraction processes, you are obtained extracts that can differ greatly even for appearance, chemical composition and biological activity [1]. This work has helped to highlight the features and current usage fields of almond and demonstrate how the almond and its derivatives in the form of extracts or scraps of traditional food processing could become the finest raw materials for the food industry and allowing farmacosmetic to open new markets for different industrial fields. It's was investigated the use of supercritical fluids as solvents in the separation and extraction techniques. This technique represents an important resource at both the scientific and industrial. The numerous areas of interest including food production, pharmaceutical, petrochemical and cosmetics. The unit operation of supercritical extraction has been placed in comparison with the conventional extraction techniques. The study of the process parameters, such as temperature, pressure and flow rate have allowed to obtain the best operating conditions. The best operating conditions for the extraction of oil and tocopherols were found to be: a temperature of 50 ± 2 degrees, a pressure of 420 ± 20 bar and a flow rate of 25 ± 5 kg h-1 using pulverized almond seeds [2].

There are obvious differences in fatty acid composition between the two methods of extraction. From the analysis, the main almond oil components results are the following (g kg-1): Oleic acid (700), linoleic acid (200) and palmitic acid (70). The composition of mono and polyunsaturated fatty acids represent about 700 and 200 g kg-1 of total fatty acids [3]. The use of SFE, offers the advantage of offering of almond oil fractions enriched in tocopherols as noted by several authors. The determinations of tochopherol and fatty acid in almond oil are very simple and rapid and gives accurate and precise results.

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PP1_64

IN VITRO POTENTIAL OF TWO LICHENS FROM DEPARTMENT TOLIMA-COLOMBIA AS ANTIOXIDANTS AND INHIBITORS OF DIGESTIVE ENZYMES

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Diseases generated by metabolic disorders are a serious public health problem, being obesity the most chronic and systemic disease with worldwide epidemic proportions. Its fundamental cause is an energy imbalance between calories consumed and calories expended, and is considered a major risk factor for other diseases such as: diabetes, multifactorial metabolic deficiency, cardiovascular diseases (mainly heart disease and stroke), musculoskeletal disorders (especially osteoarthritis), and some cancers (including endometrial, breast and colon) (WHO, 2014). In turn, oxidative stress has been linked to the etiology of many degenerative diseases: atherosclerosis, cancer, inflammatory processes, diabetes, hypertension, neurodegenerative diseases and even natural processes such as aging (Vidal, et al., 2001). Moreover, the study of enzyme mechanisms have provided an interface between organic chemistry, drug discovery (Khosla, 2000); considering enzymes as markers of pharmacological importance in the development of new therapeutic agents (Komatsu et al., 2013). In the search for new sources of bioactive compounds could be considered lichens; a symbiotic association with nutricional, cosmetic and farmacological potential. Among the biological activities exhibited by the lichen it can be mentioned: antimicrobial, antioxidant, allelopathic, antiviral, antiproliferative, antiinflammatory, antipyretic, analgesic, among other activities. The objective of this research is to establish the antioxidant capacity and inhibitory activity of digestive enzymes, to determine the therapeutic potential of lichens department of Tolima-Colombia.

The lichenic material of dried and ground *Usnea angulata* y *Parmotrema robustum* from the upper basin of the river Combeima (04°19'30'' y 04°39'57'' north latitude y los 75°10'11'' y 75°23'23'' west longitude) in the department of Tolima-Colombia, was extracted by maceration with a hydro-alcoholic mixture. Each extract was evaluated with respect to its antioxidant capacity *in vitro*, using the following colorimetric methods: DPPH scavenging, ABTS radical cation decolorization, total antioxidant capacity (TAC) and ferric reducing-antioxidant power (FRAP) (Perez & Saura, 2007). In addition, the *in vitro* inhibitory activity of the extracts was established against α-glucosidase, lipase pancreatic and the α-amylase enzymes (Luyen et al, 2014).

The antioxidant activity results showed promising activity of *P. robustum* against DPPH radical (IC_{50}= 184,7±0,63 µg/mL) and TAC (134,2 ± 1,02 mg / EAA), and of *U. angulata* against ABTS radical (IC_{50}= 154.9± 0.47 µg/mL) with a reductive capacity FRAP (555,7±1,55 µm Fe^{+2}). In most cases, the results were better than the controls used (BHT, Clorogenic acid). Concerning the effect of enzymatic inhibition, the results show that both extracts have a moderate-strong activity against two of the three enzymes tested, *P. robustum* being the most promising for pancreatic lipase (IC_{50}= 338,3±2,56 µg/mL) and *U. angulata* for α-amylase (IC_{50}= 611,5±0.62 µg/mL). In the α-glucosidase assay it was determined that both extracts have greater capacity inhibition, with inhibitory concentrations between 3 and 11 times lower than acarbose control (345 ± 11,05 µg/mL). In conclusion, based on the antiradical and antioxidant potential, the ability of enzyme inhibition revealed by species do foresees a promising future for *U. angulata* and *P. robustum* as a source of new bioactive compounds, useful as therapeutics in diseases generated by metabolic disorders, such as obesity and diabetes.

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PP1_65

CAROTENOIDS EXTRACTED FROM LYCIUM SPP PROMOTED THE ACTIVATION OF ARYL HYDROCARBON RECEPTOR

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Lycium plants are Solanaceous defoliated shrubs that primarily grow in northwest China and other parts of Asia. Their fruits (goji berries) are reported to have various biological activities and health-promoting properties and have been used in Asian countries as a traditional herbal medicine and functional food (Amagase & Farnsworth, 2011; Amagase, Sun, & Borek, 2009). Genus *Lycium* presents a high content of carotenoids and this provides a high provitamin A value and antioxidative capacity. Zeaxanthin dipalmitate represents the major carotenoid of the fruits and it is the focus of current research for prevention and treatment of atrophic age-related macular degeneration (AMD).

In this paper, a simple extraction method and a HPLC-DAD-ESI analysis for the separation and quantification of zeaxanthin dipalmitate and the other carotenoids esters in goji berries is described. Carotenoid total content in the crude samples was also determined by using UV-VIS spectrophotometric assay. The total amount of carotenoids and zeaxanthin dipalmitate were compared among fruits of different origin. The total carotenoid extract, the isolate zeaxanthin dipalmitate and pure standard zeaxanthin were tested for their capability to act as agonist ligands for Aryl hydrocarbon Receptor (AhR), a receptor involved in xenobiotic detoxification and immunoregulation.

The main components of the carotenoid fraction in all the considered samples was the zeaxanthin dipalmitate and other different carotenoid esters. It was observed that the zeaxanthin dipalmitate promoted AhR activation to a similar degree of well known AhR ligands. The same results have not been obtained with standard zeaxanthin. For the first time the reported results suggest that zeaxanthin in the dipalmitate form may mediate anti-inflammatory functions throught the involvement of AhR.

Acknowledgments: The authors are grateful to the Sud Rienergy s.r.l. farm (Corigliano Calabro, Italy) for the supply of goji berry

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PP1_66

ANTIOXIDANT CAPACITY AND PHENOL PROFILES OF SOME BEEHIVE PRODUCTS FROM UMBRIA (ITALY)

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Beehive products are easily accessible natural products which are becoming increasingly popular due to their potential role in contributing to human health, thanks to their antioxidant, antimicrobial, and anti-inflammatory properties (da Silva et al., 2016). Total phenol content, antioxidant capacity and phenol profiles of some honey, propolis, and royal jelly samples, harvested in Umbria (central Italy), were investigated.

Total phenol content was determined spectrophotometrically following the method recommended by Singleton and Rossi (1965) based on the use of Folin-Ciocalteu reagent. The analyses were carried out on filtered 1% aqueous solutions of honey and royal jelly samples, while pollen samples were extracted with 60% ethanol and then filtered (Carpes et al., 2009).

The qualitative and quantitative analysis of beehive product phenols was performed by using high-performance liquid-chromatography with diode array detector (HPLC-DAD) and, in order to identify the phenol compounds, a mass spectrometer (MS) detector was also used. Before analysis, the samples were suitably purified.

Two different *in vitro* assays, DPPH and ABTS, were used to obtain data about the antioxidant capacity of samples.

The DPPH free radical-scavenging activity of beehive products was determined according to the method described by Assimopoulou et al. (2005), while the ABTS assay was carried out following the procedure reported by Tawaha et al. (2007). All analyses were carried out in duplicate.

The highest value of total phenol content was observed for a pollen sample, and among honeys the most interesting value was found for onion honey. The same samples were characterized by higher antiradical capacity, determined both by DPPH and ABTS assays. The phenol compounds, identified and quantified by HPLC-DAD-MS, were essentially phenolic acids and flavonoids.

Acknowledgments: This work was funded by PSR Umbria 2007–2013 Mis. 1.2.4, European Fund for Rural Development, "Drink energizzante naturale di origine umbra" project.

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PP1_67

SECONDARY METABOLITES FROM CHILEAN PLANTS AND THEIR BIOLOGICAL ACTIVITY

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Plants of the Celastraceae and Rhamnaceae that growth in Chile have been studies. These plants have very interesting metabolites with epoxyeudesmane (agarofurane), benzylisoquinolinic, aporphinic and cyclopeptide skeleton. We study the biological activity associate at these compounds.

Air dried and powdered material was exhaustively extracted with MeOH. The resulting MeOH extract was filtered and concentrated in vacuum. The residue was suspended in H_2O and the sequentially extracted with solvent of increasing polarity. The resulting fraction were concentrated and chromatographed over silica gel column. Column fraction were analyzed by TLC (silica gel 60 F254), and fraction with similar TLC patterns were combined to give pure compounds. A hyphenated procedure combining HPLC-MS-NMR allows the determination of chemical structures of the isolates.

From the extracts, fractions and pure compounds for inhibitory capacity of acetylcholinoesterase enzyme and its insecticidal activity against *Cydia pomonella*, *Tenebrio molitor* and other insect pests were evaluated.

The results show that the extracts, fractions and pure compounds obtained from Chilean plants of the Celastraceae and Rhamnaceae family present an interesting insecticidal activity and inhibitory activity of acetylcholinoesterase enzyme.

Acknowledgment: To Fondecyt program grant # 1130463. The authors wish to thank to internal grant from Direccion de Investigacion Universidad del Bio-Bio DIUBB # 083009-2R, # 122509 and # 132209 GI/ C, Universidad del Bio Bio, Chillan, Chile.

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PP1_68

DETECTION OF PANCREATIC LIPASE AND ACETYLCHOLINESTERASE INHIBITORS IN EXTRACTS FROM *PIPER CF. ASPERIUSCULUM* AND *PIPER PERTOMENTELLUM* BY TLC-AUTOGRAPHY METHODS.

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Natural products have been used for thousands of years for the benefit of humans. In medicine, plants have provided various active principles which have inspired the development and synthesis of more active compounds (Fonnegra and Jimenez, 2007). Neurodegenerative diseases and obesity are pathologies that currently attract the attention of researchers due to the negative impacts that they have on the quality of life of people (González and Rodríguez, 2011; Rodríguez and Gutierrez, 2014). Thus, the wide variety of unexplored natural substances offers an alternative treatment of these diseases. Plants of genus *Piper* (Piperaceae) are species of great importance due to its use in traditional medicine, because are associating with pharmacological activities as astringent, anesthetic, antipyretic, antimalarial, antifungal and insecticide (Dallas, 2004). Despite the biological potential of the species of piper genus, there are still many species that lack of chemical and biological activity studies, as in the case of *P. asperiusculum* and *P. pertomentellum*. The objective of this research is Characterize chemically and biologically the ethanol extracts from leaves and inflorescences of *P.* cf. *asperiusculum var. glabricaule* and *P. pertomentellum* by a preliminary phytochemical study on TLC and evaluation of enzymatic inhibition of lipase and acetylcholinesterase using TLC-autograpy assays.

The preliminary chemical composition of ethanolic extracts of leaves and inflorescences was determined by a preliminary phytochemical analysis employing chromatoplates of silica gel and coloring reagents (Merk, 2000). The enzymatic inhibition on pancreatic lipase and acetylcholinesterase were evaluated by TLC-autography methods, taking advantage the capacity of the enzymes to convert α-naphtyl acetate into naphthol which reacts with Fast Blue B salt to make purple-colored background on TLC plates, producing white spots on the background where the enzymatic inhibitors are present (Hassam, 2012; Marston *et al.*, 2002). To detect acetylcholinesterase inhibitors also was used the Ellman's method on TLC plates (Yang *et al.*, 2012).

Preliminary phytochemical analysis allowed to determine the presence of flavonoids, phenols, terpenes and / or steroids in extracts of the species studied. The two methods used to detect acetylcholinesterase inhibitors provided the same results, but the method that uses Fast Blue salt is a more economical method, making it a more appropriated method for develop bio-directed studies. The extract in which are observed the largest number of zones of inhibition of acetylcholinesterase and pancreatic lipase corresponds to inflorescences of *P. pertomentellum*. The obtained results show that in the four evaluated extracts there are present more than one type of compound having inhibitory activity on acetylcholinesterase, being the most of inhibition areas related with the spots that revealed the presence of flavonoids and phenolic compounds in preliminary phytochemical analysis. In inhibiting pancreatic lipase, it is observed that this effect possibly is mainly due to triterpenes and / or steroids. This work makes contributions to the research of the species *P.* cf. *var. glabricaule asperiuculum* and *P. pertomentellum* being the first report of chemical characterization and biological activity, making evident the great potential of these species to inhibit enzymes such as pancreatic lipase and acetylcholinesterase, being important sources of study to find alternative therapies for the treatment of obesity and neurodegenerative diseases like Alzheimer.

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PP1_69

CHEMICAL-FUNCTIONAL CHARACTERIZATION OF COUROUPITA GUIANENSIS BARK

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Medicinal plants have been used in healthcare and prevention of most of the illnesses, nevertheless their uses as therapeutic approach is often underestimated. Then, considering their uses for thousand years that persist till today, it remains necessary to thoroughly investigate the intrinsic potential of the plant's Biodiversity as a source of molecules, or better phytocomplexes, able to prevent, alleviate or treat many diseases which we are still exposed.

Couroupita guianensis is a medicinal plant of the family of the Lecythidaceae and it has a wide use among Amazonian Native. It was experimentally shown many activities about this plant, like: (a scelta).

The aim of the study is to characterize the profile of secondary metabolites and the mineral content of the aqueous and ethanolic extracts of C.guianensis bark, using: qualitative colorimetric tests, quantitative tests (Folin-Ciocalteau, Fast Blue BB, Aluminium chloride), Inductive Coupling Plasma (ICP) Test.

A second set of analysis including 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, MTT assay and MTT assay in induced oxidative stress on Placental Mesenchymal Stem Cells to test the antioxidant activity and the absence of cytotoxicity on this cell line.

It has been demonstrated the presence of Triterpenoids, Reducing Sugars, Alkaloids, Saponins, Phenols and Anthroquinones in the extracts of C.guianensis. Flavonoid content was evaluated by Aluminium chloride method and resulted of 0,173-0,223 mg/ml Quercitin equivalents. Phenolic content was evaluated with FBBB Method and Folin-CiocalteauMethod. For the FBBB Method resulted to be 4,774-2,790 mg/ml Gallic acid equivalent. Using Folin-Ciocalteau Method was found 1,780-1,010 mg/ml Gallic acid equivalent. 18 several metals was quantified using ICP.

Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay that showed values of 300-437 µg/ml in terms of IC50. Citotoxic activity was measured by MTT assay at 24-48-72h from the administration of the extracts, at several concentration, on Placental Mesenchymal Stem Cells. It was demonstrated that 0,1% concentration is the most active. The same results was obtained using MTT assay in condition of induced oxidative stress.

PP1_70

COMPARISON OF TWO EXTRACTION TECHNIQUES FOR THE COMPREHENSIVE CHARACTERIZATION OF BIOACTIVE PHENOLIC COMPOUNDS IN ONION WATREUSING BY UHPLC-DAD-ESI-HRMS

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Food industry produces a large amount of onion wastes, making it necessary to search for possible ways for their utilization. One way could be to use these onion wastes as a natural source of high-value functional ingredients, since onion are rich in several groups of compounds, such as Quercetin and its glycosylated form and other flavonols [1]. These compounds are well known to be potent free radical scavengers and antioxidants, and have perceived benefits to human health [1]. The objective of this work is carried out an optimization and a comparative study between two environmentally friendly and selective extraction techniques, such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) have been carried out focusing in the bioactive phenolic compounds present in onion waste. For the analysis of the SFE and PLE extracts, a new methodology for qualitative characterization has been developed, based on the use of ultra-high-performance liquid chromatography (UHPLC), coupled with two different detection systems photodiode array detector (DAD) and of high resolution mass spectrometry (HRMS-MS) detector with an electrospray ionization interface (H-ESI). The two developed extraction method and was a useful tools for the determination and characterization of many phenolic compounds present in Onion waste that could be used as a good source of source of phenolic compounds.

Acknowledgments: this work was supported by project PON03 PE_00090_3 - Modelli sostenibili e nuove tecnologie per la valorizzazione delle filiere vegetali mediterranee.

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PP1_71

A FULLY AUTOMATED METHOD FOR DETERMINATION OF OCHRATOXIN A IN WINE AND BEER BY ULTRA HIGH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY.

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Ochratoxin A (OTA) is natural foodstuff contaminant belonging to the class of mycotoxins mainly produced by fungi of genera Aspergillus, Penicillium. This mycotoxin can be found on a wide range of agricultural commodities such as wine, beer, cereals, dried fruits, nuts, coffee [1].

The aim of this work was developed a rapid and accurate method based on automated solid-phase extraction-liquid chromatography/electrospray-tandem mass spectrometry (SPE-LC/ESI-MS-MS) for determination of Ochratoxin A in wine and beer. On-line SPE was performed by loading 100 µL of wine or beer at pH 8 through an oasis MAX cartridge (anion exchange). After a washing step to removing interfering compounds, Ochratoxin A was eluted with an isocratic mobile phase (MeOH/Water 8:2 0.5% formic) and refocused on column in order to get high chromatography efficiency and good peak shape. Chromatographic separation was achieved in less than 10 min using a kinetex C-18 reversed phase analytical column. For unequivocal identification and confirmation of analyte, two multiple reaction monitoring (MRM) transitions were acquired for OTA in the positive electrospray ionization mode (ESI+). The developed method was validated according to EU's requirements [2] and accuracy expressed as recovery ranged from 80 to 116% with method limits of quantification (LODs) in wine and beer were well below the maximum residue limits (MRLs) set by the European Union. Finally, the developed method was applied to the analysis of fifty real samples of wine and beer. This new developed method offers high sensitivity and accuracy with a minimum sample pre-treatment, offering a high throughput analysis, with a reduction of analysis time and solvent consumption

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PP1_72

IN VITRO EFFECT OF BRAZILIAN PROPOLIS ON THE EXPRESSION LEVELS OF MIR-27A-3P AND MIR-19A-3P

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Propolis is a non-toxic resinous product, collected from plants buds and exudates by *Apis mellifera* bees. Its chemical composition depends on botanical origin, geographical and climatic zone, and bee species.

Flavonoid are one of the main chemical classes, contributing greatly to its biological properties. In fact, propolis has been used since ancient times for its physiological and pharmacological properties, including antimicrobial (antibacterial, antiviral and antifungal activities), antioxidant, and anti-inflammatory activities (Salim et al.,2015 - Zhang at al., 2016). Growing evidence supports the wide applications of propolis in modern medicine. Nevertheless, in the scientific literature the mechanisms of action at the basis of propolis properties has not been completely elucidated. This study aims to investigate the ability of high quality Green Brazilian propolis extract to modulate the expression levels of miRNAs connected with oxidative stress and inflammation and its epigenetic effects.

The expression levels of two miRNAs, miR-27a-3p and miR-19a-3p, connected with oxidative stress and inflammation, respectively, was evaluated in HaCat cells (human keratinocytes) that were treated with sub-toxic increasing concentrations of green Brazilian propolis extract (prepared according to a patented technology). Moreover, the expression levels of mRNAs coding for NFE2L2 and TNF-α (validated target of miR-27a-3p and miR-19a-3p, respectively) were also determined.

The study showed that the expression levels of miRNAs and target mRNAs were influenced by green Brazilian propolis treatment. Thus, this study demonstrates that propolis exerts anti-oxidant and anti-inflammatory effects through an epigenetic mechanism.

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PP1_73

AUTHENTICATION OF HONEYS BOTANICAL ORIGIN BY UHPLC-MS/MS AMINO ACIDS DETERMINATION. A PRELIMINARY STUDY

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The Commission of the European Union (EU) has adopted a proposal to amend the Council Directive 74/409/EEC concerning honey [1]. This Directive laying down common rules and quality parameters, such as humidity, diastatic index, HMF content and pesticides levels, but some of these parameters have no relationship to geographical or botanical origin of honey. The amino acid profile could be useful tools to identify the botanical or geographical origin of honey samples. The aim of this work was the development of analytical method for determination of free amino acids in honeys. The method is based on SPE cleanup of diluted honey by a cation exchange cartridge (MCX). The purified sample was directly analysed by Hydrophilic interaction chromatography-tandem mass spectrometry (UHPLC–MS/MS) for determination of 20 amino acids. The parameters of mass spectrometry source and MRM transition were carefully optimized. Moreover, in order to achieve the best chromatography separation and a good peak resolution, different columns and mobile phases were tested. BEH Amide stationary phase using a gradient with acetonitrile and water containing formic acid, ammonium acetate and ammonium formate, has been used to obtain, in just 15 minutes, the complete separation of 20 α-amino acids investigated. The proposed method was applied to Calabrian honeys of different botanical origins (Citrus, Chestnut, Robinia, Eucaliptus and Sulla). The obtained results suggested that the developed methodology is robust and reliable for the analysis of free amino acids in honey.

Acknowledgments: This work was supported by project "ARAC –Valorizzazione dei prodotti dell'apicoltura calabrese e tecniche innovative ed ecocompatibili per il risanamento dei prodotti apistici contaminati" Regione Calabria APQ Azione 3.

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PP1_74

CUCHAMÁ LARVAE *(PARADIRPHIA FUMOSA)* EDIBLE INSECT RICH IN NUTRIENTS

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A large amount of people in developing countries live in poverty, hence they do not have an adequate food supply, situation that lead to a high index of malnutrition and other nutritional disabilities, a possible measure to overcome the problem faced by population could be the consumption of natural sources of food that nature provide such as edible insects that contain valuable nutrients, available to a large extent of population. Entomophagy cultural practice in several countries since ancient times, represent a good alternative to increase health for those social groups. Cuchamá (*Paradirphia fumosa*) butterfly inhabit in the Mixteca zone south east Puebla State, on the Manteco tree (*Parkinsonia praecox*), larvae stage, consumption represent a cheap source of nutrients for local population. The aim of this study was to analyze the nutritional value of Cuchamá insect, intake in the larva stage, and inform local people the nutraceutic benefits that insect provide in health.

Conventional sampling of larvae on the Manteco tree, were provided by local people on July 2015 and kept in plastic bags, larvae die by asphyxia, later on wash them and sundry. Raw Cuchamá samples were analyzed, moisture and macronutrients in dry basis by AOAC 1995 methods. The samples were dried at 60°C for 24 h and milled to a fine powder to analyze moisture, proteins, lipids, minerals, fiber and soluble carbohydrates. Proteins were calculated from the nitrogen content by the Kjeldahl method using the conversion factor 6.25. Lipids were determined by an extraction process with petroleum ether at 120°C for 6 h using a Goldfish apparatus, minerals were analyzed after incinerating the sample at 600°C in a muffle furance for 6h. Crude fiber by acid hydrolysis, followed by alkaline hydrolysis and soluble carbohydrates by [100-(protein+lipids+minerals+crude fiber)].

Data obtained were: moisture 6.25%; proteins 40.21%; lipids 17.07%; minerals 9.25%; crude fiber 6.45%; soluble carbohydrates 27.02%. Data may change according to biotic and abiotic conditions of the environment. Cuchamá is very low in water, high in proteins; fatty acids and minerals, however quality of proteins depend of the amino acids content, by the other hand, excess of proteins is converted in soluble carbohydrates by gluconeogenesis process consequently the amount of soluble carbohydrates can be increased. Additionally, lipids and mineral content is high but fatty acids and minerals should be analyzed individually. In conclusion, Cuchamá larvae is available to all social groups and can improve the nutritional status of local people.

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PP2_01

SECHIUM EDULE ALKALOID EXTRACT APOPTOSIS INDUCE DON HELA CELLS CERVICAL CANCER

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Cervical cancer is considered the second most common cancer in the world, if half a million new cases are reported and about 2333.400 deaths annually. In Mexico is considered the second cause of death. Because the importance of pathology chemotherapeutic treatments currently exist focused on elimination of tumor cells. Derived from the extensive knowledge of ethnobotany, chayote (*S. edule*) which is endemic in Traditional Mexican Medicine as a treatment for various ailments including considering the possible anti-tumor effect.

We propose to demonstrate the likely ability of the alkaloids obtained from chayote (*Sechium edule*) the effect of inducing apoptosis of a cell line of tumor origin, derived from cervical cancer (HeLa) . Evaluate the effect of apoptosis with inductor and on HeLa cancer cell line and get the crude chayote (*S. edule*) as well as fractions of the same extract for chromatographic separation in which apoptosis containing alkaloids. The cell was determined by Annexin tincipoon V-FITC / propidium iodide both quantified by flow cytometry in assessing each cases.

Applying alkaloids of *Sechium edule* to cells cultured HeLa cervical cancer caused a greater reduction in percentage of viable cells at all concentrations tested (2.5, 5.0, 7.5 and 10.0 .mu.L). A 2.5 uL volumes, 5.0 uL and 7.5 uL a reduction of 10, 8 and 10% of cell viability versus the negative controls, respectively, effect that was greater than that achieved by actinomycin D (positive control) was achieved. The maximal effect is achieved with a volume of 10.0 uL alkaloids, achieving a 15% reduction in cell viability. Therefore, it is demonstrated that alkaloids *S. edule* reduce cell viability, possibly by inducing apoptosis in cells cultured HeLa cervical cancer.

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PP2_02

ANTITUMOR EFFECT AND TOXICITY ACUTE ETHANOL EXTRACT OF *GANODERMA CURTISII* (BERK.) MURRILL LYMPHOMA L5178Y IN BALB / C MICE.

