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Edited by: Nicola Cicero, Valentina Mangano, Ambrogina Albergamo and Giacomo Dugo.

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# PLENARY LECTURES

# PL01- NMR methodologies in food science

Mannina L.[1,2*], Ingallina C.[1], Sobolev A.P.[1], Proietti N.[2], Di Tullio V.[2], Capitani D.[2]

luisa.mannina@uniroma1.it

[1]*Prof/Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, P.le Aldo Moro 5, CP 00185 Rome, Italy*
[2]*Istituto di Metodologie Chimiche, Laboratorio di Risonanza Magnetica "Annalaura Segre", CNR, CP 00015 Monterotondo, Rome, Italy*

## Introduction

Food is a complex matrix since it is a mixture of many primary and secondary metabolites ranging in concentration and chemical properties. Depending of the specific problem different strategies (i.e target analysis, metabolite profiling, metabolic finger printing, metabolomics) can be used to food chatactrization. The increasing ability of high field NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry being a valuable tool for qualitative and quantitative analyses[1].

## Methods

Significant aspects regarding sample preparation, experimental procedures and chemometric elaborations will be shown together with a new NMR database.

## Results / Discussion / Conclusion

The quantitative analysis of the metabolic profiling along with the application of a suitable statistical analysis has allowed food characterization in terms of geographical origin, genetic origin and farming. [2-] Some significant examples regarding olive oils[2], fruits and vegetable[3-4] and beverages[5] will be discussed. The potential of NMR spectroscopy to detect food adulterations is also shown[5].

## Bibliographic References

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# PL02- Ten year´s research with the Amazonian adaptogen plant saracura-mirá: *Ampelozizyphus amazonicus* Ducke

Leitão S. G.[1]*
*sgleitao@pharma.ufrj.br

[1]*Faculdade de Farmácia, Departamento de Produtos Naturais e Alimentos, Universidade Federal do Rio de Janeiro, CCS, Bl. A, Ilha do Fundão, 21.941-902, Rio de Janeiro, Brazil.*

*Ampelozizyphus amazonicus* Ducke (Rhamnaceae) is an Amazonian medicinal plant popularly known as "saracuramirá" that is found in the Amazon forest territories of Brazil, Venezuela, Colombia, Peru, and Ecuador. In Brazil, it is restricted to the states of Amazonas, Pará, and Roraima and grows mainly in the "terra firme" forests near waterfalls or "igarapés"[1]. An aqueous drink with reported tonic and antimalarial properties can be prepared from the bark and roots of *A. amazonicus*. This drink has a very bitter taste and forms abundant foam when shaken, due to the high saponin content in the species, which gives rise to its other popular name "cervejade-índio"[1]. Our interest in this plant as an adaptogen arose because previous investigations on the antimalarial properties of this plant have shown that it does not have a direct action on *Plasmodium* blood stage forms, but it could be effective in controlling infection induced by sporozoite forms[1,2]. Based on the findings that *A. amazonicus* does not have a direct effect upon blood stage forms of the protozoan, it might be possible to suggest that the plant could act as an adaptogen by enhancing immune system function and could alleviate the inflammatory disorders caused by malaria. In fact, ethnopharmacological studies indicate both stimulatory and energetic properties for *A. amazonicus*. Therefore, our research group engaged in the study of its immunomodulatory properties, its chemistry and its biotechnological applications. Due to the growing interest in dietary supplements with adaptogen properties and to provide a new functional ingredient, barks from *A. amazonicus* were extracted. The water extract was spray dried without drying adjuvants, resulting in a powder (SARF), which was characterized by its physicochemical properties and proximate, mineral and saponin contents. The SARF particles tended to have a spherical shape and a unimodal size distribution [3,4]. The particles also had good rehydration characteristics and high saponin content (33%) [4]. The effect of SARF on the immune response was investigated by measuring immunoglobulin production induced by immunization with the antigen TNP-Ficoll in *Plasmodium chabaudi*-infected mice, and also by measuring the levels of anti-ovalbumin, anti-LPS and anti-dextran IgM and IgG antibodies in immunized and unimmunized mice [2,4]. Our data confirmed that SART possesses immunomodulatory properties, inducing an in vivo modification of the B lymphocyte response and anti-inflammatory properties, which are partly due to a reduction in cell migration and are most likely due to an inhibition of the production of inflammatory mediators [2]. SARF also increased the basal levels of anti-ovalbumin, anti-LPS and anti-dextran IgM antibodies, and the anti-dextran IgG antibodies in unimmunized mice. No increase in antibody titers was observed after SARF treatment in immunized mice [4]. The SARF saponins were isolated into different groups by CCC and characterized by off-line ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry (HPLC-HRMS$^n$) analysis [6]. Group 1 presented mainly oleane type saponins,

and group 3 showed mainly jujubogenin glycosides, keto-dammarane type triterpene saponins and saponins with $C_{31}$ skeleton. A further purification of group 3 by CCC and HPLC-RI allowed obtaining these unusual aglycones in pure form [6]. Recent findings on the skeletal muscle performance of rats will be discussed. Taken together, these results suggest that SARF could be an interesting new functional ingredient for food applications or pharmaceutical products.

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# PL03- Gallic acid: health protective activities via epigenetic mechanisms of action

Daglia M.[1*], Tsetegho Sokeng A. J.[1], Dacrema M.[1], Habtemariam S.[2], Nabavi M.[3]

*maria.daglia@unipv.it.

[1] *Department of Drug Sciences, Medicinal Chemistry and Pharmaceutical Technology Section, Pavia University, Viale Taramelli 12, Pavia 27100, Italy;*
[2] *Pharmacognosy Research Laboratories, Medway School of Science, University of Greenwich, Chatham-Maritime, Kent ME4 4TB, UK*
[3] *Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, P.O. Box 19395-5487, Tehran 19395-5487, Iran*

Polyphenols, secondary metabolites abundantly distributed in all plants, are a topic which has gained attention for their potential health benefits in preventing chronic diseases, such as neurodegenerative and cardiovascular diseases, obesity, diabetes, and many types of cancer. Among polyphenols, gallic acid (GA) (3,4,5-trihydroxybenzoic acid) is one of the most important polyphenols due to the fact that it occurs both in plant foods and medicinal herbs. GA is found almost in all plants, in free state or as a part of more complex molecules, such as ester derivatives or polymers. In addition, GA and its derivatives are present in every part of the plant in different concentrations, ranging from milligrams to several g/kg. In the studies of polyphenol beneficial effects, bioaccessibility and bioavailability have to be investigated, because of it is known to be highly variable. Glycosylation pattern and degree of polymerization are the main factors that influence polyphenol bioavailability. Differently from other polyphenols, GA has higher bioavailability, being adsorbed by about 70% and then excreted in the urine as 4-O-methylgallic acid. The health benefits of GA and its derivatives have been under investigation since the 1990s and many properties have been ascribed to these compounds. In scientific literature, a large body of evidence shows that GA exerts antioxidant activity, cardio-, neuro- and nephroprotective activities, and cytotoxic activity against cancer cells. Over the last few years, some investigations have tried also to explain the GA mechanisms of action via epigenetic modulation. The most recent literature data on the health protective activities of GA and its derivatives will be reported, explaining the main mechanisms of action.

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# PL04- Microorganisms as sources of biobased colorants. Going deep into the rainbow of colorful fungi, yeasts, microalgae and bacteria

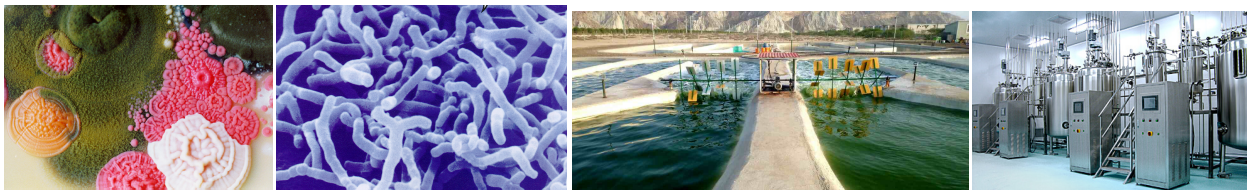
Dufossé L.[1]*

*laurent.dufosse@univ-reunion.fr

[1]*Université de La Réunion, Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, ESIROI Agroalimentaire, F-97490 Sainte-Clotilde, Ile de La Réunion, Indian Ocean, France.*

Ingredients derived from microbial fermentation or extracted from microalgae are steadily gaining ground in the food industries *(the food industry being taken here as an example of industrial application, among many others)*. Thickening or gelling agents (e.g. polysaccharides such as xanthan, curdlan, gellan), flavour enhancers (yeast hydrolysate, monosodium glutamate), polyunsaturated fatty acids (PUFAs), flavour compounds (gamma-decalactone, diacetyl, methyl-ketones), vitamins, essential amino acids, and acidulants (lactic acid, citric acid) are some examples illustrating this trend of the biobased economy. Efforts have been made and continue to be done in order to reduce the production costs of pigments produced by algal ponds and microbial fermentation, since synthetic pigments or those extracted from natural plant sources can often be produced more economically. The successful marketing of natural pigments such as β-carotene, lutein, and astaxanthin derived from microalgae (i.e. non-conventional sources) or extracted from flowering plants (conventional sources), both as food colorants and nutritional supplements, reflects the presence and importance of niche markets in which consumers are willing to pay a premium for 'natural healthy ingredients'. Among other non-conventional sources, filamentous fungi and bacteria are known to produce an extraordinary range of pigments that include several chemical classes such as carotenoids, melanins, azaphilones, flavins, phenazines, quinones, and more specifically, monascins, violacein, and indigo. The success of any class of pigment produced by fermentation depends on its acceptance by the consumers, regulatory approval, and the capital investment required bringing the product onto the market. Twenty-three years ago, influential representatives from food industry expressed doubts about the successful commercialization of microalgae-derived and fermented food grade pigments due to the high investment required for open ponds, photo-bioreactors and fermentation facilities, and the extensive and lengthy toxicity studies required by the regulatory authorities. Poor public perception of fungal-derived products for food use had also to be taken into account. Nowadays, some microbial and algal food grade pigments obtained by fermentation are existing on the market worldwide. Among them, fungal *Monascus* pigments, Arpink red™ (now Natural Red™) produced by *Penicillium oxalicum*, microalgal phycocyanin from *Arthrospira (Spirulina) platensis*, riboflavin from the mold fungus *Ashbya gossypii*, lycopene and β -carotene from the tropical mold *Blakeslea trispora*, β-carotene from the microalgae *Dunaliella salina*, and astaxanthin from the bacterium *Paracoccus carotinifaciens* and microalgae *Haematococcus pluvialis,* respectively. As an example, the production yield of β-carotene may be as high as 17g/L of the *Blakeslea trispora* culture medium. Based on proprietary research works conducted on carotenoids and azaphilones for 20 years in our laboratory, the talk will emphasize the crucial role that microorganisms and microalgae are currently playing and are likely to continue to play in

future as microbial cell factories for the production of food grade pigments and biobased colourants in general. This is due to the versatility in their pigment colour and chemical profile, amenability for easy large-scale cultivation, and a long history of production by well-investigated production strains.



# PL05- Carotenoides en especies y variedades de mamey sapote (*Pouteria* Sp [Sapoteaceae])

Murillo E.[1]*, Molinar E.[1]
*emurillo29@hotmail.com

[1]*Laboratorio de Bioquímica de Alimentos y Nutrición, Universidad de Panamá, Panamá.*

## Introducción

Mamey sapote, es el nombre común del fruto de tres especies del género pouteria; *Pouteria sapota, Pouteria viridis* y *Pouteria fossicola*. Estas especies, originarias de México y América central, se cultivan en Florida y el Caribe y son consideradas de alto valor económico. Aunque los frutos de las tres especies poseen características morfológicas que permiten identificarlas, el color de sus pulpas es muy similar (naranja-rojo). Recientemente demostramos, que el color rojo-naranja, de la pulpa de *Pouteria sapota* se debe a la presencia de carotenoides con terminación ceto K, algunos con estructuras novedosas (1). En este estudio, se identifican, por primera vez, los carotenoides de *Pouteria fossicola y Pouteria viridis.*

## Materiales y Métodos

Los carotenoides de los frutos frescos fueron extraídos y saponificados siguiendo los procedimientos recomendados por Britton (2). Los carotenoides de los extractos, fueron separados e identificados por HPLC-DAD utilizando un Cromatógrafo líquido Agilent 1100, equipado con columna C30.

## Resultados y Discusión

El análisis de los carotenoides de tres variedades de *Pouteria viridis*, diez variedades de *Pouteria fossícola* y quince variedades de *Pouteria sapota*, demostró que cerca del 96% de los carotenoides, de las tres especies son epóxidos de β-caroteno, criptoxantina y zeaxantina (amarillos) y carotenoides con anillo K (rojos). Metabólicamente, los carotenoides con anillo k se derivan de 5,6-epóxidos, en una reacción catalizada por la enzima capsanthin-capsorrubin sintasa (3). No se observaron diferencias en los perfiles de carotenoides entre las especies de mamey sapote.

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# ORAL COMMUNICATIONS

# OC01- Geographical discrimination of Italian and Tunisian herbs and spices by multi-element analysis

Di Bella G.[1*], Lo Turco V.[1], Potortì A.G.[1], Cicero N.[1], Ben Mansour H. [2], Saitta M[1]. Dugo G.[1]
*gdibella@unime.it

[1] *BioMorf Department, University of Messina, Messina, Italy.*
[2] *Research Unit of Analysis and Process Applied to Environmental –APAE UR17ES32 Higher Institute of Applied Sciences and Technology Mahdia, University of Monastir, Monastir, Tunisia.*

## Introduction

Numerous researches, published in recent years on food traceability, report that it is possible related various food products to their geographical origins developing quali/quantitative methods focused on chemical composition and applying chemometric tools to obtained results [1-4]. The present study has three objectives: (1) to establish the mineral contents in Italian and Tunisian spices and aromatic herbs to evaluate possible similarities and/or differences according to their botanical origin; (2) to apply the PCA chemometric technique to results obtained from spectrometric analysis of digested samples to evaluate if it is possible related the analyzed spices and aromatic herbs to their geographical origins; (3) to evaluate the quality and safety related to intake of these food products.

## Method

Spices (black pepper, caraway and coriander) and aromatic herbs (fennel, laurel, mint, oregano, rosemary, thyme and verbena) collected from Italy and Tunisia in 2017 year were evaluated as to their K, Ca, Mg, Mn, Na, Fe, Cu, Zn, Cr, Ni, Co, Hg, Pb, Cd, Se, and As contents by inductively coupled plasma-mass spectrometry (ICP-MS), after wet digestion by microwave system. After method validation according to EURACHEM criteria, a total of 118 samples were analyzed.

## Results / Discussion / Conclusion

The concentrations of all elements varied visibly among the spices and aromatic herbs studied. Potassium, Ca, Mg and Ni were low in laurel and rosemary; mint and thyme showed the highest Na and the lowest Se contents; arsenic and Cd levels were found highest in verbena which had also the lowest Hg content; lastly, black pepper had the highest Mn and the lowest Pb contents.
Then, the analytes concentrations were subjected to chemometric evaluation in an attempt to classify samples according to geographical origin based on their mineral concentrations. Statistical results highlight that for each spices and aromatic herbs under analysis, Italian and Tunisian samples can be split-up into two groups.
Quality evaluation, carried out according to recommended values, showed that the essential elements content was very small. Also, toxic trace element risk assessment was performed using benchmark levels. Although lead mean values were higher than maximum allowable levels for mint and thyme samples of both geographical origins and for rosemary and verbena samples from Tunisia, the achieved data indicate that Pb daily intake level reaches a maximum of 11.36% of the

protection limit. The daily intake levels for all the other toxic elements far below the tolerable intake levels. Therefore, the analyzed spices and aromatic herbs did not present any threat to consumers.

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# OC02- Evaluation of new natural ingredients for innovative functional food

Corradini C.[1,2*], Carà D.[1], Cavazza A.[1,2], Grimaldi M.[1]

*claudio.corradini@unipr.it

[1]*Department of Chemistry, Life Sciences and Environmental Sustainibility, Parma University,, Parco Area delle Scienze 17/A, 43124, Parma, Italy.*
[2]*HMI-High Materials Innovation, spin-off Parma University, Parco Area delle Scienze, 17/a, 43124 Parma, Italy*

## Introduction

The demand for nutraceuticals and functional foods is continually increasing. Consumer attention is mainly focused on health claims related to the protection from oxidative damage including all physiological conditions related to oxidative stress, as well as foods having probiotic and or prebiotic as health claims. Fructans are of growing interest as functional food ingredients because they are non-digestible carbohydrates having potential benefits effect, improving the intestinal flora, especially the bifidobacteria intestinal conditions[1-2.]

Our study was focused on the characterization of fructans composition and the main compounds responsible of antioxidant activity in vegetable such as artichoke, onion, and wild thistle.

## Method

Carbohydrate fractions were analysed by high performance anionic exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD), while phenolic compounds by reversed phase chromatography equipped with UV/DAD and mass spectrometry. Folin-Ciocalteau assay was also used for total phenolic content assessment, and Oxitest reactor allowed the evaluation of the antioxidant power by measuring the oxidative stability of a model matrix enriched with the vegetable extracts.

## Results and Discussion

For all vegetables considered, the investigation was carried out separately on different edible portions such as leaves and stems. The analysed samples were a rich source of bioactive phenolic compounds and fibre of fructans type.

The first part of the work was aimed at setting up the optimum conditions to obtain rich extracts. Details regarding experimental conditions can be found in the poster presentation of A. Cavazza and co-workers.

The oligo and polysaccharide fraction of fructans was characterized by HPAEC-PAD. The induced oxidative stability of the investigated vegetables and their by-products was carried out by Oxitest measures, which can be correlated to their antioxidant capacity. Results will be presented and discussed.

Furthermore, our study has been extended to characterize a series of new edible films, registered with the trademark FAIFF®, which have been prepared including extracts obtained from the characterized vegetables and proposed to food industry either as functional ingredients or as nutraceuticals.

This study includes the preparation of active packaging, as well as to realise biodegradable, functionalized biopolymers as promising applications to develop innovative packaging, nutraceuticals, as well as functional food products.

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# OC03- Biofortification of pasta and bread with fatty acid extracted from purslane

Di Stefano V.[1*], Pagliaro A.[2,3], Bognanni R.[2,3], Scandurra S.[3], Avellone G.[1], Melilli M. G.[3]
*vita.distefano@unipa.it

[1]*PhD/ University of Palermo -Department of Biological, Chemical, and Pharmaceutical Science and Technology (STEBICEF) - Via Archirafi 32-90039 Palermo, Italy.*
[2]*PhD/ National Council of Research, Institute for Agricultural and Forest Systems in the Mediterranean – Via Empedocle 58, 95128 Catania, Italy.*
[3]*PhD/ University of Foggia, Department of Agricultural Sciences, Food and Environment Via Napoli 25 – 71122 Foggia, Italy.*

## Introduction

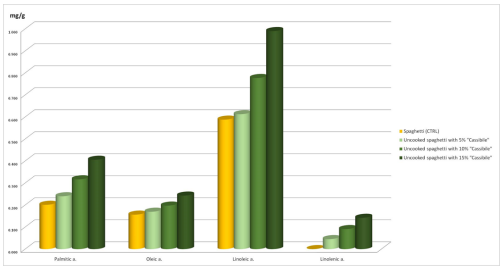
*Portulaca oleracea* L. (purslane) is an annual herbaceous plant distributed in tropical and subtropical areas[1]. In the Mediterranean regions the aerial parts of the plant can be eaten raw or cooked. Purslane possess renowned antioxidant properties, mainly acting as free radical scavenger, metal quencher and lipid peroxidation inhibitor. These effects are mainly attributed to its phenolic constituents and several fatty acids, which promote the optimum cardiovascular function, mainly by acting as anti-inflammatories and anti-nociceptive, and furthermore they could reduce the risk of human cancer[2]. Thus, purslane is a potentially valuable healthy food source and its daily consumption should be highly recommended[3,4]. In this paper, we refer about the quality characteristics of spaghetti pasta and bread produced adding different percentages of dry purslane.

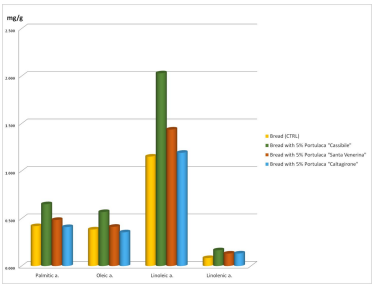
## Method

Over two years (2016-2017), three populations of wild purslane (Caltagirone, Cassibile and S.Venerina) were harvested in their sites of origin of Eastern Sicily. Plant dry material was characterized for FAMEs composition and added to flours at 5, 10 and 15% of substitution to obtain fresh pasta (spaghetti) and bread (unit of 250 g). On produced pasta (row and cooked) and bread sensory analyses evaluation (panel test scale 1-9; threshold of acceptability 5.0) and FAMEs characterization by GC/MS were performed.

## Results / Discussion / Conclusion

Among FAMEs, on averaged for populations and years of collection, the most abundant fatty acids were linoleic and linolenic acids, with 0.76± 0.26 and 0.83± 0.27 mg/g, respectively. Results about uncooked and cooked pasta with 5, 10, 15% of purslane from Caltagirone, Cassibile and Santa Venerina showed positive scores (>5.0) of Global T & O and of Global judgment. On the contrary, for the bread the panel test revealed only positive scores of Global T & O and of Global judgment at 5 % of substitution. The concentrations of FAMEs in cooked pasta is well maintained in all samples. In bread samples the substitution is well maintained only at 5%. The results showed that wild populations of dry purslane material could be used to fortify pasta and bread acting on optimum cardiovascular function.

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# OC04- Experimental NMR strategy for a data throughput toward a complete nutritional labelling of olive oil samples

Rotondo A.[1*], Salvo A.[1], La Torre G. L.[1], Mangano V. [1], Anchesi I.[1], Saitta M.[1], Dugo G.[1]
*arotondo@unime.it

[1]*Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali (BIOMORF) – Università degli Studi di Messina, Sez. SASTAS Polo Annunziata, Cittadella Universitaria Viale Annunziata, 98158 Messina*

## Introduction

The powerful features of the NMR analyses on olive oils is well established since decades ago[1] and is due to: a) lack of chemical treatments, b) constant instrumental outcome (good reproducibility), c) quick acquisition of a great amount of data, d) straightforward quantification based on a constant chemical-physical record depending just on the magnetic momentum of nuclei, this is making quantitative measurements consistent in the absolute sense[2]. For the detection of less represented compounds, the assessed sensitivity limitations, imposed by the dynamic range, can be overcome by specific strategies based on selective gradient spin-echo experiments[3] or multi pre-saturated experiments[4]. Most of the olive oil NMR studies exploit multi-variate statistical analyses in order to cluster homologous distinctive groups (according to olive kind, cultivar, soil, location, etc.); on another hand, from a scientific point of view, olive oil is an homogeneous mixture of many compounds which have to be characterized and quantified. On the basis of this last point we have focused our efforts to develop a combined protocol of three relatively quick experiments able to fit together according to an algorithm invented by us, issuing a final chemical panel for any sample (labels).

## Method

The experiments on a high resolution 500MHz NMR machine were: a) standard $^{1}$H-NMR spectrum; b) DPFGSE selective $^{1}$H-NMR to explore the aldehydic region 8-10 ppm; c) $^{13}$C-NMR experiment. These experiments are in some way complementary as the $^{1}$H experiment is very sensitive, the DPFGSE spectrum is specific and sensitive for the most important polyphenols in the olive oil (Fig.1), and the $^{13}$C, despite its intrinsic lower sensitivity reveals a surprising selectivity and efficiency for the most important fatty esters and squalene. All these data are handled by a specific algorithm procedure based on the theoretical NMR principles.

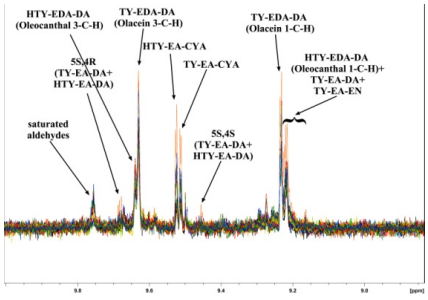


**Fig. 1**: $^{1}$H-DPFGSE spectrum at 500 MHz with the assignment of tyrosol and hydroxyl tyrosol derivate with aldehydic or dialdehydic (DA) groups

## Conclusion

The specific data throughput provides a coherent chemical composition corresponding to different olive oils, beyond the comparable qualitative profiles, samples are clearly distinguished by the quantitative compositions.

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# OC05- Levels of heavy metals in muscle and digestive gland of *Octopus vulgaris* from the southern Tyrrhenian Sea

Ariano A.[1*], Smaldone G.[1], Zaccaroni A.[2], Marrone R.[1], Campani E.[1], Anastasio A.[1], Severino L.[1]
*andrea.ariano@unina.it

[1]*University of Naples Federico II, Department of Veterinary Medicine and Animal Production, via Delpino 1, 80137, Naples, Italy*
[2]*Department of Veterinary Sciences, University of Bologna, Italy*

## Introduction

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 non-essential elements. In general, feeding is considered the primary pathway for trace element bioaccumulation in cephalopods and second seawater. Furthermore *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 and digestive gland of *Octopus vulgaris* collected in two different sites from the southern Tyrrhenian Sea in Italy and to assess the health risk related to human consumption.

## Method

Samples (n.42) of *Octopus vulgaris* were caught along the coast of the southern Tyrrhenian Sea between May and October 2017, in two sites of Campania region, at Napoli and Castellammare di Stabia. Weight, mantle length and sex were determined for each individual. Organisms were stored in individual plastic bags and immediately frozen and kept at -25°C until dissection. The muscle from arm and mantle and the digestive gland from each animal were individually separated and homogenized by means of a laboratory mixer. Aliquots of each sample (0.50 ± 0.02 g) were digested in 7 ml of ultrapure 65% $HNO_3$ and 3 ml of 30% $H_2O_2$ in a microwave digestion system (Milestone). The final volume was obtained by adding ultrapure milliQ water. Metal concentrations in the digested samples were determined with an atomic absorption spectrometer (Analyst 600, Perkin- Elmer).

## Results / Discussion / Conclusion

Mean concentrations of heavy metals in sample of muscle and digestive gland of *Octopus vulgaris* were summarized in Table 1. Data are expressed as the mean concentration of elements with standard deviations (SD).

All samples of digestive gland of octopus showed the highest Cd and Pb concentration, confirming the primary role of this district in the bioaccumulation and detoxification processes of Cd and Pb and confirming the presence of these metals at both sampling areas. No statistical differences were reported for Cd, Pb and Hg concentrations in digestive gland of octopus between *Site A* and *Site B*. Levels of Cd and Hg were very low in all samples of octopus muscle and were

below the legal limit for human consumption. In contrast, average concentration of Pb was generally high in samples of muscle from site B (0.537±0.668 mg/kg w.w.) and were above the maximum concentration level of 0.3 mg/kg excluding this product to human consumption (Reg CE 1881/2006). Results showed significantly higher concentration of Pb in muscle of octopus in *site B* than *site A* ($P < 0.05$). No statistical differences were reported for Cd and Hg concentrations in muscle of octopus between *Site A* and *Site B*. To establish possible human health implications related to consumption of octopus, the Pb estimate weekly intakes (EWI) were subsequently compared with the provisional tolerable weekly intake (PTWI) of 25 μg/kg of body weight. Considering the level of Pb, the consumption of octopus muscle from site B 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 results obtained in the current study showed the presence of Pb and Cd, 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 metals in these areas.

**Table 1.** Mean concentration (mg/kg w.w.) of heavy metals muscle and in digestive gland of *Octopus vulgaris* from Campania coast and their SD.

| Species | Sampling sites | Muscle | | | Digestive gland | | |
|---|---|---|---|---|---|---|---|
| | | Cd | Pb | Hg | Cd | Pb | Hg |
| *Octopus vulgaris* | Site A (Napoli) n=21 | 0.012± 0.029 | 0.046± 0.069 | <0.003 | 2.969± 4.902 | 1.063± 1.647 | 0.101± 0.188 |
| | Site B (Castellammare di Stabia) n=21 | 0.007± 0.015 | 0.537± 0.668 | 0.011± 0.032 | 2.643± 3.801 | 1.874± 2.015 | 0.040± 0.091 |

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# OC06- Plasticizers and BPA in spices and aromatic herbs of Mediterranean areas

Lo Turco V.[1*], Di Bella G.[1], Potortì A.G.[1], Ben Mansour H. [2], Saitta M.[1], Dugo G.[1]
*vloturco@unime.it

[1] *BioMorf Department, University of Messina, Messina, Italy.*
[2] *Research Unit of Analysis and Process Applied to Environmental –APAE UR17ES32 Higher Institute of Applied Sciences and Technology Mahdia, University of Monastir, Monastir, Tunisia.*

## Introduction

Spices and aromatic herbs have always used worldwide to flavor dishes. Several researches about their beneficial effects for human health, linked to their antioxidative activity, can be found in literature, whereas about plasticizer and BPA residues only a limited number of studies are available[1-2]. Today, the migration from plastic packing are considered as among the major sources of human exposure to these contaminants[3-4], which however could also resulted from pollutants of the marine and terrestrial environment and/or agriculture practice[5]. Plasticizers and BPA are considered endocrine disruptors[6], for this reason various risk assessments of plasticizers have been performed[7-11]. This research is carried out in order to characterize the actual contamination status in spices and aromatic herbs from Mediterranean areas by plasticizers and BPA on the belief that these contaminants are ubiquitous environmental pollutants. Also, their dietary intake based on herbs and spices consumption is evaluated.

## Method

Spices (black pepper, caraway and coriander) and aromatic herbs (fennel, laurel, mint, oregano, rosemary, thyme and verbena) collected in 2017-2018 years from Italy (n=53), Tunisia (n=65) and Algeria (*n=26*) were evaluated as to their plasticizers and BPA contents by GC-MS after solid phase extraction and purification. The analyses were carried out a Shimadzu GC-2010 gas chromatograph, dedicated to these particular analyses only. Analytes were separated in temperature programmed on a Supelco SPB-5MS capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness). The acquisition was executed in full scan and in selective ion monitoring (SIM). Three characteristic mass fragments for each analyte were used.

## Results / Discussion / Conclusion

DMP, DiBA, DBA, DPrP, BB, DPhP and BPA residues were lower than their LOQ in all analyzed samples. Algerian samples seem to contain fewer residues then Italian and Tunisian samples, precisely only DMA, DBP, DcHeP were found at concentration $\leq 0.40$ $\mu g \cdot g^{-1}$ whereas DEHS is always $\leq 1.23$ $\mu g \cdot g^{-1}$. Among the Italian samples, only aromatic herbs, precisely mint, oregano, and laurel, were contaminated by DMA, DEP, DiBP, DBP, DEHP, DEHT and DEHS. In particular, residues higher than 1 $\mu g \cdot g^{-1}$ were detected only in mint (DiBP) and in oregano (DEHS). In general, all Tunisian samples showed at last five plasticizers residues: the caraway, among the spices, and the rosemary, among the aromatic herbs, are found to contain more residues. DEHP and DEHT were

found in all types of samples at concentration next to 1 $\mu g \cdot g^{-1}$; also DMA was always found but constantly lower than 0.5 $\mu g \cdot g^{-1}$. DEHS was detected only in caraway, coriander, oregano e rosemary but with values higher than others. All other phthalates and adipates were rarely detected. Intake of these contaminants by spices and aromatic herbs under analysis seems not to constitute a dangerous risk to the consumers.

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# OC07- Valorisation of Sicilian quality agro-food products

Ristuccia M.E.[1]

[1]*Co.Ri.Bi.A. Consorzio di Ricerca sul Rischio Biologico in Agricoltura- via Gino Marinuzzi 3, 90129, Palermo, Italy*

## Introduction

For typical products, the territorial component represents the fundamental element of differentiation and therefore the main lever on which to act. The first aspect that qualifies a territory is that which refers to strictly environmental variables (climatic, pedological characteristics and so on). Sometimes the food product acts as an attractor for the valorization of the territory; in other times, it's the territory to be used as an enrichment of the image of the food product, for the purpose of its valorization[1]. Starting from the indissoluble link between agro-food products and the territory, the territorial marketing is a tool that allows to enhance the image of the territory also through the promotion of some of these products. In the food product is contained a piece of the territory as well as the typical food product is an essential component of a territory [1].To demonstrate this, the evolution and development of the agricultural sector in Sicily have always been characterized by a close connection with the territory, having regard to the tradition associated with it, indeed in various sicilian territories are produced valuable local products, some of which are marked by quality trademarks and others recognized as traditional products and others not adequately valued. The attention of consumers is increasingly oriented towards a healthy lifestyle, so having products related to the land that produces them, genuine and traced, is a guarantee to the demand for quality and food safety.

## Method

Agriculture is the economic activity that is most organized and adapts to the environmental characteristics of a territory; so, products with quality trademarks, and all the others that it produces, represent the identity card, their showcase, and, consequently, promoting them, means to promote the territory that produces them.

The pilot project envisaged the creation of a network of products coming from the 9 territories (former provinces) of Sicily to facilitate access to consumption through knowledge. A group of companies voluntarily involved showing motivation of the entrepreneur, his interest and his will to connect to the initiatives to promote the territory, and through their involvement, their market experience, and through the study activity of Co.Ri.Bi.A., has been implemented a pilot way to give the consumer a better visibility and accessibility of typical products present in the regional territory.

## Results / Discussion / Conclusion

The meeting between demand and offer does not always necessarily take place within the market context2. By comparing the demand side with the offer profile, there is a real risk that the information coming from market may once again be misaligned between producers and consumers". So, it's necessary to give a contribution to a widespread dissemination of the culture of traditional/tipical products in consumers and producers.

The pilot idea involved the creation of structured web pages in a simple and intuitive way to immediately capture the attention of stakeholders. One of the weaknesses, in fact, detected during a meeting with the companies is to prepare the consumer for purchase by educating him to distinguish a typical product from the traditional one. Therefore the construction of a website aims to train and help the consumer to make knowledge-based purchase; it highlights information about country, historical information, seasonality, nutritional and health aspect.
A broader knowledge-based attitude of consumers and producers could expand the market for the typical / traditional Sicilian food chain, thus improving the ability of the food system to innovate with the diffusion of sustainable dynamics in the food system.

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# OC08- Use of phycobiliproteins from Atacama cyanobacteria as a food colorant

Galetović A.[1*], Gallardo V.[2], Graves R.[2], Seura F.[2], Sanzana S.[2], Vega M.[2], Gómez-Silva B.[1]
*alexandra.galetovic@uantof.cl

[1]*Laboratorio de Bioquímica, Depto. Biomédico, Facultad Ciencias de la Salud y Centre for Biotechnology and Bioengineering (CeBiB), Universidad de Antofagasta, Avenida Angamos N° 601, Antofagasta, Chile.*
[2]*Departamento Ciencia de los Alimentos y Nutrición, Universidad de Antofagasta/ Avenida Angamos N° 601, Antofagasta, Chile.*

## Introduction

Phycobiliproteins (PBP) of cyanobacteria are colored water-soluble proteins covalently bound to phycobilins and are part of the photosynthetic apparatus. PBP (phycoerythrin PE, phycocyanin PC and allophycocyanin APC) have antioxidant and anti-inflammatory properties in animals. Currently, the interest for natural pigments in the food industry has grown to replace artificial dyes for alternative natural colorants. Our objective was to evaluate the stability of PBP from Andean cyanobacteria versus different pH and temperature values, in order to elaborate a functional dairy product.

## Method

Two cyanobacteria (CAQ-15 and LLC-10) isolated from wetlands at the Andean highlands were grown and collected at their exponential phase of growth. The cells were disrupted by ultrasound, the homogenate was centrifuged and the supernatant containing PBP was recovered. The pigments PE and PC were separated by ammonium sulfate precipitation (0-35% and 35-60% saturation, respectively). After dialysis, each PBP extract was loaded on a DEAE column to optimize their purification and the PE and PC fractions were collected and freeze-dried. Chemical stability of each PBP solution was tested in a range of pH (1-14) and temperature (0-80°C) by observing the changes on their absorption maxima. Functional food products were prepared adding PE or PC to skim milk to a final concentration of 30-140 mg% w/v. Also, a sensory test was carried out for each skim milk preparation containing PBP by ten untrained volunteers, using a hedonic scale.