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Cancer is the uncontrolled proliferation of abnormal cells, that is, loss of normal patterns of cell behavior[1]. These illnesses are a major cause of death in the world[2]. Malignant tumors in México ranks third in mortality[3] and for the Hidalgo State ranks second[4]. Is necessary searching one natural alternative for the treatment of these diseases, one natural choice cheap, accessible, and safe. *Ganoderma* is a genus of mushroom used over 4000 years ago in eastern cultures because of medicinal properties. In China, your name is Reishi and is called "the mushroom of the 10,000 years"; and Ling Zhi "mushroom of immortality". Mannetake and shitake in Japanese and Korean,Young Zhi [5]. The population uses *Ganoderma* for treatment of cancer [6]. The aim of the research is to evaluate the antitumor effect of ethanol extract of *Ganoderma curtisii* in lymphoma L5178Y (ATCC.CRL-9518) mouse model *in vivo* and acute toxicity in Balb / c mice.

The collection was made in the municipality of Acaxochitlán Hgo., in forest Quercus-Pinus and taxonomic identification. The ethanol extract was obtained by rotary evaporation (40 °C). For antitumor test, 7 groups of 6 male Balb/c mice 30 g were formed, subjected to adjustment period for seven days, weighed and marked. L5178Y lymphoma was inoculated intraperitoneally into each mouse. The toxicity test was conducted based on the Lorke method; nine male mice were used to form three batches with three mice each, indicating low dose 1600 mg / kg, 2900 mg average dose / kg and high dose 5000 mg / kg.

Days survival of mice after inoculation L5178Y lymphoma and treatment with low dose of *G curtisii*. (0.0128 g / kg) were on average of 11.22 days; and for medium dose (0.128 g / kg) were 11.8 days, but the high dose (0.64 g / kg) caused immediate death of the mice. According to statistical analysis (ANOVA), no significant statistical difference between the low dose and the average dose compared with the control group. No anti-tumor effect was found in the doses used compared with the control group. Results indicate that *G.curtisii* did not show antitumor effect on lymphoma L5178Y. *G.curtisii* was very toxic from 1600 mg / kg.

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PP2_03

SYNTHESIS AND EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF OPTICALLY ACTIVE TRIAZINES

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It is known that most of the potential chiral synthetic drugs are generally obtained as racemates. This fact could be a drawback because it is documented that in several cases one of such enantiomers reduces the activity of the other or produces undesirable side effects. For that, the searching for enantioselective synthetic approaches in drug discovery programs continue being an issue of high priority.[1] The present research work is directed toward the development of an enantioselective synthetic approach of novel pyrrolidinetriazines with potential anti-inflammatory activity, mediated by the use of L-proline as the key starting material.

The synthesis of our target molecules **4** was planned as depicted in the following Scheme.

Figure 1. Proposed synthetic sequence for the novel chiral pyrrolidinetriazines 4

The L-proline methyl ester **2** was obtained by treatment of L-proline **1** with $SOCl_2$ in MeOH at reflux, by following a previous report.[2] Then, ester **2** was subjected to reflux in MeOH with phenylhydrazine affording the hydrazide **3** (R^1 = H). Finally, the treatment of hydrazide **3** with benzaldehydes in EtOH at reflux in the presence of AcOH as catalyst, afforded the expected chiral products **4** in good yields. Three new products **4** (derived from 4-ClC_6H_4CHO, 4-$MeOC_6H_4CHO$ and 3,4-$Cl_2C_6H_3CHO$) have been obtained through this simple methodology. Looking for a wider scope, the synthesis of further products **4** is currently in progress starting from diverse phenylhydrazine and benzaldehyde derivatives. In addition, experiments are in progress in order to evaluate de anti-inflammatory activity of **4** and its derivatives, using murine macrophages (RAW 264.7 cell line) stimulated with bacterial lipopolysaccharide (LPS) at different time points.

Acknowledgments to Universidad del Valle and Universidad Tecnológica de Pereira (Grant 9-16-3) for financial support.

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PP2_04

COLON-AVAILABLE DIETARY MELANOIDINS EXHIBIT CYTOTOXIC ACTIVITY ON HUMAN COLON ADENOCARCINOMA CELL LINES

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Emerging evidence from *in vitro* and *in vivo* studies suggested that the gastro-intestinal tract might be the key site for the biological action of food melanoidins [1]. Recently, Vitaglione et al. [2] reviewed the possible mechanisms by which coffee melanoidins may influence the risk of colorectal cancer development. In this work, we tested the water-soluble high molecular weight melanoidins (HMWM) obtained from different food sources for their cytotoxic activity against colon cancer cell lines.

HMWM were obtained by ultrafiltration (cut-off 10 kDa) from instant coffee, cocoa brew, dark beer and instant barley coffee beverages [2]. HMWM have been chemically characterized for their content in total polyphenols, protein, and polysaccharides as well as their spectroscopic properties. The value of $K_{mix\ 420nm}$ (defined as the absorption at 420nm of a solution of HMWM at a concentration of 1 g/L) provides information on the relative amount of melanoidins ($K_{mix\ 420nm}$) respect to other compounds found in the HMWM of beverages. Cytotoxic activity against two colon cancer cell lines (Caco-2 and SW480) was assayed by MTT assay. HMWM obtained from the above beverages submitted to *in vitro* gastro-intestinal digestion [3], were subjected to the same chemical analysis and assayed for their cytotoxic activity.

Barley coffee had the highest HMWM content (83.3g/100g of dry matter) followed by coffee (34.4g/100g of dry matter), cocoa (28.1g/100g of dry matter) and dark beer (20.0g/100g of dry matter). The HMWM of coffee had the highest $K_{mix\ 420nm}$ value, suggesting that this fraction is relatively rich in melanoidins. On the contrary, despite the highest content in HMWM, barley coffee was the sample with the lowest melanoidins content. Protein content of HMWM ranged from 6.1% (dark beer) to 10.6% (coffee) whereas polysaccharides content ranged from 42% (dark beer) to 61% (cocoa). Cocoa and coffee HMWM had the highest phenolic content (6 and 12%, respectively). The *in vitro* digestion caused a general decrease in the polysaccharide and protein content of all the analysed HMWM because of the action of amylolytic and proteolytic enzymes. The analysis of the content of polyphenols showed a decrease in these components as a result of the digestion. On the contrary, the *in vitro* digestion caused an increase in the $K_{mix\ 420nm}$ of all the HMWM investigated suggesting that this process resulted in an enrichment in melanoidins. The effect of HMWM on the human colon adenocarcinoma Caco-2 and SW480 cell cultures was investigated. Un-digested HMWM had no significant cytotoxic effect against both the cell lines. However, the *in vitro* digestion greatly increased the cytotoxic activity of HMWM against both the cell lines. Coffee HMWM were the most active against Caco-2 cells whereas cocoa HMWM were found to be most active against SW480 cells. Considering that the colon accumulates its content over at least 24 h in a maximum volume of 2 l, it is possible to estimate the concentrations of HMWM reaching daily the colon with diet according to the different food matrices [1]. Data showed that, the consumption of coffee, cocoa and barley coffee beverages may result in intestinal concentrations of HMWM, which are cytotoxic against Caco-2 and SW480 cell lines.

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PP2_05

A FACILE SYNTHESIS OF 6-AZASTEROIDS

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Azasteroids have been described to possess relevant biological activities such as 5α-reductase inhibitors [1]. In this context, some 6-azaesteroids have been described to exhibit equivalent inhibitory activity against 5α-reductase compared to finasteride (4-azasteroid), which is the first drug accepted by US to treat benign prostatic hyperplasia [1]. A recurring methodology to prepare 6-azasteroids from 3-hydroxy-Δ^5 steroids consists of a route of at least six steps. These procedures usually employ chromium-based oxidants or ozone to afford a 6-carboxy-secosteroid, which is the key precursor in this procedure [2]. Herein, we introduce a novel methodology to synthetize two 6-azaesteroids using cholesterol and diosgenin acetate as starting materials without the use of polluting oxidants.
In order to insert a nitrogen-containing motif at the C-6 position of cholesterol or diosgenin acetates, we planned to obtain the oximes **2a** or **2b** as key precursors, which in turn could be transformed into the 5,6-seconitrile derivatives **3a** or **3b**. Subsequent reactions on the nitrile function could afford the target 6-azasteroids **4a** and **4b** (Figure 1).

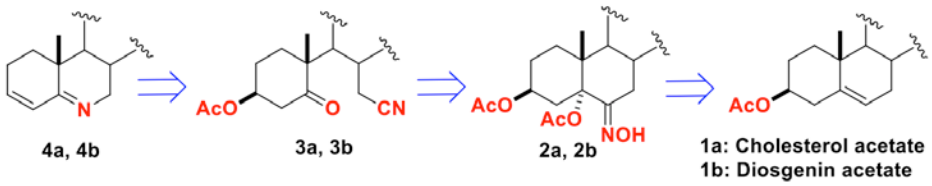

Figure 1: General method for the synthesis of 6-azasteroids.

Key precursors **2a** or **2b** were obtained following a two-step sequence: 1) Treatment of cholesterol or diosgenin acetate with $NaNO_2$, $BF_3{\cdot}OEt_2$ in AcOH afforded 3β,5α-diacetoxy-6-nitroimine derivatives [4]. 2) The nitroimines moieties were transformed into the corresponding oximes with NaOAc and $NH_2OH.HCl$ in refluxing ethanol. Cleavage of B ring was achieved through a second order Beckmann rearrangement of the oximes affording seconitrile derivatives **3a** or **3b**. The nitriles were partially hydrolyzed to the corresponding amides [5]. Finally, one pot Hoffmann rearrangement/cyclocondensation reaction was tested to obtain the 6-azasteroids.
In summary, we have developed a novel procedure to obtain 6-azaesteroids avoiding the use of polluting oxidants or ozone.

Acknowledgments: We thank CONACYT for the scholarship to R.M.P. and for the financial support via to the project 240329.

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PP2_06

ANTI-INFLAMMATORY AND ANALGESIC EVALUATION OF FLOWERS *SEDUM PRAEALTUM* DC

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Sedum praealtum DC (Crassulaceae) is a plant widely used in the Mexican traditional medicine to treat bucal ulcers, gums and eyes irritation and it has beneficial effect in teeth pain and burns. In experimental models, extract of stems and leaves, and the lyophilized from this plant have demonstrated a high anti-inflammatory activity in cotton pellet granuloma model in rats and this response was dose-dependent. Also, it has been reported that main components of extract are alkaloids, flavonoids and terpines (Meléndez et al., 2002; Castro-Mussot et al., 2011). Inflammation is widely related with pain, because these process are pathophysiological responses of the body to harmful stimuli and some signaling pathways are well correlated. Based on the above, the objective of the present was evaluate the anti-inflammatory and analgesic effects of flowers ethanol extract of *S. praealtum* in murine models.

Female mice 30 ± 5 g and Wistar rats 200 ± 20 g of body weight (b.w.) were used. Flowers ethanol *S. praealtum* extract (SEE) was prepared to administrate 100 and 200 mg/kg doses (SEE 100 and SEE 200 groups). Preliminary phytochemical analysis was realized. Animals were grouped and results were compared with sham group. Acetic acid induced writhing response and hot plate models were perform to evaluate analgesic activity. Cotton pellet-induced granuloma formation were used to evaluate anti-inflammatory activity. Indomethacin 5 mg/kg of b.w. (IND) and acetylsalicylic acid 100 mg/kg of b.w. (AAS) were used as anti-inflammatory reference drugs.

Pain is a complex unpleasant phenomenon composed of sensory experiences that include time, space, intensity, emotion, cognition and motivation originating from damage tissue or abnormal physiological condition. In acetic acid induced writhing, the animal reacts with a characteristic stretching behavior due to irritation of serous membranes while hot plate model produces two behavioral components than can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supra-spinally integrated responses (Milind and Monu, 2013). Abdominal constrictions induced by intraperitoneal injection of 0.6 % acetic acid were reduced with SEE 100 and 200 ($P < 0.05$ and $P < 0.001$, respectively). Similar effect was observed in hot plate model, in which SEE 100 was capable to decrease latency time at 2 h ($P < 0.001$) with the highest inhibition percentage (68.5 %) compared with all groups). SEE 200 induced a decrease of latency time at 1, 2 and 3 h ($P < 0.001$, $P < 0.001$ and $P < 0.01$, respectively). These results demonstrated that SEE is capable to down-regulated the nociception process at peripheral and central level, since the mechanism involved in the first model is related with release of arachidonic acid metabolites via cyclooxygenase (COX) and prostaglandin biosynthesis (Franzotti et al., 2000) and the second with central response to thermal stimuli.

For the other way, we evaluated SEE 100 in cotton pellet-induced granuloma formation model, observing that the extract decrease the wet, dry and final granuloma weight ($P < 0.05$, $P < 0.001$ and $P < 0.01$, respectively), similarly to IND. Unlike IND, SEE did not produce stomach or duodenal injury. This results revealed that SEE is decrease transudative, exudative and proliferative phases of the response to a subcutaneously implanted cotton pellet (Swingle and Shideman, 1972). With the above, we can conclude that flowers ethanol *S. praealtum* extract possesses anti-inflammatory and analgesic in murine models.

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PP2_07

Morus nigra L. LEAVES EXTRACTS STANDARDIZED IN CHLOROGENIC ACID, RUTIN AND ISOQUERCITRIN: TYROSINASE INHIBITION

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Melanogenesis is a process responsible for melanin production, which is stored in melanocytes containing tyrosinase. This enzyme is responsible for skin hyperpigmentation due to the overproduction of melanin. Inhibition of this enzyme is a target in the cosmetics industry, since it controls undesirable skin conditions. Plant species of the *Morus* genus are known for the beneficial uses offered in different parts of the plant, including tyrosinase inhibition. Thus, this project aimed to study the inhibitory activity of tyrosinase by extracts from *Morus nigra* leaves, as well as the characterization of the chromatographic profile of its extract and viability of incorporation in cosmetics from their cytotoxicity, in order to become a new therapeutic option from a natural source.

M. nigra leaves were collected, pulverized, equally divided into five batches and the standardized extract was obtained by passive maceration. The voucher specimen was deposited in the Herbarium of the University of Brasília, number Fagg CW 2302. In order to assess extraction reproducibility, the total solids content, yield and moisture content were measured for each batch. Tyrosinase enzymatic activity was performed for each batch according to Khatib et al. (2005) with modifications (1), providing the percentage of enzyme inhibition and IC_{50} values, which were obtained by constructing dose-response curves and compared with kojic acid, a well-known tyrosinase inhibitor. A High Performance Liquid Chromatography (HPLC) was used to determine chlorogenic acid, rutin and isoquercitrin levels in *M. nigra* ethanol extract. A Dionex UltiMate 3000 liquid chromatography system equipped with a Diode Array Detector DAD-3000 operating in 280, 330 and 354 nm was used.

The standardized *M. nigra* leaves extract showed no significant difference between batches for total solids content, yield and moisture content, which shows good reproducibility of the extraction process, also demonstrated by TLC chromatographic profile. High inhibition of tyrosinase activity was observed (above 90 % at 15,625 µg/mL) and IC_{50} values ranging from 5.00 µg/mL ± 0.23 to 8.49 µg/mL ± 0.59, comparable to kojic acid (3.37 µg/mL ± 0.65). According to ICH guidelines, the chromatographic method employed using High Profile Liquid Chromatography (HPLC) was validated. HPLC analysis revealed the presence of isoquercitrin as its major compound, followed by rutin and chlorogenic acid, with similar chromatographic profiles on all batches. Thus, *M. nigra* leaf extract was standardized using these polyphenols as markers, in which isoquercitrin concentration is, on average, 2.34 times greater than chlorogenic acid and, on average, 1.54 times greater than rutin in all batches. This study demonstrated the potential of *M. nigra* extract as a promising whitening agent of natural source against skin hyperpigmentation.

Acknowledgments: Authors are grateful to National Council of Technological and Scientific Development (CNPq), Support Research of the Federal District Foundation (FAPDF), Higher Education Personnel Improvement Coordination (CAPES), Scientific and Technological Enterprise Foundation (FINATEC) and University of Brasilia (UnB) for providing funds to perform the present research.

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PP2_08

ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY OF *SALVIA LERIIFOLIA* BENTH. EXTRACTS

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Salvia species have been used since ancient times in folk medicine in the treatment of cardiovascular diseases. Several pre-clinical studies point towards promising effects of *Salvia* species on one of the most important cardiovascular risk factor: hypertension. Angiotensin Converting Enzyme (ACE) inhibitors are widely use for the treatment of hypertension (Shlipak et al. 2001). As a part of our research for a natural therapeutic approach to the treatment of high blood pressure, *in vitro* screening has been conducted to evaluate the ACE inhibitory activity of *S. leriifolia* extracts.

S. leriifolia aerial parts were extracted by maceration with methanol and successively partitioned with dichloromethane, ethyl acetate and *n*-butanol. The ACE inhibitory activity was measured using the method described by Hansen et al. (1995). Folin-Ciocalteau method was used to determining the total phenols content.

The methanol extract inhibited ACE enzyme with an IC_{50} value of 199.7 µg/mL. After fractionation, ethyl acetate fraction demonstrated the highest ACE inhibitory activity, with an IC_{50} value of 104.3 µg/mL, followed by dichloromethane fraction (IC_{50} value of 158.7 µg/mL). The ethyl acetate fraction a high total phenols content. The ACE inhibitory activity of several *Salvia* species was previously demonstrated. *S. acetabulosa* methanol extract inhibited ACE with IC_{50} value of 52.7 µg/mL (Loizzo et al. 2008). More recently, Jiménez-Ferrer et al. (2010) demonstrated the *in vivo* ACE inhibitory activity of *S. elegans* hydroalcoholic extract (50.27%). This extract at the dose of 0.75 mg/kg significantly decreased systolic blood pressure and even had an antihypertensive effect that was greater than that treatment with losartan.

In conclusion, our result confirm the potential use of *Salvia* extract for the treatment of hypertension. Further study are necessary to identify *S. leriifolia* bioactive constituents.

Acknowledgments: Authors thank Farsad Nadjafi, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, GC, Tehran, Iran, for supplying the plant material.

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PP2_09

SYNTHESIS OF (*E*)-1-(FURAN-2-YLMETHYLENE)-2-PHENYLHYDRAZINE

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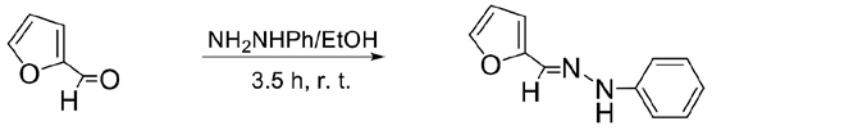
Cancer is a major burden of disease worldwide [1]. Each year, 10 millions of people are diagnose with cancer around the world, and more than half of the patients eventually die from it. In many countries, cancer ranks the second most common cause of death following cardiovascular diseaseas.[2] With significant improvement in treatment and prevention of cardiovascular diseases, cancer has or will soon become the number one killer in many parts of the world. As elderly people are most susceptible to cancer and population aging continues in many countries, cancer will remain a major health problem around the globe.



furan-2-carbaldehyde (*E*)-1-(furan-2-ylmethylene)-2-phenylhydrazine

Phenylhydrazine (3.12 mmol, 577 mg) was dissolved in ethanol, a chemical equivalent (300 mg) of aldehyde which was previously dissolved in the same solvent and it was added drop by drop stirring constantly. The action mixture was kept at room temperature and was monitored by TLC, and then vacuum filtered. The hydrazones were recrystallized by a continuous and controlled process until light yellow crystals with adequate size and purity were developed in order to obtain X-ray studies and characterization was performed by m. p., U.V, I.R. ^{1}H NMR, ^{13}C NMR.[3]

(*E*)-1,1'-Diphenyl-2-(thiophen-2-ylmethylene) hydrazine.

Light Yellow crystals; yield: 75% at 25 °C, mp. 117–119 °C. Anal.calc. for $C_{17}H_{14}N_2S$ (MW = 278 g mol^{-1}) C, 73.3; H, 5.1; N, 10.1. Found: C, 73.2; H, 5.0; N, 10.15%. UV λmax = 412 nm. FT. IR(film): (cm $^{-1}$): 3097 ν(C –H), 1667–1920 ν(Ph), 1585 ν(C=N),1490 ν(C=C), 695 ν(C–S). ^{1}H NMR 400 MHz, $(CD_3)_2CO$: (δ/ppm,J/Hz): 7.44 (m, 4H, C3'), 7.37 (d, 1H, C5), 7.36 (s, 1H, C=N), 7.21(m, 2H, C4'), 7.17 (dd, 4H, C2') 7.02 (dd, 1H, C3), 6.98 (dd, 1H,C4). ^{13}C NMR (300 MHz, $(CD_3)_2CO$): (δ/ppm): 143.39 (C2), 141.67(C1'), 130.52 (C5), 129.89 (C3'), 127.32 (C4), 127.01 (C3), 125.61(C=N), 124.64 (C4'), 122.24 (C2'). MS-EI: m/z = 278.37M^{+}.

Our investigation work group has made an experimental approach with the evaluation of synthetized hydrazones as ((*E*)-1,1'-Diphenyl-2-(thiophen-2-ylmethylene) hydrazine presented IC_{50} of 0.98 μM, that represent s the best potency of cell growth inhibition with respect to metronidazole (IC50 = 6.8 μM)[4].

Moreover, our group is working on the synthesis of metallic complex in order of intensify the pharmacological activity, in particular the amoebicidal activity.

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PP2_10

SYNTHESIS OF (*E*)-1,1-DIPHENYL-2-(THIOPHEN-2-YLMETHYLENE)HYDRAZINE AND CELL EVALUATION AGAINST AMIBIASIS

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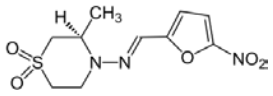
The Chagas disease is a public health problem, being in the fifth place within the top ten most important parasitic diseases worldwide and takes the second place only after malaria in the American continent that can be transmitted by insects.[1] Hydrazones and derivatives are most frequently used as drugs because they are nitroheterocyclic compunds as nitrofuran (nifurtimox) and nitroimidazole derivative (benznidazole), which are used in the treatment option for T. cruzi infections[2]. Our investigation group designed some compounds that have structural similarities with Nifurtimox, which is one of two most used drugs for treatment of parasitic *Trypanosoma cruzi.*[3] On the other hand, Nitroimidazol or its derivatives as common drugs, like Metronidazole, are well recognized for their amoebicidal properties that can heal bacterial infections of the vagina, stomach, skin, joints and respiratory tract. Nitroimidazole derivatives have been assayed as amoebicides and their activities are associated with the redox biotransformation of the nitro group that generates a short-lived nitroso free radical.[4]

493 mg (2.67 mmol) diphenylhydrazine were dissolved in ethanol and acetic acid (0.5 mL) and were slowly added into this solution while stirring; 300 mg (2.67 mmol) of thiophene-2-carbaldehyde were added drop by drop into the above solution with strongly stirring and the resulting mixture was kept at room temperature until it became a light yellow transparent solution. After one and a half hours the orange solution precipitated. The reaction was monitored by TLC, aluminum Alugram Sil G/UV254. The mixture was separated by filtration in *vacuo* system and the precipitate was washed three times with cold methanol. Recrystallization was performed three times with ethanol to obtain orange crystals for X-ray analysis and characterization was performed by m. p., U.V, I.R. ^{1}H NMR, ^{13}C NMR.

Light Yellow crystals; yield: 75% at 25 °C, mp. 117–119 °C. Anal. calc. for $C_{17}H_{14}N_2S$ (MW = 278 g mol^{-1}) C, 73.3; H, 5.1; N, 10.1. Found: C, 73.2; H, 5.0; N, 10.15%. UV λ_{max} = 412 nm. FT. IR (film): (cm^{-1}): 3097 (C –H), 1667–1920 (Ph), 1585 (C=N), 1490 (C=C), 695 (C–S). ^{1}H NMR 400 MHz, $(CD_3)_2CO$: (/ppm, *J*/Hz): 7.44 (m, 4H, C3´), 7.37 (d, 1H, C5), 7.36 (s, 1H, C=N), 7.21 (m, 2H, C4´), 7.17 (dd, 4H, C2´) 7.02 (dd, 1H, C3), 6.98 (dd, 1H, C4). ^{13}C NMR (300 MHz, $(CD_3)_2CO$): (/ppm): 143.39 (C2), 141.67 (C1´), 130.52 (C5), 129.89 (C3´), 127.32 (C4), 127.01 (C3), 125.61(C=N), 124.64 (C4´), 122.24 (C2´). MS-EI: m/z = 278.37M^{+}.



Nifurtimox

Our work group has made an experimental approach with the evaluation of synthetized hydrazones as (*E*)-2-(2-nitrobenzylidene)-1,1-diphenylhydrazine presented IC_{50} of 7.6 M, that owns the same potency of cell growth inhibition as metronidazole (IC_{50} = 6.8 M).[3]

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PP2_11

SYNTHESIS OF (*E*)-2-((5-(2-NITROPHENYL)FURAN-2-YL)METHYLENE)-1,1-DIPHENYLHYDRAZINE AND ANTICANCER CELL EVALUATION

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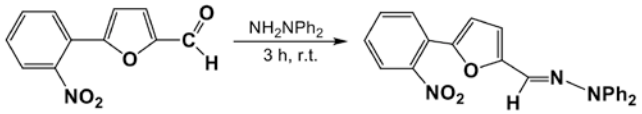
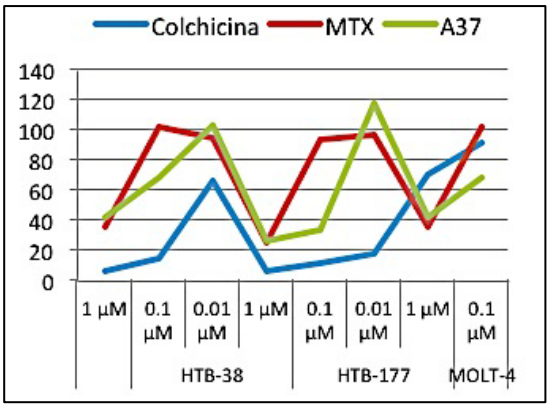
Breast cancer is the most common type of cancer affecting more than 1 million women and causing high mortality worldwide. It is estimated that there have been 232,620 new cases of breast cancer and 39,970 deaths occurring in the USA in 2011.[1] A large number of breast cancers express estrogen receptor (ER) as well as progesterone receptor (PR)[2] and respond to hormonal therapy or aromatase inhibitors. However, there is a group of patients (12–17%) who do not respond to such treatment because of the absence of these two receptors as well as the receptor HER-2/neu (ErbB2); this group represents a highly aggressive breast cancer subtype, known as triple-negative breast cancers (TNBC),[3] that are difficult to treat. In recent years, TNBC has gained attention due to its aggressive behavior, poor prognosis and lack of targeted therapies.