## Results/Discussion/Conclusion

PBP purified from two Andean cyanobacteria showed chemical stability under pH 5 to 8, and temperatures between 0°C to 50 °C. The highest score at the sensory test was obtained by skim milk fortified with PE. Thus, a functional food based on skim milk containing cyanobacterial PBP can be considered a reasonable alternative in replacement to added artificial colorants. (Grants: SI-5305, Universidad de Antofagasta, Chile; CeBiB F-0001, CONICYT, Chile)

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# OC09- Seasonal trend of Anisakidae infestation in south Mediterranean bluefish

Cammilleri G. [1*], Costa A.[1], Graci S. [1], Collura R. [1], Glaviano A. [1], Buscemi M. D. [1], Giangrosso G. [1], Pulvirenti A. [2], Ferrantelli V. [1]
*gaetano.cammilleri86@gmail.com

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", via Gino Marinuzzi 3, 90129, Palermo, Italy.*
[2]*Dipartimento di Scienze della Vita, Università degli studi di Modena e Reggio Emilia, via Università 124, 41121 Modena, Italy*

## Introduction

The nematodes belonging to the Anisakidae family are parasite of interest for human health due to their high zoonotic potential. These nematodes if ingested alive with raw fish can cause Anisakiasis, a zoonoses which can compromise the entire gastro-intestinal tract, with several symptoms such as nausea, vomiting and gastroesophageal reflux. A recent EFSA report (2010) urges agencies involved in food safety to get more information about the biology and ecology of these organisms, in order to implement the prevention tools for consumer's protection. Anchovies are one of the most infested fishes by Anisakidae larvae. Furthermore, fish belonging to this species are prevalently consumed uncooked (marinades). In this study it was evaluated the seasonal trend of Anisakidae infestation in anchovies, in order to assess a possible correlation between fish ecology and infestation degree. cinquecentododici

## Method

A total of 1352 anchovies (*Engraulis encrasicolus*), sardines (*Sardina pilchardus*), mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*) and silver scabbard fish (*Lepidopus caudatus*) samples were analyzed for Anisakidae larvae detection by visual inspection. The sampling was carried out during the whole 2015, in order to obtain a balanced number of samples for each month. The collected larvae were subjected to morphological (by optical microscopy) and molecular (by RFLP-PCR method) investigation in order to confirm the belonging to the Anisakidae family.

## Results / Discussion / Conclusion

Five hundred and twelve fish samples showed the presence of Anisakidae larvae, with infestation prevalence between 9% (sardines) and 100% (silver scabbardfish). The infestation prevalence was divided monthly. The results showed peak of the prevalence values during the summer season for sardines and anchovies and during spring for mackerels and horse mackerels. A bimodal infestation trend was found for the silver scabbard fish samples. The Kruskal-Wallis test showed a significant difference in the seasonal trend of infestation ($p<0.05$). This significant difference can be attributed to the ecology of the fish species examined. During the cold season *Engraulis encrasicolus* inhabits the deeper parts of the water column and reducing its predatory activity. During the reproductive period in summer, anchovies move up to the water column and intensify their predatory activity increasing the chance of ingesting Anisakidae larvae intermediate hosts of

their life cycle as copepods and other crustaceans. The results of this work could be useful for a comprehensive overview of the ecology of anisakid nematodes and to give more instruction for the fishing of the fish species cited above

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# OC10- Fatty acid profile of dried chili seed oil (*Capsicum annum*)

Téllez-Vizcaya S.Y.[1], Vázquez-Arriaga O.[2], Guadarrama-Lezama A.Y.[1], Colín-Cruz M.A.[1]*, *macolinc@uaemex.mx

[1] *Universidad Autónoma del Estado de México, Facultad de Química, Paseo Colón S/N, Toluca, Edo. de México, México, C.P. 50120*

[2] *Instituto Nacional de Investigaciones Nucleares. Carretera México-Toluca s/n, Ocoyoacac, Edo. de México, México C.P. 52750*

## Introduction

Chili has been part of the basic diet of Mexico for more than 5000 years[1]. Archaeological discoveries estimate that this fruit was cultivated since 7000 a. C. in the southeast and northwest of Mexico[2]. *Capsicum annuum* variety is the most cultivated in the world and the most consumed in Mexico, either fresh or dry. The seeds of dried chili have been consumed since prehispanic times and are used to prepare traditional Mexican dishes. Results of a previous study showed that chilli seeds have a high nutritional value[3]. The objective of this study was to identify the fatty acids content in four varieties of *C. annuum* seeds oil in order to know their nutritional value.

## Method

The seeds of 4 varieties of widely consumed *C. annuum* (Ancho, Guajillo, Mulato and Pasilla) were physicochemicaly analized: moisture, ash, protein, fat. For protein determination, the seeds were treated as follows: oven dried at 45 ° C for 48 h, whole and ground; warm distilled water washing, sunny and oven drying, 4 h and 45 ° C for 48 h, respectively and ground. Protein was also determined in the seed without treatment. The oil was extracted from the seeds with solvents (Soxhlet technique). Different extraction times were evaluated (3h 26 min, 2h 25 min and 1h 24 min), as well as two methylation ways (in oil and seeds). The fatty acid composition was determined by gas chromatography coupled to mass spectrometer.

## Results/Discussion/Conclusion

The moisture content ranged 6.6 to 7.7%, the ashes 3.0 to 3.42%, both without significant differences ($p < 0.05$) among varieties. The protein content ranged 16.9 to 17.81%, without significant differences; but in dried seeds protein content increased 1% in all the varieties. In the Ancho variety fat content was 21.29%, 20.31% in Guajillo, 20.67 in Mulato % and 20.22% in Pasilla; and there was a significant difference among the Ancho, Mulato and Pasilla varieties. No significant difference was found between the fatty acids content identified in the seed and in the oil, nor among the chili varieties. The fatty acid profile of the four *Capsicum* varieties is shown in the table.

| Fatty acid | | Fatty acids methyl esters % (SD) | | | |
|---|---|---|---|---|---|
| | | **Mulato** | **Guajillo** | **Ancho** | **Pasilla** |
| Palmitic | **C16:0** | 13.86 (±1.05) [a] | 14.03 (±0.28) [a] | 13.51 (±0.35) [a] | 16.0 (±0.52) [a] |
| Palmitoleic | **C16:1** | 0.24 (±0.08) [a] | 0.32 (±0.05) [a] | 0.26 (±0.05) [a] | 0.33 (±0.04) [a] |
| Stearic | **C18:0** | 3.96 (±0.34) [a] | 3.90 (±0.20) [a] | 3.69 (±0.53) [a] | 4.45 (±0.18) [a] |
| Oleic | **C18:1** | 12.31 (±0.53) [a] | 11.4 (±0.86) [a] | 12.43 (±0.94) [a] | 12.81 (±0.13) [a] |
| Vaccenic | **C18:1** | 1.49 (±0.12) [a] | 1.49 (±0.19) [a] | 1.53 (±0.57) [a] | 1.62 (±0.04) [a] |
| Linoleic | **C18:2** | 67.94 (±1.98) [a] | 68.68 (±0.83) [a] | 68.14 (±1.70) [a] | 64.55 (±0.23) [a] |
| Linolenic | **C18:3** | 0.22 (±0.03) [a] | 0.23 (±0.05) [a] | 0.26 (±0.10) [a] | 0.26 (±0.01) [a] |
| Arachidic | **C20:0** | ND | ND | 0.3 | ND |

ND= not detected

[ab] means with different superscripts in a row differ significantly ($p < 0.05$), ($n = 3$).

Cheul *et al*. (2010) reported similar results for fatty acids in the red pepper seed oil variety of *C. annuum*:13.7% palmitic, 70.63% linoleic and 0.40% arachidic[4]. Results showed that fatty acids content in the four varieties of *C. annuum* seeds oil are a good source of ω3 (0.24%), 6 (67.32%) and 9 (12.23%). As a food source, chilli seeds are valuable because of its protein, fat and fiber content. Furthermore, they have a cultural importance.

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# OC11- Development of value added products from agricultural food waste

Gervasi T.[1,2*], Pellizzeri V. [1,2], Cicero N.[1,2], Dugo G.[1,2]
*tgervasi@unime.it

[1]*Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy.*
[2]*Science4Life, Spin Off Company, University of Messina, Italy.*

## Introduction

In food processing industries, up to 30 % of incoming raw materials becomes waste rather than value-added products. Waste remaining after the production of citrus juice production are known to contain significant amounts of biomolecules with interesting nutritional and biological proprieties. Citrus presents various possibilities of biomass utilization. This biomass is rich in bioflavonoids, insoluble and soluble fibers. Starting from a research focused on the use of dry spray technology on food matrices, the method has been applied to the formulation of new functional foods, a probiotic fruit juice powder, starting from food waste.
In the current industrial landscape, the use of spray dry is an innovative and cheaper technique. The application of this technology allows, in fact, to transform liquid food products into dry powder of high quality and at low cost.

## Method

The study started by investigating strategies for the biomolecules recovery from citrus waste. The separation of soluble fiber and bioflavonoids from insoluble fiber was obtained by heating it up to ebullition under vigorous stirring, the mixture was filtered in order to separate soluble fiber and bioflavonoids from insoluble fiber. Bioflavonoids were desorbed on polymer resin the soluble fiber was concentered by heating it up to ebullition and dehydrated by spray dry, the insoluble fiber was dehydrated by dryer. In the second step of the study, soluble fibers, represented by pectins, were used as drying agent in spray drying process.
The orange juice was prepared as follow, total soluble solids content of the juice was adjusted to 1.5% (w/v), the drying agent obtained from citrus waste, the pectins, was added to the juice at 2% (w/v), the probiotic microorganisms were added to juice in in numbers about $10^8$-$10^8$. The drying of orange juice with each probiotic culture and pectins was carried out in a pilot-scale Spray Dryer. The solutions, stirred continuously at room temperature , were fed into the chamber at a flow rate of 25 mL/min and at inlet air temperature and outlet temperature ere adjustedto 150 °C. and 70 °C respectively. The dried powders were collected in a single cyclone air separator system. Three replicates were conducted for each experiment.

## Results and Discussion

In this work, the formulation of value-added products by using ingredients obtained from agro industrial waste and by implementing new technologies with low environmental impact and high economic sustainability was investigated

Starting from a research focused on the use of dry spray technology on food matrices, the method has been applied to the formulation of “green powder” by using biomolecules obtained from citrus peel waste.

The study of the formulation of "green powders" was obtained, therefore, thanks to the implementation of spray dry technology. Specifically, the project allowed the production of powdered juices with a recovery of 92% of Vit C, probiotic microorganisms and prebiotic substances. represented by the drying agent ,the pectin, obtained from citrus waste.The implementation of the spry dry technology, in correlation with other pre-treatment used for some waste products, could allow the recovery of bioactive molecules from waste that could be redirected to food, nutraceutical and cosmetic fields. The “green powders" project is therefore focused towards the support of applied research and technological innovation, that is aimed at economic development, technological implementation and product innovation in the field of functional biomolecules, and, mostly, in the one of industrial by-products recovery.

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# OC12- Comprehensive two-dimensional liquid chromatography in food and natural products analysis

Cacciola F.[1*], Dugo P.[2,3,4], Mondello L.[2,3,4]
*cacciolaf@unime.it

[1]*Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, University of Messina, Via Consolare Valeria, 98125 Messina, Italy*
[2]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, Polo Annunziata, Viale Annunziata, 98168 Messina, Italy*
[3]*Chromaleont s.r.l., c/o Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali", University of Messina, Polo Annunziata, viale Annunziata, 98168 Messina, Italy*
[4]*Department of Medicine, University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy*

## Introduction

Chemistry of food and natural products is continuously involved in the assessment of quality and authenticity, with a special focus on the characterization of molecules with a possible beneficial effect (nutraceuticals) or a toxic effect on human health. In this context analytical methods should be capable to allow the determination of the main components of food and natural products samples, but can also be selective and sensitive enough to determine minor components.

## Method

In this contribution, comprehensive two-dimensional liquid chromatography (LC×LC), emerged in the last two decades as an interesting alternative to analyze complex samples, was employed. The LC×LC technique involves the combination of two or more independent or nearly independent separation steps, increasing significantly the separation power of the corresponding one-dimensional liquid chromatography (1D-LC) techniques. Partially porous reversed-phase columns were employed in the second dimension, in combination with photodiode array and triple quadrupole mass spectrometry (LC×LC-PDA-MS/MS) detection.

## Results / Discussion / Conclusion

The performance of conventional, full-in-fraction and shifted secondary gradients were compared, in the latter case allowing to effectively reduce the co-elutions occurring in conventional 1D-LC. Further, the selectivity and sensitivity of the multiple reaction monitoring operation made "target" analyte quantification more robust, as demonstrated for the determination of a selected polyphenols in the food and natural product samples investigated.

**Acknowledgments**: The research was performed within the context of the project AGER2-Rif 2016-0169 "Valorizzazione dei prodotti italiani derivanti dall'oliva attraverso tecniche analitiche innovative".

# OC13- The aroma of Sicilian red garlic of Nubia as affected by drying methods

Cincotta F.[1*], Tripodi G.[1], Merlino M.[1], Verzera A.[1], Condurso C.[1]

*fabcincotta@unime.it

[1]*University of Messina, Department of Veterinary Sciences, Polo Universitario dell'Annunziata, 98168, Messina, Italy.*

## Introduction

Garlic (*Allium sativum L.*) and garlic supplements are one of the oldest food flavoring also used for centuries in folk medicine for its antioxidant, anti-inflammatory, antimutagenic, antihypertensive effects[1]. These biological activities can be mainly recognized to many organosulphur compounds deriving by S-(2-propenyl)-1-cysteine-S-oxide due to the enzyme allinase. Nubia garlic is traditionally cultivated in the western Sicily (South Italy), mainly within the municipality of Paceco, in the Integral Natural Reserve of Saline. Despite its valuable quality, Nubia garlic is mainly traded as fresh product on the local market. However, the manufacturing of processed garlic products could rise new perspectives for its valorization by reaching markets far from the places of production. The aim of the research was to assess the effects of two different drying methods, namely hot air- and microwave-drying, on the quality and the amount of aroma volatile compounds, with particular attention to those exhibiting a biological activity.

## Materials and Method

Fresh red garlic of Nubia bulbs, harvested during the 2017 crop season, were supplied by a local producer in May 2017 and used in the experiments. Volatile aroma compounds were determined both on fresh and dried samples. Dried samples were obtained using: 1) a tray dryer at 70 °C per 60 min; 2) a microwave oven at 400 W per 3 min. Volatiles have been analyzed by Headspace-Solid-Phase-Microextraction (HS-SPME), using a 50/30-µm film thickness DVB/CAR/PDMS fiber, coupled with a Gas-Chromatograph-Triple Quadrupole Mass Spectrometer (Shimadzu GC 2010 Plus-TQMS 8040). Each volatile compound was identified using mass spectral data, NIST' 17 (NIST/ EPA/NIH Mass Spectra Library, version 2.3, USA) and FFNSC 3.0 (Shimadzu) database, linear retention indices (LRI), literature data and the injection of standards were available.

## Results / Discussion / Conclusion

More than one hundred volatile compounds were identified, mainly sulfur compounds followed by aldehydes, alcohols, terpenes and esters. Qualitative and quantitative differences resulted among fresh, air-dried and microwave-dried samples. Diallyl disulfide was the most abundant compound in all samples, even if in a different amount. The dehydration processes determined an increase of the total amount of monosulfides, trisulfides, tetrasulphides and of almost all the classes of cyclic sulphur containing compounds. Opposite, the total amount of disulfides decreased after dehydration; in particular, diallyl disulfide and allyl-(*E*)-1-propenyl disulfide, generating by the action of allinase on allicine, resulted the main volatile compounds in fresh samples. In dried

samples, diallyl sulfide, diallyl trisulfide and diallyl tetrasufide, 1,2-dithiolane, 2-vinyl-4H-1,3-dithiin, 3-vinyl-4H-1,2-dithiin and 5-methyl-1,2,3,4-tetrathiane prevailed. They are considered key compounds for the garlic aroma and their anti-cancer activity against prostate epithelial cells has been dimostrated[2]. These compounds also showed beneficial effect on diabetes mellitus, anti-microbial, antiviral, anti-inflammatory and cardiovascular effect[3]. The amount of trisulfides, tetrasulfides, thiophenes, thiolanes and of the cyclic octaatomic sulfur compound resulted statistically higher in the microwave-dried samples if compared with the air-dried ones. Since it has been demonstrated that the pharmacological activity of allium sulphur compounds increase when the number of sulphur atom increase[4], the microwave drying method could be a more efficient technology than hot air-drying not only for reducing the processing time but also for enhancing the benefic health effects of garlic.

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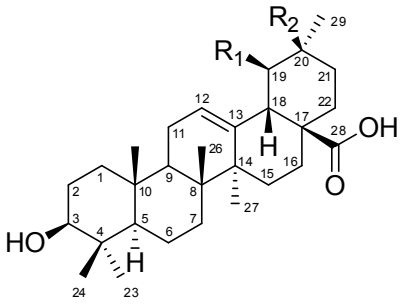
# OC14- Evaluation of NF-κB involvement in the cytotoxicity of oleanolic and ursolic acid semisynthetic derivatives toward hepatocellular carcinoma

Fontana G.[1], Bruno M.[1], Notarbartolo M.[1], Labbozzetta M.[1], Poma P.[1], Spinella A.[2], Rosselli S.[3]
*sergio.rosselli@unipa.it

*1Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Viale delle Scienze, ed. 17, I-90128 Palermo, Italy.*
*[2] Centro Grandi Apparecchiature (CGA) – ATeN Center, University of Palermo, via F. Marini 14, 90128, Palermo, Italy.*
*[3] Dipartimento di Scienze Agrarie, Alimentari e Forestali (SAAF), Università degli Studi di Palermo, Viale delle Scienze, ed. 4, I-90128 Palermo, Italy*

## Introduction

3β-hydroxy-urs-12-en-28-oic acid, known with the common name of ursolic acid (UA) and 3β-hydroxy-olean-12-en-28-oic acid, known as oleanolic acid (OA) are ubiquitous pentacyclic ursane- and oleanane-type triterpenes that possess the same functional groups. The only difference between ursane and oleanane carbon framework lies in the position of 30-methyl ($R_1$ in UA and $R_2$ in OA, see figure). Both compounds were shown to possess several interesting biological properties, such as anti-inflammatory[1], antimicrobial[2], antidiabetic activities[3]. Ursolic acid is present in a large number of vegetables popularly known for their supposed tumor-preventive properties such as apples and cranberry[4] while OA can enter into a normal daily diet through the consumption of other putative cancer-preventive foods, such as berry fruits[5] and olives (fruit and oil)[6].




**UA** $R_1$ = Me $R_2$ = H
**OA** $R_1$ = H $R_2$ = Me

## Method

From 1.0 Kg of *Olea europea* leaves, after extraction by Soxhlet apparatus using EtOAc as solvent, 1.30 g (0.13 %) of pure OA were obtained.after purification by repeated column chromatographies.
2.0 Kg of *Malus domestica* yielded 1.45 g (0.07 %) of pure UA applying the same isolation procedure as above. The pure triterpenoic acids were used as starting material for simple chemical transformations.

## Results / Discussion / Conclusion

A set of derivatives of the two compounds with a modified oxidation state and lipophylicity at C-3 and C-28 positions, were prepared and tested as anticancer agents versus the lines HepG2, Hep3B and HA22T/VGH of hepatocarcinoma, a strongly aggressive tumor that is not responsive toward the standard therapies. Two molecules containing a three carbons side chain on the C-3 position were synthetized in both stereoisomeric forms by the Barbier-Grignard procedure and were found to be active toward all of the three targets. The implication of the κB nuclear factor in the

mechanism of action was assessed for the more active compounds in the set, as HCC cyto-types are known to overexpress NF-κB.

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# OC15- *In vivo* and *in vitro* toxicity of a bioactive *Cyrtocarpa procera* methanol extract

Gomez-Chang E.[1*], López-Bañuelos L.[2], Galot-Linaldi J.[2], Estrada-Muñiz E[2]., Vega L.[2], Romero I.[1].
*ergoc@bq.unam.mx

[1]*Universidad Nacional Autónoma de México, Facultad de Medicina, Departamento de Bioquímica. Av. Universidad 3000, Coyoacán, Ciudad de México, CP 04510, México.*
[2]*Instituto Politécnico Nacional. Centro de Investigación y Estudios Avanzados. Departamento de Toxicología. Av. IPN, 2508, San Pedro Zacatenco, Ciudad de México, CP 07360, México.*

## Introduction

Previous studies have suggested the preclinical potential of the methanol extract of *Cyrtocarpa procera* bark (CpMet) as a candidate for the development of an integral therapy for the treatment of gastritis and gastric ulcers. The CpMet seems to display polypharmacological effects, such as anti-*Helicobacter pylori* activity in *vitro*, moderate anti-inflammatory activity, high gastroprotective activity and a remarkable capacity to achieve a significant increase in the histological quality of resolution of gastric ulcers in murine models[1,2]. Taking into account that phytopharmaceuticals are very popular among populations as important alternatives to allopathic medicines, it is imperative to assure the safety of these products, besides studying their quality and efficacy, as it has been promoted by the World Health Organization's Traditional Medicine Strategy 2014-2023. In respect thereof, the objective of this research was to determine the possible toxic effects associated to CpMet.

## Method

*In vivo* acute oral CpMet toxicity (LD50) was determined by Lorke's method[3]. Moreover, acute intraperitoneal (i.p.) and intravenous (i.v.) CpMet toxicity was assessed following the dosing procedure recommended by the OECD guidelines related to the Acute Oral Toxicity -Up-and-Down- Procedure[4]. *In vitro* CpMet cytotoxicity was tested in normal human peripheral blood mononuclear cells (PBMC), as well as in 5 transformed cell lines (HeLa, HaCaT, AGS, WRL68, and 4T1), by the MTT reduction assay.

## Results / Discussion / Conclusion

Following the Lorke's protocol, the oral LD50 obtained was > 5000 mg/kg, a value that is regarded as non-toxic. The LD50 values obtained by the i.v. and i.p. administration routes were 9.92 mg/kg, and 144.91 mg/kg, respectively. Both LD50 values are considered as highly and moderately toxic for the i.v. and i.p. routes[5], respectively. Concerning the MTT assay, after a 48-hour incubation period of PBMC in the presence of CpMet, the IC50 was of 313.4 μg/ml. In the case of the other tested cell lines, the IC50 value was very similar between themselves (IC50 mean = 177 ± 25.9 μg/ml).

Seen as a whole, the results generated with respect to the acute toxicity assessment indicate that, the exposure route is an important factor that influences the level of toxicity. In this context, we conclude that CpMet orally administered seems to be non-toxic, and this result affords practical guidance for selecting safe doses that might be used in further clinical trials. Regarding *in vitro* cytotoxicity assays, it appears that CpMet mainly targets cells with high proliferation rates. This apparent selectivity of the extract against highly proliferative cells, opens up the potential of studying its effects as an antineoplastic agent, however further studies should be done to explore this approach.

**Acknowledgements.** This work was partially supported by DGAPA-PAPIIT IN214317.

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# OC16- Preparation of highly functionalized himachalanes by oxidation of longipinene derivatives

Chacón-Morales P.A.[1,2*], Amaro-Luis J.M.[2]
*pablo.chaconm@ug.edu.ec

[1]*PhD/ Faculty of Chemical Sciences, University of Guayaquil, Ciudadela Universitaria Av. Delta y Av. Kennedy s/n, C. P. 090112, Guayaquil, Ecuador*
[2]*PhD/ Natural Products Laboratory, Department of Chemistry, Faculty of Science, University of Los Andes, Av. Alberto Carnevalli La Hechicera, C.P. 5101, Mérida, Venezuela*

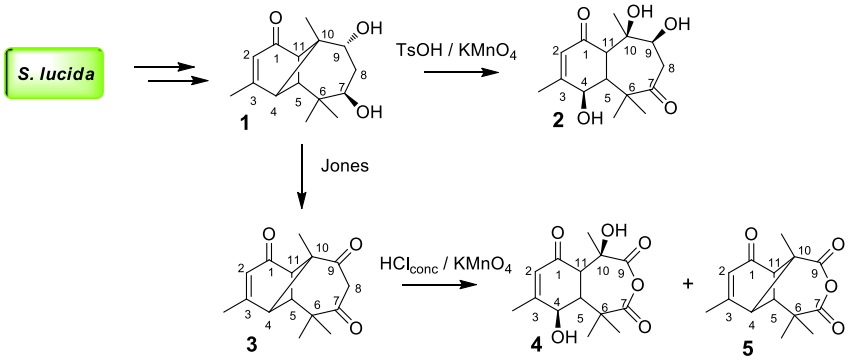
## Introduction

The coleopter insects are included in the family Chrysomelidae, they are important plagues of the plants of *Phaseolus* genus (beans productors). There are several species of Chrysomélidos, specifically of the subfamily Galerucinae (*Andrector arcuatus*, *A. ruficornis*, *Gynandrobrotica equestris*, etc.) that attack beans, used as edible in the basic diet of the Latin American people[1]. In several studies, it has been determined that the sesquiterpene derivatives of himachalane type are a pheromonic component of *Phyllotreta cruciferae*, *Aphthona flava* and other species of Chrysomelidae[2], for this reason, the synthesis and functionalization of himachalane derivatives may have applications in the pesticide industry[3]. In connection with the above, the present paper describes the synthesis of hydroxylated derivatives of himachalane, using as starting material tricyclic sesquiterpenes of the longipinene series.

## Method

Dichloromethane extract of dried uncrushed leaves and stems of *Stevia lucida* Lag. (Asteraceae), which it was highly rich in complex mixtures of 7b,9a-dihydroxylongipinene diesters, was hydrolyzed with KOH / MeOH by boiling under reflux for 30 minutes in order to obtain the corresponding diol [(4*R*,5*S*,7*R*,9*R*,10*R*,11*R*)-7,9-dihydroxylongipin-2-en-1-one **1**]. Treatment of diol **1** with a mixture of $KMnO_4$ / TsOH led to the formation of 4,9,10-trihydroxyhimachalan-2-en-1,7-dione **2**. The oxidation of **1** with Jones reagent produced the 7,9-dioxolongipin-2-en-1-one **3**. The trione **3** was treated with $KMnO_4$ / $HCl_{conc}$, to generate the anhydride **4** in mixture with the anhydride **5**. The compound **4** was identified as 8-*nor*-4,10-dihydroxyhimachalan-2-en-1,7-dione (**4**). The compounds **2**, **3** and **4** were separated and purified by preparative chromatography using silica gel plates. These compounds were identified by IR and NMR (1D and 2D).

## Results / Discussion / Conclusion




General Scheme

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# OC17- Natural and semisynthetic polymer blended oral fast dissolving films of citalopram

Shahzad Y.[1*], Maqbool M.[2], Hussain T.[1], Yousaf A.M.[1], Mahmood T.[2]
*yshahzad@ciitlahore.edu.pk

[1]*PhD/Department of Pharmacy, COMSATS Institute of Information Technology, Lahore, Pakistan.*
[2]*PhD/Faculty of Pharmacy, University of Central Punjab, Lahore, Pakistan.*

## Introduction

The use of natural polysaccharides has gained considerable attention in pharmaceutical industry for the preparation of various dosage forms such as topical gels, emulsion and matrix tablets[1] (Ghori et al., 2014). Due to their availability, biocompatibility, biodegradability and non-toxicity, polysaccharides are capable to compete with synthetic and semi-synthetic polymers[2] (Emeje et al., 2011). More recently, oral fast dissolving films have attracted interest because of various advantages such as improved patient compliance especially for the patients having difficulty in swallowing the tablets, a condition known as dysphagia[3].

## Method

Fast dissolving films were prepared by dissolving the ingredients in 10 mL of distilled water, as given in Table 1. Ludiflash as superdisintegrant, citric acid as salivary agent and fructose as sweetener, and PEG 400 as plasticizer was used in the specified quantities. Finally, the homogenous mixture was casted on a petri dish having 9 cm diameter. The mixture was dried by placing petri dish in hot air oven at 45 °C for 24 hrs. The films were carefully removed from petri dishes and cut into 2×2 $cm^2$ size to deliver a 10 mg equivalent dose of the citalopram HBr. The prepared films were extensively characterized for physical, mechanical and solid-state properties.

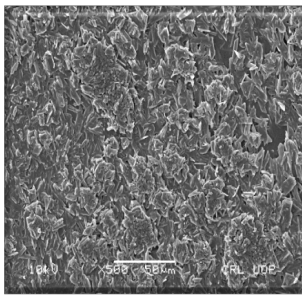
**Table 1:** Composition of blended films

| Formulation | HPMC K15 (mg) | HPMC K4M (mg) | Okra gum (mg) | Pullulan (mg) | Moringa gum (mg) | Citalopram (mg) | Ludiflash (mg) | Citric acid (mg) | Fructose (mg) | PEG 400 (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| **F1** | 360 | 320 | - | - | - | 160 | 80 | 80 | 80 | 320 |
| **F2** | 320 | - | 128 | | - | 160 | 80 | 80 | 80 | 320 |
| **F3** | - | - | 128 | 400 | - | 160 | 80 | 80 | 80 | 320 |
| **F4** | 160 | - | - | - | 400 | 160 | 80 | 80 | 80 | 320 |

## Results / Discussion / Conclusion

The prepared films showed excellent physical, mechanical and solid-state properties, as summarized in the table and figure below. IR spectroscopy revealed no interactions while X-ray diffraction revealed amorphous nature of drug in the films.

| **Formul-ations** | **Thickness (mm)** | **Weight variations (mg)** | **Disintegration time (sec)** | **%age drug content** | **Folding endurance** | **Tensile strength** ($N/mm^2$) |
|---|---|---|---|---|---|---|
| F1 | 0.18 ± 0.03 | 65 ± 0.02 | 19 | 98.90 ± 0.06 | 220 ± 2.31 | 2.6 |
| F2 | 0.18 ± 0.02 | 61 ± 0.05 | 25 | 97.87 ± 0.03 | 222 ± 4.51 | 4.3 |
| F3 | 0.19 ± 0.02 | 68 ± 0.04 | 11 | 102.05 ± 0.02 | 240 ± 1.99 | 4.6 |
| F4 | 0.21 ± 0.01 | 73 ± 0.03 | 18 | 99.95 ± 0.05 | 230 ± 2.00 | 1.8 |



It was noteworthy that combination of okra gum with HPMC K15 and okra gum with pullulan produced films with highest tensile strength, thus endorsing their suitability as potential citalopram oral fast dissolving films.

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# OC18- The influence of surfactant and cosurfactant in the formation of liquid crystals containing gemfibrozil

Alonso-Durán J.[1], Cañarte-Soledispa P.A.[2], Lilibeth G.[2], Pibaque D.[2], Ferrín García D.G.[2], Kolenyak-Santos F[2,3*]
*fernandakolenyak@outlook.com

*[1]MSc/, Universidad Estadual Paulista, UNESP, Instituto de Química Departamento de Química Analítica. Rua Profesor Francisco Degni, Araraquara, São Paulo, Brasil, C.P. 14800-060.*
*[2]MSc/Facultad de Ciencias Químicas, Av. Delta y Av. Kennedy, Universidad de Guayaquil, C. P. 471, Guayaquil, Ecuador.*
*[3]PhD/Universidad Estadual Paulista, UNESP, Programa de Nanotecnología Farmacéutica, Facultad de Ciencias Farmacéuticas, Departamento de Fármacos y Medicamentos Rodovia Araraquara-Jaú, Araraquara, São Paulo, C.P.14800-903.*

## Introduction

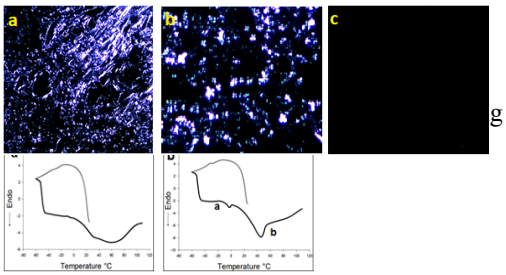
Hypercholesterolemia is a disorder caused by increased plasma levels of cholesterol of low density lipoprotein (LDL), Gemfibrozil (GMF) is an anti-lipid agent, a lipid regulator derived from fibric acid, which reduces triglycerides in the blood, although widely used, gemfibrozil (GMF) has some characteristics that limit its use, such as poor solubility in water. The aim of this work was evaluated the influence of surfactant and cosurfactant in the formation of liquid crystals containing gemfibrozil (GMF).

## Method

The phase diagram was developed with the mixture of oleic acid, tween 80® and water Milli Q®. All the sample the samples were maintained at 25 ± 0.1 °C for 24 h to complete system equilibration. The liquid crystals (LC31) were characterized by Polarized light microscopy, Texture profile analysis (TPA), Thermal Analysis Differential Scattering Calorimetry (DSC).

## Results / Discussion / Conclusion

**Fig. 1** Polarized light microscopy




g Calorimetry (DSC).

**Table 1** Texture profile analysis (TPA)

| Sample | Hardness | Compressibility | Adhesiveness | Cohesiveness |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **LC 807** | 0.0209± 0,006284 | 107.1025± 2.43022 | 36.8625± 5.59 | 98.83898± 0.413958 |
| **LCGFZ-807** | * | * | * | * |

*It was not possible to collect these data.

It was possible to obtaining hexagonal (Fig.1 a) lamellar (Fig. 1b) phases (LC-807) incorporation of the GFZ (LC-GFZ-807) led to the presence of a black field (Fig. 1c), which can be indicative of microemulsions. DSC analysis showed an interaction of the drug with the liquid crystal (Fig 2a) due the emergence of a new fusion event and the drug fusion occurred in a distinct temperature and non-crystalline material (Fig 2b) due absence of peak. GFZ decreased the values of hardness and compressibility for all sample (Table 1) with can indicate greater fluidity. The results are confirmed by the values of adhesiveness with can indicate that the system does not need force to compress. The high values of cohesiveness may indicate that the sample does not break during the compression. These systems can be potential carriers for GFZ administration, providing potential advantages over conventional pharmaceutical forms.

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# OC19- Cyanidin-3-O-glucoside ameliorates palmitate-induced inflammation and insulin resistance in 3T3-L1 hypertrophic adipocytes

Molonia M.S[1]., Muscarà C.[1,2], Bashllari R.[1], Cristani M.[1], Saija A.[1], Cimino F.[1]; Speciale A.[1] , *mmolonia@unime.it

[1]*Dept. of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;*
[2]*Foundation Antonio Imbesi Messina, Italy;*

## Introduction

Obesity, one of the most common worldwide health disease, is a metabolic disorder correlated with an elevated morbidity and mortality rates, and increased risk of numerous pathologies, such as cardiovascular diseases and type 2 diabetes (Kaufer *et al*., 2001). It is associated with increased free fatty acids accumulation, alteration of adipokine levels and adaptive immune system that promote lipotoxicity and cause oxidative stress, inflammation, and insulin resistance in adipose tissue (McLaughlin *et al*., 2014). Several studies suggest that anthocyanins, natural polyphenols commonly present in food and vegetables from Mediterranean Diet, exert significant cardiovascular health-promoting effects probably through the modulation of specific cellular signalling pathways (Cimino *et al*., 2013). The aim of this work was to evaluate the *in vitro* protective effect exerted by cyanidin-3-*O*-glucoside (C3G), a widely distributed anthocyanin, on inflammation and insulin resistance induced by Palmitic Acid (PA) in mouse adipocytes (3T3-L1).

## Method

Fully differentiated 3T3-L1 adipocytes were pretreated with different concentrations of C3G (5-10μM) for 24 h and then exposed to high concentrations of PA (1 mM) for 24 h in order to induce cellular hypertrophy. To evaluate the insulin-resistance condition, cells were subsequently treated with insulin 100 nM. NF-kB and insulin pathways were evaluated by means of Western blot and Real-time PCR techniques.

## Results / Discussion /Conclusion

Our results confirmed that PA was able to induce NF-κB proinflammatory pathway through the activation of IKK and the increase of nuclear accumulation of p65/NF-κB. Furthermore, PA induced mRNA up-regulation of NF-κB–modulated proinflammatory cytokines, such as IL-6 and TNF-α. Interestingly, C3G pretreatment was able to prevent the activation of NF-κB pathway and downstream cytokines mRNA levels. Moreover, PA induced insulin-resistance by the specific impairment of insulin IRS1/P3K/Akt signaling pathway, followed by a downstream reduction of the glucose transporter GLUT-1 and adiponectin. Interestingly, C3G pretreatment effectively reversed the effects of PA on IRS1/PI3K/Akt axis. In particular, we observed that C3G restored AdipoQ and GLUT1 expression altered by PA so improving insulin sensibility from hypertrophic adipocytes. These findings demonstrate that C3G ameliorates inflammation and insulin-resistance conditions induced by PA, thus suggesting new potential roles for this molecule in the prevention and treatment of pathological conditions linked to obesity.