Diphenylhydrazine (1.38 mmol, 254 mg) was dissolved in a solvent, a chemical equivalent (300 mg) of aldehyde which was previously dissolved in the same solvent and it was added drop by drop stirring constantly and which was developed with greener procedure. The action mixture was kept at room temperature and was monitored by TLC, and then vacuum filtered. The hydrazones were recrystallized by a continuous and controlled process until wine crystals with adequate size and purity were developed in order to obtain X-ray studies and characterization was performed by m. p., U.V, I.R. ^{1}H NMR, ^{13}C NMR.[4]



5-(2-nitrophenyl)furan-2-carbaldehyde

Wine crystals; yield: 90.6% at 25 °C, mp. 393-395 K. Anal. calc. for $C_{17}H_{14}N_2S$ (MW = 278 g mol $^{-1}$) C, 73.3; H, 5.1; N, 10.1. Found: C, 73.2; H, 5.0; N, 10.15%. UV λ_{max} = 412 nm. FT. IR (film): (cm^{-1}): 3097 ν(C-H), 1667–1920 νν(Ph), 1585ν(C=N), 1490 ν(C=C), 695 ν(C-S). ^{1}H NMR (400 MHz, $(CD_3)_2CO$: (δ/ p.p.m., J/Hz): 7.77 (dd, H-3, *J* =7.94 H3 –H4 *J* =1.20 coupling W H3 –H5); 7.64(dd, H-6, *J* =8.10 H6 –H5 and *J* =1.16 coupling W H6 –H4); 7.54 (td, H-5, coupling H-4 H-6 *J* =7.74 and coupling W H-3, *J* =1.20); 7.42 (t, H-3); 7.36 (td, H-4 coupling H-3 H-5, *J* = 7.74, coupling W H-6, *J* = 1.36); 7.20 (m, 4H,H-2, H2,H-4); 6.99 (s, H-i); 6.69 (d, H-4 coupling H-3, *J* =3.60); 6.58 (d, H-3 coupling H-4, *J* = 3.60). ^{13}C NMR (100 MHz, $(CD_3)_2CO$): (δ/ p.p.m.): 153.0 (C2), 147.56 (C5), 147.17 (C2), 142.92 (C1), 131.77 (C5), 129.84 (C3), 128.50 (C3), 127.97 (C4), 124.87 (C2), 124.79 (iminic-C), 123.81 (C1), 123.69 (C6), 122.40 (C4), 111.91 (C4), 110.10 (C3).



Our work group has made an experimental approach with the cell evaluation in order to measure the anticancer activity against HTB-38, HTB-177, Molt-4.[3]

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PP2_12

INFLUENCE OF GRANULAR PREPARATION METHOD AND TABLET HARDNESS IN THE DISSOLUTION OF A PHYTOPHARMACEUTICAL PRODUCT

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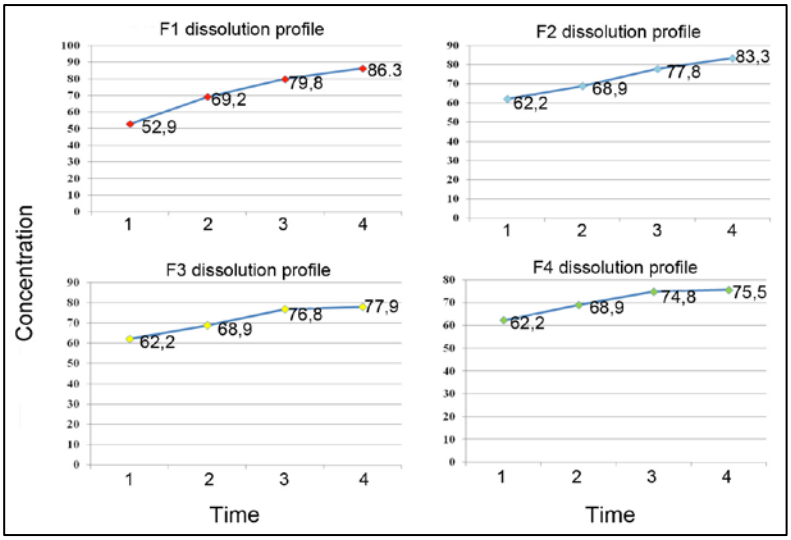
The growing demand for herbal medicine products observed in the last decade, sustained the need to look for technological-pharmaceutical improvements in the preparation of such products. In the present study, we have analyzed and simultaneously verified changes that tablet hardness (fracture resistance) and granulate preparation method may exert on in product disintegration and dissolution. It is in fact important to underline that the quality of these products is based on the characteristics that define and determine their suitability, allowing them to be classified as therapeutically useful.

Tablets used in the present study were prepared according to formulas in Table 1. Granules production was obtained using four methods: process of "simple mixture" (F1), process of "double compression" (F2), process of "wet granulation" (F3) and the process of "mixed method" (F4). Mechanical, physical and technological properties of tablets were evaluated in order to compare the influence of these processes on tablet hardness (resistance to fracture), disintegration and solubility.

Table 1. Formula used to make the phytodrug

1.	Plant Extract soft problem	60 mg
2.	Lactose	80 mg
3.	Avicel ph 101	200 mg
4.	Calcium carbonate	50 mg
5.	Cornstarch	50 mg.
6.	Magnesium stearate	1 % dry granules

As illustrated in graphs of the dissolution profiles, changes in the hardness of tablets exert an increase in the disintegration time and a decrease in the average concentration of components from the dissolved phytodrug. Results suggest that changes in these parameters may critically influence the efficiency of herbal medicinal products.



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PP2_13

IN VITRO VALIDATION OF ANTI-INFLAMMATORY, HEALING AND ANTI-LEISHMANIAL ACTIVITY OF *TABEBUIA CHRYSANTHA*, *TABEBUIA ROSEA CALENDULA OFICINALIS MATRICARIA CHAMOMILLA*, *THYMUS VULGARIS* AND *OREGANUM VULGARE*

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Cutaneous leishmaniasis (CL) is a worldwide disease, which mainly occurs in the poorest countries. The diseases affect million people causing high morbidity. Despite CL does not cause death *per se*, it is highly associated with social stigma and discrimination. Unfortunately, lack of therapeutic alternatives, decreased efficacy, side effects and high costs, emphasize the need for advance in the development of new and better drugs. Natural products remain as an important source of novel chemical structures and therapeutic medicines. However, bio-guided studies to develop innovative structures with therapeutic potential are required. Given that CL is a chronic inflammatory disease that allows parasite persistence in the host and facilitates the progression of lesions, an useful strategy is the combination of anti-inflammatory and healing properties of the drug candidates with the capability to kill *Leishmania*. The ability of regulating the inflammatory response and stimulating the healing activity, could accelerate the resolution of inflammation and scarring, which in turn would lead to a treatment with better benefits either by the shorter scheme or improvement of the scar appearance. Thus, this study was aimed to determine the possible effect of various natural products and synthetic derivatives with activity against *L. (V) panamensis* on the inflammatory and healing processes.

Ethanol extracts from flowers of *Tabebuia chrysantha*, *T. rosea* and *Calendula oficinalis* and essential oils of *Matricaria chamomilla*, *Thymus vulgaris* and *Oreganum vulgare* were tested for cytotoxicity in U937, BHK-21 and Detroit 551 cell lines and primary cultures of monocyte-derived macrophages (MDM) using the MTT method. The anti-leishmanial activity was evaluated *in vitro* against *L. (V) panamensis* amastigotes by flow cytometry. The effect of these products on inflammation and healing processes was determined quantifying the production of IFN TGF, MCP-1, COX-2 and various dermal growth factors in culture supernatants of human monocyte-derived fibrocytes (FDM) by ELISA. Lastly, the scarring capability was tested *in vitro* according to the effect of these compounds on the fibrocyte migration using the cytoselect™ system.

The anti-leishmanial activity but also the production of immunological factors associated with inflammation and scarring varied among extracts and essential oils. The ethanol extract of *C. oficinalis* and the essential oil of *M. charantia* were the most effective compounds which meet all the characteristics pursued in the main objective of this work and could be postulated as "hit" to continue the drug development process.

Acknowledgement to University of Antioquia (CIIEs – CIDEPRO).

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PP2_14

ANTI-INFLAMMATORY ACTIVITY OF A *PHYSALIS ANGULATA* L. DICHLOROMETHANE FRACTION IN DSS-INDUCED COLITIS

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Physalis angulata is a plant commonly used in folk medicine of Latin American countries to treat inflammation related pathologies [1]. In this work, we evaluated the anti-inflammatory potential of an enriched fraction obtained from its calyces using a Dextran Sulfate Sodium (DSS)-induced colitis model in mice and Lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages *in vitro*.

The total ethanolic extract of the *P. angulata* calyces was fractioned using liquid-liquid partition with petroleum ether, dichloromethane and methanol. The dichloromethane fraction proved to have the highest anti-inflammatory potential *in vitro*, and was therefore selected for the next experiments. In the DSS model, the fraction was administered to mice for 7 days, after two DSS/Water cycles of 7/10 days, respectively. Weight changes, stool consistency and disease activity index were monitored throughout the experiment, while colon and mesenteric lymphatic nodes (MLN) were extracted at the end to assess the level of local and systemic inflammation. *In vitro*, the fraction was evaluated on LPS-stimulated RAW 264.7 macrophages, to ascertain its influence on the expression levels of pro-inflammatory genes like IL-1β, NOS2, COX2 and TNF-α, as well as genes that promote an anti-inflammatory, tissue repairing environment like ARG1, IL-10 and TGF-β1, using Real Time-Polymerase Chain Reaction (RT-PCR)[2].

The dichloromethane *P. angulata* fraction reduced the disease activity index and colon shortening induced by DSS. The fraction showed an important effect on the expression of the evaluated genes, downregulating the pro-inflammatory and upregulating the anti-inflammatory genes. This work confirms the therapeutic potential of *P. angulata* metabolites to modulate inflammatory processes.

Acknowledgments: This study was supported by Colciencias (grant 110772553569) and the University of Cartagena (grant 003-2015).

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PP2_15

CYTOTOXIC EFFECT OF ETHANOLIC EXTRACT AND FRACTIONS FROM *CROTON MALAMBO* BARK AGAINST MDA-MB-231 CELL LINE.

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Breast cancer is the most common in women worldwide, is a serious public health issue with nearly 1.7 million new cases diagnosed in 2012[(1)]; therefore an effective management and treatment of this disease is undoubtedly crucial. In this sense the ethnopharmacology research of botanical species has proved to be a useful approach to find potential anti-cancer agents. In this work, we evaluated the cytotoxic effect of ethanolic extract of *Croton malambo* bark and its fractions on the MDA-MB-231 breast cancer cell line, using the MTT method.

The ethanolic extract obtained of the *C. malambo* bark was loaded on a column chromatography over silica gel, and eluted with solvents in crescent order of polarity, under a bio-guide scheme based on the cytotoxic activity against MDA-MB-231 cells, employing the methyl-tetrazolium bromide colorimetric method (MTT)[(2)]. The extract and fractions were classified into three categories as: active (LC_{50}≤20 µg/ml), moderately active (20<LC_{50}<100 µg/ml) and inactive (IC_{50}≥100 µg/ml)[(3)].

Chromatographic fractionation of *C. malambo* total extract, led to obtain six major fractions identified as CM-F1, CM-F2, CM-F3, CM-F4, CM-F5 and CM-F6, which were evaluated in the biological model. The total extract was moderately active on MDA-MB-231 cells (LC_{50}=63.68 µg/mL), this activity was maintained in two of six fraction with a better cytotoxic effect (CM-F5 - LC_{50}=43.45 µg/mL and CM-F6 - LC_{50}=43.35 µg/mL), which were eluted with ethyl acetate. These fractions represent a promising source of cytotoxic compounds that should be further explored, aiming to obtain new active compounds that improve the therapeutic arsenal available for breast cancer treatment.

Acknowledgments: This study was supported by Colciencias and the University of Cartagena (Grant 110756933390). Daneiva Caro is deeply grateful to Colciencias and the University of Cartagena for her PhD fellowship through the National Program for Doctoral Formation (Grant 647-2014).

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PP2_16

ANTICONVULSANT EFFECT OF *TANACETUM PARTHENIUM* (L.) SCHULTZ-BIP ON PENTYLENETETRAZOLE-INDUCED SEIZURES IN MICE

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In Danish and South Africa folk medicine, *Tanacetum parthenium* is used to treat epilepsy and seizures (Jäger *et al*., 2006). Based on this background, there have been several studies *in vitro* of the extracts of this plant or its components, suggesting an anticonvulsant effect through interaction with the GABAergic system in the CNS (Viola *et al*., 1995; Jäger *et al*., 2006; Jäger *et al*., 2009). However, it is not known with certainty the components responsible for the anticonvulsant activity or mechanism through which they operate.

The main objective of this study was conducting *in vivo* tests allowing to characterize the anticonvulsant effect of aqueous and aqueous-alcoholic extract of *T. parthenium* and explore its possible mechanism of action, using a model of acute administration of pentylenetetrazole (PTZ).

Chemical characterization of aqueous and hydroalcoholic extracts of *T. parthenium*, respectively was performed. The presence of total polyphenols and flavonoids were quantified, and identified the main compounds by HPLC-DAD-ESI-MS analysis. Acute toxicity tests for determining the LD_{50} were performed. The acute model of convulsive activity induced with PTZ in mice was used for the evaluation of anticonvulsant effect.

The hydroalcoholic extract of *T. parthenium* produced the best effect ($p < 0.01$) at doses of 100 mg kg reducing the number and duration of tonic-clonic seizures induced by PTZ. The aqueous extract at dose of 100 mg/kg, significantly decreased the number of myoclonic seizures ($p < 0.001$) compared with the control group and against the reference drug (Valproic acid) ($p < 0.05$), and also increased the latency to tonic-clonic seizures, although this increase was not statistically significant. For the chemical characterization of the extracts two major compounds were isolated: santin and santamarin. The latter was tested in the model of PTZ, producing his best anticonvulsant effect at the dose of 1 mg/kg by reducing the number ($p < 0.05$) and duration ($p < 0.01$) of tonic-clonic seizures and number of myoclonic seizures ($p < 0.01$) compared with the control group, and an LD_{50} greater than 10 mg/kg. The LD_{50} for the aqueous hydroalcoholic extract was greater than 5000 mg/kg.

The aqueous and hydroalcoholic extracts of *T. parthenium* and the sesquiterpene lactone santamarin exercised their anticonvulsant effect in mice treated with PTZ. These results suggest that the anticonvulsant effect of these products is mediated at least in part by the GABAergic system, in particular for $GABA_A$ receptors in the CNS from a possible synergistic activity of its components (Sucher y Carles, 2015). So that, the potential of *Tanacetum parthenium* is proposed for its use as a therapeutic alternative accessible, and low toxicity, for the treatment of epilepsy.

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PP2_17

LC/MS ANALYSIS AND HPLC QUANTIFICATION OF MAIN COMPOUNDS OF FIVE *HYPERICUM* SPECIES INDIGENOUS IN THE PELOPONNESE

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Hypericum genus (Hypericaceae) is widely known because of *Hypericum perforatum* L., a species with numerous traditional uses especially treating mild depression; action mainly attributed to hyperforin, a prenylated phloroglucinol. Naphthodianthrones are found exclusively in aerial parts of *Hypericum* genus along with flavonols and flavonols glycosides, biflavonoids, and other phenolic compounds (as organic acids)[1]. In Greece except *H. perforatum* L., other 40 taxa of *Hypericum* genus are indigenous, moreover one third of them endemic [2]. However their phytochemical composition has not been investigated sufficiently.

In this study we focused on the chemodiversity of five species of *Hypericum* growing wild in Peloponesse (Greece), *H. perforatum* L., *H. perfoliatum* subsp. *veronense* (Schrank) A. Fröhl, *H. triquetrifolium* Turra, *H. empetrifolium* Willd. subsp. *empetrifolium* and *H. vesiculosum* Griseb. Aerial parts of the species were collected during the flowering period and dried at room temperature. The phytochemical composition of the dry methanolic extracts was studied using "Ultra Performance" Liquid Chromatography (UPLC) coupled to Q-TOF (quadrupole - time of flight) Mass Spectrometry. Several organic acids, flavonoids, naphthodianthrones and phloroglucinols were identified by LC-MS. Total flavonoids, naphthodianthrones and prenylated phloroglucinol derivatives as well as chlorogenic acid, rutin, isoquercitrin, hyperoside, quercetin, I18-I3-biapigenin, pseudohypericin and hypericin were quantified in all extracts by High Performance Liquid Chromatography (HPLC - DAD), on a C-18 column, with a method previously developed and validated in our laboratory [3].

The majority of the main compounds were common among the examined species; however some differences were detected especially as regards the phloroglucinol derivatives. *H. perforatum* had the highest quantity of compounds per mg of dry extract, double than the other species, and the highest % concentration of total flavonoids and phloroglucinol derivatives. *H. vesiculosum* had the highest content of napthodianthrones, while *H. empetrifollium,* had the lowest content of napthodianthrones and flavonoids, and the highest content of phenolic acids. The results of this study shed a light on *Hypericum* chemodiversity and may even reveal other taxa which could be valuable as sources of the medicinal preparations.

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PP2_18

OPTIMIZATION AND VALIDATION OF TWO METHODS OF ENZYME INHIBITION RELATED TO ANTI-HYPERGLYCEMIC ACTIVITY

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In vitro microscale assays are fast, simple, inexpensive and reduce reagents, residues and the consumption of experimental animals in the initial screening of biological activity. However, they have low reproducibility and low correlation with *in vivo* models, possibly by changes made in each laboratory that can affect accuracy. In this work were optimized and validated *in vitro* microscale assays related with antihyperglycemic activity.

Optimization was carried out using a fractional factorial design and a basic sequential simplex method, taking into account the best inhibition percentage and the absorbances of the controls. With the optimized experimental conditions the methods were validated.

The optimized conditions of α-glucosidase method were: enzyme concentration of 0.55 U/mL, substrate concentration of 111.5 uM, 17.5 min incubation at 37 ° C. In the validation of this method, a linear range between 100 and 310.2 ug/mL for acarbose with a value of R^2 0995, was established. The percentage coefficient of variation were less than 2% and error percentages were below 3%, indicating good precision and accuracy. The Z factor was greater than 0.96. Optimal conditions for the α-amylase method were: enzyme concentration of 0.5 U/mL, substrate concentration of 1.11 mM, 7.2 min preincubation, incubation of 5.5 min and 15.6 min in water bath. The linear range was established between 0.5 and 12 ug/mL of acarbose. The% CV were below 5% and error % were lower 6%. A Z-factor greater than 0.94 was calculated.

In conclusion *in vitro* microscale methods, precise and accurate able to determine the potential antihyperglycemic activity in natural extracts methods were developed.

PP2_19

THEORETICAL STUDY OF THE CONVERSION (E)-□,□-UNSATURATED OXAZOLIDINONES

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Stereochemistry has been studied in the conversion of (E)-□□□-unsaturated oxazolidines to the □□□-unsaturated. The Z/E ratios of the resulting □□□-unsaturated compounds vary according to □-substituens of the (E)-□□□-unsaturated oxazolidines. It is assumed that this reaction occurs by interaction of □->□* or via 6□-electron aromaticity, which is called the *syn effect*.

The reaction ilustrated in Figure 1 consiting in the conversion of (E)-□□□-unsaturated oxazolidinones to □□□-unsaturated was studied using computational methods. Gaussian09 hasbee used to perform the electronic structure calculation, whereas molecular orbital calculations were done using polarized and diffuse basis set. Molecular orbitals were displayed using MOLDEN. Intrinsic reaction coordinates were also measured.

The stereochemistry of the conversion of (E)-□□□-unsaturated oxazolidines to the corresponding □□□-unsaturated was suggestive for a *syn-effect* and it can be proposed that the relative degree on the □-substituents consists of the interaction between orbitals □-> □.

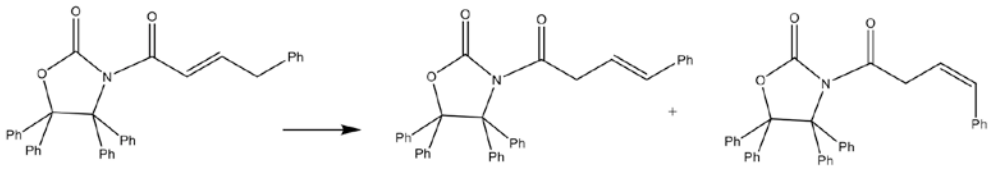

Figure 1. Conversion of (E)-□□□-unsaturated oxazolidines to the corresponding Z/E □□□-unsaturated,

Looking at MOLDEN-generated graphics it could be suggested that molecular orbitals of the three molecules exhibit significant differences. It was also observed that the MOs are bigger in the molecules □□□-unsaturated. For □□□-unsaturated oxazolidines, the □-substituents gave different ratios according to the substituent. The IRC gave also some insight of the moment of conversion.

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PP2_20

COMPUTATIONAL ANALYSIS ON VITAMIN D RECEPTOR

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The Vitamin D Receptor (VDR) is a member of nuclear receptor superfamily, which mediates the biological actions of vitamin D, the hormone 1, 25-dihydroxivitamin D_3 [1, $25(OH)_2D_3$]. Also a receptor for Lithocholic acid (LCA), is a potential enteric carcinogenic, which induces its metabolism trough VDR interaction. Are examined theoretically the interaction of LCA and D_3 on VDR.

It has been used 4Q0A y 1DB1 structures from Protein Data Bank to compare the electronic interactions, and geometrical comparisons between 1α,25-dihydroxyvitamin D_3 and Lithocholic acid (LCA), which both stimulate the Vitamin D Receptor. This theoretical study was carried on using Spartan and VMD suite of programs.

The purpose is to identify what kind of structural, electronic or electrostatic differences are present between these two molecules.

The molecules studied are presented in figure 1. The molecule 1, $25\text{-}(OH)_2D_3$ interacts with VDR nuclear receptor when is in the nuclear receptor, in a space of 26.508Angstrom large, 10.553 high and 16.618 wide. Two molecules of Lithocholic Acid interact with VDR, one of them among nuclear receptor and other by outside. The distance between the two LCA are for both C=O is 11.784 and for $CH_3$19.432 Angstrom. Electronic and energetic properties are different for the metabolites.

Acknowledgments: this project was supported by VIEP/BUAP

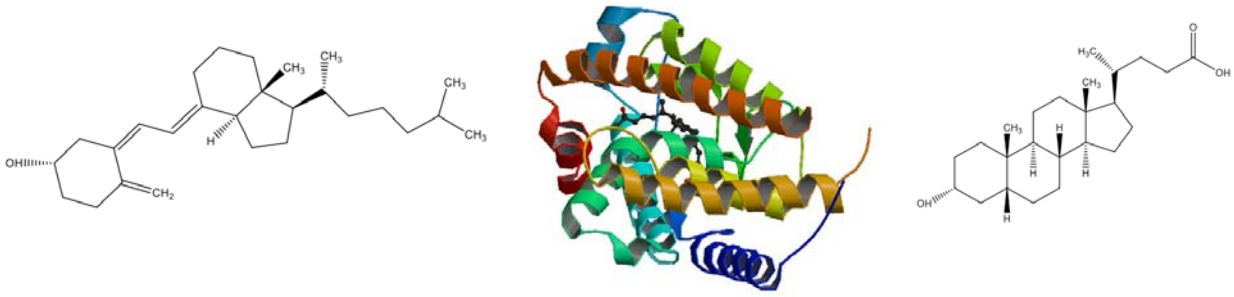

Figure 1. a) 1, $25\text{-}(OH)_2D_3$, b) Vitamin D and VDR, c) Lithocholic Acid.

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PP2_21

MEDICAL APPLICATIONS OF OZONIZED OIL. AN UP-DATE

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Early evidence on the clinical use of ozonated oils first appear in scientific literature in 1859. This lecture reviews the general and main clinical applications of ozonated oils that have appeared in scientific literature between 1859-2016.

An in-depth screening of primary sources of information online - via Scopus databases as well as Embase, PubMed, and the Cochrane Database of Systemic Reviews - was performed. The most significant papers of the last 25 years are presented and their proposals critically evaluated.

The main applications of these agents are for infectious diseases. The oxidation products generated after the reaction of ozone with fatty acids and other substrates can acts as a germicide, immune stimulant and tissue restoration agent. Some of the advantages of ozonated oils are: low cost, similar or superior effects compared to traditional antibiotics, a broad antimicrobial spectrum and a low rate of adverse events. The stability of the ozonated oils allows the development of standard formulations that deliver the benefits of ozone, in a product that can be conventional stored for future clinical use. This is in contrast to the therapeutic application of the ozone gas, where the gas must be applied immediately following its production. The main clinical studies that support the use of ozonized oils apply ozonized sunflower oil or ozonized olive oil in different clinical conditions. The applications are essentially for external use, however there is evidence of immune-stimulating and repair effects when used orally. A deep study of the biological effects of various formulations already on the market will support the scientific basis of the use of ozonized oil in different pathologies.

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PP2_22

BIOSAFETY ASPECTS IN THE REGULATION OF MODERN BIOTECHNOLOGY IN PLANTS

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The RNA interference (RNAi) technology, involving small non-coding RNAs (sRNA) is currently one of the main tools of modern biotechnology to develop resistant plants, especially against virus, and with desired characteristics (Zhou et al., 2013). microRNAs (miRNA), a type of sRNA, had been used in a new strategy for developing plant for food (Sherman, et al. 2015). Of extreme interest of this new technology is the fact that it enables genetic modification (GM) of the plant with no production of transgenic protein. Nevertheless, these advances should not ignore the potential risks to human, animal, and to the environment (Cascuberta et al., 2013). In this sense, it is necessary to discuss miRNA-GM plants risk assessment in the regulatory framework. This work aims to promote an analysis on the Biosafety regulation of the GM techniques using miRNA.

The question "Is there a specific biosafety regulation for miRNA technique or strategy for plants?" was pursued for the descriptive and comparative analysis of the food biosafety regulation about the miRNA technique in the legal frameworks of Brazil (http://ctnbio.mcti.gov.br), European Union (EU) (http://eur-lex.europa.eu/homepage.html) and United States (US) (https://www.loc.gov).

In the US, there are five GM plants developed with the RNAi technology approved to market, namely, apple, potato, tomato, tobacco and soybean. The risk assessment of these GM plants is similar to that used for GM plants that produce transgenic proteins. The environmental impact is determined empirically. Nevertheless, the US are reviewing the legal framework process on this subject since 2015. In the EU, which adopts the precautionary principle, the list of genetic engineering technologies does not mention any of the RNAi-based strategies. Hence a debate has started in the EU with participants from academy, industry, government and non-governmental organizations. Although, neither a consensus about which criteria should be employed nor a way to regulate such plants has been reached at the moment, two GM plants using RNAi were approved in the EU, potato and soybean, but potato is no longer marketed. In Brazil, which also adopts the precautionary principle, the regulation establishes a case-by-case analysis and does not describes specific criteria for GM plants using RNAi-based strategy. A GM plant using this strategy, a bean resistant to the Golden Mosaic Virus, was recently approved in the country and it was subjected to risk assessment without application of differential criteria. The dialogue on risk assessment and updating the regulatory GM plants using new technologies have already started in all legal frameworks studied; the regulation in the US and Brazil remain defining the application of the same biosafety criteria for both GM plants using RNAi-based strategy and other GM plants produced by other techniques of modern biotechnology. These results suggest a political and conservative position instead of science-based. The current scientific evidence indicates a degree of risk differentiation (or safety) between the RNAi techniques or strategies and those with other techniques of modern biotechnology. We conclude that this evidence could support the application of differential (specific or simplified) criteria in the risk assessment that has not been applied in the legal frameworks studied.

Acknowledgments: we thank CAPES, CNPq and FAPES for financial support.

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PP2_23

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *LEPIDIUM SATIVUM* EXTRACTS

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Lepidium sativum, commonly known as Habarachad, is an edible grass with fast growth potential belonging to the *Brassicaceae* family. The seeds of this medicinal plant are diuretic and tonic; they are used to treat bacterial and fungal infections. The main aim of this study is to evaluate the phenolic composition, the antioxidant and antimicrobial activities of extracts prepared from *L. sativum* seeds. The yield of the extraction was 12.09% and 0.87% for Methanol (MeOH) and Aqueous (Aq.) Extracts respectively. The Aq. extract presented the highest content of total polyphenols (39.38 ± 0.001 mg. Gallic Acid equivalent /g. of extract), followed by the MeOH extract (31.38 ± 0.024 mg G.A.E. /g. of extract), whereas the contents of flavonoids were 14.12 ± 3.69 and 7.10 ± 7.86 mg. Rutin equivalent /g. dry extract for MeOH and Aq. extracts respectively. Moreover, the antioxidant activity was evaluated by using DPPH test [1], whereas the antimicrobial activity was assessed via disc-diffusion method [2]. The results revealed that the two extracts showed significant effects on the radical DPPH; with an IC_{50} for the Aq. Extract (0.104 ± 0.006 mg/ml) then the MeOH extract (0.166 ± 0.007 mg/ml). Furthermore, the study of the antimicrobial activity of the two extracts showed some effects against *Aspergillus flavus* (28.33 mm. in diameter for the Aq. extract) and *Aspergillus niger* (21 mm. in diameter for the same extract). However, no effect on the bacterial strains was recorded for both extracts.

Acknowledgments: The authors would like to thank the DGRSDT, Ministry of Higher Education and Scientific Research of Algeria for financial assistance.

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PP2_24

TRITERPENES-ENRICHED FRACTIONS OF *EUCALYPTUS TERETICORNIS* MODULATE GENE EXPRESSION ON HUMAN ADIPOSE TISSUE CELL LINES MODELS

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Obesity is associated with adipose tissue inflammation that eventually results in insulin resistance and it is linked with cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (Khan, SE. et al., 2006; Esser N. et al. 2014). Previous studies established the beneficial effects of enriched-triterpenes fractions from *Eucalyptus tereticornis* (Eu) on insulin resistance, glucose intolerance and hyperglycaemia in a diabetic mouse model (Guillén A., et al. 2015). In addition, they showed a reduction in levels of pro-inflammatory cytokines in adipose tissue (Guillén A., et al. 2015). Our aim is to evaluate the effect of triterpenes-enriched fractions from Eu on human adipocyte and macrophage cell lines models.

A crude methanolic extract (OBE100) from leaves of Eu was partitioned with ethyl acetate, concentrated and purified by Sephadex LH-20. The fractions containing triterpenes were pooled (OBE104), and used for biological assays. Adipose tissue-derived cells were obtained by enzymatic digestion of adipose tissue from healthy patients. These cells were induced to differentiate and treated for 7 days with OBE100 and OBE 104. Fatty acid content was evaluated by oil red staining. Gene expression levels of PPAR-γ, C/EBPα, FABP4, Glut4, adiponectin and leptin were analyzed by real-time RT-PCR. Human macrophage cell line U937, differentiated and activated with lipopolysaccharide (LPS) and INF-γ, was used to evaluate OBE100 and OBE104 anti-inflammatory action. Gene expression levels of IL-6, IL-1β and MCP-1 were detected by real-time RT-PCR. Both cell lines viability was determined using MTT assay.

Both fractions of Eu (OBE100-OBE104) presented no toxicity on macrophage or adipocyte cells. We observed a dose-dependent reduction of the intracellular fatty acid content in adipocytes treated for 7 days with Eu fractions. OBE 100 and OBE 104 increased the genetic expression of PPAR-γ in adipocytes. It was also observed a significant reduction of leptin and Glut4 mRNA levels when we treated these cells with the extracts. However, the expression of C/EBPα, FABP4 and adiponectin genes was regulated differently by OBE 100 and OBE 104. OBE 100 increased the expression of these genes and OBE 104 decreased their expression, this effect was significantly observed when we treated the adipocytes with a high concentration of the extract. Both fractions reduced mRNA expression of pro-inflammatory cytokine IL-6 in human activated macrophages, with no modification of the expression of MCP-1and IL-1β genes.

Conclusions: This study demonstrates that the treatment of differentiated human adipocytes with triterpenes-enriched fractions of *Eucalyptus tereticornis* reduces their fatty acid content. These plant extracts also alter the expression of genes involved in adipocyte differentiation and macrophage inflammatory response. We have confirmed in a human cellular model the effects of *Eucalyptus tereticornis* observed previously in mice models.

Acknowledgements: This research was supported by the Colciencias grant 111565740656

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PP2_25

ACTIVITY AGAINST THE FUNGUS *COLLETOTRICHUM LINDEMUTHIANUM* OF PHASEOLLIN ALONE OR IN COMBINATION WITH STRUCTURALLY RELATED COMPOUNDS, PHENYLPROPANOIDS AND AROMATIC MONOTERPENES

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The common bean (*Phaseolus vulgaris* L.) is the most important edible legume in the world; it represents 50% of the grain legumes consumed worldwide. Unfortunately, this crop is seriously affected by numerous fungal pathogens that can cause severe yield losses. In Colombia, the most widespread and destructive disease in common bean is the anthracnose (caused by *Colletotrichum lindemuthianum* Sacc. & Magnus; Scribner). Traditionally, the disease has been acceptably controlled by the application of conventional synthetic fungicides, some of which can present potential harmful effects on the environment and human health (Tripathi & Dubey, 2004). Nowadays, one of the alternatives that are being intensely explored to control phytopathogenic fungus is based in the natural protective mechanisms of plants, which include the accumulation of low molecular weight antimicrobial compounds called phytoalexins. In common bean, the main phytoalexin correspond to the pterocarpan phaseollin. In the present work, the inhibitory effect against *C. lindemuthianum* of phaseollin, alone or in combination with genistein, daidzein, chromone, 4-thiochromanone, 4-chromanone, homopterocarpin, eudesmin, carvacrol, thymol, eugenol, and anethole was evaluated.

The fungus was isolated from diseased *P. vulgaris* pods and characterized by morphological analysis. The toxicity of compounds against *C. lindemuthianum* was evaluated through the poisoned food technique (Grover & Moore, 1962). Evaluations were made in sterile Petri dishes of 70 mm diameter to which was added 7 mL of potato dextrose agar (PDA). The compounds were added to a concentration of 200 µg/mL; ethanol was used as solvent (< 2 µL/L). Petri dishes with and without ethanol were used as controls. The Petri dishes were incubated at room temperature and mycelial growth was measured each 24 h (during 96 h). The relative growth inhibition of the treatments compared to the controls was calculated as percentage, using the formula: Inhibition (%) = {1–[radial growth of treatment (mm)/radial growth of control (mm)]}x100. Additionally, fungitoxicity of combinations of thymol, carvacrol, homopterocarpin, eudesmin, anethole, eugenol, 4-chromanone, chromone, 4-thiochromanone with phaseollin, in relation 5.5:1.5, 3.5:3.5, and 1.5:3.5, v/v) was evaluated.

The results show that eudesmin, carvacrol, and 4-thiochromanone exhibited the highest fungistatic effect (>97%). Phaseollin displayed a relatively high inhibitory effect against the fungus (between 87 and 69%). However, the inhibition was increased (additive interaction) by combining the phytoalexin with eugenol, carvacrol and eudesmin. This effect was dependent on specific structural requirements in the compounds evaluated. On the other hand, a subtractive interaction was exhibited for mixtures of phaseollin and anethole, thymol, 4-thiochromanone, 4-chromanone, genistein, daidzein, and chromone. The results presented can provide useful information for the development of antifungal agents more selective and friendly to the environment, to control bean anthracnose.

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PP2_26

CORDIA CURASSAVICA: PRIMARY SOURCE FOR DISCOVERING A NATURAL MEDICINE FOR DENGUE TREATMENT

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Dengue poses a substantial economic and disease burden in endemic countries around the world. Severe dengue has significant hospital costs that increase during epidemics. There is currently no specific antiviral therapy available for dengue treatment. Herbal therapy for reduction of virus load and the production of inflammatory mediators may be beneficial in attenuating severity of dengue. Colombia has a rich herbal repertoire and the Vademecum on Medicinal Plants prescribes medicines for a variety of diseases, but dengue as such is not included.

The current work was carried out in order to identify anti-dengue activities in essential oils (EOs) from plants grown in Colombia.

Nine plants species were selected. EOs extraction was carried out by using m*icrowave-assisted hydrodistillation.* A systematic bioassay-guided screening approach was used to identify samples with relevant inhibitory effects on virus infection and production of inflammatory mediators. An EO, which showed an IC_{50} value ≤ 50 µg/ml, SI (selectivity index) ≥ 4.0 and reduced inflammatory mediators by more than 25%, was designated to be active as an inhibitor of dengue virus (DENV) cellular infection.

An MTT assay showed that EOs obtained from *Cordia curassavica*, *Baccharis decussata*, *Lippia origanoides*, *Piper holtonii*, *Psidium sartorianum*, *Calycolpus moritzianus*, *Wedelia calycina* and *Zornia brasiliensis* had no discernible cytotoxicity (CC_{50} > 80 µg/ml) on four cell lines derived from human (HepG-2 and HEK-293) and animal (Vero, and B16F10) organs. EO from *Tagetes caracasana* resulted toxic (CC_{50} between 60 and 26 µg/ml) on animal cells. All EOs (30 µg/mL) were screened for antiviral activities in DENV-1-infected Vero-cells by using an area-fraction output method with ImageJ freeware. Only *Cordia curassavica* reduced (46.6%) cellular death caused by virus infection, and inhibited DENV-2 (IC_{50}: 38.2 µg/ml; SI: 5), DENV-4 (IC_{50}: 10.0 µg/ml; SI: 19) and DENV-1 (IC_{50}: 69.7 µg/ml; SI: 2.7) in a virus protein (NS1) reduction assay. A reduction in the production of prostaglandin E2 (42.4%) and the IL-6 cytokine (37.6%), but not (< 20%) in inflammatory mediators such us NO, TNF-α and IL-1β, was observed in murine macrophage cells (Raw 264.7) treated with *C. cursavvica*.

Analysis of biological properties of indigenous species is an option for identifying starting material for discovering both herbal medicines and synthetic antivirals for dengue treatment. This study has resulted in the identification of *Cordia curassavica* EO, which presents inhibitory effect on cell infection caused by DENV serotypes. The EO could be selected for protective efficacy studies in an animal model in order to confirm antiviral, immunomodulation and toxicity activities.

Acknowledgments: This research was carried out thanks to financial support from the Colombian Institute of Science and Technology (Colciencias), Grant RC-245-2011 (Patrimonio Autónomo del Fondo Nacional de Financiamiento para la Ciencia, Tecnología e Innovación, Francisco José de Caldas).

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PP2_27

ESSENTIAL OILS AND THEIR COMMON METABOLITE B-CARYOPHYLLENE AS PRIMARY SOURCE FOR DISCOVERING AN ANTIVIRAL AGENT AGAINST DENGUE VIRUSES

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Dengue Virus (DENV) causes millions of infections in tropical countries around the world. There is currently no specific antiviral therapy available for dengue treatment. Analysis of biological properties of products derived from medicinal plants is an option for identifying starting samples for discovering of herbal medicines and synthetic antivirals. Colombia has a rich herbal repertoire, but knowledge about anti-DENV activities is limited.

The current work was undertaken to investigate anti-DENV activities of essential oils (EOs) from plants commonly used in traditional folk medicine in Colombia, and to assess further the anti-DENV activity of their most common metabolite.

Seven plants species were selected for EOs extraction by using microwave-assisted hydrodistillation. Chromatographic (GC/FDI, GC/MS) and statistical analyses were applied for identification of EO metabolites. A systematic bioassay-guided screening approach in human liver (HepG-2) and animal kidney (Vero) cells was followed for screening of antiviral activities by using an area-fraction output method (with ImageJ freeware), and virus-antigen reduction tests.

EOs from *Cordia curassavica*, *Piper marginatum, Piper medium, Baccharis decussata*, *Lippia origanoides*, *Hyptis brachiate*, and *Zornia brasiliensis* had no discernible cytotoxicity on four cell lines from human and animal organs (CC_{50} > 80 µg/ml for all cells) in a MTT assay. Only EOs from *Cordia curassavica* and *Piper marginatum* reduced (46.3 – 60.2%) cellular death caused by virus infection, and reduced cellular infection of all four DENV serotypes (IC_{50} between 10.0 and 86.5 µg/mL; SI between 30 and 1.6) in virus-antigen reduction assays. Twenty-one EOs constituents were identified, and *β-caryophyllene was* the only metabolite common to all oils. *β-Caryophyllene* was active against all four DENV serotypes (IC_{50} between 8.4 and 15.6 µM with SI between 22.2 and 41.3), and time-of-drug-addition assays revealed that it inhibited at very early stages of virus-infection (0 – 4 h after virus adsorption).

Products derived from plants used in local traditional folk medicine are source of compounds for discovering of medicine drugs. This study identified *EOs and their metabolite β-caryophyllene with promising anti-DENV activities, which* could be used as starting points for consideration in designing effective herbal medicines and/or synthetic antivirals for dengue treatment. Studies on protective efficacy in an animal model are necessary in order to confirm antiviral and toxicity activities.

Acknowledgments: this research was carried out thanks to financial support from the Colombian Institute of Science and Technology (Colciencias), Grant RC-245-2011 (Patrimonio Autónomo del Fondo Nacional de Financiamiento para la Ciencia, Tecnología e Innovación, Francisco José de Caldas).

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PP2_28

PHARMACOLOGICAL INVESTIGATIONS ON *KIGELIA AFRICANA* (LAM) BENTH., FRUITS EXTRACT

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Kigelia africana (Lam) Benth., syn. *K. pinnata* (Bignoniaceae) is abundant in the tropics and is widely used in Southern, Central and West Africa as a herbal remedy for various ailments such as malaria, eczema, rheumatism. The fruits in particular has a wide reputation in folk medicine for the treatment of infertility, sexual impotence, postpartum hemorrhages, but there is no scientific evidence that *Kigelia* is effective in these case [1, 2, 3]. Chemical constituents of *K. africana* include some naftochinons, iridoids, phytosterols, isocumarin, lignan, flavonoids, phenolic acids, alkaloids, saponins and tannins [4, 5, 6, 7]. Pharmacological studies of different *Kigelia* extracts have shown antibacterial activity of the fruits [8] and antiossidant activity of the fruit and leaves [9]. In the present study the *Kigelia* hydroethanolic fruits extract was studied for its possible uterotonic activity, on spontaneous and agonists-induced contractions in isolated non-pregnant human myometrium in order to validate its traditional use in the treatment of post-partum bleeding.

Authenticated fruits of *K. Africana,* collected in Limakole and Siby villages (Mali), were extracted in Soxhlet with ethanol 60%. Myometrial strips, taken during the follicular stage of the menstrual cycle, were obtained from hysterectomy specimens of premenopausal women. The longitudinal muscle strips of 1.5 cm were dissected and mounted vertically in a 20 ml tissue baths under physiological conditions to record their isometric contractions by a force transducer. The responses of cumulative concentrations of plant extract on spontaneous contractions in the presence and absence of α-adrenoceptor blocker (prazosin), muscarinic receptor blocker (atropine) or cyclooxygenase inhibitor (indomethacin), and on agonist-induced contractions, were investigated. The effects of extract on myometrium may be compared with those provoked by PGE_2, used as positive control, in the same experimental conditions.

Kigelia extract increased, in a concentration-dependent manner, muscular basic tonus and amplitude of spontaneous uterine contractions at concentrations ranging from 50-400 µg/ml. Pretreatment with prazosin, atropine or indomethacin did not affect the uterine responses to *Kigelia* extract. Furthermore, *Kigelia* extract increased the contractile response induced by vasopressin and acetylcholine. These investigations indicating that plant extract exert uterotonic effects of muscolotropic type on human myometrial muscles and his justifies the traditional use of this plant in the treatment of post-partum haemorrhage.

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PP2_29

ANTILEISHMANIAL ACTIVITY AND *IN VIVO* TOXICITY OF FOUR CHEMOTYPES OF *LIPPIA* (FAM. VERBENACEAE) ESSENTIAL OILS

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Essential oils (EOs) from plants distributed in Colombia and belonging to the genus *Lippia* are interesting candidates for cutaneous leishmaniasis pharmaceutic systems. The EO-chemotypes of *Lippia alba* and *L. origanoides* characterized by their major compounds namely citral (EO1), thymol (EO2), carvacrol (EO3), and phellandrene (EO4) were tested against *Leishmania* and their toxicology profile was determined in BALB/c mice.

Essential oils were obtained by microwave radiation-assisted hydrodistillation and characterized by gas chromatography coupled to mass spectrometry [1]. Activities against *Leishmania (Viannia) panamensis, L. (V.) braziliensis* and cell toxicities on THP-1 cells were determined by standard methodologies and IC_{50} and CC_{50} were calculated [2]. Skin irritation was studied after topical treatment with 10, 50 and 100% of EO (w/w in olive oil) and acute toxicity after oral administration of 2000 mg/kg. The contact hypersensitivity was determined by pre-treatment/challenger with EOs and the EO-induced genotoxicity after oral administration of 100 mg/kg/14 days by comet and micronucleolus (MN) assay. Cyclophosphamide and ethyl methanesulfonate were used as controls [3].

All EOs were active against *Leishmania* promastigotes (IC_{50} 0.39-11.18 µg/mL) and EO1 and EO3 were active against *L. (V.) panamensis* (IC_{50} 42.66 and 63.87µg/mL) and *L. (V.) braziliensis* (IC_{50} 9.19 and 15.43 µg/mL) amastigotes. The EO-cell toxicities on THP-1 cells were in the range of CC_{50} 26.27-52.55 µg/mL.

EO2 and EO3 used at 100% (pure) induced oedema, severe erythema and crusting with histopathology features of hyperkeratosis, parakeratosis, spongiosis, and exocytosis of inflammatory cells. No irritation signs were observed using lower EO-concentration. No changes of weight, food intake and serum levels of urea (34.16 to 70.0 mg/dL) and AST/GOT (69.55 to 80.10 U/L) were observed after oral treatment. Increased levels of alkaline phosphatase were observed after EO2 and EO3 treatment (82.32 and 559.80 U/L versus 23.95-55.46 U/L) suggesting a possible transient-liver damage. Compared with the positive control (1-fluoro-2,4-dinitrobenzene), neither increase in ears thickness (0.15 versus 1.0 mm) nor histological inflammatory changes were induced in EOs-sensitized animals after EOs-challenger. No DNA damage was observed after treatment with serial doses of EOs. The % of DNA damage was 22-28.75% (Control: 74.38%) and for MN was 0.5-3.17 (Control: 175-200 MN) in polychromatic erythrocytes.

All EOs chemotypes showed *in vitro* anti-parasitic activity. At the higher concentration used of thymol (EO2) and carvacrol (EO3) chemotypes, they showed irritation and some signs of acute toxicity. Lower EO-concentrations than the tested in this study could be used safety in case of pharmacological formulations.

Acknowledgement: This study was conducted thanks to the "Patrimonio Autónomo, Fondo Nacional de Financiamiento para la Ciencia, Francisco Jose de Caldas," contract RC-0572-2012- Bio-Red-CENIVAM and to the Universidad Industrial de Santander.

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PP2_30

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *LEPIDIUM SATIVUM* EXTRACTS

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Nowadays, the use of officinal plants is still at top of medical practices in many countries, it is estimated that 80% of the world population use a wide variety of herbs and regional plants to treat illnesses with a great sureness of their remedial effectiveness (Sandhya et al., 2006). Therefore, this study aims to evaluate the antioxidant and antimicrobial activities of *Lepidium sativum,* a worldwide known species and largely utilized in Mediterranean folks medicine.

Lepidium sativum seeds were rinsed, dried and stocked in a room temperature conditions. The aqueous extract of *Lepidium sativum* seeds was obtained following the procedure described by Divekar et al. (2010), and the methanolic extract was got following the procedure of Markham (1982). The phenolic content of the extracts was measured by the Folin-Ciocalteu method (Li et al., 2007), the Flavonoid content was measured following the $AlCl_3$ method (Bahorun et al., 1996); for the antioxidant activity the DPPH test was carried out as described by Rapisarda et al. (1999), and for the antimicrobial activity the disc-diffusion test was performed as described by Zaidan et al. (2005).

The phytochemical study shows that the MeOH extract of *Lepidium sativum* seeds got better yield and was richer with flavonoids, whereas the aqueous extract had better content of total phenolic compounds, which claim the therapeutic importance of the plant and its role as a source of bioactive compounds. Moreover, the results of the DPPH assay show that the aqueous extract of *Lepidium sativum* seeds had a better scavenger effect than the MeOH extract, but both extract got weak antioxidant activity regarding the standards, which may be due to the weak content of the active compounds within the extracts. Furthermore, the results of the disc diffusion test reveal that both extracts have no antibacterial effect against the tested strains. However, both extracts of *Lepidium sativum* seeds showed a good antifungal activity against *Aspergillus flavus* and *Aspergillus niger*, and this may be due to the properties of polyphenols found in the extracts of the seeds, notably the aqueous extract.

Results from the present study encourage for more research on the nature of the compounds found in the seeds of *Lepidium sativum* as well as the toxicological aspect of the plant.

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PP2_31

QUANTITATIVE HPLC- UV/DAD ANALYZES OF THE MAIN C-GLYCOSIDE FLAVONOIDS IN THREE DIFFERENT METHANOLIC EXTRACTS OF *ALTERNANTHERA TENELLA* COLLA AERIAL PARTS

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Alternanthera tenella Colla, belonging to the Amaranthaceae family, is a bush widely distributed in Latin America, and used in Brazilian traditional medicine for the treatment of infections and inflammatory diseases (Ferreira et al., 2003; Salvador et al., 2004). Several methods of extraction can be employed to obtain plant extracts and their quality depends on the procedure used. This work compares three different extracting methods: ultrasound, packed-bed reactor and maceration in order to prepare *A. tenella* Colla standardized extracts. The C-glycoside flavonoids (2"-O-α-L-rhamnopyranosylvitexin and vitexin) were analyzed by means of high-performance liquid chromatography coupled with diode array detection (HPLC –UV/DAD).

Alternanthera tenella Colla was collected in Ribeirão Preto (SP, Brazil) and a voucher specimen was kept in the FFCLRP/USP Herbarium (reference SPFR 02968). The aerial part of the plant was powdered, air-dried and submitted to a packed-bed reactor (PR) and ultrasound-assisted (US) 40 kHz, for 30 and 60 min., 30°C and maceration (MAC) for 720 and 1440 min., 30°C. Methanol was used as extracting solvents (1:20, m/v). The HPLC-UV-DAD analysis were carried out in a LC-20-AT Shimadzu Prominence Liquid Chromatograph using a C-18 (Kinetix - Phenomenex) column (5 µm, 150 x 4.6 mm id) with a guard column C 18 (Phenomenex, 5µm, 2.1 x 4.6 mm id). The mobile phase was composed of distilled water: formic acid (99:1, v/v) and acetonitrile:THF (tetrahydrofuran) (95:5, v/v). The separation was achieved by using a gradient elution as follows: 0-15 min from 15% to 22% B; 15-22 min from 22% to 20% B; 22- 25 min from 20% to 40% B; 25 – 35 min from 40% to 15% B; which was held for 5 min. The flow-rate was 0.65 mL/min, injection volume was 20µL, at 25 °C. Chromatograms were acquired at 350 nm. The validation of the HPLC-UV/DAD method was performed in agreement with the international guidelines for analytical techniques for the quality control of pharmaceuticals (ICH guidelines). The standards used were previously isolated in out laboratory from this same plant family.

The HPLC-UV/DAD method developed linearity was (r^2 > 0.99) for the reference standards: 2"-O-α-L-rhamnopyranosylvitexin, and vitexin. The limit of detection: 0.156 µg/ml, limit of quantification: 0.625 µg/ml, repeatability <2.0%, accuracy 94-102%. This method was applied to quantify the standards. The concentration of 2"-O-α-L-rhamnopyranosylvitexin, identified in the *A. tenella* metanolic extract found in µg/g was: US 30 min: 17.36±0.06; US 60 min: 21.22±0.12; PR 30 min: 25.36±0.07; PR 60 min: 23.81±0.36; Mac 720 min: 27.50±0.19 and MAC 1440 min: 36.80±0.05.The concentration of vitexin was (µg/g): US 30: 3.88±0.01; US 60: 4.48±0.03; PR 30: 4.70±0.01; PR 60: 4.07±0.15); Mac 720: 5.75±0.05 and MAC 1440: 6.73±0.03. De Santana Aquino *et al.* (2015) showed that 2″-O-α-L-rhamnopyranosylvitexin, significantly inhibited paw edema and reduced both leukocyte migration and the leakage of protein into the pleural cavity. The maceration extraction presented the highest amount of flavonoids content, however the packed-bed reactor extraction time was lower and showed around 50-90% of the flavonoid quantity found in maceration extracts, as well as exhibited higher content of the C-glycoside flavonoids in comparation with the amount found in the extracts obtained from the ultrasound technical. The packed-bed reactor and ultrasound-assisted methods employed in this study improved the extraction process of *A. tenella*, showed a good yield of mass transfer in a shorter time, when compared to traditional maceration method.