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# OC20- Phytochemical composition and biological activities of essential oils from two *Cannabis sativa* L. biotypes

Smeriglio A.[1,2], Denaro M.[1,2], Alloisio S.[3], Cornara L.[4], Grassi G.[5], Trombetta D.[1], Circosta C.[1]
asmeriglio@unime.it

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, V.le SS. Annunziata, 98168 Messina, Italy;*
[2]*Foundation Prof. A. Imbesi, University of Messina, Italy;*
[3]*ETT Spa, via Sestri 37, 16154 Genova, Italy;*
[4]*Department of Earth, Environment and Life Sciences, University of Genova, Italy;* [5]*CRA-Research Institute for Industrial Crops (ISCI), 45100 Rovigo, Italy.*

## Introduction

*Cannabis sativa* L. (Cannabaceae) has been an important source of food, fiber, dietary oil and medicine for thousands of years in Europe, Asia and Africa[1,2]. It also contains an essential oil composed primarily of terpenoids also if some phytocannabinoids may be present[3]. It seems likely that differences in terpenoids composition of essential oil could be responsible of distinctive medicinal properties of different *Cannabis* biotypes[4]. The aim of this study was to examine the essential oils (EOs) composition of two different *C. sativa* biotypes, evaluating their antioxidant and anti-acetylcholinesterase properties as well as the effects on Nervous System by means of microelectrode arrays (MEAs) electrophysiology.

## Materials and Methods

EOs of dried flowering tops of a new Chinese accession (G-309) and one *fibrante* variety of the *C. sativa* with low $\Delta^9$-THC content (< 0.2%) were extracted by hydro-distillation with a Clevenger-type apparatus according to the European Pharmacopoeia. After that, the volatile fractions were characterized by GC-FID and GC-MS analyses and their antioxidant and anti-acetylcholinesterase properties investigated by *in vitro* assays. In addition, the ability of EOs to inhibit spontaneous electrical activity on Human iPSC-derived CNS.4U® neurons and astrocytes growing on MEAs was evaluated. Samples of dried flowering tops from both *C. sativa* biotypes were observed by scanning electron microscopy (SEM) in order to highlight any micromorphological differences.

## Results and Discussions

The quali-quantitative GC analysis of both EOs showed that in the Chinese accession EO, sesquiterpenes represent the most abundant class (58.06 %) followed by sesquiterpene oxygenated (23.48 %), phytocannabinoids (8.57 %), monoterpenes (8.52 %), monoterpene oxygenated (0.17 %) and other compounds (1.19 %). Major compounds include Caryophyllene (20.98 %), alfa-Bisabolol (15.41 %), Caryophyllene oxide (6.98 %), γ-Catinene (6.23 %); $\Delta^9$-Tetrahydrocannabivarin (THCV) is the most representative (6 %) of non-psychotropic phytocannabinoids. In the *C. sativa var. fibrante* EO, sesquiterpenes also represent the most abundant class (67. 92 %) followed by sesquiterpene oxygenated (17.93 %), monoterpenes (6.20 %), phytocannabinoids (3.84 %), monoterpenes oxygenated (0.60 %) and others (3.51 %).

However, a substantial difference in the relative abundance of the main compounds between the two EOs investigated was detected. In fact, the major compounds of *C. sativa var. fibrante* EO include Caryophyllene (43.44 %), α-Caryophyllene (16.50 %), Caryophyllene oxide (15.87 %) and (-)-trans-Cannabidiol (3.52 %).

Both EOs showed a remarkable antioxidant and free-radical scavenging activity with the following order of potency: ORAC > TEAC > Folin-Ciocalteu > β-carotene bleaching > DPPH > FRAP for Chinese accession and β-carotene bleaching > ORAC > TEAC >DPPH > FRAP > Folin-Ciocalteu for *var. fibrante*, respectively. The *C. sativa* EOs exhibited also significant inhibition of acetylcholinesterase activity with $IC_{50}$ values of 74.64 µg/mL (C.L.= 61.705-90.286) and 57.307 µg/mL (C.L.=29.544-111.158) for Chinese accession and *var. fibrante* respectively. Furthermore, both EOs induced a concentration dependent inhibition of spontaneous electrical activity and, in particular, the *fibrante* variety was the most effective one in provoking a rapid block of it. The different degree of biological activities observed suggesting that this could be mainly ascribed to the different EOs terpenoids composition. Furthermore, the SEM analysis of both *Cannabis* biotypes showed micromorphological differences. In particular, on bracteoles of Chinese accession are present, other than long, multicellular, stalked glandular trichomes, small glandular trichomes with bicellular head and unicellular stalk. Furthermore, many cystolithic trichomes with the characteristic bear claw shape on Chinese accession leaflets were observed.

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# OC21- Hypoglicemic and hypolipedimic effects of *Spilanthes oleraceae* var *oleraceae* in streptozotocin-induced diabetic rats

Bach E.E.[1*], Hi E.B.M.[2], Martins A.M.C.R.P.F. [3], De Marco R.M.[1], D'Elle H.[4], Wadt N.S.Y.[5]
*ernabach@gmail.com

[1] *PhD/UNINOVE (Universidade Nove de Julho), Health Department, Biomedical Sciences, SP, Brasil,*
[2] *Ms/UNILUS, Experimental Biochemistry Academic Nucleum (NABEX), Santos, SP, Brazil*
[3] *PhD/Biological Institute, São Paulo, Brazil*
[4] *PhD/UNINOVE (Universidade Nove de Julho), Department of Medicin, SP, Brazil*
[5] *PhD/UNIP (Universidade Paulista), Health Sciences Institute, SP, Brasil*

## Introduction

The *Spilanthes oleracea* L., popularly known in Brazil as Jambu or Agrião-do-Para is considered a domestic vegetable used as food and cosmetic. It is used as an anesthetic, anticonvulsant, analgesic, antimicrobial agent, among others uses. In diabetic patients, the function as a diuretic may help and the objective of the present study is to evaluate the potential of Jabu extract as a glycemic controller in a model of diabetes mellitus.

## Method

Aqueous extract was made with 90g of Jambu leaves of plants with 60 days of germination for proteins and phenols analysis. Male Wistar rats (250 to 280 g) were obtained from UNINOVE vivarum with the approval of the animal ethical committee (process 20/2012). Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (50 mg/kg) in citrate buffer. The experiment involved four groups of rat; Control (non-diabetic rats); DM (diabetic rats); Control+Extract (1mL extract/200g rat); and DM+Extract (diabetic group treated with 1mL of extract). Blood glucose levels were measured 48 hours after the STZ injection, and animals were considered diabetic when blood glucose levels were, at least, 500mg/dL. The groups were evaluated for 30 days, the animals were euthanized, and blood was collected for measurement of glucose, total cholesterol, triglycerides (TAG), urea, creatinine and analysis of amino acids tryptophan (Try), methionine (Met). Blood serum was precipitated with ammonium sulfate, followed by dialysis and submitted to HPLC for amino acids analysis. Pancreas was collected for histological analysis.

## Results / Discussion / Conclusion

Aqueous extract presented 653.66 mg of protein and 568.60 mg of phenol. HPLC test was carried out to evaluate phenol presence and was observed in the extract just 2 peaks at the HTPC analysis representing caffeic acid and p-coumaric acid. Control animals and treated with the extract retained the glycemic rate varying from 89 to 94mg/dL, DM rats presented severe hyperglycemia (473 to 524 mg/dL), and with Jambu extract showed a reduced average glucose serum value (201mg/dL). Evaluating creatinine, urea and lipids, total diabetic animals manifested high rates when compared to control and treated group. DM presented creatinine concentration of 102mg/dL and TAG 101mg/dL, DM+Extract presented 66mg/dL and TAG 90mg/dL. Plasma from control

and treated rats presented Try and Met, however, these amino acids were undetectable in diabetic's rats, being that in these was detected kynurenine (Kyn) in plasma. Treatment with Jambu extract reestablished the Try and Met levels, decreasing Kyn. Analyses of the pancreatic tissue shown a loss in pancreatic islet total area in diabetic rats and Jambu extract reestablished it. The mechanism that promotes the effect may be due to the Caffeic acid found in the extract promotes glucose use to generate energy and can regulate beta cell function as well as exerts anti-degenerative effect on the islets. In conclusion aqueous extract of *S. oleraceae* ameliorates glycemic and lipidemic exacerbation in the STZ-model, pointing to an alternative treatment for diabetes mellitus. Acknowledgments: Financial Support from CNPq 474681 / 2013-0.

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# OC22- Anti-angiogenic activity of *Alnus glutinosa* (L.) Gaertn. (Betulaceae)

Germanò M.P.[1*], D'Angelo V.[1], Raimondo F.M.[2], Smeriglio A.[1,3]
germanom@unime.it

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy.*
[2] *Department STEBICEF/Section of Botany and Plant Ecology, University of Palermo, Via Archirafi 38, 90123-Palermo, Italy.*
[3]*Foundation Prof. Antonio Imbesi, University of Messina, P.zza Pugliatti 1, 98122 Messina, Italy.*

## Introduction

*Alnus glutinosa* (L.) Gaertn. (Betulaceae) (black alder, European alder) is a tree found in Europe, southeastern Asia, Caucasus mountains, and western Siberia. Many studies have shown that extracts from *A. glutinosa* bark display antimicrobial, anti-inflammatory and anticancer activities[1,2]. Considering that angiogenesis inhibition might be a promising approach for anticancer therapies and other diseases, the aim of our study was to investigate the effects of a black alder bark extract on angiogenesis.

## Method

A crude extract (80% aqueous MeOH, AGE) was obtained from the fresh bark of *A. glutinosa*[3]. The quali-quantitative analysis carried out by RP-HPLC-DAD analysis highlighted a marked presence of the diarylheptanoid oregonin (418.45 μg/mg of dry extract)[4]. This result lead us to investigate the anti-angiogenic properties of AGE and oregonin (AGE-isolated compound) by two *in vivo* models monitoring the new blood vessel formation: *Danio rerio* (zebrafish) embryo and the chick chorioallantoic membrane[5].

## Results / Discussion / Conclusion

The anti-angiogenic activity of AGE was preliminarily evaluated by the quantitative endogenous alkaline phosphatase (EAP) assay in zebrafish embryos. Results showed that treatment with AGE (50 μg/ embryo) induces a strong inhibition of vessel formation by 57% as compared to control. A marked reduction on capillary growth (56.37% of inhibition) was also observed in the microvasculature of chorioallantoic membranes (CAMs) after treatment with AGE at 100 μg/egg. In addition, the biological screening showed that the diarylheptanoid oregonin produces a good anti-angiogenic response in the CAM assay (54.02% of inhibition at 30 μg/egg).
In conclusion, based on the preliminary results here presented, the bark of black alder can be considered a valuable source of angiomodulators.

**Figure 1.** *Alnus glutinosa* (L.) Gaertn. (Betulaceae) bark (a) and oregonin (b).

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# OC23- Pistachio hull extract as source of Ideain: absorption, transport and anti-inflammatory studies on Caco-2 transwell model

Denaro M.[1,2*], Smeriglio A.[1,2], Trombetta D.[1]
*denaromarcella.md@gmail.com

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, V.le SS. Annunziata, 98168 Messina, Italy;*
[2]*Foundation Prof. A. Imbesi, University of Messina, Italy*

## Introduction

Pistachio hull (*P. vera* L., Bronte variety) is an attractive source of health-promoting compounds potentially helpful in preventing the onset of various oxidative stress-related disorders[1]. Recently we described the nutraceutical, antioxidant and cytoprotective activity of several phenol extracts of pistachio hull[1,2]. In particular, among the flavonoid class, we identify the Ideain (Cyanidin-3-*O*-galactoside) as the most abundant compound. The richness and purity of this anthocyanin, which possess remarkable antioxidant, free radical scavenging and cytoprotective properties with respect to widely used synthetic antioxidants, makes the pistachio hull a valuable raw material for nutraceutical employment[2]. However, up to date, absorption efficiency and anti-inflammatory properties of this anthocyanin are completely missing.
The aim of this study was to investigate the potential absorption and the transport mechanisms of Ideain across Caco-2 transwell model. Moreover, anti-inflammatory properties of Ideain were evaluated by lipopolysaccharide (LPS)-induced inflammation.

## Method

A pre-assay quality control of the monolayer integrity was carried out by measuring the transepithelial electrical resistance (TEER). The bidirectional transport and the effect of time (0-120 min), drug concentration, P-Glycoprotein (P-gp) inhibitor (Verapamil, 100 μM) and EDTA-$Na_2$ (tight junction modulator, 1-5 mM) on the absorption efficiency of Ideain (10 μM) were investigated. Drug concentration was monitored by RP-LC-DAD-MS instrument and the absorption efficiency was calculated. IL-8 release, post Ideain pre-treatment (10 μM, 4 h) and LPS (10 μg/ml, 20 h)-induced inflammation, was evaluated using a sandwich ELISA method. Cytotoxicity evaluation as well as post-assay quality control of the barrier system were carried out by MTT and Lucifer yellow paracellular permeability assays, respectively.

## Results / Discussion / Conclusion

Transport studies showed an Ideain absorption efficiency of about 11% according to previous investigations on other glycosylated anthocyanins in the same experimental model. Absorption efficiency observed was not related to the Ideain concentration, confirming an active transport involvement by sodium-glucose carriers (SGLT 1 and 2) across the Caco-2 cell monolayer[3]. No absorption efficiency alteration was observed after Verapamil apical treatment; on the contrary, an increase of Ideain absorption efficiency (>50%) was observed after Verapamil basolateral

treatment. This result confirms that Ideain, under physiological conditions, is expelled from the basolateral compartment by the P-gp, showing an asymmetric transport[4]. EDTA-treatment at lowest concentration (1 mM) no alters significantly the Ideain behavior while at higher concentrations (2.5-5 mM) a significant absorption efficiency decrease (about 50%) was observed. This effect could be due to hydrophobic interactions between EDTA and Ideain, which is no longer recognized by SGLTs[5]. Moreover, Ideain pre-treatment of Caco-2 cells decreases of about 20% the LPS-induced IL-8 release with respect to the control without showing any cytotoxicity or alteration of the barrier system.

In conclusion, our results show that Ideain absorbtion, likewise other glycosilated anthocyanins, is mediated by SGLTs as well as by the P-gp and that Ideain pre-treatment is able to modulate, already at pharmanutritional dose, the LPS-induced inflammation, which makes it a potentially useful molecule for nutraceutical use.

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# OC24- Phytochemical composition and antioxidant activities of *Melicoccus bijugatus* Jacq fruits

Russo D.[1], Faraone I.[1], Sinisgalli C.[1], Di Stefano M.R., Saluzzi R.[1], Bisaccia D.[1], Milella L.[1]

[1]*Dipartimento di Scienze, Università degli Studi della Basilicata, viale dell'Ateneo lucano 10, 85100 Potenza*

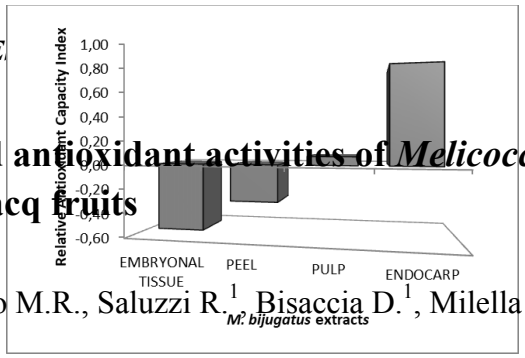


**Figure 1**. Relative Antioxidant Capacity Index of different parts of *M. bijugatus* fruit

## Introduction

*Melicoccus bijugatus* Jacq (Sapindaceae family) is a fruit species called also "mamoncillo" [1] native of South America. The ethnopharmacological use of this species varies according to the geographic region. Cuban people uses mamoncillo for the treatment of hypertension, in Nicaragua it is used to treat the stomach aches, in Puerto-Rico for constipation and respiratory problems [2, 3]. The seeds can be consumed after roasting and they are used to treat the diarrhea by the indigenous people of Columbia and Venezuela [2, 3]. Fruits are usually consumed raw or processed in jelly, pies and in fresh drinks [4]. Although the large use of the fruits, little is known about its chemical composition and antioxidant activity of different fruit parts. The aim of study was to investigate the phytochemical composition and antioxidant activities of peel, pulp, endocarp and embryonal tissue from fruits of *M. bijugatus*.

## Method

Peel, pulp, endocarp and embryonal tissue from fruits of *M. bijugatus* were extracted with methanol by maceration technique. The content of polyphenols was determined by Folin-Ciocalteu reagent, whereas antioxidant activity by using different *in vitro* assays to test radical-scavenging ability, reducing power and inhibition of lipid peroxidation; results of *in vitro* antioxidant assay were used to calculate Relative Antioxidant Capacity Index (RACI) [5, 6]. The chemical composition was analyzed by HPLC-DAD. The extract effect and the reactive oxygen species scavenging activity was also tested on cell lines.

## Results / Discussion / Conclusion

Different parts of *M. bijugatus* fruit were analyzed and the chemical composition allowed to identify hydroxybenzoic and hydroxycinnamic acids, tannins and flavonoids.

Tannin derivatives were found in all parts of the fruit, mainly in endocarp and embryonal tissue. Leaves were rich in flavonoid derivatives and hydroxycinnamic acid derivatives. Endocarp reported the highest content of polyphenols by Folin-Ciocalteu reagent and the highest radical scavenging activity and reducing power. Peel extract reported the highest lipid peroxidation inhibition. According to results, endocarp was the fruit part with the highest RACI (Fig. 1). The antioxidant activity of fruit tissues was also reported on tested cell lines.

Fruits of *M. bijugatus* can be considered as source of bioactive compounds to be applied in nutraceutical and pharmaceutical field.

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# OC25- Acute toxicity in mouse CD1 *Amphipterygium adstringens* (Cuachalalate)

Figueroa Gutiérrez A.H.[1], Montejano Almaguer B.V.[2], Isidoro González I.J.[2], Almaguer Vargas G.[3], Sánchez Arenas J.A.[4], Montejano Rodríguez J.R.[3]*
*jrmontejano@yahoo.com.mx

1 *Professor University Hidalgo State/ Medicine Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México*
2. *Bachelor student in Pharmacy at the University Hidalgo State/Pharmacy Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*
3. *Professor University Hidalgo State/ Pharmacy Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*
*4 Bachelor student in biology at the University Hidalgo State/ Center for Biological Research, Academic Area of Biology, Institute of Basic Sciences and Engineering. Highway Pachuca Tulancingo, Pachuca de Soto, Hgo.*

## Introduction

Medicinal plants are a therapeutic resource that needs to be studied, it is estimated that around 10,000 medicinal species are used in the world for medicinal purposes. In developing countries, they represent the only therapeutic resource available to the most disadvantaged sectors of this population. Due to the above, health authorities worldwide have increased their attention to herbal therapeutics considerably, since they are the only medicine available in developing countries and, they have become a popular alternative in developed countries[1].