Acknowledgements: to CAPES, CNPq, FAPEAM, FAPESP and FAEPEX-UNICAMP

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PP2_32

SPECTROSCOPY CHARACTERIZATION OF (*E*)-2-(2,6-DICHLOROBENZYLIDENE)-1,1-DIPHENYLHYDRAZINE

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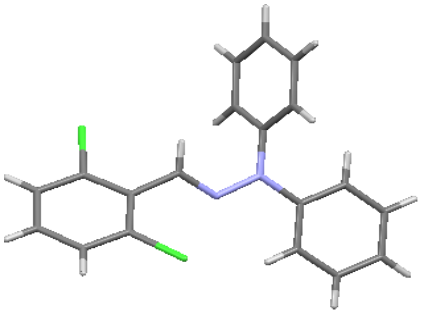
The hydrazones are an important group which has a biological activity. Hydrazones are very important group of analytical regents for the determination of various metal ions by using various analytical techniques. Besides this use of hydrazones are also having biological activities also. Lakshmi et al discussed about the chemical nature of hydrazones and their biological activities and focused on the analytical applications spectrophotometric and spectrofluorimetric of hydrazones.[1]

Moreover Hydrazones derivatives of pyridine are versatile class of an important group of organic compounds that are used as bactericides, fungicides, anticancer agents.[2]

Light green crystals; yield: 74% at 25°C, mp. 100-102°C. UV λ_{max}=349nm. FT. IR (film): (cm-1): 3134 (C-H), 1465, (C=N), 1300 (C=N), 1231 (arC-H ip), 881 C-Cl) 746 (τ -CH_2. ^{1}H NMR (300 MHz, $(CD_3)_2CO$: (ppm, J/Hz): 7.46 (m,4H,C2', 2H,C4'), 7.38 (s,1H,C=N), 7.29 (s,1H,C3), 7.26(m,6H,C4,5,3'). ^{13}C NMR (300 MHz, $(CD_3)_2CO$): (ppm): 143.43 (C1', C=N), 134.12 (C2), 131.70 (C1), 130.55 (C5), 130.21 (C3'), 129.44 (C4), 129.37 (C3), 125.31 (C4'), 122.57 (C2'). MS-EI: m/z = 341.24 M^+. $C_{19}H_{14}Cl_2N_2$

Diphenylhydrazine (1.42 mmol, 263.01 mg) was dissolved in a solvent, a chemical equivalent (1.42 mmol, 250 mg) of 2,6-dichlorobenzaldehyde which was previously dissolved in the same solvent and it was added drop by drop stirring constantly and which was developed with greener procedure. The action mixture was kept at room temperature and was monitored by TLC, and then vacuum filtered. The hydrazones were recrystallized by a continuous and controlled process until wine crystals with adequate size and purity were developed in order to obtain X-ray studies.



Our work group has been synthetizing many interesting structures of hydrazones that have been characterized by m. p., U.V, I.R. ^{1}H NMR, ^{13}C NMR.[3] (*E*)-2-(2,6-dichlorobenzylidene)-1,1-diphenylhydrazine.[4]

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PP2_33

IN VITRO AND *IN VIVO* ANTILEISHMANIAL ACTIVITY OF *ARTEMISIA ANNUA* LEAVES POWDER AND POTENTIAL UTILITY IN THE TREATMENT OF UNCOMPLICATED CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a tropical disease that affect about 12 million people around world. Although still effective, the currently used drugs to treat this disease have serious side effects and therefore, alternatives drugs are urgently needed. Because 80% of the world population use traditional medicine and plant derived materials, the World Health Organization has prioritized the validation of traditional medicine products. Recently, a mixture of *Artemisia annua* powder capsules was developed as a new galenic formulation of dried herb powder ("*totum*") for the oral treatment of malaria and other parasitosis.

The antileishmanial activity and cytotoxicity of *A. annua* powder capsules was determined by *in vitro* and *in vivo* studies. A preliminar evaluation of the therapeutical potential as antileishmanial treatment in humans was done in two patients with uncomplicated cutaneous leishmaniasis (CL).

The *A. annua* powder capsules showed moderate in vitro activity against amastigotes of *L. panamensis* and no cytotoxicity in U-937 macrophages nor genotoxicity in human lymphocytes was observed. Five of 6 hamsters (83.3%) treated with the *A. annua* leaves powder at a dose of 500mg/kg/day during 30 days cured at the end of the study. Indeed, two patients with uncomplicated CL caused by *L. panamensis* treated with capsules of *A. annua* leaves powder at 30 g total showed complete cure after 45 days of treatment ended without adverse reactions. Both patients remain cured at 22 and 20 months after have finished the treatment, respectively.

In conclusion, *A. annua* powder capsules may represent an option for treating uncomplicated CL and the evaluation of the efficacy and safety of this herb product in CL by randomized controlled trials are necessary.

Acknowledgments: Authors thank the patients for agreeing to participate in the study. This work was supported by COLCIENCIAS (CT-695-2014) and the University of Antioquia (CIDEPRO-CIIEs-2009-2014).

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ANTILEPTOSPIRAL POTENTIAL OF MEDICINAL PLANTS COMMONLY USED FOR THE TREATMENT OF INFECTIOUS DISEASES

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Leptospirosis is a zoonosis caused by *Leptospira* spp, which is transmitted to humans by direct contact with the urine of wild or domestic animals infected with the bacteria. The disease occurs worldwide but is most often in places where sanitary conditions are poor, having an impact on human health, livestock and agriculture. This disease can represent up to 20% of febrile diseases of unknown origin, it has an endemic overall average annual incidence of 5 per 100,000 inhabitants, about 17% of people hospitalized with leptospirosis suffer acute lung injury and of these 25% die; in addition, in the case of the Americas the incidence is 12.5 per 100,000 population, and in Colombia of 3.05 cases per 100,000 inhabitants. Additionally, there is no differential diagnosis of leptospirosis which has led to failures in controlling the disease and treatment decisions favoring the bacteria resistance to antimicrobial as trimethoprim/sulfa, neomycin, and fluoroquinolones. That is why it requires low-cost treatment options, less toxic and more effective to control the disease; in this sense, a novel alternative and little studied for treatment of leptospirosis is the use of natural antibiotics. Therefore, the objective of this study was to identify which of the medicinal plants commonly used to treat infections in Colombia, can be used to control leptospirosis. For this it 11 plants were selected, with the greatest consensus of use for the treatment of infectious diseases, which were extracted by cold maceration with 96% ethanol and its antimicrobial effect by the microdilution (MDT) was evaluated against *Leptospira* spp serovar Canicola and Hardjo it was used as revealer viability of Alamar blue. As a result of study identified that *Parietaria officinalis*, *Achyrocline bogotensis*, *Equisetum giganteum* and *Pulmonaria officinalis* presented antileptospiral potential to 30mg/mL against the two serovars of *Leptospira* spp while *Polypodium calaguala* only showed activity against Hardjo and *Phytolacca bogotensi* against Canicola. This type of study contributes to knowledge about the susceptibility of strains *Leptospira* spp against natural antimicrobials, allowing evaluate the use of medicinal plants with potential for control of Leptospirosis. Well as studies aimed at obtaining bio-active principle for the prevention and control of this zoonosis.

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PP2_35

HYPOGLYCEMIC EFFECT OF ETHANOLIC EXTRACT OF *PARMENTIERA ACULEATA* (KUNTH) SEEM FRUIT AND BARK IN MALE WISTAR RATS

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Diabetes Mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances in carbohydrates, fats and proteins metabolism associated with absolute or relative deficiency in insulin secretion and/or their action. It is estimated that 366 million people had DM in 2011 and by 2030 this would have risen to 552 million (Olokoba *et al*, 2012). In this sense, many plants used in traditional medicine such as *Cissus sicyoides*, *Argemone mexicana*, *Juglans regia* L. are using to treat this syndrome. In this sense, *Parmentiera aculeata* (Kunth) is a tree commonly known in Mexico as "cuajilote", it belongs to the family of Bignoniaceae. Fruit is used for the treatment of several illnesses in the Mexican folklore like diabetes, headache, gallstones, deafness and diarrhea. From bark and fruit have been isolated several secondary metabolites such as β-sitosterol and stigmasterol regarded as hypoglycemic. Although in some places of México is usually used for the treatment of diabetes (Andrade-Cetto and Heinrich, 2005; Juárez-Vázquez *et al*., 2013), there are not reports about the biological activity of the bark. For these reasons, in the present work we decided to evaluate the ethanolic extract (EtOH-E) from bark and fruit of *Parmentiera aculeata* using an oral glucose tolerance (OGTT) in order to corroborate its ethnomedical use.

Bark and fruit were collected in Misantla, Veracruz State, Mexico. Plant material were cut, dried and extracted by exhaustive sequential maceration (hexane, chloroform and ethanol). Phytochemical analysis of extracts was undertaken using standard qualitative methods. Glucose tolerance test was performed in 32 male Wistar rats (weighing 250-300 g). In each group eight randomly selected animals were used. The dose of 200 mg/kg body weight of EtOH-E bark and EtOH-E fruit was given p.o. in volume 2 mL/Kg. Glibenclamide (Gli, 20 mg/Kg) was the pharmacological control and water with tween 80 (5 %) and polyethylene glycol (5 %) as vehicle (Veh). All treatments were dissolved in vehicle solution. Animals were 12 h overnight fasted and *ad lib* access to water only. At morning, a first blood sample by tail bleeding was taken at 0 min (t = 0). Immediately the corresponding treatments were administered. After 30 minutes an oral dose of glucose was given (50% w/v, 5 mL/Kg). Blood samples were taken at 0.5, 1, 2, 3, 4 and 24 h after glucose loading, and blood glucose level (BGL) were measured.

Phytochemical screening revealed the presence of phenolic compounds (flavonoids and coumarins) and sterols in both extracts; terpenoids only were detected in the extract of bark and quinones only in fruit. No were detected presences of alkaloids in both extracts. Treatment with ethanolic extract of bark significantly decreased the BGL like Gli; however, no significant differences were found when ethanolic extract of fruit was used with respect to the vehicle group ($F = 11.375$, $p \leq 0.0001$). Statistical analysis revealed significant differences on interaction time per treatment ($F = 2.227$, $p \leq 0.010$). The BGL of all groups prior to treatment administration (0 hour) showed no apparent difference compared to each other. All groups, however, showed increase in BGL 30 min following glucose loading (one hour after oral treatment administration), confirming the induction of hyperglycemia. EtOH-E bark statistically decreased the BGL one hour after the glucose loading, and hypoglycemic effect was sustained up to 4 hours. However, the hyperglycemia with glucose challenge was not significantly brought down with EtOH-E fruit. These results are important considering that traditional medicine is often used mainly the fruit and leaves of this plant as an infusion for the treatment of diabetes.

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DEVELOPMENT OF REPELLENT WITH ESSENTIAL OILS FROM MEDICINAL PLANTS

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Essential oils extracted from several natural resources are usually used as effective repelent against mosquitoes that could be a vector for tropical diseases such as: malaria, dengue, chikungunya and zika. Those natural products constitute an organic alternative to synthetic compounds comercially available for this purpose, since they are generally less friendly to the environment and in recent years have generated controversy due to increased resistance developed by mosquitoes. This tropical diseases such have a high mortality rate in some countries, so the use of repellents is a point of vital importance for their control. In this work, chemical composition of sixteen essential oils from plants cultivated in Mexico were analyzed in order to develop an effective repellent against mosquito based on statistical analysis by PCA (Principal Component Analysis). Species studied were: Tea tree (*Melaleuca alternifolia*), orange (*Citrus aurantium*), cinnamon (*Cinnamomum* spp.), Citronella (*Cymbopogon* spp.), Cloves (*Eugenia caryophyllata*), eucalyptus (*Eucalyptus* spp.), "Hierba del burro" (*Hyptis mutabilis*), lavender (*Lavender angustifolia* and *Lavender* spp.), lime (*Citrus aurantifolia*), lemon (*Citrus medica*), mint (*Menta arvensis* and *Menta piperita*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*) and verbena (*Verbena officinalis*).

Analysis of essential oils were carried out in a gas chromatograph Agilent Technologies ® model 6890M, using a DB-5 column phenylmethylpolysiloxane, 5%, with a length of 60 m, 0.25 mm id, 0.25µm film thickness, this equipment was coupled to a mass spectrometer. Individual identification of components was performed by comparison with the mass spectra of the database version 2.0 NIST05 MS. Samples were injected in triplicate and the results were expressed as mean ± S.E.M of the area under the curve of each component. Information obtained from the GC-MS analysis was ordered and cataloged in a matrix that allowed statistical analysis by the PCA method with Statistica 2.1 program (variation coeficient 0.95, 0.7 and 0.4.) in order to select the essential oils with the best features of repellency.

Seventy five different compounds were determined in analyzed essential oils and forty-two of them have reports about repellent activity. D-limonene, α-pinene, β-pinene, caryophyllene, eucaliptol and myrcene were the most common terpenes present in samples. The analysis of variables in this study using PCA allowed to define the components that explain most of the observed variability. The distribution of data for the analysis was carried out in 3 stages, taking into account the following factors: repellant properties, presence in the largest number of samples and the interaction between these factors, using the coefficients of variation obtained by PCA model. This scheme of analysis resulted in two possible combinations of repellent mixtures based on the similarity of its components, each of which brings together 6 essential oils. The combination A and B have in common citronella oils, orange blossom, lime, eucalyptus and verbena but differ in one component, while A has lavender essential oil B contains Peppermint. From the results obtained we proceeded to the preparation of two repellents solutions which sensory acceptance was assessed (odor) using a hedonic scale of 5 points being the solution A the most accepted without differences by age group or gender. We are currently working on the development of a standardized formulation that allows the evaluation of the repellent properties, stability and viability.

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PP2_37

EFFECT OF COMBINATION OF CHIA (*SALVIA HISPANICA*) AND SPIRULINA (*ARTHROSPIRA MAXIMA*) ON DIABETES

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Type 2 diabetes (T2D) is a progressive metabolic disorder with diverse pathological manifestations associated with abnormal lipid and carbohydrate metabolism. T2D is a public health problem at worldwide level and as a result of its high prevalence, there are high costs and high rates of morbidity and mortality. Considering the limitations of current treatments, it is crucial to identify new therapies whith low undesirable effects and higher efficacy than conventional therapies. Natural products are a valid alternative. In the present study, two natural products, namely chia (Salvia hispanica) and spirulina (Arthrospira maxima), were evaluated for their effect on glucose concentration and lipid profile in diabetic mice.

Diabetes was induced by a unique alloxan injection. On the 3th day (72 h later), mice with blood glucose concentration above 200 mg/dl were used. Animals were separated into five groups: a) no diabetic, b) diabetic, c) diabetic-chia, d) diabetic-spirulina e) diabetic-combination. Glucose concentration was measured weekly. After 28 days of treatment, blood samples were taken from the retro-orbital sinus and centrifuged at 3,000 g for 15 min to isolate serum for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) determination.

All mice had free access to food and water and were kept on a 12 h light, 12 h dark cycle. Animal care and experimental procedures were carried out according to the official mexican norm NOM-062-ZOO-1999 entitled "Technical specifications for the production, care and use of laboratory animals".

After 28 days, the diabetic state (D) significantly modified glucose concentration and some serum lipid profile parameters compared to the negative control (ND) group of mice. In particular, total cholesterol increased, whereas triglycerides and HDL cholesterol decreased. Addition of chia to the drinking water (DCH) significantly decreased glucose concentration, increased triglycerides and HDL levels and decreased the amount of total cholesterol compared to untreated diabetic animals (D). Similarly, administration of the spirulin (DSP) significantly decreased glucose concentration, increased HDL levels and decreased total cholesterol compared to diabetic mice (D). No significant changes were oberved on triglycerides after spirulin supplementation. Interestingly, combined administration of spirulina-chia to diabetic mice (DCHSP) produced no significant changes on glucose, triglycerides, total cholesterol or HDL.

Results indicate that the combination of these two natural products do not exert the same therapeutic effect of chia and spirulina when they are administered alone, thus suggesting that these compouns may compete with each other.

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ORAL ANTI-INFLAMMATORY ACTIVITY OF DICHLOROMETHANE FRACTION OF *LACISTEMA PUBESCENS* MART. LEAVES

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Lacistema pubescens Mart. (Lacistemataceae) is a plant species originally from Brazil and widely distributed in Latin America. The leaves are traditionally used to treat several diseases, including rheumatism, body aches and fever (Roumy et al., 2007). Preliminary studies performed by our group showed a marked anti-inflammatory potential of the dichloromethane fraction (DF) of the leaves obtained from the methanol crude extract, which encouraged further *in vivo* evaluation of its oral anti-inflammatory activity.

The croton oil-induced ear edema test was performed as described by Schiantarelli et al. (1982). Mice were orally treated with DF (100 and 300 mg/kg), indomethacin (10 mg/kg), used as positive control, and vehicle (12% Tween in saline). Next, 20 µL of croton oil (2.5% in acetone) were applied on the inner surface of the right ear of each mouse. The left ear was treated only with acetone. The animals were euthanized 6 hours later, and a fragment of 6 mm diameter was removed from both ears using a metallic punch. The weight difference between the fragments indicated the edema (inflammation reaction). The data were expressed as mean ± SEM. ANOVA followed by Newman-Keuls test was used for statistical analysis using the Prism 5.0 (GraphPad Software Inc.) statistic program.

DF 100, DF 300 and indomethacin were able to significantly reduce the edema by 54%, 52% and 44%, respectively. Croton oil is an inflammatory agent that induces an intense cutaneous vasodilatation and erythema, followed by the increase of ear thickness as a result of cell extravasation. This inflammatory process involves the activation of protein kinase C, the vascular permeability increasing and the synthesis of arachidonic acid (AA) metabolites, COX-2, IL-1β, TNF-α and adhesion molecules (Saraiva et al., 2010). For this reason, glucocorticoids and drugs capable to interfere with AA metabolism, as COX inhibitors and/or LOX, may efficiently respond to this test (Gábor, 2000). Thus, these findings strongly suggested that DF is endowed with natural compounds with anti-inflammatory potential.

Acknowledgments: This work was supported by the grants from FAPEMIG, CAPES and CNPq.

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PP2_39

WOUND HEALING ACTIVITY OF GELS CONTAINING AN EXTRACT OF *CECROPIA PACHYSTACHYA* LEAVES

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Wound is a rupture in the epithelial integrity of the skin which resulted from violence or trauma and maybe followed by disruption of the structure and function of underlying normal tissue (Attama et al., 2011). Efforts are being made all over the world to discover agents that can promote a good healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications (Mahmud et al., 2010; Malla et al., 2015). So, the aims of this study were to evaluate the *in vivo* wound healing of gels containing extract of *Cecropia pachystachya* (ECP) and to perform the chemical fingerprint of the extract.

The wound healing activity of the gels was evaluated for 21 days, using the excision model in rats. After anesthetized and being placed in the prone position, the animals were shaved and a cylindrical skin fragment was removed from the medium line of the dorsal region with a 10 mm diameter biopsy punch. The depth of the skin wound included the epidermis, derme, hipoderm and muscular layer, so that the *fascia superficialis* was exposed. After the surgery, the skin wounds were topically treated with 200 µL of carbopol gel 1% (control group), ECP 2% or ECP 5%. The animals were treated once a day, during pre-established periods of 3, 7, 14 and 21 days for each subgroup. At the end of each treatment, the animals were euthanized and the elliptical excision encompassing the area of the healing process was removed from each animal. The material was subjected to histological process and hematoxylin and eosin staining. The slides were histometrically analyzed using the software Image Pro Plus 6.0®. To identify the chemical markers, a HPLC analysis was performed. The study was approved by the Brazilian College of Animal Experimentation (COBEA) protocol nº 050/2012.

The group treated with ECP 5% presented edema and inflammatory infiltrate with less intensity than ECP 2% and control groups. In addition, neovascularization was significantly lower in ECP 5% group, indicating that the inflammatory reaction was not prolonged and the process of tissue repair was more advanced in this group. Both ECP 2% and 5% gels showed less neovascularization and cellularity, and better tissue repair when compared to the control, which showed a younger and homogeneous tissue. The present study has demonstrated that the ECP gels promoted the acceleration of the healing process when compared to the control group. Wound contraction, angiogenesis, epithelialization and the collagen deposition support further evaluation of C. pachystachya leaves in the topical treatment and management of skin wounds. The compounds orientin, isoorientin and chlorogenic acid identified in the extract had already been reported with healing activity (Aragão et al., 2010).

Acknowledgments: This work was supported by the grants from FAPEMIG, CAPES and CNPq.

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ANTIOXIDANT ACTIVITY OF *CECROPIA PACHYSTACHYA* TRÉCUL (URTICACEAE) IN NORMAL AND DIABETIC RATS

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Diabetes mellitus is caused by the increase of glycemia. In the long term, hyperglycemia is associated with increased oxidative stress, contributing to the development of diabetic complications (Tiwari and Rao, 2002). Many plant species have been studied since they have a high content of antioxidants (Chun et al., 2005). Thus, this study aimed to evaluate the antioxidant effect of the ethyl acetate extract of *Cecropia pachystachya* Trécul (Urticaceae) (ECP) in normal and diabetic rats.

Wistar rats were divided into 5 groups (n=6): diabetic animals receiving vehicle (negative control), diabetic animals receiving ECP 80 mg/kg, diabetic animals receiving glibenclamide solution 3 mg/kg (positive control), normal animals receiving the vehicle and normal animals receiving ECP 80 mg/kg. Diabetes was induced by streptozotocin (40 mg/kg) intravenously. The treatment occurred daily at 7 h am for a period of six months. At the time of euthanasia, liver was removed for analysis of the antioxidant activity of ECP. The enzymes catalase, superoxide dismutase, and glutathione reductase were evaluated in a 96-well plate. ANOVA followed by Tukey test was used for statistical analysis.

Both normal and diabetic animals treated with ECP presented the levels of the antioxidant enzymes close to normal. For diabetic animals treated with ECP, the levels of catalase, superoxide dismutase, and glutathione reductase were 6.16 ± 0.45; 13.97 ± 0.82 and 7.77 ± 0.62 U/mL, respectively while normal animals showed 5.95 ± 0.89; 11.76 ± 0.40 and 7.64 ± 0.90 U/mL, respectively. For diabetic animals not treated with ECP, the level of enzymes remained much lower (0.28 ± 0.06; 0.57 ± 0.20 e 0.68 ± 0.21 U/mL, respectively). It is important to highlight that glibenclamide, used as positive control, failed to increase the level of enzymes (0.73±0.23; 1.25±0.36; 0.65±0.13 U/mL, respectively). This fact reflected the great stability and integrity of the antioxidant system in animals treated with ECP. Those results can be partially attributed to the presence of the antioxidant compounds chlorogenic acid, isorientin and orientin, previously identified in ECP (Aragão et al., 2010).

Acknowledgments: This work was supported by the grants from FAPEMIG, CAPES and CNPq.

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PP2_41

PREPARATION OF PARVIFOLINE DERIVATIVES AS TUBULIN INHIBITORS

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Nowadays, cancer treatments have many pharmacological targets. One of them is the structural protein tubulin which forms highly organized polymeric structures named microtubules. They are involved in cellular functions as signaling, vesicular transport, migration, and proliferation.[1] Microtubules maintain a dynamic equilibrium among shortening, growth, and stabilization of the length, depending on specific cellular conditions. This phenomenon can be replicated and studied *in vitro*[2] to find new substances with modulatory effect on this protein and its functions. Tubulin interacting agents have a wide structural diversity; although in many of them the presence of aromatic rings and electronegative atoms can be recognized, as in colchicine, which has a benzene moiety fused to a seven-membered ring, as well as seven electronegative atoms. In this work we prepared and studied several derivatives of parvifoline[3] [(–)-(6Z,10R)-5,8,9,10-tetrahydro-3,6,10-trimethyl-2-benzocyclo-octenol] which share some structural features with colchicine and therefore could interact with tubulin inhibiting its polymerization process.

Parvifoline was isolated from the roots of *Acourtia humboltii*. The reactions for the preparation of its derivatives included epoxidations with *m*-chloroperbenzoic acid, esterifications with benzoyl chloride, methylations with dimethyl sulfate, and transpositions with $Et_2O{:}BF_3$. Purifications were carried out using column chromatography and the structure elucidations for new compounds were done by 1D and 2D NMR and mass spectrometry. *In vitro* analyses were performed with tubulin using the protocol described by Shelanski *et al*.,[4] but the wavelength for readings was changed from 340 to 450 nm. Colchicine was employed as the positive control for inhibition of polymerization.[5] Each molecule was modeled in Spartan'04 and optimized using Density Functional Theory at the B3LYP/DGDZVP level in Gaussian 03. The optimized structures were used for docking studies in AutoDock 4.

A series of 14 new parvifoline derivatives were prepared by reaction sequences that comprised combinations of epoxidations, hydroxylations or incorporation of ethers or ketones into the eight-membered ring. In one group of compounds the phenolic hydroxyl group of parvifoline was left unchanged, while in two other collections the hydroxyl group was either esterified or methylated to afford three different sets of compounds. According to the docking analysis, the inhibitory activity of parvifoline and some of its derivatives on tubulin polymerization was in agreement with the hydrophobic interactions found in the colchicine binding site located in α-tubulin, mainly with Phe 296, Tyr 312, Lys 341 and Ile 311. Parvifoline itself also displayed two hydrogen bonds, one of them between the phenolic oxygen atom and the hydrogen atom of Tyr 312 amino group, and the other one between the phenolic hydrogen and Gln 342 amidic oxygen atom. Parvifoline benzoate showed nonpolar interactions with Lys 338, Pro 298, Pro 307 and polar interactions with His 309 and Gln 342, while *O*-methylparvifoline sustained hydrophobic interactions with Pro 298 and Gln 342, as well as a polar contact with Gly 310.

Acknowledgement: This work was supported by Conacyt-Mexico (grant 241053).

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PP2_42

GC/MS ANALYSIS AND BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OIL FROM THE DRIED AND FLESH LEAVES OF *ANNONA CACANS* (ANNONACEAE)

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Several *Annonaceae* species are used in folk medicine and biological properties of essential oils have been reported, including antioxidant, antimicrobial, anti-trypanosoma, larvicidal, anti-inflamatory and anti-tumoral effects. As parts of a research project focusing on phytochemical investigation of Brazilian medicinal plants searching for new bioactivity of natural products, in this study, our aim was to report the chemical analysis of the essential oil from the dried and flesh leaves of *Annona cacans* from São Paulo, Brazil by GC/MS and evaluation of their antimicrobial activity against bacteria, fungus and protozoan.

The essential oil was obtained by hydrodistillation from dried and fresh leaves of *Annona cacans* and analyzed by gas capillary (GC/MS). Antimicrobial and antileishmanial activities were evaluated *in vitro* according to SIQUEIRA *et al*, (2015). Thirty-one compounds were identified from fresh leaves and twenty-seven compounds were identified from dried leaves, representing 60.78% and 57.44% respectively of the total of the oil. The main compounds identified in the essential oil of the flesh leaves were spathulenol (24.02%), β-(E)-ocimene (21.70%), germacrene B (13.26%) and linalool (10.52%), and essential oil from dried leaves were germacrene B (28.25%), spathulenol (20.83%) and linalool (10.29%). Antimicrobial activity of the oils were evaluated and good results were verified against *Escherichia coli* ATCC-10799, *Enterobacter aerogenes* and *Staphylococcus aureus* ATCC-14458. Moreover, the oils also showed promising antileishmanial activity, giving results against *Leishmania* (L.) *chagasi* with IC_{50} between 25.0 and 40.0 $\mu g.mL^{-1}$ for dried and fresh leaves oils. This is the first report on the analysis of the volatile constituents from the dried and flesh leaves of *A. cacans* and evaluation of their antimicrobial activity. Both essential oils from the dried and flesh leaves of *A. cacans* exhibited promising activities against *L.* (L.) *chagasi*, were negative against fungi strains evaluated and shown to be sensible against some bacteria, eliminating between 80% and 90% of the tested strains. The significant biological activities presented by the essential oils suggest that these species are a rich source of biologically active compounds. This study also confirms the importance of chemical and biological investigations of essential oils of Annonaceous species, in particular *Annona* spp. in the search for new and safer antibacterial and antileishmanial agents.

Acknowledgments: CAPES, CNPq, FAPEAM, FAEPEX-Unicamp and FAPESP.

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PP2_43

CHEMICAL COMPOSITION AND ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FROM *PLATYMISCIUM GRACILE* BENTH. SAWDUST

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Platymiscium gracile Benth. (Fabaceae), is a large tree from tropical rainforests. This is mainly distributed in the Amazon rainforest of Peru and Colombia (Herrera and Ferreira, 2006). In South Colombia it is widely used for the production of handicrafts and furniture due to the high quality of its wood and resistance to fungi and insects. During the processes of cutting, grinding, drilling, and sanding, a large amount of sawdust is produced as a by-product and usually discarded. Other Platymiscium species have been reported to contain isoflavonoids, which have shown to have important antioxidant and antimicrobial properties (Reyes-Chilpa *et al*., 1998). As part of our search program of bioactive natural products from agro-industrial waste, in the present study the chemical composition and antifungal, antibacterial and antioxidant activities of P. gracile Benth. sawdust, were analyzed.

Four extracts from sawdust of *P. gracile* were prepared by successive percolation with *n*-Hexane, dichloromethane, ethyl acetate and methanol. Then, eight compounds were obtained by chromatographic techniques, and their structures assigned by spectroscopic methods (NMR) corresponding to scoparone, homopterocarpine, calycosin, medicarpin, coniferyl alcohol, coniferyl aldehyde, isoliquiritigenin, and naringenin. The antifungal activity was carried out by the poison food technique (Grover and Moore *et al.*, 1962) using the fungi *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* Simmonds. isolated from infected tamarillo fruits (*Solanum betaceum* Cav., Solanaceae). The results are expressed as percentage of inhibition of mycelial growth in relation to a negative control. All concentrations were tested in triplicate. The antibacterial activity was evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876 and *Enterococcus faecalis* strains by the broth dilution technique. MIC calculating was made by a stain with 2,3,5-Triphenyl-2H-tetrazolium chloride (TTC) (Balouiri *et al*., 2016). The antioxidant activity and free radical inhibitory (FR) was performed by DPPH, FRAP, ORAC and ABTS assays by spectrophotometric methods. The calculations were made taking as reference Trolox (DPPH, ORAC, ABTS) and ascorbic acid (ORAC). Quantification of total phenolic content (TPC) in each extract was made by the Folin-Ciocalteu method using gallic acid as standard (Preciado *et al.*, 2015).

The *n*-hexane-soluble fraction exhibited the greatest inhibitory effect against both fungi. Homopterocarpin has a strong antifungal activity against *C. acutatum* and *C. gloeosporioides* with inhibition percentages at 24 h ranging from 40 to 70% and 24 to 54%, respectively. The TPC values for fractions ranged from 3235 ± 221 (ethyl acetate) and 36350 ± 3049 mg gallic acid / 100 g extract (*n*-hexane). The evaluation of the antioxidant activity (ORAC) revealed that the highest values were 9601 ± 613 (isoliquiritigenin) and 5928 ± 430 trolox µmol / 100g (medicarpin). The fractions and isolated compounds from *P. gracile* did not show antibacterial activity under the conditions used. The results show that *P. gracile* sawdust is a promising source of compounds with antifungal and antioxidant activity.

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PP2_44

EVALUATION OF TOTAL BETALAINS OF *STENOCEREUS SPP* AND CHARACTERIZATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY

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The daily intake of fruits and vegetables is related to the decrease of radical species of oxygen and nitrogen that cause oxidative stress for its antioxidant properties and its adjuvant action in various degenerative pathogeneses (Simirgiotis & Schemeda-Hirschman, 2011). In recent years, the content of bioactive compounds from natural products have been incorporated in food formulations, dietary supplements, edible films and coatings considered as functional products. Betalains are red pigments present in some Mexican cactaceae (red tunas, xoconostle and pitaya) recognized for its antioxidant, hypoglycemic, hypocholesterolemic, and antinociceptive effects (Li Chen Wu, et. al, 2006); however, little is said about its toxicity. Therefore, the aim of this study was to evaluate the acute toxicity of an acetonic extract of betalains from the fruit of Pitaya in a mouse model and characterized the content of phenolics and total flavonoids in the exocarp and mesocarp, respectively.

Exocarp and mesocarp (50 g each) were extracted separately with acetone (1:1 w/v) using a Blender. The slurry was filtered through a Whatman n°1 filter paper by vacuum suction using a Buchner funnel. The sample was re-extracted with 70% (v/v) aqueous acetone two more times. The filtrates were combined and transferred to a separatory funnel, mixed with 2 vol chloroform and gently mixed. The samples were stored in a dark room until a clear separation between the two phases was obtaneid. After partition the aqueous phase was transferred to a boiling flask and the residual acetone/chloroform was removed using a rotary evaporator at 40°C under vacuum and proceeded to freeze drying. In order to obtain aqueous extracts the lyophilized was washing with acid citric solution 0.1% in relation 1:20 (w/v). The acute toxicity LD_{50} was performance according to Lorke method (1983). For characterization of the extracts of total phenols content it was determined in different parts of the fruit by the method described by Chen et. al., 2007. The determination of total flavonoids was performed according to described by Osorio et. al., 2013. While the scavenging effect of the radical of each piece of fruit was evaluated by the DPPH method and compared with catechin.

Mice given the concentrated extract total betalains showed no macroscopic or histopathological toxicological signs. The LD_{50} was higher than 5000 mg / kg, therefore the extract is considered according to the classification of toxicity as practically nontoxic. The betalains content was highest in the aqueous extract lyophilized pulp (386.26±42.75 mg eq. betacyanin/100 g), as well as phenolic compounds and flavonoids (535.41±11.65 mg eq. A.G/100 g y 139.50±7.29 mg eq. cat./100 g) compared to cetonic pulp and peel extracts (47.19±11.12 mg eq. betacyanin/100g, 0.203±0.01 mg eq. betacyanin/100 g) respectively. Despite the low-betalains in pericarp this presented a high antioxidant capacity (88.28 ± 0.46% inhibition of DPPH) and a content of total phenols and flavonoids (38.78 ± 0.44 mg / 100g and 22.49 ± 1.91 mg / 100 g) compared to fresh pulp present a percent inhibition of 25.57 ± 10.27%, the content of flavonoids and phenolic compounds was (43.611±0.71 mg eq.A.G/100g y 7.461±0.470 mg eq. cat./100 g). The results indicate that the pulp lyophilized pitaya contains the highest concentration of compounds of biological interest compared to solvent extraction, although none of the extracts is toxic, lyophilization is a safe method for obtaining this functional ingredient.

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PP2_45

ANTIOXIDANT EFFECT OF BETALAINS FROM *STENOCEREUS SPP*. AGAINST N-NITROSODIETHYLAMINE IN MICE

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Nitrosamines are nitrogen compounds found in nature, smoked foods, meat products, alcoholic beverages, some cosmetics and certain drugs. Recent studies have pointed to a correlation between cancer and nitrosamines exposure. Nitrosamines require metabolic bioactivation by a β-hroxilation. This reaction is catalized by cyp450, producing alquilan agents that damage DNA due to the reactive oxygen species leading to oxidative stress. Epidemiological studies have shown that intake of fruits and vegetables provide to the diet diverse phytochemicals that possess antioxidant properties. Recently, it has been demonstrated that betalains from beetroot and sour prickly fruits amount other red-colored fruits are powerful antioxidants. Thus, our goal was to evaluate the antioxidant capacity of betalains extracted from red pitaya (*Stenocereus* spp.) in *in vitro* and *in vivo* studies against n-nitrosodiethylamine.

The betalains extraction and identification was carried out according to Osorio *et al*., (2011). Antioxidant capacity *in vitro* was performed by ABTS and DPPH methods (Re, 1999; Osorio et al., 2011). Oxidative injury was evaluated in mice. Mice were intragastric gavage 28 days with betalains extracts at 0, 20, 60 and 180 mg/kg/day. On the 27th day, the mice were administered a single dose of NDEA (150 mg/kg). Several parameters such as Lipid peroxidation, Carbonilades Proteins, Super OxideDdismutase and Catalase enzymes were evalated in blood serum.

The betalains content in methanolic extract was found to be 17.5±0.3 mg betanin equivalents/100 g wet basis. Antioxidant activity *in vitro* was 10.95 ± 0.53 µmol trolox equivalents/g and 9.06± 0.27 mg GAE with ABTS and DPPH method, respectively. We observed antioxidant protective effect from betalains *in vivo.* The betalains extract showed a significant decrease on oxidative effect produced by NDEA in groups treated with betalains extracts compare to control group. These results suggest that fruits from S*tenocereus spp* are a good source of phytocompounds which are capable to protect against oxidative damage produced by high consumption of food rich in nitrites and nitrates.

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PP2_46

CANNABIS SATIVA L.: DEVELOPMENT AND VALIDATION OF A NEW HPLC METHOD WITH UV/DAD AND ESI-MS^n DETECTION FOR THE ANALYSIS OF NON-PSYCHOACTIVE CANNABINOIDS

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C. sativa L. is gaining a renewed interest, thanks to the biomedical relevance of the phyto-cannabinoids [1]. In general, the main phytocannabinoids present in fiber type plants are the acids, mainly cannabidiolic acid (CBDA), followed by their neutral forms, mainly cannabidiol (CBD) [2]. Cannabinoids are usually analyzed by using gas-chromatographic techniques, which involve high operating temperature, leading to the decarboxylation of the native acidic compounds to their neutral forms and to an alteration of the real cannabinoid profiling of the plant material [2,3].
The aim of the work was the development and validation of a reliable method for the *metabolite profiling* of the main bio-active compounds in hemp cultivars of different origins, in order to select those that could be applied for the preparation of extracts with high pharmaceutical value. The study was also addressed to the isolation of phytocannabinoids from specific hemp varieties, with particular attention to the bioactive cannabidiol (CBD).
The *metabolite profiling* of hemp non-psychoactive phyto-cannabinoids was performed by developing a RP-HPLC method coupled with UV/DAD and ESI-MS^n detection taking again advantage of the fused-core stationary phase.
The purification of non-psychoactive cannabinoids from hemp samples was carried out by means of normal phase preparative liquid chromatography (NP-LC) with silica gel as the stationary phase and chloroform and methanol as the mobile phase. The structure of the two isolated compounds was confirmed by the use of NMR spectroscopy.
The analytical method optimized in this study was completely validated for linearity, sensitivity, accuracy and precision to show compliance with international requirements (ICH guidelines) [4] and successfully applied to several hemp varieties for the selection of those with a higher content in the bioactive phytocannabinoids. Two of the analyzed samples were found to be highly rich in CBD and CBDA and, thus, they were selected for the isolation of the aforementioned compounds. Two of the analyzed samples were found to be highly rich in CBD and CBDA and, thus, they were selected for the isolation of the aforementioned compounds.
Preparative LC allowed us to obtain the isolation of two fractions with a high content in CBD (99.6%) and CBDA (92.5%), respectively, with a mean yield of 1.2% w/w from the raw material. the two purified compounds were confirmed to be CBD and CBDA by means of NMR spectroscopy.

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PP2_47

BRONCHODILATOR AND ANTITUSIVE EFFECTS OF *BLEPHAROCALIX SALICIFOLIUS* ("ANACAHUITA")

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The tree of *Blepharocalyx salicifolius* Kunth O. Berg (Myrtaceae) grows near the costs of Rio de la Plata, it is known as "anacahuita" and the leaves are used to alleviate cough, broncospasm and diarrhea, in part due to the presence of 1,8-cineol in the essential oil (EO-B.s) and tincture (T-B.s) (1, 2). Nevertheless, there are not pharmacological reports about these effects and the mechanism of action. In a previous communication we showed that both EO-B.s and T-B.s non-competitively inhibited the contractility induced by carbachol in the isolated small bowel (3, 4). The aim of this work was to evaluate the direct effects of the essential oil and those of 1,8-cineol on the isolated rat trachea. Also, the antitussive effect was assessed in the model of cough induced by ammonia chloride in mice.

Leaves of B.s were collected in the 2013-2014 summer and dried at air. The essential oil was isolated by hydrodistillation. The EO was diluted in DMSO before dilluting in Tyrode the day of the experiment. Concentration-Relaxation curves (CRC) of the EO-B.s were done in rat isolated trachea, over the contracture obtained by carbachol and compared with papaverine (Pap). Contractility was measured by a force transducer and acquisted on a computer. For studying the mechanism on the smooth muscle, the EO was also evaluated on CRC of calcium (Ca^{2+}) in a high [K^+] media in isolated small bowel (4). In mice, the EO was injected intraperitoneally 30 min before exposing the mouse to nebulization of ammonia chloride and sodium hydoxide to form volatile ammonia. Number of coughs were counted during 5 minutes. A positive control of codeine phosphate was used.

In rat isolated trachea, the EO relaxed the contracture obtained by carbachol (IC50: 1.5 ± 0.2 µg/mL, n= 11), which was lower (that is, 8 times more potent) than the CI of papaverine (IC50: 12 ± 1.2 µg/mL, n= 5). Also the maximal bronchodilation of EO-B.s was about 140% of the maximal carbachol contraction while papaverine relaxed to about 70% of the carbachol contracture. The EO-B.s also inhibited in a non-competitive way the concentration-response curves (CRC) of calcium (Ca^{2+}) in a high [K^+] media in isolated small bowel finding a IC50 of 1.77± 0.34 µg/mL for the EO and 23.7±5.5 µg/mL for 1,8-cineol. In mice, 90 mg/Kg EO-B.s reduced the cough frequency to about 25% (n=11), as well as codeine 30 mg/kg (n=7).

Results suggest that the EO of *Blepharocalyx salicifolius* are effective for treating cough and bronchospasms, because it is a more potent relaxant than papaverine, associated to inhibition of Ca influx to the smooth muscle. Also, the EO of *Blepharocalyx salicifolius* is a good central antitussive, as well as codeine.

Acknowledgements: grant UNLP X-642.

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PP2_48

PHENOLIC PROFILE AND CYTOTOXIC PROPERTIES OF POLAR EXTRACTS FROM BASAL LEAVES AND FLOWERS OF *ISATIS TINCTORIA* L.

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Isatis tinctoria L. (Brassicaceae) is an ancient European dye and medicinal plant. Historically, *I. tinctoria* has been cultivated for the production of indigo dye (blue colour) in Europe, and traditionally used for the treatment of wounds, ulcers and tumours, haemorrhoids, snake bites and various inflammatory ailments (Hamburger, 2002). Although *I. tinctoria* is not considered as an edible vegetable worldwide, rural people living around Vulcan Etna (Sicily, Italy) consume boiled flower buds of this plant as ingredients for salads and omelets (Galletti et al., 2008). Many authors have reported the anti-inflammatory and anti-cancer properties of lipophilic extracts and isolated compounds from *I. tinctoria*, whereas only a few studies have been carried out to investigate the biological activities of polar constituents present in this species. In continuation of our researches, this work was designed to characterize the phenolic profile and to evaluate the cytotoxic properties of the polar extracts from basal leaves (It-B) and flowers (It-F). of *I. tinctoria* grown wild around Acireale (Catania, Sicily, Italy).

Plant material was lyophilized and sequentially extracted with dichloromethane and 70% methanol at a temperature of 50 °C. Characterization of the phenolic profile of the hydroalcoholic extracts was attained by HPLC-PDA-ESI-MS analysis. *Artemia salina* lethality bioassay was carried out to investigate the potential cytotoxicity of It-B and It-F (Miceli et al., 2016). To test *in vitro* the anti-proliferative effects of the extracts, a human acute monocytic leukemia (AML-M5a) cell line (MOLM-13) was used. After 24 and 48 hours of treatment, cells were stained with Annexin V and 7-amino-actinomycin D and fluorescence was evaluated by flow cytometry.

By HPLC-PDA-ESI-MS analysis 13 and 8 compounds were successfully separated and identified for It-B and It-F, respectively. *I tinctoria* extracts have shown a pretty similar phenolic fingerprint; the total amount of the identified phenolics was higher in It-F (19.95 mg/g extract) than It-B (10.13 mg/g extract). Flavonoids represented the main class of compounds, with luteolin-glucuronide and stellarin-2 being present in the greatest amount in It-B and It-F, respectively. Cinnamic acids were more abundant in It-B than in It-F.

In the *A. salina* lethality bioassay both extracts resulted non-toxic to brine shrimp larvae (LC_{50} > 1000 µg/ml). It-B extract demonstrated good cytotoxic effect against MOLM-13 cells at both time points; particularly, after 48 h exposure, a reduction of viability close to 100% was observed at the highest tested concentration (1 mg/ml). It-F extract showed lower activity, causing about 40% growth inhibition.

Further investigations are needed to better explore the promising cytotoxic properties of the polar extract from *I. tinctoria* basal leaves and to determine its active components.

Acknowledgments: The authors wish to thank the "University of Messina" within the "Research and Mobility" Project.

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PP2_49

ANTI-GLYCANT AND ANTI-DIABETIC ACTIVITIES OF AROMATIC GUANYLHYDRAZONE DERIVATIVES

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In 2015 were estimated 415 million people living with diabetes, remaining on the ranking of the most important global diseases today. Vascular complications that lead to heart attacks, blindness and amputations are generated from biochemical disorders due to Advanced Glycation End Products (AGEs) accumulation.

Some aromatic systems were introduced at aminoguanidine structure, known as an anti-AGE, resulting in 19 derivatives. To evaluate the anti-glycant activity, AGEs were produced, *in vitro*, from combination between glucose and bovine serum albumin (BSA). Derivatives with best *in vitro* response were subjected to test of acute *in vivo* toxicity at concentrations of 2 mg kg^{-1}, 10 mg kg^{-1} and 100 mg kg^{-1}, showing the anti-glycant and anti-diabetic potentials in diabetic rats (*Wistar*), induced by streptozotocin.

In *in vitro* anti-glycant assay, 13 of these derivatives showed more than 50% of inhibition in the AGE-formation at concentration of 2 mg ml^{-1}, when compared to aminoguanidine molecule. In serial dosage, 0.5 to 2.5 mg ml^{-1}, checking the inhibition percentage every seven days during 49 days, the LQM13 derived reached to 94.6% inhibition, while aminoguanidine inhibited up to 74% AGE-formation, at concentration of 1.0 mg ml^{-1}. The *in vivo* studies showed that LQM3, LQM13 and LQM15 were not cytotoxic. In tests for anti-diabetic activity and anti-glycant, *in vivo*, these three derivatives were able to reduce blood glucose levels up to 65%, when compared to glimepiride drug. Furthermore, the levels of fructosamine and glycated hemoglobin levels remained lower in treated groups with these compounds.

Starting from results, we conclude that the studied derivatives demonstrate low cytotoxicity, show anti-glycant potential (AGEs-BSA) and are able to reduce blood glucose and fructosamine levels and glycated hemoglobin in diabetic rats.

Acknowledgments: FAPEAL, CNPq, CAPES and FINEP for financial support.

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PP2_50

A STUDY OF GUANYLHYDRAZONE DERIVATIVES WITH VASORELAXANT ACTIVITY USING DYNAMIC SIMULATIONS, MOLECULAR DOCKING AND 2D-QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS

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Cardiovascular diseases represent a major public health problem, which stand out hypertension, responsible by one-third of all deaths worldwide.[1] Actually, different chemical structures from guanidine-guanylhydrazones classes are used in the antihypertensive treatment, *i.e.* Clonidine, Brimonidine, Guanfacine, Guanabenz (GBZ), Guanethidine, Guanazodine and Tizanidine.[2] Due the structural similarity of these compounds with the GBZ drug, now we have investigated the vasorelaxant potential effects in spontaneously hypertensive rats (SHR), an experimental model of genetic hypertension similar to the human form of increased blood pressure.[3] In addition, we are reporting the Dynamic Simulations and Molecular Docking and 2D-QSAR analysis.

All experimental procedures were carried out in accordance with the internationally accepted principles for laboratory animal use and care as contained in European Community guidelines.[4] Dynamic simulations were performed on DESMOND® software by Schrödinger Suite. The coordinates for the possible target determined by X-ray crystallography was applied to obtain the initial system for dynamic simulations studies.[5] The crystal structure of β_2-adrenergic G protein-coupled receptor (PDB entry: 2HR1) was preliminarily analyzed by using GOLD® software. The modeling analysis, calculations, and visualizations for 2D-QSAR were performed by using the VLife Molecular Design Suite 4.4.

Guanylhydrazone derivatives (LQM01-15) were investigated, where LQM07 (pD2= 5.5±0.09), LQM08 (pD2= 4.75±0.07) and LQM14 (pD2= 5.6±0.1) presented similar potency to GBZ (pD2= 6.49±0.06). The β_2-receptor was considered as target involved. Dynamic simulations were used as relaxation method, leading to RMSF satisfactory values. Molecular docking demonstrated these compounds are able to interact with ASP113, VAL114, VAL117, PHE193, PHE289, PHE290, and ASN312. Finally, 2D-QSAR studies were performed to investigate the structural requirements of β_2-receptor antagonists. Two statistically significant models (r^2= 0.99, q^2= 0.99, *pred_*r^2= 0.99) were selected for predictive analysis. The information generated by 2D-QSAR models may lead to a better understanding of structural requirements to aid the design of novel antihypertensive agents.

Compounds LQM07 and LQM14 showed the best activity profile, with efficacies comparable to GBZ, although with lower potency than the latter. In addition, molecular dynamics and docking studies suggest that this chemotype may interact with β_2-adrenergic receptors, as probable mechanism of action, since interactions with key residues for carazolol binding may be observed for them. Lastly, the built 2D-QSAR models for that series of compounds showed good predictability for guanylhydrazones used an external validation set (test set 1 and 2), with activity nearly similar to experimental data.

Acknowledgements: to FAPEAL, CNPq, CAPES and FINEP for financial support.

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PP2_51

Baccharis trimera (LESS.) DC (ASTERACEAE) EXTRACTS ARE PROMISING ANTIBACTERIAL AND ANTIOXIDANT AGENTS

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Baccharis trimera (Less.) DC (Asteraceae), popularly known in Brazil as "carqueja", is a South America's native medicinal plant. This vegetal specie is traditionally used as hypoglycemic, hepatoprotective, digestive, anti-inflammatory, analgesic and also to treat skin wounds (Lorenzi; Matos, 2008). The current study aimed to characterize the chemical profile and the antioxidant and antibacterial activities of hexane (HE), ethyl acetate (EAE) and ethanol (EE) extracts of *B. trimera* aerial parts.

Phytochemical screening was carried out by identifying chemical reactions (Matos, 2007). Total phenolic and flavonoids were determined by Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively (Singleton et al., 1999; Sobrinho et al., 2008). The chemical profile was established by reverse phase HPLC-DAD. The antioxidant activity was explored by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and β-carotene Bleaching Test (BCBT) assays (Koleva et al., 2002; Mensor et al., 2001; Oyaizu, 1986). The antibacterial activity was assessed by the Minimal Inhibitory Concentration (MIC) using microdilution method followed by the Minimal Bactericidal Concentration (MBC), which allows classification of the antibacterial effect as bacteriostatic or bactericidal (Andrews, 2001; CLSI, 2012). *Staphylococcus aureus* subsp. *aureus* (ATCC® 29213™), *Escherichia coli* (ATCC® 10536™), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC® 13311™) and *Pseudomonas aeruginosa* (ATCC® 9027™) were tested.