*Amphypteryngium adstringes* is used to treat infections and cancer; popularly called cuachalalate, cuachalala or chalalate[2]. Due to the attributed properties and the popularity that this plant is reaching, we proceeded to perform acute toxicity in CD-1 mice. The objective of the present work was to carry out, through the ethanolic extract of *Amphypteryngium adstringes* (Cuachalalate), the acute toxicity in CD-1 mice using the Lorke methodology.

## Material and methods

The specimen was obtained in the market of barreteros located in the Central Colony of Pachuca Hidalgo, Mexico and its taxonomic identification was made in the Institute of Biological Sciences of UAEH. The bark of *Amphipterygium adstringens* was placed in pieces of one to two cm2, it was left to marinate at room temperature for 7 days in 70% ethanol.

For the toxicity test the ethanolic extract was started of *Amphypteryngium adstringes*, the procedure was performed according to the methodology of acute toxicity (LD50) of Lorke in CD-1 mice, the doses used due to previous studies were 1600; 2900 and 5000 mg/kg[3,6] of live weight of the ethanolic extract that were administered in batches of three animals each, which were observed 15 days and

subsequently the necropsy was performed[3]. This procedure was carried out in the UAEH bioterium. The zootechnical management and the slaughter of the animals used in this research were carried out in accordance with NOM-062-ZOO-1999[4]. Waste and handling of Infectious Biological Hazardous Waste were processed according to NOM -087- ECOL- SSA1_2002[5].

## Results

At a dose of 2800 mg/kg of weight, toxic effects were observed, the animals presented hyperpnea, tachycardia, agitation, hyperesthesia, this followed by lethargy, cyanosis, disorientation and death. Necropsy showed right ventricular hypertrophy (apple-shaped heart), hepatomegaly accompanied by hepatic congestion and hemorrhage, intestinal intussusception in the small intestine, typhlitis, gastric mucosal defacement with projection of gastric contents into the esophagus, nephritis and death in two mice with the dose of 1600 mg/kg, and with that of 2900 three animals. The LD50 corresponds to 1,743.5 mg/kg.

## Conclusions

In the present work for *Amphipterygium adstringens* it was observed that the LD50 corresponds to 1,743.5 mg/kg of live weight[6].

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# OC26- Fitochemistry and acute toxicity in Balb/C mouse of *Decatropis bicolor*

Montejano Almaguer B.V.[1], Montejano Rodríguez J.R.[2], Figueroa Gutiérrez A.H.[3], Zamudio López J.L.[1], Almaguer Vargas G.[2]*
*almaguergina@gmail.com

1. *Bachelor student in Pharmacy at the University Hidalgo State/Pharmacy Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*
2. *Professor University Hidalgo State/ Pharmacy Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*
3. *Professor University Hidalgo State/ Medicine Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*

## Introduction

It should be considered that for the population of scarce resources the use of medicinal plants more than a fashion is a necessity, for which a broader knowledge about this resource for health will originate a safer and more effective therapy that will be able to provide a better quality of treatment and lifetime. *Decatropis bicolor* is a shrub from which dried and cut leaves are popularly used to treat wounds and the decoction is used for cancer[1,2]. The study of this plant is important because *D. bicolor* leaflets are sold in markets and tianguis in the state of Hidalgo, Mexico. Therefore, the objective of this work was to perform the qualitative phytochemistry and the acute toxicity of the ethanolic extract of the leaves of *Decatropis bicolor*.

## Material and methods

The plant was collected in Ixmiquilpan Hidalgo, the roasted leaflets were macerated during 7 days in a 70% ethanol solution, evaporated at 70°C. A qualitative evaluation was carried out performing the tests of Dragendorff, Mayer, Wagner, Lieberman- Burchard, Salkowski, Rosemheim, Bortrager, Baljet, Legal, Erlich, Fehling, Benedict, Ferric Chloride, Ninhydrin, Sulfuric Acid, Shinoda, Rosemheim. The acute toxicity test was performed according to the Lorke methodology in the UAEH bioterium, using three male mice, Balb/c of 20 g for each group, the doses used were 10, 100 and 1000 mg/kg[4,5]. Subsequently necropsy of the mice was performed. The handling, care and use of the mice was carried out in accordance with Mexican federal standards NOM-062-ZOO-1999[6]. Regarding the elimination and handling of Infectious Biological Hazardous Waste was processed according to NOM -087- ECOL-SSA1_2002[7].

## Results

The qualitative preliminary phytochemistry of the ethanolic extract of *D. bicolor* leaves showed the presence of alkaloids, coumarins, flavonoids, triterpenes. At the dose toxicity of the aforementioned ethanol extract from 100 mg/kg, anxiety, open mouth, ataxia, dyspnea, abdominal breathing, cyanosis, piloerection, abdominal contractions, squeaking were observed. At the end of the 15 days of observation, the necropsy was performed and congested liver and kidney, splenomegaly and low weight were observed. When administering the dose of 1000 mg/kg, the mice showed incoordination, tachycardia, apnea and death of two mice at four hours and 24 hours

later the third died, which presented dark stools, dehydration, cyanosis, low weight. At necropsy, hepatic and renal congestion, hemorrhagic gastric mucosa, intestine with dark content, concentrated urine were observed. The LD50 was 376.22 mg/kg live weigth in this work.

## Conclusions

The preliminary phytochemistry of *D. bicolor* showed the presence of alkaloids, coumarins, treterpenoids, flavonoids. The LD50 was 376.22.mg/kg live weigth[5].

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# OC27- Mechanisms of interaction between flavonoids present in *Citrus bergamia* juice and the AMPK/SIRT-1 axis: an *in silico*, *cell-free* and *in vitro* study

Maugeri A.[1,2*], Ferlazzo N.[1], Gitto R.[1], De Luca L.[1], Navarra M.[1]
*amaugeri@unime.it

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy*
[2]*Foundation "Prof. Antonio Imbesi", Messina, Italy.*

## Introduction

Different studies demonstrated that flavonoids, secondary plant metabolites, possess noticeable biological properties, and induce significant pharmacological effects, among which some are currently exploited in common therapeutic protocols. Recently, the juice of *Citrus bergamia* (bergamot), an endemic tree of the Calabria region (Italy), has drawn the attention of the scientific community for its lipid-lowering, antioxidant, anti-inflammatory and anti-cancer activities[1,2,3]. Previous study performed by our research group indicated that the anti-inflammatory effect of a flavonoid-rich extract of bergamot juice (BJe) is mediated by the activation of SIRT-1 enzyme[4]. This is a histone deacetylase belonging to the family of human sirtuins, and it is involved in various physio-pathological processes such as cellular metabolism, inflammation, immunity and tumorigenesis[5]. In addition, numerous scientific evidences suggested that AMPK, a kinase fundamental for cellular homeostasis, plays a pivotal role in the enzymatic activation of this sirtuin[6].

## Method

Based on these observations, our attention focused on the role played by SIRT-1 in the anti-inflammatory effect of BJe and its major flavonoids in THP-1 cells exposed to lipopolysaccharide (LPS), employing computational techniques (*in silico*) as well as abiotic and *in vitro* models.

## Results / Discussion / Conclusion

Results of molecular docking analyses showed that flavonoids quantitatively most representative of BJe (neohesperidin, NHP; naringin, NRG; neoeriocitrin, NER; hesperetin, HSP; naringenin, NAR) can interact directly to SIRT-1 enzyme in two different allosteric sites: that of activators as well as that of inhibitors. In particular, NAR and HSP bind better in the site of inhibitors, whereas glycosylated flavonoids (NHP, NRG and NER) seem to possess more affinity toward the site of activators. In order to get inside their interaction with SIRT-1, the activity of these flavonoids was tested through a cell-free model, employing the isolated recombinant enzyme. Results obtained with this methodology indicated that BJe as well as each single flavonoid inhibited SIRT-1, yet with different effectiveness.

With the aim of clarifying the conflictual results obtained in the present study and those reported by Risitano and co-workers (2014)[4], we employed an *in vitro* experimental model to evaluate the role of flavonoids hereby tested in the activation/inhibition of SIRT-1. Treatment of THP-1 cells with LPS 500 ng/ml for 3 h significantly reduced SIRT-1 activity, that was hindered by the pre-

incubation (30 min) with BJe or each single flavonoid, thus indicating an indirect activation of SIRT-1 in the complex cell environment. These data were further confirmed by real-time PCR analysis. Through the employment of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and dorsomorphin, AMPK activator and inhibitor, respectively, we could highlight the involvement of this kinase in the activation of SIRT-1 induced by flavonoids of BJe in the entire cell.

In conclusion, the results of our study demonstrated that BJe, along with its major flavonoids are able to activate or inhibit SIRT-1 depending to the model employed. Moreover, in the whole cell, we showed that their activity is mediated by AMPK. Our study represents an advance in the knowledge on the anti-inflammatory activity of BJe.

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# OC28- Reduction of atherosclerotic lesions in ApoE KO mice treated with lycopene extracted from Sicilian tomatoes

Irrera N.[1], Mannino F.[1], Pizzino G.[1], Pallio G.[1], Squadrito F.[1], Altavilla D.[2], Bitto A.[1]

[1]*Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy.*
[2]*Department of Biomedical, Dental, Morphological and Functional Sciences, University of Messina, Messina, Italy.*

## Introduction

Lycopene is a carotenoid found in tomatoes with a potent antioxidant and anti-inflammatory activity. The Mediterranean diet is particularly rich in lycopene with well known beneficial effects on cardiovascular health. So, the aim of this study was to evaluate lycopene effects in an experimental model of atherosclerosis.

## Methods

Lycopene was extracted from Sicilian tomatoes (that have shown the highest concentration of lycopene per gram) and its effect was evaluated in ApoE knock out mice fed with a high fat western diet for 14 weeks. Lycopene was administered by an oral suspension every day from week 3 to week 14, at the human equivalent dose of 60 mg/day (0.264mg/mouse/day) which was previously reported to be effective in a clinical trial. Body weight, food intake, cholesterol and triglyceride levels were recorded every week; thoracic aorta, liver, and blood were collected at the end of the experiment.

## Results

Lycopene supplementation reduced the extent of the atherosclerotic plaques and also triglycerides and cholesterol levels. Lycopene mechanism of action was related to Nuclear factor erythroid-2-related factor 2 activation, as demonstrated by immunoistochemistry in aortic sections. PPAR-alpha and SREBP-1 mRNA expression was significantly affected by lycopene supplementation ($p<0.05$ vs untreated ApoE mice) in liver and an increased expression of the AMPK-alpha kinase was observed by western blot analysis, thus demonstrating that animals treated with lycopene probably had an increased cell metabolism ($p<0.05$ vs untreated ApoE mice).

## Conclusions

These results further support the hypothesis that lycopene extracts might be used to reduce atherosclerosis.

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# OC29- Application of batch and simulated moving bed chromatography for food and natural products processing

Luebbert M.*
*luebbert@knauer.net

*PhD/KNAUER Wissenschaftliche Geraete GmbH, Hegauer Weg 38, 14163 Berlin, Germany*

## Introduction

Chromatography is an established purification technology for chemicals, pharmaceutical ingredients, and biomolecules. While operating costs are relatively high (usage of solvents and stationary phase, evaporation of solvent from product), the wide range of mobile and stationary phases as well as the mild operating conditions make chromatography a very flexible tool for a wide range of purification tasks.
Simulated Moving Bed chromatography (SMB) is an implementation of chromatography that allows a fully continuous feed supply and product recovery. It is widely used in sugar separations, i.e. for the separation of fructose and glucose.

## Technology and Applications

Starting from the physical/chemical background, the principles and technical implementation of chromatographic are shown. An additional focus is set towards applications. Examples from sugar, ω-3 fatty acids and vitamins are shown. Systems from micro-scale laboratory units to multi-ton bulk purification are available on the market.

## Conclusion

Due to their flexible operating modes, batch and continuous chromatography are excellent tools for the purification of bulk to high-value products. Their high selectivity and efficiency can even turn waste streams into valuable products.

# OC30- Use of phytotherapics in wounds skin healing at SUS

Wadt N.S.Y.[1]*, Palomo B.R.[2], Lima L.F.[2], Soares V.L.S.O.[2], Trindade B.L.[3], Batista E.R.N.[3], Wadt M.[1], Cavalini F.[4]
*nilwadt@gmail.com

*1 PhD/ University Paulista (UNIP) -Health Sciences Institute- Farmacy- Av. Armando Giassetti, 577 - Jundiaí - SP -*
*2 Student/ University Paulista (UNIP) -Health Sciences Institute- Farmacy*
*3 Doc/ Municipal Health Secretary/ Valinhos Prefecture*
*4 Esp/ Municipal Health Secretary/ Valinhos Prefecture*

## Introduction

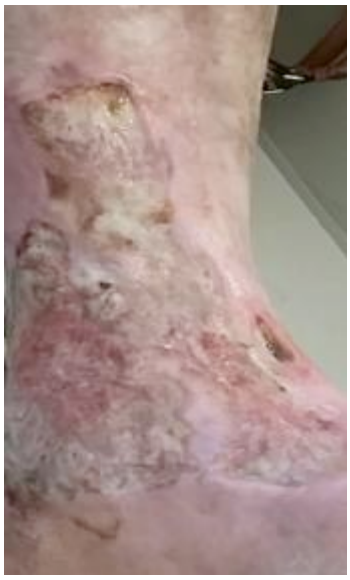
In Brazil, there are politics that encourage the use of phytotherapics at the Health Sole System (HSS)[1]. This study reports the implantation of phytotherapics in the Neuropathic and Vascular Injury Specialized Service (SELVEN) associated with already standardized coverage in Valinhos-SP[2]. The wounds, mainly vascular ulcers and diabetes foot, are a great challenge to SUS, due to them being of complicated skin healing, and cause pain and discomfort to the patient, besides its high cost to the health service[3].

## Method

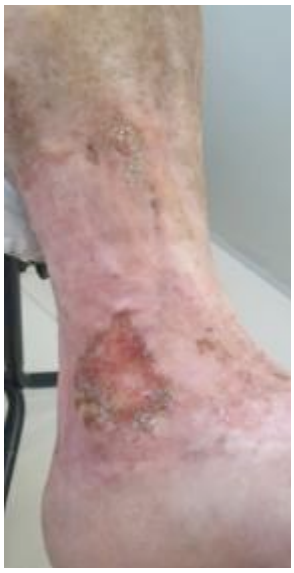
The project was approved by the in Humans Ethics Committee (CAAE: 60579916.7.0000.5512) and was performed at SELVEN in Valinhos-SP. Patients were divided in 2 groups (control and phythoterapics), with the phythoterapics used being decoction of guava tree and pitanga tree leaves. The decoction was prepared boiling 10 leaves of guava and pitanga tree in 1 L of water for 2 minutes. After cooling, the decoction was applied in wound by baths or humidified compresses, keeping touch with the same for 30 minutes. The patients did not dry the wound and bandages used were standardized by SELVEN.

## Results / Discussion / Conclusion

Patients treated with the phythoterapics presented pain improvement, reduced secretions, odor and consequently, infections related to the wounds. The average time of treatment was reduced in about 40% and the majority of patients did not return to SELVEN, indicating that the guava and pitanga tree leaves decoction promotes a skin healing process more effective than normal dressings. The ease in obtaining the leaves of both plants helped for the adhesion of patients, since Valinhos has commercial planting of guava, and pitanga grows naturally in the city, and both are typical from the region. It was possible to conclude that the guava and pitanga tree leaves when used in decoction are effective in the skin healing process of wounds.



Beginning



After 4 month

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# OC31- Tumorigenic effect of *Thevetia peruviana* in wistar rats

López González Y.[1], Almaguer Vargas G.[3], Betanzos Palmeros Z.[2] ,Torres Ortiz C.[1], Montejano Rodríguez J.R.[3]*
*jrmontejano@yahoo.com.mx

*1. Bachelor student in Pharmacy at the University Hidalgo State/Pharmacy Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*
*2. Science teacher, Professor University Hidalgo State/ Nutrition Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México.*
*3. PhD, Professor University Hidalgo State/ Pharmacy Academic Area, former hacienda circuit conception s / n, Pachuca road Actopan. San Agustín Tlaxiaca. Pachuca, Hidalgo, México*

## Introduction

The irrational consumption of some plants can cause health disorders that lead to death, such as the seed of *Thevetia peruviana* which is used by the population to treat obesity, its use has been extended that even in Mexico products such as Capslim and Easy figure[3,4] are commercialized, cardiac glycosides are found among their metabolites[5], it is known that their adverse effects can be rhinorrhea, headache, polyuria, polydipsia, paraparesis, hypokalemia, apnea, bradycardia even death[6]. Some people referred to this work the growth of small painful masses located in the large dorsal and infraspinatus muscles, which have not been reported yet in the literatura. For which the objective of the present investigation was to evaluate the tumorigenic effect of the *Thevetia peruviana* seed in a Wistar rat model.

## Material and methods

The seed of *Thevetia peruviana* was purchased in the market 1° de mayo of the State of Hidalgo, Mexico. Its taxonomic identification was made in the Institute of Biological Sciences of the UAEH. The work was done in the UAEH bioterium, 20 Wistar female rats of 250 g PV were used on average, 4 batches were randomly made with 5 rats each: control, and three batches with different doses 0.3 mg / kg three times a week; 0.9 mg / kg two and three times a week, intragastric administration. These doses correspond to what a 70 kg person consumes on average. At the end of 90 days, euthanasia was performed with Xylazine and sodium pentobarbital. Subsequently, the necropsy was performed and the tumors found were extracted, weighed, photographed and measured. The handling, care and use of the rats was carried out according to Mexican federal regulations NOM-062-ZOO-199924. Regarding the elimination and handling of Infectious Biological Hazardous Waste, proceeded according to NOM -087- ECOL- SSA1_2002.

## Results

At necropsy, three tumor masses located in subcutaneous tissue were found, located dorsally to the large dorsal muscle, with a spherical shape, encapsulated, highly irrigated, with a smooth surface, glandular appearance, smooth consistency, adhering to the skin and presenting different areas between them. (From 20 cm2 to 64 cm2), the weight of these masses was between 26 and 137 g. They were found in two rats with the dose of 0.3 mg /

kg every 3 days and the other tumor mass was found with the dose of 0.9 mg/kg administered three times a week, causing the death of this rat.