Tannins, flavonoids, coumarins, terpenoids and steroids were detected. Total phenolic and flavonoid contents ranged from 0.22 (HE) to 8.66 (EE) g/100g and 0.00 (HE) to 4.62 (EE) g/100g, respectively. UV spectra obtained from chromatographic analysis of HE, EAE and EE suggested the presence of chlorogenic acid and flavonoids, especially flavonols and flavones. DPPH and FRAP assays revealed EC_{50} values of 61.61 (EE) to 390.75 (HE) µg/mL and 218.95 (EAE) to 566.75 (HE) µg/mL, in this order. According BCBT, I% varied from 28.64 (EE) to 41.27 (HE) %. Considering *S. aureus* (ATCC® 29213™), HE, EAE and EE exhibited MIC values of 5000, 1250 and 5000 µg/mL, in this order, with bacteriostatic effect. EE also revealed MIC value of 5000 µg/mL against *E. coli* (ATCC® 10536™), with the same effect. These results suggest that *B. trimera* is a source of bioactive substances, especially flavonoids, with antioxidant and antibacterial properties.

Acknowledgments: This investigation was supported by UFJF, FAPEMIG (CDS-APQ-04680-10), CNPq and CAPES.

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PP2_52

MOLECULAR DESCRIPTORS AS PREDICTING CRITERIA IN THE SEARCH OF ACETYLCHOLINESTERASE (AChE) ISOFLAVONE-LIKE INHIBITORS

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Acetylcholinesterase (AChE) is an enzyme that catalyzes the hydrolysis of acetylcholine, a neurotransmitter in synaptic half processes. Acetylcholine deficiency impedes neuronal communication and the nerve impulses transmission which leads neurodegenerative diseases, e.g., Alzheimer **(1).** These diseases are characterized because they are not cured, but treated to improve the quality of life of people affected by **(2)**; within the alternative treatments is the use of natural products such as isoflavones, which reported as AChE inhibitors **(3)**. Additionally, these compounds are good antioxidants and could prevent neuronal apoptosis and oxidative stress-induced brain damage **(4)**.

A group of isoflavones (reported in the Fabaceae family) were assessed at *in silico*-level through molecular docking using Autodock/Vina. Results were expressed as Vina Scores (affinity in kcal/mol). The ligands possessing the best docking results were studied by molecular dynamics simulations within the active site of AChE during 5000 ps using Gromacs. Thus, for the most-stable isoflavone-enzyme complexes, a detailed structural interactions analysis and their relevance were also documented. The affinity values were analyzed with molecular descriptors (CDK generated) for each ligand by principal component analysis (PCA) and partial less square regression (PLS) analysis.

Docking results let to the identification of two hits for AChE inhibition. Particular interactions into de active site were also observed to explain the complex stability. In addition, crucial residues for the enzyme:ligand interaction were identified by molecular dynamics and its stability was found to be constant accross time. PCA allowed finding differences and correlations from molecular descriptors for those studied isoflavones. Relationships (PLS) between affinities for each enzyme-isoflavone complex respect to some descriptors were likewise observed. This fact could be used as predictors in the search of AChE inhibitors as alternatives in the treatment of neurodegenerative disorders like Alzheimer.

Acknowledgments: The present work is a product derived by the Project INV-CIAS-2050 financed by Vicerrectoría de Investigaciones at UMNG - Validity 2016.

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PP2_53

IN-SILICO STUDIES ON PTEROCAPARNS AS AN ALTERNATIVE IN TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder affecting 33.6 million people worldwide [1]. There are several hypotheses about the etiology of AD such as the cholinergic hypothesis, accumulation of protein β-amyloid and tau, genetic factors, lifestyle and environmental factors. One of the most used AD treatments is the use of acetylcholinesterase (AChE) inhibitors-based therapy; this enzyme catalyzes the acetylcholine hydrolysis, a neurotransmitter that mediates synaptic nervous system activity [2]. Among the compounds reported as AChE inhibitors are flavonoids-like compounds such as pterocarpans, which are produced as phytoalexins in plants of the Fabaceae family [3].

A group of pterocarpans (reported in the Fabaceae family) was assessed at *in silico*-level through molecular docking. Test compounds were structurally optimized at DFT level in SPARTAN. Docking calculations in Autodock/Vina per each AChE:ligand complex were performed ten times and the resulting data (affinity in kcal/mol and RMSD in Å) were analyzed by multivariate analysis. In addition, better interactions were analyzed at molecular level as selecting criteria. The affinity values were correlated with molecular descriptors (CDK generated) for each ligand by partial less square regression (PLS) analysis.

Each pterocarpan-type compounds exhibited particular interactions (mainly polar contacts) with the residues into the AChE active site. However, some of them were found to have higher affinity and good convergence (RMSD < 2 Å). Affinity and RMSD values were also correlated with some of the molecular descriptors through multivariate statistical analysis, indicating that those descriptos could be used as predictors for finding alternatives based on AChE inhibitors in the treatment of AD. *Acknowledgments: The present work was performed within the Project INV-CIAS-2050 granted by Vicerrectoría de Investigaciones at UMNG - Validity 2016.*

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PP2_54

NEW MULTITARGET DRUGS DERIVED FROM NATURAL POLYPHENOLS

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Polyphenols are secondary metabolites particularly abundant in the vegetal kingdom, and featured with relevant biological properties; in this context, they have been reported to be strong antioxidants, anti-inflammatory, antimicrobial, neuroprotective, or antiproliferative agents [1]. Therefore, polyphenols constitute an attractive family of potential drugs against a series of severe pathologies like chronic inflammatory processes, neurodegenerative disorders, or cancer.
Moreover, the complex and multifactorial etiology of such diseases made it necessary to change the classical point of view in Medicinal Chemistry; in this context, multi-target drugs appeared as a promising approach for targeting simultaneously several biological receptors [2].
Concerned by the increasing rates of Alzheimer's disease and cancer, we designed two new families of multi-target drugs based on polyphenols. In both cases, dual molecules were prepared as two pharmacophores were incorporated: a polyphenolic motif, for tackling oxidative stress, one common feature of such diseases [3], and an specific motif for each pathology (a selenoureido group for cancer, and a tacrine residue (1,2,3,4-tetrahydroacridin-9-amine) for Alzheimer's disease).
All the derivatives were found to be strong antioxidant agents, capable of efficiently scavenging *Reactive Oxygen Species*, like free radicals, or peroxides, responsible for deleterious oxidative stress. Moreover, the combination of polyphenols with selenoureido motifs afforded strong antiproliferative agents in the low micromolar range.
On the other hand, in Alzheimer's patients, a common feature is the particularly low levels of the neurotransmitter acetylcholine, leading to a progressive and sever decline in cognitive functions; therefore, acetylcholinesterase inhibitors, like tacrine, the first marketed drug in this context, are claimed to be potentially useful drugs against memory loss in this neurodegenerative disease [4].
We have accomplished the synthesis of *N*-alkyl tacrine derivatives decorated with a polyphenolic motif; such compounds turned out to be submicromolar inhibitors of acetylcholinesterase, with a potency of the same order compared to parent tacrine, and also capable of reducing the oxidative stress provoked by tacrine, which leads to hepatotoxicity [5].

Acknowledgments: We thank the Dirección General de Investigación (CTQ2011-28417-C02-01, CEI10/00018), Junta de Andalucía (FQM134, P11-CVI-7427), Instituto de Salud Carlos III (PI11/00840), FEDER funds, 7thI EU Framework Programme (FP7-REGPOT-2012-CT2012-31637-IMBRAIN) for the financial support.

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PP2_55

EVALUATION OF GEL PRODUCTION AND ANTIRADICALAR ACTIVITY IN SEVERAL *ALOE* SPECIES

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Genus *Aloe* (Family *Liliaceae*) is worldwide known genus with 600 species naturally from Africa. Aloes have been long used in traditional medicine, specially the gel produced, and nowadays are used for several purposes – like cosmetics and dietary supplements. Nowadays some species, namely *Aloe barbadensis*, are cultivated as raw-material for agro-food, cosmetic or even pharmaceutical industries. Nevertheless, as indicated, many Aloe species exists that might be also used for these purposes.

In this work our goals were i- evaluate several aloe species concerning gel production and antioxidant activity, and ii- compare eventual differences between species grown in greenhouse or open field conditions.

A total of 19 different *Aloe* species were evaluated for gel production capacity, in percentage of fresh epidermal part of leaf (skin, peel) and fresh parenchyma transparent mucilaginous gel (gel), and for their antioxidant capacity (by ABTS method). The species were cultivated in equivalent conditions in the same field, in Santarem (Portugal). Additionally, some species (10) were grown both outside (ext) and in a greenhouse (int) conditions. Samples (leaves) from different plants (3) of each species and condition (int or ext) were collected in February and June of 2015, in a period that does not rain for at least a week. Fresh leafs were weighed, and the gel and peel collected separately and weight. Gel and peel were lyophilized and stored at -20 °C, until the processing.

In order to evaluate antiradicalar capacity, aliquots of lyophilized gels were solubilized in DMSO (1 mg/mL). ABTS assay was used for antiradicalar activity evaluation and comparison among species and growth conditions.

The lowest percentage of fresh gel (8.2% ± 0.6) was measured in *Aloe zebrina* (ext). The highest percentage of fresh gel (69.2% ± 1.4) was obtained in hybrid of *Aloe arborescens x Aloe barbadensis* (int). The productions of gel were affected by growing plants outside and in greenhouse, but no clear tendency was observed.

Aloe chabaudii, and *Aloe inyangensis*, both at greenhouse conditions (int), showed the highest antiradicalar activities with 57.1% ± 0.4 and 39.9% ± 0.6, respectively. The lowest scavenging activity was assessed in *Aloe zebrina* (int): 2.3 % ± 1.7. *Aloe barbadensis* (ext) and (int) showed an activity of 16.5%± 1.3 and 5.9% ± 0.1, respectively. *Aloe arborescens x Aloe barbadensis* hybrid (int) and (ext) was 33.2% ± 1.3 and 3.9% ± 1.2. So the variability among *Aloe* species, concerning gel production and antiradicalar activity, is high and *Aloe barbadensis* might not be the best species in terms of these properties for agriculture practices.

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ANTILEISHMANIAL ACTIVITY OF *GUAREA GUIDONIA* (MELIACEAE)

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Meliaceae family is chemically highlighted due to abundance and diversity of those limonoids their plants can produced [1]. The use versatility of these limonoids (commonly called as meliacins) has motivated its continuous search and discovering, since some plants of the Meliaceae family (which are well-recognized as a source of tetranortriterpenoids) have been traditionally used for pest control and for medicinal purposes. For example, some plants of the *Guarea* genus have ethnomedicinal records for the control of Leishmaniasis [2]. However, the chemistry and biological activity of plant-derived materials from this genus could be considered unexplored. Thus, as part of our research on Meliaceae plants, a LC-MS-based chemical study and an antileishmanial activity evaluation were performed with *G. guidonia,* a neotropical tree possessing few chemical studies.

Leaves, seeds, wood and bark of test plant were collected in Vaupés, Colombia. These plant materials were then dried, pulverized and subjected to maceration process with ethanol. Chemical profiles were recorded by LC-MS analysis of the resulting crude extracts. In order to explore the effectiveness and security of the extracts as parasiticide agents, crude extracts were then tested against extracellular (promastigotes) and internalized (amastigotes) *Leishmania panamensis* parasites and two macrophages cell lines (murine J774 and human macrophages). Cell viability of promastigotes and macrophages, under treatments with several doses of extracts in comparison to a control, was determined by Alamar Blue assay. The activity of extracts against internalized amastigotes was evaluated by fluorescence microscopy previous marking with SYBR green under exposition to various doses of extracts. Activity results were expressed as EC_{50} (parasites) and LC_{50} (macrophages) in µg/mL (three replicates).

Ethanol-soluble extracts were found to be very rich in limonoid-like compounds and other kind of metabolites by LC-MS analysis. This fact indicated that this kind of phytoconstituents could be possibly responsible for the activity of *G. guidonia*-derived extracts (promastigotes EC_{50} 46-200 µg/mL range; amastigotes EC_{50} 54-200 µg/mL range). Additionally, some materials exhibited low cytotoxic effect against both macrophages cell lines (LC_{50} > 200 µg/mL) and showed selectivity indexes > 2 as well.

Acknowledgments: This work was supported by grants from the Fondo de Investigación en Salud, Francisco José de Caldas (Contract No. RC463-2012) administered by Colciencias.

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PP2_57

UFLC-ESI-MS-BASED PROFILING, CYTOTOXICITY AND ANTILEISHMANIAL ACTIVITY OF EXTRACTS OF *Azadiractha indica* SEEDS

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Azadirachta indica (also known as neem) is a tree typically grown in tropical and semi-tropical regions. This plant was introduced in Colombia due to the excellent pesticide properties among others (1). There are some studies directed on the evaluation of the parasiticide activity of *A. indica* seeds (e.g., against *Leishmania amazonensis* parasites) (2). However, there is a lack of information about the effect of *A. indica* on *L. panamensis*. Thus, as part of our research on Meliaceae plants, the present study was directed to the phytochemical exploration and the evaluation of cytotoxicity and antileishmanial activity (against *L. panamensis* parasites) of *A. indica* seeds-derived extracts.

Seeds of *A. indica* were collected in Huila department, Colombia. This plant material was then dried, pulverized and subjected to several extraction processes including S-L extraction (using 96% ethanol), Soxhlet extraction and extrusion. Resulting crude extracts were chemically characterized by LC-ESI-MS-based analyses employing a validated method for profiling herbal extracts. Extracts were also tested against extracellular (promastigotes) and internalized (amastigotes) *Leishmania panamensis* parasites and two macrophages cell lines (murine J774 and human macrophages) in order to examine the effectiveness and security of the extracts as parasiticide agents Cell viability of promastigotes and macrophages, under treatments with several doses of extracts in comparison to a control, was determined by Alamar Blue assay. The activity of extracts against internalized amastigotes was evaluated by fluorescence microscopy previous marking with SYBR green under exposition to various doses of extracts. Activity results were expressed as EC_{50} (parasites) and LC_{50} (macrophages) in µg/mL (three replicates).

Extracts were found to have limonoid-related metabolites. However, particular differences were observed in the LC-MS profiles regarding the presence/absence of some metabolites. Extracts exhibited EC_{50} values for promastigotes and amastigotes in the 77-200 and 143-200 µg/mL ranges, respectively. Ethanol-soluble crude extract from *A. indica* seeds was then the most potent antileishmanial material. In addition, low cytotoxiciy against J774 and human macrophages was exhibited for all extracts (LC_{50} > 150 µg/mL) revealing selectivity indexes > 2. Thus, a bioguided fractionation leading to obtain the bioactive compounds is currently in course. The present study is the first report on the activity of *A. indica* against *L. panamensis*.

Acknowledgments: This work was supported by grants from the Fondo de Investigación en Salud, Francisco José de Caldas (Contract No. RC463-2012) administered by Colciencias.

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PP2_58

ANTI-INFLAMMATORY ACTIVITY OF EXTRACS FROM AN *IN VITRO* CULTURE OF *SAMBUCUS NIGRA* L.

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Sambucus nigra L. (Acanthaceae) is a plant used in traditional Mexican medicine to treat a number of health disorders including cough, fever, flu, diarrhea, dysentery, stomach pain, swelling, and found accumulate active compounds as anthocyanins, flavonols, phenolic acids with antioxidant, antiviral and antibacterial activities. However, this plant does not have studies that support that *in vitro* culture of *S. nigra* produces the same compounds as wild plants and also preserve their biological activity. In addition, due to that is collected and sold indiscriminately could be reduce its population and possibly endanger of extinction. The aim of this study was evaluated the anti-inflammatory activity of extract from *in vitro* culture of *S. nigra* previously establishment as homogeneous plant material source for bioactive compounds production.

The *in vitro* culture callus were induced from explants (flowers, leaves, buds and nodes) on MS medium (Murashige and Skoog), and growth regulators combination, NAA (naphthalene-1 acid), BAP (6-benzylaminopurine), IBA (indole butyric acid) and 2,4-D (dichlorophenoxyacetic acid), at different concentrations (Cruz et al., 2015). For anti-inflammatory activity of *in vitro* culture and wild plant extracts of *S nigra* (hexane, ethyl acetate, methanol and aqueous, doses 1.6 mg/ear) we used TPA-induced mouse ear oedema assay (12-O-tetradecanoyl phorbol-13-acetate; dose 2.5µg/ear) (Paya et al., 1993). Dexamethasone was used as a positive control (1.0 mg/ear). Furthermore, were evaluated the doses-response of active *S. nigra* extracts from wild plants at doses of 0.4, 0.8 and 1.6 mg/ear, both ethyl acetate and methanol extracts. The extracts were further subjected to phytochemical studies (Chaovanalikit & Wrolstad, 2004; Dewanto *et al*., 2002).

The topical administration of *S. nigra* extracts at dose of 1.6 mg/ear produced anti-inflammatory effect. Ethyl acetate and methanol extract from wild plants showed inflammation inhibition about 88.5% and 57.1% respectively with ED_{50} = 1.01 mg/ear for ethyl acetate extract. The *in vitro* culture extracts, also shown inflammation inhibition, the methanol extract with 93.5% of inhibition similar to ethyl acetate extract from wild plants and to times more that dexamethasone, which is our drug reference. The chemical profile by TLC of ethyl acetate extracts from wild plant showed the presence of different bands (phenolic compounds and flavonoids) in comparison to TLC of the *in vitro* extract; and its total secondary metabolites content was high compared with the other extracts. The *in vitro* culture extract of *S. nigra* showed greater decrease of inflammation than our reference drug dexamethasone, and are an alternative source of anti-inflammatory compound to wild growing plants. This activity was associates with its traditional use for inflammation treatment.

Acknowledgements: Financial support from CONACyT (grant 183958). Proyecto 3212 Catedras CONACyT (N° 245640). Cruz-Cruz is indebted to CONACyT for fellowship awarded (N° 330824).

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PP2_59

ANTI-INFLAMMATORY ACTIVITY OF *SAMBUCUS NIGRA* EXTRACTS FROM CALLUS *IN VITRO* CULTURE

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PP2_60

ACUTE TOXICITY "IN VIVO" OF METHANOLIC EXTRACT OF *RHIPSALIS BACCIFERA*

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Rhipsalis baccifera is an epiphytic cactus known as "mistletoe cactus" and grows in different parts of Mexico, Central and South America. This plant belongs to the Cactaceae family and is commonly used in the treatment of fractures, to promote hair growth, strengthening the intestinal flora, as antiparasitic and antidote for snakebite.

In our Laboratory, we perform hypoglycemic studies with the methanolic extract of *Rhipsalis baccifera*, so our objective is in this work was the evaluation of acute oral toxicity of the plant, according to the OECD Guidelines, 2001. Mice were weighed, marked and separated into five experimental groups: Group 1, control; 2, vehicle (water); groups 3, 4 and 4 were administered with different doses of methanolic extract of the plant (1, 10 and 100 mg/kg). Administration was intragastrically, the mice were observed for 1, 12, 24, 48 and 72 h post-administration. We performed HPLC analysis of the extract.

Methanolic extract of the plant up to 100 mg/kg did not produce any toxic effects in mice. Our results of chromatographic profile indicated that the methanolic extract contains a wide variety of polar secondary metabolites; HPLC of the extract shows two abundant peaks, which they may be associated with secondary metabolites responsible of the hypoglycemic effect of the extract.

Acknowledgments: Supported Project for the Programa de Apoyo a Investigación y Posgrado (PAIP). Facultad de Química. UNAM. Clave: Technical support Q.F.B. Luis Antonio Montoya Salas. Universidad de Coahuila. México.

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PP2_61

HYDROALCOHOLIC EXTRACT OF *ERYNGIUM CARLINAE* FOR THE TREATMENT OF HYPERCHOLESTEROLEMIA IN MICE

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Hypercholesterolemia is a problem of public health in Mexico. The main factors for the development of this metabolic disease are obesity and a fat rich diet. Eryngium carlinae is a plant belonging to the Umbelliferae family and has important ethnobotanical uses for the treatment of lipid diseases.

We examined the effect of hydroalcoholic extract of *Eryngium carlinae* in serum lipids concentration on male CD1 mice fed with a hypercholesterolemic diet (cholesterol 2%, cholic acid 0.5%). The experimental groups (n=5) consisted in: normal diet, hypercholesterolemic diet, ezetimibe (10 mg/kg) and hydroalcoholic extract of the plant (100 mg/kg). Histopathological studies of the liver were performed and concentrations of total cholesterol, HDL and non-HDL were measured.

After 4 weeks, the hypercholesterolemic diet significantly increased plasmatic cholesterol concentration in mice; it caused increases in LDL cholesterol concentration; the levels of HDL cholesterol decreased significantly. In mice fed with a cholesterol rich diet increased liver weight. In addition, there was moderate infiltration of neutrophilic granulocytes in the liver during the entire study period. After treatment with the plant extract (for 4 weeks), there was a decrease in total cholesterol and non-HDL cholesterol concentration and there was an increase in levels of HDL ($p<0.05$). Liver tissue showed vacuolar degeneration. The plant extract increased the expression of the intestinal sterol transporter Abcg8 without altering the expression of Abcg5 in mice. The same results were observed in the treatment with ezetimibe at 10 mg/kg; The hypocholesterolemic effect of *Eryngium carlinae* may be associated with the increased expression of the transporter Abcg8, which facilitates an increase in intestinal cholesterol efflux.

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PP2_62

SYNTHESIS AND BIOLOGICAL ACTIVITY OF ALKOXYLATED CHALCONE DP7 ON IMPORTANT PLANT PATHOGENS

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Chalcones are natural compounds that are widely distributed in a variety of plant species. Their diverse biological activity (including antimicrobial, antifungal and anticancer activity) and the simplicity in the preparation of synthetic chalcones have attracted the attention of pharmacologists (Xiao-Guang, 2014) as well as phytopharmacologists. 1,3-diphenyl-2-propen-1-one is a central core for all chalcones and the efficiency of their bioactivity depends on side groups, substitutions and concentration of the agent (Lahtchen et al., 2008). In order to evaluate the biological potential of this compounds on plant pathogens; the alkoxylated chalcone DP7 was synthetized and tested, by using different bioassays, on *Colletrotichum gloesporioides* and *Ralstonia solanacearum*, both phytopathogens that attacks important crops such as tomato and wheat crops worldwide.

The ((E)-1-alkoxyphenyl-3-arylprop-2-en-1-one derivatives were prepared with acceptable yields starting with 3,4-dimethoxyacetophenone or 4-phenoxyacetophenone and aromatic aldehydes in the presence of NaOH (solid) and ethanol under ultrasonic irradiation at room temperature during 5–15 minutes. The yield of the reaction was estimated by stoichiometric analysis of the starting materials and the final weight of the final product was purified by recrystallization with ethanol and then subject to complete drying (compared to the stoichiometric expected quantity). The structures of the synthesized compound DP7 was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and MS. Bioassays were conducted in order to determine the antimicrobial activity on the pathogens *Colletrotichum gloesporioides* and *Ralstonia solanacearum* by using routinely antimicrobial test including diffusion and broth microdilution methodology.

Chalcones are flavonoid precursors with a diverse bioactivity present in various plant species. These compounds could be a nature-friendlier alternative as anti-phytopathogenic agents thus replacing pesticides now in use. Our results showed bioactivity of tested chalcone (DP7) against the species that were used in the experiment. Fungistatic activity of the assayed molecule was increasing until the third day of incubation but dropped in the last day according to PIC analysis. Previous studies showed that chalcones are very efficient at suppressing not only the growth of fungus and bacteria but also cancer cells. Additionally, the results show moderate inhibition at different concentrations on *R. solanacearum* growth in comparison with the negative control (DMSO). In conclusion, the efficiency of chalcones is relative. Its biological activity depends on the cell wall density, concentration and susceptibility. However, this kind of screening is necessary in order to design new products that could be used as alternative method to control important plant pathogens under sustainable practices.

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PP2_63

KALANCHOE BRASILIENSIS CAMB. (CRASSULACEAE): AN INTERESTING SOURCE OF BIOACTIVE SUBSTANCES TO TREAT INFECTIOUS DISEASES CAUSED BY *SALMONELLA* STRAINS

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Kalanchoe brasiliensis Camb. (Crassulaceae) is a Brazilian vegetal species popularly known as "saião" and "courama branca" traditionally used to treat injuries, abscesses, enlarged ganglia and inflammatory processes (Costa et al., 1994; Mourão et al., 1999). However, there are few reports about the pharmacological activities of this medicinal plant, mainly about its possible antibacterial activity. The current study was designed to explore the chemical composition and the antibacterial activity against *Salmonella* reference and routine strains of hydroethanolic extracts (HEE) obtained from fresh leaves of *K. brasiliensis* collected before blooming, using three different concentrations of ethanol [30% (HEE230), 50% (HEE250) and 70% (HEE270) v/v].

The chemical characterization was established by reverse phase HPLC-DAD. The antibacterial activity was assessed by the Minimal Inhibitory Concentration (MIC) using microdilution method followed by the Minimal Bactericidal Concentration (MBC), which allows the classification of the antibacterial effect as bacteriostatic or bactericidal (Andrews, 2001; CLSI, 2012). Two reference strains *Salmonella enterica* subsp. *enterica* serovar Choleraesuis (ATCC® 10708™) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC® 13311™) and five routine strains *Salmonella enterica* subsp. *enterica* serovar Enteritidis 1406591, *Salmonella enterica* subsp. *enterica* serovar Enteritidis 1418594, *Salmonella enterica* subsp. *enterica* serovar Enteritidis 1628260, *Salmonella* spp. 1266695 and *Salmonella* spp. 1507788 isolated from clinical human specimens were tested.

UV spectra obtained from chromatographic analysis suggested the presence of the same six flavonols in HEE230, HEE250 and HEE270. Considering the antibacterial activity, HEE230 and HEE250 were active against *S.* Choleraesuis (ATCC® 10708™) and *S.* Typhimurium (ATCC® 13311™), with MIC values of 5000 µg/mL and bacteriostatic effect. However, HEE270 inhibited all *Salmonella* strains tested, with MIC values of 5000 µg/mL and bacteriostatic effect, being the most active extract among those investigated. These results indicate that *K. brasiliensis* is an interesting source of bioactive substances, being effective against *Salmonella* strains, including antibiotic-resistant ones.

Acknowledgments: This investigation was supported by UFJF, FAPEMIG (CDS-APQ-04680-10), CAPES, and PAEC OEA-GCUB.

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PP2_64

IN VITRO ANTIBACTERIAL ACTIVITY OF *HYPOESTES FORSSKAOLII* VAHL. ROEM & SCHULT. (ACANTHACEAE) AGAIST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: PRELIMINARY RESULTS

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Hypoestes forsskaolii is a perennial herb belonging to Acanthaceae family, which shows several beneficial properties as antioxidant, antibacterial, anticancer, antifungal and antiparasitic activity (Mothana et al. 2009; Ojo-Amaize et al. 2007; Rasoamiaranjanahary et al. 2003).