## Conclusions

In the present work it was observed that the chronic administration of the *Thevetia peruviana* seed in Wistar rats, caused the growth of tumor masses.

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# OC32- Chemical profile and biological activities of *Achillea moschata* Wulfen

Vitalini S.[1*], Pezzani R.[2,3], Argentieri M.P.[4], Vallone L.[5], Sisto F.[6], Zorzan M.[3], Collazuol D.[3], Iriti M.[1]
*sara.vitalini@unimi.it

[1]*PhD/Università degli Studi di Milano, Dipartimento di Scienze Agrarie ed Ambientali, via G. Celoria 2, 20133 Milano, Italy.*
[2]*PhD/AIROB, Associazione Italiana per la Ricerca Oncologica di Base, Padova, Italy*
[3]*PhD/Università di Padova, O.U. Endocrinology, Dipartimento di Medicina (DIMED), via Ospedale 105, 35128 Padova, Italy.*
[4]*PhD/ Università degli Studi di Bari "Aldo Moro", Dipartimento di Farmacia-Scienze del Farmaco, via Orabona 4, 70125 Bari, Italy.*
[5]*PhD/Università degli Studi di Milano, Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, via G. Celoria 10, 20133 Milano, Italy.*
[6]*PhD/Università degli Studi di Milano, Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, via Pascal 36, 20133 Milano, Italy.*

## Introduction

*Achillea moschata* Wulfen grows on siliceous rocks, screes and stony pastures, along the Alps from 1800 m a.s.l.[1]. It is a herb used in traditional and modern recipes for its aromatic traits and in several remedies - both in human and veterinary medicine – to treat various ailments, as documented in some ethnobotanical studies[2-3]. Despite its long tradition of collection and use, *A. moschata* has been poorly studied to date. The only oil volatile composition was characterized more than 20 years ago[4-5]. The aim of the present work was the investigation of the aerial part secondary metabolite content of different extracts as well as of their *in vitro* antioxidant, antibacterial and antiproliferative properties.

## Method

The aerial parts of *A. moschata* were subjected to steam-distillation and the GC/FID and GC/MS analyses were carried out on the obtained essential oil (EO). Aerial parts were also defatted with petroleum ether (PET) and successively extracted with dichloromethane (DCM), and methanol (MeOH). Inspection of the metabolic profile was done by combining HPLC-DAD and ESI-MS/MS data. The antioxidant capacity of the different extracts was evaluated by employing $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical scavenging assays[6]. The disk diffusion method[7] and assay for minimum inhibitory concentration (MIC) were used to assess antibacterial activities against Gram-positive (3 strains) and Gram-negative bacteria (4 strains). Different procedures - MTT and SRB assays[8] - evaluated the effects of *A. moschata* on viability of NCI-N87 and OE21 cells used as a gastroesophageal model.

## Results / Discussion / Conclusion

The EO is characterized by camphor (27.16%), 1,8-cineole (10.69%), and bornylacetate (6.21%) as the main constituents. It was able to better act toward $DPPH\cdot$ radical ($IC_{50}$=47.7±0.78 mM) than against $ABTS\cdot+$ (5.9±0.01 μmol Eq Trolox/g). Otherwise, the MeOH extract was the only

significantly effective sample against both synthetic radicals ($IC_{50}$=3.2±0.09 µM and 502.4±0.01 µmol Eq Trolox/g, respectively). Its HPLC and ESI-MS/MS analyses evidenced the presence of glycosylated flavonoids with luteolin and apigenin as the main aglycones. Among them, the major compound was 7-*O*-glucosyl apigenin (33.57±0.93 µg/mg). Caffeoyl derivates were other phenolics identified (12.68 µg/mg). All extracts showed a broad spectrum of antibacterial activity with growth inhibition diameters ranging from 8 to 24 mm against *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, and MIC values from 8 to 168 µg/ml against *Helicobacter pylori*. Lastly, about cytotoxic ability, only DCM extract demonstrated pronounced inhibition of cell viability, with an $IC_{50}$ of 168±22 µg/ml and 281±15 µg/ml in NCI-N87 and OE21 cell lines, respectively.
These preliminary results suggest that *A. moschata* can be considered a good source of bioactive compounds potentially exploitable in the pharmaceutical field, and support some of the documented ethnobotanical uses.

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# OC33- Advances in the development of phytomedicines for treating cutaneous leishmaniasis

Robledo S.M.[1*], Echeverri F.[2], Lutgen P.[3], Quiñones W.[2], Archbold R.[2], Torres F.[2], Escobar G.[2], Arboleda M.[4], Quintero J.[1], Velez I.D.[1]
*sara.robledo@udea.edu.co

[1]*PhD/Facultad de Medicina, Universidad de Antioquia, Calle 70 # 52-71, Medellín, Colombia. C. P. 050010*
[2]*PhD/Instituto de Química, Universidad de Antioquia, Calle 70 # 52-71, Medellín, Colombia. C. P. 050010*
[3]*PhD/ IFBV BEL-HERB, Luxembourg*
[4]*Instituto Colombiano de Medicina Tropical, Carrera 98 Nro. 106-176, Apartadó, Colombia*

## Introduction

Leishmaniasis is a worldwide disease mainly affecting million people in tropical and subtropical countries. Cutaneous leishmaniasis (CL) is the most common form of the disease, among other forms such as visceral (VL) and mucocutaneous (MCL) leishmaniasis. Despite CL is not associated with death, the disease is cause of discrimination and therefore, social stigma, suffering and psychological damage[1]. Although leishmaniasis is a treatable and curable disease, drugs used for treatment have many drawbacks. Besides their high toxicity, prolonged schemes and high cost, they are not included in the lists of national essential medicines, a single manufacturer produces most of them and, the shortage is frequent. On the other hand, the lack of options, decreased efficacy, side effects and high costs, emphasize the need for work in the development of new and better drugs. Plants remain as the most important source of new medicines and many patients seek herbal therapy, which is cheaper and affordable. During the last three decades, numerous published works have evaluated the antileishmanial activity of extracts, fractions and metabolites form plants. However, only a very few of these products have advanced to stages of development and preclinical evaluation and even less to clinical evaluation in humans. Since 2006, we are working on the development of medicines for the leishmaniasis from natural products and other strategies. In the present work, we show the results obtained with three products developed from extracts of *Sapindus saponaria*, *Caesalpinia spinosa* and *Artemisia annua,* commonly named "chumbimbo", "tara" and "ajenjo", respectively.

## Method

Starting from the available ethnomedical knowledge, we have evaluated the antileishmanial and cytotoxic activities of extracts and fractions of several plants by *in vitro* and *in vivo* systems. Extracts from *S. saponaria* and *C. spinose* were formulated for topical use and *A. annua* was formulated for oral administration. The antileishmanial activity *in vitro* was evaluated in human macrophages infected with *L. panamensis* or *L. braziliensis* and the therapeutic response was evaluated in hamsters experimentally infected with the same species of *Leishmania*[2]. The formulations were developed and characterized according to the International Conference of Harmonization guidelines and the toxicological profile was determined according to the guidelines of the Organization for Economic Cooperation and Development[3,4].

**Results / Discussion / Conclusion**

Up to now, our formulations are effective and safe in hamsters with CL, with cure rates ranging from 80 to 100%. Likewise, they are able to cure patients with uncomplicated LC without adverse effects. These results provide important basis to suggest that these formulations are good candidates as a new alternative therapy for CL and therefore, the clinical trials are being initiated to validate the use of these phytomedicines in patients with CL. In conclusion, once again, is confirmed the usefulness of plants as a source of medicines for the treatment of human diseases.

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# OC34- Chemistry and biology of chilean genus *Colletia* and *Discaria*

Alarcón-Enos J.E.[1]*, Quiroz-Carreño S.M.[1], Céspedes-Acuña C.L.[1]
*jualarcon@ubiobio.cl

[1]*PhD/Facultad de Ciencias, Dpto. Ciecnias BasicasQuímicas,Universidad del Bio-Bio,Chillán, Chile.*

## Introduction

Some South American Discaria and Colletia (Rhamnacea) genus investigated for their secondary metabolites and the presence of alkaloids, triterpenes, flavonoids, fatty alcohols and organic acids were stablished. In the course of our research on Chilean plants, we examined the aerial parts of *Discaria serratifolia*, *D. trevervis*, *D. chacaye*, *Colletia spinossisima*, *C. spinosa* and *C. histrix*. These plants are shrub that occurs in the south central of Chile. In this communication, we report the isolation, structural determination and biological activity of compounds obtained from these plats.

## Method

Dried milled aerial or roots portions of the plants exhaustively extracted with MeOH in a Soxhlet apparatus for 12 h. The resulting MeOH-extract filtered and concentrated under vacuum to obtain a raw extract. The raw extract divided in two parts. Part A suspended in $H_2O$ and sequentially extracted five times each with n-hexane and EtOAc. The *n*-hexane, and EtOAc fractions and the remaining aqueous solution, submitted upon evaporation yielded deep brown syrups. Part B used for alkaloids extraction procedures. Isolation and purification of compounds performed by chromatographic methods. The characterization was performed by m. p., U.V, I.R. $^{1}$H NMR, $^{13}$C NMR and x-Ray.

## Results / Discussion / Conclusion

The study allowed isolating and identifying a set of triterpene pentacyclic compounds with skeletons of the oleanone and ceanothane type. In addition to alkaloids of benzylisoquinolinic and aporphinic nucleous (fig 1). Total extract, fraction and compounds show insecticidal activity. Additionally, some compounds showed inhibitory activity against acetylcholinesterase, probably this activity explain the insecticidal activity observed.

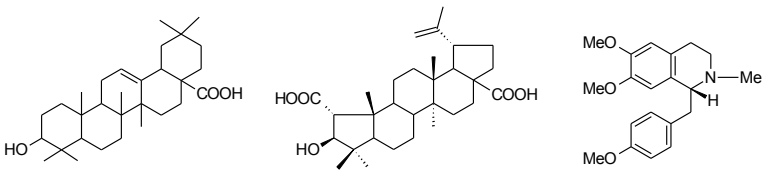


**Fig. 1** Compounds isolated from Chilean genus *Discaria* and *Colletia*

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# OC35- Anticonvulsant effect of *Anacyclus pyrethrum* on pilocarpine induced generalized seizures: possible involvement of cholinergic mechanism

Bezza K.[1]*, Laadraoui J.[1], El Gabbas Z.[1], Aboufatima R.[2], Chait A.[1].
*kenzabezza@gmail.com

[1]*Laboratory of Pharmacology, Neurobiology and behavior, Semlalia Faculty of Sciences, cadi Ayyad University, Marrakech, Morocco*
[2]*Laboratory of Génie Biologique, Sultan Moulay Slimane University, Faculty of Sciences et Techniques, Beni Mellal, Morocco*

## Introduction

Epilepsy is a serious neurological disease; approximately 1% of the world's population suffers from epilepsy. The current anticonvulsant drugs are being used alone or in combination for the treatment of epilepsy. However, antiepileptic drugs are associated with side effects.
*A.pyrethrum* is used in folk medicine for treatment of different diseases like anti-inflammatory and seizure. The present study was undertaken to evaluate the anticonvulsant activity of roots of *A.pyrethrum* by using pilocarpine experimental model of epilepsy in rat, and to determine its possible anticonvulsant mechanism.

## Method

Methanolic extract (200 and 400mg/kg p.o) from *A.pyrethrum* administered orally 45 min before pilocarpine (400mg/kg)) induced seizures. The possible anticonvulsant mechanism was investigated by testing the effect of the muscarinic acetylcholine receptors (mAChRs) antagonist atropine (2 ml/kg). The scoring of seizure severity and the seizures time latency were recorded.

## Results / Discussion / Conclusion

Methanolic extract (200 and 400mg/kg p.o) from *A.pyrethrum* prolonged significantly the onset time of seizure and decreased significantly the duration of seizures compared to control group ($p < 0.001$). The percentage of seizure protection was 100%.
The co-administered of MEAP with atropine has completely abolished the pilocarpine induced seizures.
These findings suggested that the methanolic extract from *A.pyrethrum* had anticonvulsant activity against pilocarpine induced seizures.

# OC36- Effects of *Kigelia africana* (Lam) Benth. fruits extract on the development and maturation of the reproductive system in immature male rats

Micheli V.[1], Sanogo R.[2], Occhiuto F.[3]*
*focchiuto@unime.it

[1]*Fundation A. Imbesi, University of Messina. Vill. SS. Annunziata 98168 Messina, Italy*
[2]*Department of Medicine Traditional, University of Bamako,* B.P. 1746 *Bamako, Mali, Africa*
[3]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina. Vill. SS. Annunziata 98168 Messina, Italy.*

## Introduction

*Kigelia africana* (Lam) Benth. (Bignoniaceae) is a plant used in Africa as a herbal remedy for various ailments. The fruits are employed by traditional healers also in the treatment of infertility, poor libido, sexual asthenia and erectile disfunction, but there is no scientific evidence that *Kigelia* is effective in these case (1, 2) and no work has been carried out to characterize the effects of this medicinal plant on the development and maturation of the male reproductive system of immature rats. In the present study, we investigated the effects of *K. africana* fruits hydroethanolic extract in prepubertal male Sprague-Dawley rats, to evaluate the influence of the extract on the reproductive system, on gonadal and adrenal steroid hormones production, and on the hypothalamic-pituitary-testicular axis function.

## Methods

Experiments were conducted using the rodent pubertal male assay (3), according to the EDSTAC and EPA protocols with some modifications, and approval by animal welfare Committee University of Messina (ID 16/2016) and Ministry of Health (authorization number 814/2016 PR). 50 immature Sprague-Dawley male rats (21 days of age) were used for the study, divided into 5 groups (n = 10 in each group): control group (saline), testosterone group (1 mg/kg,b.w.,s.c.), *K. africana* fruits extract groups (200, 400 and 800 mg/kg b.w., orally). Changes in body weight, age at preputial separation, endpoints associated with the development of male sex organ and secondary sexual characteristics including reproductive organs weights and histoarchitecture of organs, and hormonal status exposure to plant extract or positive control compound from post-natal date 21 to post-natal 53 days were examined.

## Results

Results obtained indicate that preputial separation (PPS) was significantly anticipated in the 400 mg/kg plant extract dose group. PPS was also anticipated in the testosterone group, although significantly higher than plant extract group. The rats were weighed and killed on PND 53, and testes, epididymides, vas deferens, seminal vesicles, and prostates were removed. Plant extract (400 mg/kg) treatment resulted in a significant increase in body weight and body muscular mass, and in accessory sexual organs: testes, seminal vesicles and prostate weights. In particular, the effect on seminal vesicles weight was remarkable, whereas, vas deferens and epididymal weights

were slightly increase in the *Kigelia* group and in the positive control group. Apparent increase in serum testosterone in the *Kigelia* group was not statistically significantly on PND 53.

These results indicate that *Kigelia* extract anticipates puberty in the male rat and its mode of action appears to be due to stimulation of the secretion of steroid hormones or to a stimulating effect on their receptors and having subsequent effects on the development of the reproductive tract, which appear to be due, at least in part, to *Kigelia* activating effects on the hypothalamic-pituitary-testicular axis function.

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# OC37- Chronic *Salvia officinalis* treatment and rosmarinic acid alleviates neuropathic pain in mice sciatic nerve chronic constriction injury model

El Gabbas Z.[1*], Bezza K.[1], Laadraoui J.[1], Aboufatima R.[2], Chait A.[1]
*elgabbas.zineb@gmail.com

[1]*Laboratory of Pharmacology, Neurobiology and behavior, Semlalia Faculty of Sciences, cadi Ayyad University, Marrakech, Morocco*
[2]*Laboratory of Biotechnology, Protection and valorization of plant Resources, Phytochemistry and Pharmacology of Aromatic and Medicinal plants unit. Department of Biology, Faculty of Sciences Semlalia, Cadi AyyadUniversity,Marrakech, Morocco*

## Introduction

The treatment of neuropathic pain remains a challenge. The therapeutic response on the pharmacological treatment of neuropathic pain is rather poor. More recently, herbal medication could be useful in the management of painful neuropathy. One such herb with possible therapeutic utility on neuropathic pain is Salvia officinalis. The present study aimed to investigate the curative effects of *Salvia officinalis* and its major compound rosmarinic acid on peripheral neuropathic pain in mice

## Method

Chronic constriction injury (CCI) was produced by loosely ligating the sciatic nerves in mice. Von Frey Hair, acetone drop, and Hot plate tests, were performed to assess degree of mechanical, chemical, and thermal sensation, at different time intervals i.e.,(day 0, 1, 7, 14, and 21). Methanolic extract of *Salvia officinalis* (S.O, 100 and 200 mg/kg, p.o.) rosmarinic acid (20,40mg/kg, i.p.) and clomipramine (5mg/kg, i.p, a positive control) were administered for 21 consecutive days from the day of surgery.

## Results

CCI produced significant development in mechanical and thermal hyperalgesia, cold allodynia in mice. Oral administration of *Salvia officinalis* (100 and 200mg/kg) and rosmarinic acid (20,40mg/kg, i.p.) for 3 weeks and clomipramine (5 mg/kg ,i.p), significantly reversed the decreased withdrawal threshold intensity and withdrawal latency in Von Frey and hot plate tests respectively. The significant anti-allodynic effect was also observed in the acetone test. Taken all together, our data suggest that S.O leaves extract can attenuate neuropathic pain.

## Discussion

*Salvia officinalis* prevented CCI induced neuropathy which may be attributed to its multiple actions including anti-oxidative, anti-inflammatory, and neuroprotective effects. However, other mechanisms may also involve. S.O leaves may be the potential novel adjuvant therapy for neuropathic pain management.