Plants produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Samie et al. 2010). A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs (Edwards 2004).

In order to develop innovative antibacterial compounds with potencial applications in preventing microbial proliferation, in this study we evaluated the antibacterial activity of *H. forsskaolii* against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591.

The crude extracts of *H. forsskaolii* were obtained through a polarity solvent maceration process (*n*-hexane, chloroform, chloroform : methanol (9:1 v/v) and methanol of 326 g of roots.

The extract concentrations used for this study ranged from 50 µg/mL to 500 µg/mL.

The antimicrobial activity was measured by suspending MRSA (one single porous bead, a carrier to support microorganisms in Microbank vial containing cryopreservative) in Brain Heart Infusion Broth (Oxoid) for 24 h at 37°C; thereafter, the Optical Density at 600 nm (OD 600) of the bacterial suspension was adjusted to 0.1 with the addition of sterile broth. Tests were carried out in microtiter plate with the bacterial suspension by adding the extracts at different concentrations (50, 70, 80, 100, 200, 500 µg/mL). The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of the extracts able to prevent the microbial growth. The analyses were performed in triplicate on three independent experiments.

We demonstrated that only the chloroformic extract was effective in growth inhibition of the tested MRSA strain using a concentration up to 100 µg/mL.

The antimicrobial activity reported could be due to the terpenoids which are present in the chloroformic extract. However, further studies are needed to better define the effectiveness of in vitro activity of *H. forsskaolii* chloroformic extract.

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PP2_65

SEASONAL VARIATION AND CHEMOTYPE IDENTIFICATION IN VOLATILE AND NON-VOLATILE FRACTIONS FROM *Schinus molle* LEAVES BY CHROMATOGRAPHIC PROFILING COUPLED WITH CHEMOMETRICS

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Schinus molle is a plant commonly distributed around the world, which is used for purely ornamental purposes. Despite its wide distribution and its great adaptation ability to grow under several environmental conditions, studies on the biological potential and chemical composition of this species is currently limited. In fact, only few reports about the chemical composition from fruit essential oils have been published [1], whereas chemical components from other parts and non-volatile fractions remain almost unexplored. Therefore, as part of our research on metabolomics from plant species growing in Colombia, variability on chemical composition of volatile and non-volatile fractions from *S. molle* leaves was evaluated using GC-MS and LC-DAD-ESI-MS coupled with chemometric techniques.

A total of 48 specimens of *S. molle* were collected around Bogotá, Colombia. Each sample was submitted to extraction of both volatile and non-volatile components. The first one was accomplished by supercritical fluid extraction while the latter one was carried out by ultrasound-assisted extraction. Subsequently, chromatographic profiling by GC-MS and LC-DAD-ESI-MS for volatile and non-volatile fractions, respectively, was performed. The corresponding profiles were independently aligned, normalized and scaled, and afterwards statistically compared under unsupervised and supervised models, including PCA and OPLS-DA. Total phenolic and total flavonoid contents were also determined for non-volatile extracts by conventional spectrophotometric methods.

Comparison of Total Phenolic Contents for the analyzed samples showed to be significantly different among them as indicated by ANOVA ($p<0.05$). For its part, Total Flavonoid Contents resulted to be less changing, although significant differences could be also observed. PCA and HCA demonstrated samples can be classified in four different groups according to their LC-UV-profiles, which means significant variations in composition along the whole set of samples was therefore evident. OPLS-DA using collection date as supervision variable afforded clear differentiation of samples, indicating marked compositional changes with the season, which could help out defining chemotypes in *S. molle*. On the other hand, the variations on phenolic content were related to changes in chemical profiles as demonstrated by OPLS. In contrast, any relationship between flavonoid contents and chromatographic profiles could not be define. In case of the volatile fractions, besides they were characterized as sesquiterpene-rich oils, four different groups by HCA according their profiles were observed. Moreover, seasonal variations in their chemical compositions were demonstrated as well, supporting the above mentioned results for non-volatile components. In summary, statistical models let demonstrate the existence of chemotypes among *S. molle* specimens growing in Bogotá, which can be attributed to seasonal metabolic variations. Identification of chemical markers for each chemotype are currently in progress.

Acknowledgments: *The present work was performed within the Project INV-CIAS-2050 granted by Vicerrectoría de Investigaciones at UMNG - Validity 2016.*

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PP2_66

PHOTODYNAMIC ANTIMICROBIAL ACTIVITY OF *SYZYGIUM CUMINI* EXTRACTS ON *KLEBSIELLA PNEUMONIAE*

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The genus *Klebsiella* frequently causes human nosocomial infections [1]. The most important specie from this genus is *Klebsiella pneumoniae,* a bacteria responsible of infections as pneumonia and septicemias, that require intense health care [2]. It is characterized by a capsule (virulence determinant), siderophores and filamentary projections (Pilus) present on the bacterial surface which favour adhesion to host organisms. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. Recently, new types of strains, called extended-spectrum-β-lactamase (ESBL) producers, represent a challenge in the search of effective alternatives for the management of these infections [3]. Reactive oxygen species (ROS) are molecules involved in a variety of chemical, biological and environmental processes [1-3]. ROS generation is promising in antimicrobial photodynamic therapy since their presence leads to damages of the microbial membrane and to protein degradation. However, synthesis of these compounds requires a variety of reagents expensive and sometimes toxic to humans and to the environment. For this reason, with the aim to select new compounds with potential biological activity on *K. Pneumoniae,* extracts obtained from the plant *Syzygium cumini* were evaluated by using photodynamic therapy and bioassays. Ethanol extract of *Syzygium cumini* fruit was obtained according to the following protocol: fresh grapes were picked, cleaned with distilled water and heated at 45°C for 5 days until constant weight. Dry samples were then isolated from shell. An amount of crushed material was placed into a percolator; the solvent ethanol was added to completely cover the material (380 mL). Percolation process was performed for 1 day, thereafter 350 mL of the mixture were collected. Solvent was recovered by simple distillation and returned to percolator. Then a proportional amount of the extract-ethanol mixture at the bottom was collected and the percolation process performed until constant weight. The antimicrobial activity of ethanol extract of *Syzygium cumini* fruit was evaluated on *Klebsiella pneumoniae* ATCC 70503 by using broth microdilution protocol in 96 well microplate at 37°C. Different concentrations of irradiated and non-irradiated extracts were used as treatments (51.8, 41.4, 25.9, 15.5 and 10,4 µg/µl). The final volume was 100µL and the final concentration of DMSO (negative control) in the assay did not exceed 1%. The OD600 was monitored and the inhibition percent and Minimal Inhibitory Concentration (MIC) was calculated following the recommendations of the CLSI-2012 for microdilution (IC50) by using the Graphpad Prism 5.0 statistical package. Data were compared with those using Gentamicin (20µg/mL) as positive control. Results show statistically significant differences between treatments and negative and positive controls. In addition, irradiation treatment increased the biological activity sustaining the use of photodynamic therapy. We observed a positive correlation between extract concentration and bacterial growth inhibition, values ranging between 41,6% and 90,5% for irradiated treatments. Non irradiated treatments showed inhibition ranging between 0% and 58,6%. The MIC values in case of irradiated treatments was 7.25µg/µL. The incidence of ESBL-producing strains among clinical *Klebsiella* spp. isolates has been steadily increasing over the past years. Limitations in therapeutic options demand new measures to counteract hospital infections. For this reason, natural sensitizers represent practible alternatives for selecting new compounds with antibacterial activity [4]. In addition, these natural sensitizers are cheap, accessible, abundant and have lower environmental impact compared to synthetic compounds. In nature, fruit, flower and leaf of plants show different colors from red to purple due to the presence of natural dyes, which can be used in different application. It can be suggested that natural pigments from plants as *Syzygium cumini* will be used as potential antibiotics representing a valuable opportunity to counteract this kind of multidrug-resistant bacteria.

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PP2_67

IDENTIFICATION OF PHENYLPROPANOID GLYCOSIDES FROM ALOYSIA POLYSTACHYA (GRISEB. ET MOLDENKE) BY HPLC-MS ANALYSIS

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Data on the chemical composition of *Aloysia polystachya* (Griseb. et Moldenke) are poor in the literature. Phenolic compounds characterization in plant matrix can be very difficult, owing to bond to different sugar moieties, or conjugation to form complex structures. Despite a great number of investigations, also the separation and the simultaneous determination of polyphenolics of different groups remain difficult. Among the different methods available, high-performance liquid chromatography (HPLC) is preferable for the separation and quantification of polyphenolics. The HPLC-MS characterization of the phenylpropanoid glycosides fraction, purified by Solid Phase Extraction (SPE), in *Aloysia polystachya* (Griseb. et Moldenke) extract was performed.
Two different MS techniques have been coupled to HPLC, to identify the compounds present in the extract, and support tentative structure identification: ion trap mass spectrometry (Ion Trap LC/MS), and the quadrupole-time of flight high resolution mass spectrometry (Q-TOF HRMS).
Nine phenylpropanoid glycosides were identified in methanolic extract of the Aloysia polystachya (Griseb. et Moldenke) leaves. Among them, the compounds forsythoside A, plantainoside C, purpureaside D, martynoside and its two isomers were identified here for the first time.
The results present here could be helpful in the future developing and validating of an analytical method to quantify the isolated compounds, and to assess the quality of Aloysia polystachya (Griseb. et Moldenke) leaves. Knowledge of the content of phenylpropanoid glycosides could further contribute to the chemotaxonomy of the genus.

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PP2_68

ESSENTIAL OILS AS POTENTIAL FUMIGANT AGENTS FOR THE CONTROL OF *SITOPHILUS ZEAMAIS*

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Grains like rice, wheat and maize are considered fundamental by the World Health Organization (WHO) to ensure the global food security. Due to accelerated world population growth, by 2050 is estimated that global demand for stored grains will increase around 40% of current production, exceeding the 300 million tons per year. Despite the great importance of these cereals, in developing countries like Colombia the production for own consumption is insufficient, in the case of maize 4.5 million tons are imported annually. The main factors affecting maize production are the low agricultural technology and crop losses caused by insects and diseases attack (FAO 2016). The postharvest insects are the most responsible for the damage of the stored product. Coleoptera of *Sitophilus* genus are a cosmopolitan plague that affects stored grains and cereals, within them is *S. zeamais* known as maize weevil (Pingali and Pandey, 2001; García and Bergvinson, 2007). The control of these insects is done with synthetic and systemic pesticides, which are expensive, highly toxic and are becoming less effective as result of the emergence of resistant species. Therefore the discovery and development of new effective and safety biocontrol agents is necessary. The botanical volatile metabolites like essential oils are a potential source of new agrochemicals due to high volatility, rich chemical composition and high distribution (Rees D. 2004). In this work was performed a screening of fumigant activity of essential oils against *Sitophilus zeamais*.

The plant material of 52 native and introduced species was collected or acquired in different parts of the Colombian Andean region. From the fresh material the essential oils were extracted by steam distillation technique. The chemical characterization of essential oils was performed by GC-MS on HP-5 and Carbowax columns, the components were identified by comparison of retention times and mass spectra. The fumigant activity of essential oils was determined against *S. zeamais* using the vial into vial method. Filter papers were impregnated with essential oil at calculated doses to give a final fumigant concentration of 500µL/L in air. The impregnated filter papers were disposed in a 1,5 ml vial, these were placed in a 22ml vial, which contained 10 adults insect. To determine the fumigant activity two variations of the assay were evaluated, in the first assay the insect had the possibility to come in contact with the impregnated disc. Otherwise, in the second test was avoided the contact covering the top of the small vial whit a semipermeable membrane (Pascual et al., 2004; Prieto J.A. 2012).

In the preliminary screening of fumigant activity 24 essential oils of the 52 tested showed insecticidal activity against *S. zeamais*. By comparison to the mortality rates obtained by the two methods were found variations in the activity of some oils. *Pimpinela anisum, Ocimum basilicum* y *Artemisia vulgaris* essential oils presented activity mainly by contact, in the first assay they showed mortality rates of 80%, 63% and 40% respectively, while in the second test they were totally inactive. Moreover, the *Lavandula stoechas, Rosmarius officinalis y Lippia alba* essential oils showed fumigant action predominantly with mortality rates higher than 85% in both methods assays. Through the screening of preliminary activity was determined the insecticide potential of 24 essential oils and as well as the methods by which conducted the effect by fumigant ability or as contact insecticides.

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PP2_69

SYNTHESIS OF BIOACTIVE CHROMENES INSPIRED ON THOSE ISOLATED FROM PIPER CF. CUMANENSE KUNTH (PIPERACEAE).

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Chromenes, also known as benzopyrones, are organic compounds of a cyclic fused structure between an aromatic ring and a pyrone. Such compounds are widely distributed in nature and exist as two types of isomers: 2H-chromene (2H-1-benzopyran) and 4H-chromene (4H-1-benzopyran). Such metabolites are biologically interesting compounds that have shown antiviral, mutagenic, antimicrobial, antitumor, anti-HIV, and antifungal activity. Furthermore, they are also active in the central nervous system, among others (1). They have been isolated from various genuses belonging to the families Apiacae, Asteraceae, Fabaceae, Rutaceae and Piperaceae, mainly. In the Piperaceae family they have been generally reported in species of the genus Piper and Peperomia (2). Chromenes found in the genus Piper are characterized by different alkyl chains at position 2, a double bond between 3 and 4, carboxyl or ester group at position 6 and sometimes substituents at position 8 hydroxyl type or aliphatic chains like prenyl, geranyl and farnesyl are also common. Recently, we have demonstrated that some isolated chromenes from Piper cf. cumanense Kunth (Piperaceae) show potent antifungal activity (3,4). In order to establish the structure-activity relationship, we started the synthesis of several analogs of chromenes inspired on those isolated from the genus Piper and preliminary results are described.

The synthesis of the chromenes is based on protocols previously described for different precursors (5). We started with substituted phenols, subjected to an acetylation reaction on the hydroxyl group, followed by a Fries rearrangement to form 2-acetophenones (with different substituents). Subsequently a condensation with different ketones was performed, and therefore the generation of the benzopira-2-one ring was conducted. Finally the obtained products were subjected to reduction, dehydration and/or to prenylaciones to generate the different analogs. The method developed is efficient, yields are very good providing a new class of chomenes and analogs. Furthermore, the obtained core allows structural modifications and the introduction of differente substituents. Applying this procedure, 15 analogues with different electron-donating and electron-whitdrawing groups and different chains and allylic halogen substituents were synthesized. Results contributes to research development on chromenes mainly isolated from the genus Piper, thus providing the basis for structure-activity studies aiming to find substances with greater antifungal activity.

Acknowledgments: Authors greatfully acknowledge financial supports provided by Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, (Contract No. 0459 –2013, RIFRUTBIO) and by Colciencias (1101-05-17783), Universidad Nacional de Colombia and Universidad de los Andes.

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PP2_70

CYTOTOXICITY OF DQ-35 IS MEDIATED BY G2-ARREST AND MICRONUCLEI FORMATION WITHOUT NUCLEAR DAMAGE

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Quinones represent a diverse family of naturally occurring secondary metabolites. Interest in these substances has intensified in recent years due to their pharmacological importance [1]. In particular, many clinically important antitumor agents possess a planar ring system containing the quinone nucleus. In a previous study, we identified QD-35, a thiophene-naphthoquinone, as a candidate molecule to develop colorectal cancer treatment [2]. Here, we demonstrated that the cytotoxic effect of this compound is mediated through G2-arrest without nuclear damage.

QD-35 was obtained by chemical synthesis using the method described by Zuse *et. al.* [3]. Structure was confirmed by spectroscopic data (FT-IR, MNR and MS). Colorectal cancer cells, HT-29 (ATCC®-HTB-38™), were treated with QD-35 (LC_{50}≈1.8 µM) for 48 h. Thereafter, genotoxic effect was evaluated by cytokinesis-block micronucleus (MN) assay with some modifications [4], as well as comet assay in neutral conditions as described by [5]. In addition, the effect of test compound on cell cycle progression was assessed by flow cytometry using propidium iodide staining.

MN formation is a hallmark of property of drug-induced genotoxicity. Treatment of HT-29 cells with QD-35 significantly increased the MN frequency. In contrast, test compound did not induce comet formation. QD-35 treatment resulted in a remarkable increase in the proportion of 4N cell population, and significant decrease of 2N population, suggesting an induction of G2/M arrest. Moreover, we found a remarkable increase of >4N population, which is consistent with the induction of MN. In summary, our results indicate the ability of QD-35 to induce chromosomal damage and genome instability in HT-29 cells without inducing DNA damage.

Acknowledgments: This study was supported by Colciencias and the University of Cartagena (Grant 110756933930).

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PP2_71

SECONDARY METABOLITES FROM CHILEAN PLANTS AND THEIR BIOLOGICAL ACTIVITY

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Plants of the Celastraceae and Rhamnaceae that growth in Chile have been studies. These plants have very interesting metabolites with epoxyeudesmane (agarofurane), benzylisoquinolinic, aporphinic and cyclopeptide skeleton. We study the biological activity associate at these compounds.

Air dried and powdered material was exhaustively extracted with MeOH. The resulting MeOH extract was filtered and concentrated in vacuum. The residue was suspended in H_2O and the sequentially extracted with solvent of increasing polarity. The resulting fraction were concentrated and chromatographed over silica gel column. Column fraction were analyzed by TLC (silica gel 60 F254), and fraction with similar TLC patterns were combined to give pure compounds. A hyphenated procedure combining HPLC-MS-NMR allows the determination of chemical structures of the isolates.

From the extracts, fractions and pure compounds for inhibitory capacity of acetylcholinoesterase enzyme and its insecticidal activity against *Cydia pomonella*, *Tenebrio molitor* and other insect pests were evaluated.

Results show that the extracts, fractions and pure compounds obtained from Chilean plants of the Celastraceae and Rhamnaceae family present an interesting insecticidal activity and inhibitory activity of acetylcholinoesterase enzyme.

Acknowledgments: To Fondecyt program grant # 1130463. The authors wish to thank to internal grant from Direccion de Investigacion Universidad del Bio-Bio DIUBB # 083009-2R, # 122509 and # 132209 GI/ C, Universidad del Bio Bio, Chillan, Chile.

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PP2_72

ETHANOL CRUDE EXTRACT FROM LEAVES OF *SINNINGIA SCHIFFNERI* (GESNERIACEAE) AND THEIR FRACTIONS AS NATURAL PHOTOSENSITIZER IN PHOTODYNAMIC CHEMOTHERAPY

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The Photodynamic chemotherapy (PDT) has been used for many purposes, such as antitumor, performing as a non-invasive therapy and moderate or minimal side effects. Photosensitizers are agents that, under illumination at a certain wavelength and in the presence of oxygen, produce reactive oxygen species that interact with biological systems and are cytotoxic to the target cells (Brown et al., 2004; Andreazza et al., 2015). The *Sinningia schiffneri* belong to Gesneriaceae family what comprises around 150 genera and 3500 species, distributed in tropics and subtropics around the world. In Brazil, the family is represented by 27 genera and 211 species, being *Sinningia* the most important genus. Phytochemical studies with *Sinningia* reported the identification of phenolic glycosides, anthocyanins and anthraquinones with biological properties (Scharf et al., 2016; Verdan et al., 2015). It is known that anthraquinone as hypericin are natural photosensitizers with application in PDT (Wainwright, 2009). This prompted us to investigate the potential of extracts from *S. schiffneri* for employment as natural photosensitizer in PDT against human melanoma cell line.

The leaves of *S. schiffneri* was dried at 40° C and the powder subjected to the maceration procedure with ethanol, and partitioned with organic solvents in order of increasing polarity (hexane, dichloromethane, ethyl acetate and n-butanol). Then, was determined the absorption profile of the crude extract and of the fractions in the visible region of interest PDT (400-850 nm). The production of singlet oxygen was determined using the method of the photodecomposition of 1,3-diphenylisobenzofuran, 1,3-DPBF (Andreazza et al., 2015). The antiproliferative activity of the crude extract against human melanoma cell lines (UACC 62) was assessed colorimetrically by the reduction of a tetrazolium salt (MTT). Absorbances were expressed as percentages relative to untreated controls. As control, was used the chemotherapeutic Doxorubicin (positive control), the diluent (negative control) and the methylene blue (positive control of the photosensitizer), and a non-irradiated plate. The bioassays were carried out in triplicate. One plate in each assay was irradiated while the other was not irradiated, the antiproliferative effect was investigated, and data analyzed by the Tukey test. The chemical composition of the extract was determined by ESI-MS/MS and HPLC-MS/UV-DAD techniques.

The crude ethanol extract from the leaves of *S. schiffineri* and their hexane, dichloromethane and ethyl acetate fractions were obtained and showed absorption from 600 to 700nm. Also, singlet molecular oxygen ($^1O_2$) production (type II photosensitization reaction) was examined, and the results show that 1,3-DPBF photodegradation was greatly enhanced in the presence of ethanol crude extract of *S. schiffineri* and their hexane and dichloromethane fractions indicating production of singlet oxygen. Laser irradiation alone at 660nm using diode laser, output power of 35mW, and energy of 28J/cm^2, or non-irradiated crude extracts in sub-inhibitory concentration did not reduce the cell viability significantly, whereas irradiated ethanol extract of *S. schiffneri*, in sub-inhibitory concentrations, exhibited antiproliferative effect against UACC 62 cell lines. It is suggested the presence of substances with photosensitizing potential in bioactive samples. Naphthoquinones, anthraquinones and phenolic compounds were identified in the bioactive extract by ESI-MS/MS and HPLC-MS/UV-DAD analysis. Despite the positive results, further investigations are necessary to confirm the potential of this natural product as photosensitizer in PDT.

Acknowledgments: Financial Support from Fapesp, Faepex-Unicamp, Capes and CNPq

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THE SYNERGISTIC EFFECTS OF ANTIOXIDANTS IN THE CONTROL OF REDOX BALANCE IN CULTURED FIBROBLASTS AND POSSIBLE INFLUENCE ON PATHOLOGIC CALCIFICATION

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In the last decades lifespan is progressively increased, but, at the same time, higher incidence of age-associated pathologic conditions as cancer, cardiovascular diseases, diabetes and Alzheimer were also observed [1]. Both genetic and environmental factors, including dietary habits, can contribute to modulate the susceptibility of individuals to the occurrence and severity of age-related diseases [2]. Reactive oxygen species (ROS), including those physiologically produced by cellular metabolism, are known to accumulate with aging, thus contributing to damage nucleic acids, lipids and proteins and thereby altering their functions [3-4]. This observation suggested that antioxidant supplementation can restore redox balance, and therefore great attention is paid to increase the antioxidant properties of dietary products eventually adding antioxidant polyphenols, vitamins, long-chain unsaturated fatty acids and carotenoids [2]. However, there is a large body of evidence demonstrating that ROS can also act as signalling molecules, thus posing the question whether cellular homeostasis can be efficiently improved by a combination of antioxidants either in physiologic or in pathologic conditions. Moreover, the observation that ectopic calcification, a frequent complication of age-related disease (namely atherosclerosis, diabetes, uremia), is associated to increased oxidative stress, suggested that antioxidants may interfere with the calcification process [5-7].

The present study investigates in human dermal fibroblasts cultured from healthy adult individuals if a combination of antioxidants at low and high concentrations (Table) can modulate the expression of different parameters of redox balance [hydrogen peroxide, anion superoxide, lipid peroxidation (LPO), protein carbonylation and total antioxidant status (TAS)]. Furthermore, fibroblasts were also cultured in vitro in a calcifying medium and alkaline phosphatase activity as well as mineral deposition were evaluated.

ANTOXIDANTS	[LOW]	[HIGH]
Quercetin	2,5 μ M	25 μ M
Kaempferol	2,5 μM	25 μM
Vitamin C	1 μM	10 μM
Vitamin E	5 μM	50 μM

Results indicate that: a) reduction of ROS was observed only with the antioxidant mix at high concentration; b) LPO was decreased by antioxidants at both low and high concentrations; c) surprisingly, protein carbonylation appeared increased in cells cultured with antioxidants at high concentration, d) TAS was never increased by treatment; e) treatments determined an increase of alkaline phosphatase activity and of the number of calcified areas. To be noted that high doses of antioxidants for several days are associated to cell death, calcification being, at least in part, the consequence of the necrotic process.

These data indicate that a combination of quercetin, kaempferol and vitamins C and E, although capable to modify the redox balance, are not only inefficient in counteracting ectopic calcification, but on the contrary, seem to favour the calcification process also at low doses in the absence of evident cytotoxic effects. Therefore, antioxidant mix exerts many additional effects, beside those on redox balance, and therefore their use may require a careful evaluation especially when are given as supplements.

Acknowledgments: work supported by Fondazione CRMO(#2015-0306)

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FLASH CHROMATOGRAPHY MS-TARGETED ISOLATION OF NATURAL PRODUCTS UNDER NORMAL PHASE CONDITIONS

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Reversed phase liquid chromatography (RP-LC) is widely used for the metabolite profiling of complex natural extracts and is now more and more used for targeted MS isolation of biomarkers. Normal phase chromatography (NP-LC) is well suited for the purification of lipophilic secondary metabolites, also offering some advantages compared to RP, like low operating pressures and cheaper stationary phases. The complementary usage of both of stationary phases and MS detection for metabolite purification at the preparative scale using generic separation methods has been investigated on an innovative Flash chromatography system integrating MS and ELSD detection (PuriFlash®- MS). A mixture of representative natural product standards was chosen and analysed under both normal phase/reverse phase conditions. All parameters were carefully optimized for both separation and detection (gradient system, split rate, flow rate, temperature, inj. volume, column length, ionization source parameters). A special care was taken to find MS ionization and splitting conditions that provide good detection and preclude source contamination. The HPLC analytical gradient was transferred to flash chromatography following a geometric gradient transfer method after calibration of the chromatographic systems.[1] MS, in complement to UV detection, enabled the monitoring of NPs with weak and strong chromophores and the selectivity of MS was of great help for a precise collection of partially co-eluting compounds. APCI-MS detection with optimized splitting and post-column elution of appropriate solvent was found robust and well-suited for purifications in both NP and RP modes. This strategy allows an efficient and rational targeted isolation of tens to hundreds mg of compounds for further structural identification or bioactivity characterization studies.

Acknowledgements: Interchim (Monluçon, France) for loan of instrument and technical support

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