# OC38- Ethnopharmacology of Southamerican medicinal plants with cardiovascular effects

Consolini, A.E.[1*], Matera, S.[1], Bayley M. [1], Colareda, G.A. [1,2], Ragone, M.I. [1,2], Bonazzola, P.[2,3]
Namuncurá M.S.[4], Alcalde Bahamonde S.M. [5], Córdoba O.L. [6], Flores M.L. [4]
*dinamia@biol.unlp.edu.ar

*[1]PhD, Farmacología, Dpto Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, La Plata, Buenos Aires, Argentina. [2]PhD, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
*[3]PhD, Facultad de Medicina-ININCA Universidad de Buenos Aires, CABA, Argentina*
*[4]PhD, Farmacognosia, [5]PhD, Farmacología I, y [6]PhD, Química Biológica II; Facultad de Ciencias Naturales y Ciencias de la Salud, GQBMRNP y AAI – CRIDECIT, Universidad Nacional de la Patagonia San Juan Bosco, Km 4, s/N°, 9000, Comodoro Rivadavia, Chubut, Argentina.*

## Introduction

The Ethnopharmacology has been defined as an interdisciplinary scientific study of the biologically active agents traditionally used by human communities. Some hypothesis about the biological activity of the plant appear from the empirical use, and the evaluation needs experimental models that resemble the medical effect. In South America there is a wide phytotherapy based on the traditional use, but only few of them have been pharmacologically validated. In our laboratory the cardiovascular effects of some medicinal plants described as cardiotonic (e.g. *Melissa officinalis* and *Cecropia pachystachya,* ambay*)* or other natural products were studied[1,2,3,4]. Other plants were reported to contain phytoestrogens (rooth of *Lepidium meyenii,* maca, and leaves of *Medicago sativa,* alfalfa) and other contains flavonoids (*Chiliotrichum diffusum,* mata negra)[5]. The soy isoflavone genistein is a phytoestrogen cardioprotective in the "stunning" due to ischemia and reperfusion (I/R)[6], and flavonoids reduce calcium influx and overload. So, we evaluated whether these plants with phytoestrogens or flavonoids were cardioprotective in rat hearts exposed to I/R.

## Methods

*Lepidium meyenii* (Brassicaceae) rooth powder was orally administered in water at doses of 1 g/kg daily to Sprague-Dawley rats of both sexes during 1 week before the *ex vivo* experiment in isolated hearts. *Medicago sativa* (Fabaceae) leaves ethanolic extracts were dilluted in Krebs (at 1, 3 and 10 mg/100 ml) and perfused in isolated hearts before the I/R. In other group, the alfalfa extract was orally administered to rats in drinking water during 2 days before isolating the heart. *Chiliotrichum diffussum* (Asteraceae) aerial parts ethanolic extract was dilluted at 30 µg/ml and perfused to hearts before the moderate I/R. Genistein 5 mg/kg was orally administered to rats 1 day before the I/R experiment and used as a positive control.

*Model of stunning due to I/R:* after anesthesia the rat hearts were isolated and perfused with Krebs solution inside a flow-calorimeter at 37°C and electrically stimulated at 3 Hz. The contractile (intraventricular pressure, LVP, in mmHg) and calorimetrical (total heat rate, Ht, in mW/g) performances were continuously measured. After stabilization hearts were perfused in the absence

(control group) or the presence of extracts, then exposed to 20 or 30 minutes of no-flow ischemia (I) followed by 45 minutes of reperfusion (R). During this intervention, hearts decrease their contractility (P) and increase the diastolic tone (ΔLVEDP), in a characteristic dysfunction. The reduction of contractile recovery without infarct during R is called "cardiac stunning", and was estimated as % of initial P, % Ht, total muscle economy (P/Ht).
*Pharmacognosy*: ethanolic extracts and their ethyl acetate fractions of the three species were analyzed by qualitative chemical reactions and chromatographic profiles (TLC and RP-HPLC-DAD).

**Results**
Oral administration of *Lepidium meyenii* powder increased the postischemic contractile recovery (PICR to 37±7% in males and 44±8% in females versus 14±2% in control, $p<0.05$) and the muscle economy (P/Ht). This behavior was similar to that exhibited by genistein, which increased PICR to 51±5% in males and 34±5% in females ($p<0.05$ vs control) as well as muscle economy (P/Ht).
*Medicago sativa* leaves did not demonstrate cardioprotection but increased the dysfunction, since PICR resulted 26±5% in perfusion and 24±8% in oral administration (NS vs control), the ΔLVEDP was higher and arrithmias were developed (64±15 episodes during R). The *Chiliotrichum diffussum* extract was negative inotropic, and it reduced the PICR to 32±11% vs 77±3% in control group after 20 min I-15 minutes R, and finally recovered about 65%, similarly to it. It also increased ΔLVEDP.
By chemical characterization in *Lepidium meyenii* powder were found carbohydrates, flavonoids and alkaloids. The analysis of phenolic compounds by RP-HPLC-DAD evidenced isoflavones similar to orionin and 5,7-dihydroxyisoflavone. *Medicago sativa* leaves showed carbohydrates, peptides, aminoacids, flavonoids (glycosides and genins e isoflavones but in minor proportions), phenolic acids, steroids, saponins, cianoglycosides and alkaloids. *Chiliotrichum diffussum* aerial parts showed phenols (flavonoids and phenolic acids) and carbohydrates fundamentally.

**Conclusions**
With Ethnopharmacology we have validated the cardioprotective effects of *Lepidium meyenii* powder rooth which was comparable to that found with genistein, and could be associated to the content of isoflavones. In contrast, *Medicago sativa* leaves did not demonstrate cardioprotection but it was arrhythmogenic, which could be due to complex chemical composition. The ethanolic extract of *Chiliotrichum diffussum* was negative inotropic in agreement with flavonoids (quercetin and their glycosides) and phenolic acids content but it was not cardioprotective in I/R. In summary, only the presence of isoflavones was associated to cardioprotection in these models of stunning due to ischemia and reperfusion.

**Acknowledgements:** grants from Universidad Nac. de La Plata (UNLP 11X-795) and UNPSJB (PI 1201).

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# OC39- Comprehensive data evaluation (CDE) of $\delta^{13}C$ for quality assessment and traceability of natural compounds

*Bonaccorsi I.[1], Schipilliti L.[1]
*ivabonaccorsi@unime.it ; lschipilliti@unime.it

[2]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, Polo Annunziata, Viale Annunziata, 98168 Messina, Italy*

The issue related to geographic or botanic origin of food, food ingredients, flavors etc. is always a challenge. Many methods are proposed for the geographic or botanic differentiation of natural products, and among the many different ones, Isotope Ratios determined by mass spectrometry is one of the most successful. Usually this approach consists of the evaluation of the results based on few selected components separated by GC or MDGC and plotting their isotope ratios using the internal standard approach, proposed by Mosandl's group in the 90s. [1] Today we propose a new analytical method that will extend the evaluation to the entire samples' volatiles and plotted vs one selected compound typical of the sample analyzed. The present research reports a new evaluation approach of the data relative to the carbon isotope analysis of natural compounds. This is based on the comprehensive data evaluation (CDE) of the entire volatile's carbon isotope ratio (volatile bulk) determined by proper statistics of the GC-C-IRMS results plotted vs. the $d^{13}C$ of a selected pure compound. The results will allow to cluster the samples of identical nature based on their origins.

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# OC40- Antimalarial transmission blocking activity of angeloylated germacranolides from *Daucus* sp.

Rigano D.[1*], Sirignano C.[1], Formisano C.[1], Snene A.[2], Hammami S.[2], Tapanelli S.[3], Habluetzel A.[3], Taglialatela-Scafati O.[1]
[*]drigano@unina.it

[1]*Department of Pharmacy, University Federico II, Via D. Montesano, 49, I-80131 Naples, Italy;*
[2]*Research Unit Applied Chemistry and Environment 13ES63, Monastir University, Faculty of Sciences of Monastir, 5000 Monastir, TUNISIA*
[3]*School of Pharmacy, University of Camerino, Piazza dei Costanti, 62032 Camerino, MC, Italy*

## Introduction

The genus *Daucus* (family Apiaceae) includes more than 80 accepted species distributed mostly in Europe, North Africa and West Asia; about five *Daucus* species are widespread in Tunisia. *Daucus* species have been the focus of intense phytochemical investigations, with special attention paid to the best known member of the genus, the wild carrot (*D. carota*). Along with polyacetylenes, phenylpropanoids and carotenoids, several sesquiterpenes have been characterized from these studies, mainly belonging to the daucane, guaiane, and eudesmane types[1]. *Daucus virgatus* (Poir.) Maire grows in Northern Africa as an herbaceous annual or biennial species and is used in folk medicine in Tunisia[2]. The first phytochemical screening on *D. virgatus* aerial parts, collected during the flowering stage, yielded to the isolation of several new germacranolides called daucovirgolides. To the best of our knowledge, this is the first report of a *Daucus* extract dominated by germacranolides. The isolated compounds were assayed for their transmission blocking effects on *Plasmodium berghei* early sporogonic development.

## Method

The molecules were identified and their stereostructures determined by a detailed MS and NMR analysis. The effects of daucovirgolides on the development in vitro of *P. berghei* early sporogonic stages, i.e., on gametes, zygotes, and ookinetes developing within 24 h in the mosquito midgut after ingestion of gametocytemic blood, were evaluated by employing the rodent malaria strain *P. berghei* $CTRP_p$.GFP and the ookinete development assay, according to Delves et al.[3] with slight modifications.

## Results / Discussion / Conclusion

Daucovirgolide G (Fig. 1) proved to be the single member of this family to possess a marked inhibitory activity (92% at 50 μg/mL) on the development of *Plasmodium* early sporogonic stages, the nonpathogenic transmissible stages of malaria parasites, devoid of general cytotoxicity.

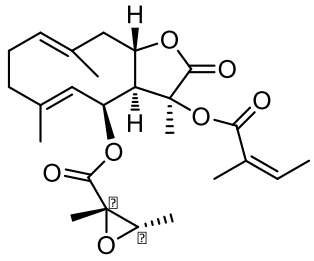

**Fig. 1**: Daucovirgolide G

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# OC41- Antioxidant activity and chemical constituents of *Astragalus monspessulanus*

Bourezzane S.[1]; Benkhaled M.[1]*; Long C.[2]; Haba H.[1.]
*mbenkhaled@yahoo.fr

[1]*Prof, Batna-1 University, Chemistry Department, Laboratory of Chemistry and Environmental Chemistry LCCE, Batna, Algeria*
[2]*Doctor,USR 3388 CNRS-Pierre Fabre, 3 Avenue Hubert Curien, Toulouse, France*

## Introduction

The genus *Astragalus* is represented in Algeria by about 40 species, including *Astragalus monspessulanus*. Many of the *Astragalus* species are famed in traditional medicine as anti-perspirants, diuretics as well as tonics and for the treatment of nephritis, diabetes, leukemia and uterine cancer. Previous phytochemical investigations on *Astragalus* genus revealed the presence of saponins, polysaccharides, phenolics and alkaloids. *Astragalus monspessulanus* is very common in the semiarid land and˝Tell˝ in Algerian Atlas (Quezel and Santa, 1963). The chemical investigation of the ethyl acetate and *n*-butanol extracts of the aerial parts of this species resulted in the isolation of thirteen compounds **1**–**13**. As well as, the antioxidant activity of the *n*-BuOH extract was evaluated using DPPH radical scavenging and ferrous ion chelating assays (Bourezzane et al., 2018).

## Method

The structures of the isolated compounds were 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*. *monspessulanus* (aerial parts; 1 Kg) was macerated three times (10 l x 3, each 72 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, 400 mL x 3). 7 g of *n*-butanol extract were submitted to vacuum liquid chromatography (VLC) on RP-18, using a gradient of $H_2O$/MeOH (80:20 to 0:100) as eluent to obtain 15 fractions (Fr1–Fr15). Further chromatographic separation and purification using CC, TLC, HPLC and precipitation on fractions Fr1 (5.53 g) and Fr4 (60 mg) allowed the isolation of 10 compounds **1-10**. 7 g of ethyl acetate extract were subjected to RP-18 vacuum liquid chromatography (VLC) using a gradient system of $H_2O$/MeOH (80:20 to 0:100) to afford 9 fractions (Fr1-Fr9). Fractions Fr9 (232 mg), Fr6 (695 mg) and Fr7 (780 mg) were chromatographed on successive silica gel CC followed by precipitation to provide 3 compounds **11-13**.

## Results / Discussion / Conclusion

Column chromatography over silica gel ($SiO_2$), preparative TLC, sephadex LH-20 and HPLC (RP-18) afforded seven flavonoids, one lignan and two saponins from the *n*-BuOH extract, two sterols and one triterpenoid from the ethyl acetate extract. These results, in agreement with previous studies performed on *Astragalus* species, confirm the occurrence of flavonoids and

saponins. The present study reports for the first time the isolation and identification of triterpenic saponins in *A. monspessulanus*. In agreement with the literature data, this is also the first occurrence of the compounds (Calendoside III, kaempferol 3-*O*-(4-α-L-rhamnopyranosyl)-β-D-glucopyranoside, quercetin 3-*O*-(2,6-α-L-dirhamnopyranosyl)-β-D-glucopyranoside, 3',5'-di-*C* glucopyranosylphloretin, isolariciresinol 9'-*O*-β-D-glucopyranoside and hovetrichoside C in the genus *Astragalus*.

The antioxidant activity of the *n*-butanol extract of *A. monspessulanus* was assessed *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferrous ion chelating activities. The crude extract possessed a moderate scavenging effect ($IC_{50}$ = 63.60 ± 0.01436 µg/mL) compared to ascorbic acid as a standard (IC50 = 3.15 µg/mL). This antioxidant activity may be due to the presence of phenolic compounds, including flavonoids and lignan.

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# OC42- New acylated triterpenoids from *Euphorbia pterococca* with biological activities

Haba H.[1]*, Benabdelaziz I.[1], Gómez-Ruiz S.[2], Benkhaled M.[1], Carralero S.[2], Schenker P.[3], Salm A.[3], Gertsch J.[3]
*haba.hamada@yahoo.fr

[1] *Université de Batna-1, Laboratoire de Chimie et Chimie de l'Environnement (L.C.C.E), Département de Chimie, Faculté des Sciences de la matière, Algérie.*
[2]*Universidad Rey Juan Carlos, 28933 Móstoles Departamento de Biología y Geología, Física y Química Inorgánica, E.S.C.E.T., Madrid, Spain.*
[3]*University of Bern, Bühlstrasse 28, CH-3012 Bern, Institute of Biochemistry and Molecular Medicine, Switzerland.*

## Introduction

The genus *Euphorbia* is one of the largest genera of the family Euphorbiaceae, with about 2000 species grown mainly in tropical, subtropical and warm temperate regions[1]. In our ongoing research program on *Euphorbia* species from the flora of Algeria, we are particularly interested in phytochemical and biological investigations of *Euphorbia pterococca* Brot. which is a perennial, multicolored plant[2]. *E. atlantica*, locally named "hanγut", is a medicinal plant used by the Aures population of Algeria to extirpate thorns and warts. This study reports the isolation and identification of four new acylated cycloartanes **1**–**4**, as well as, nine known tetracyclic triterpenes. Their structures and configuration were determined on the basis of extensive spectroscopic analysis and together with a comparison with literature data. In addition, different biological tests of the new isolated compounds have been carried out to determine their potential biological activities.

## Method

All compounds **(1-13)** were isolated from the acetonic extract of the plant *E. pterococca* by the use of various chromatographic techniques: vacuum liquid chromatography (VLC), column chromatography (CC) and thin layer chromatography (TLC). These compounds were identified from 1D and 2D NMR analysis, ESI-MS spectral techniques, hydrolysis and by comparison with literature data. In order to profile prospective biological activities, new cycloartanes **1-3** were tested in different biological assays. First, we screened the cytotoxicity of **1-3** in the three different cancer cell lines HeLa, Neuro2A and RAW264.7. The triterpenes **1-3** were also tested on *Trypanosoma cruzi*, the parasite causing Chagas disease in humans. We tested also the tetracyclic triterpenes **1-3** bearing α,β-unsatureated fatty acid moieties on ABHD6 and ABHD12 as well as MAGL.

## Results / Discussion / Conclusion

The phytochemical investigation of *E. pterococca* led to the identification of 13 tetracyclic triterpenes including four new cycloartanes: cycloartenyl-2'*E*,4'*E*-decadienoate (**1**), cycloartenyl-2'*E*,4'*Z*-decadienoate (**2**), 24-methylene-cycloartanyl-2'*E*,4'*Z*-tetradecadienoate (**3**), and 24-oxo-29-norcycloartanyl-2'*E*,4'*Z*-hexadeca-dienoate (**4**) and nine known compounds with cycloartane,

and ergostane skeletons. Their structures were established mainly by extensive use of spectroscopic techniques, including 1D ($^{1}H$ and $^{13}C$) and 2D homo- and heteronuclear NMR experiments (COSY, HSQC, HMBC and NOESY), and mass spectrometry (HRESIMS), chemical transformation, and by comparison with data reported in the literature. In addition, the new compounds **1-3** have been tested for cytotoxicity, trypanocidal effects and on enzymes involved in endocannabinoid degradation. While inactive in all assays up to 100 μM, compound **1** showed selective inhibition of α/β-hydrolase 12 with an $IC_{50}$ = 11.6 ± 1.9 μM.

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# OC43- Profile characterization of VOCs on *in vitro* propagated plants of *Hedeoma multiflorum* and its comparison with wild plant

Goleniowski M.E.[1]*
*mgoleniowski@gmail.com

[1]*CCT-CONICET Córdoba, Argentina.*

## Introduction

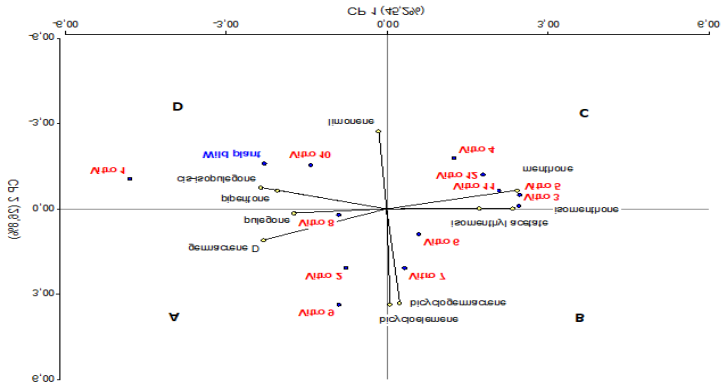
*Hedeoma multiflorum* Benth (Lamiaceae) known as Tomillo de las Sierras, grows wild in the foothills of the Sierras de Córdoba and it is a specie rich in aromatic essential oils. Is used in ethnopharmacology as digestive, anti-rheumatic and abortive, and for its culinary properties is also employed for the production of juices, bitter drinks ("bitter") and "yerba mate composed" (1-2). Due to this, *H. multiflorum* has been subjected to intense harvesting causing devastation of native areas and put in risk survival of remaining populations in natural habitats (3). The aim of this work was to study the effect of the *in vitro* culture conditions of plants of *H. multiflorum* on the production of volatile organic compounds (VOCs) to find the better laboratory culture conditions that can provide specimens with similar characteristics to plants that grow wild.

## Methods

Twelve different concentrations and combinations of growth regulators (PGRs) on Murashige Skoog medium at half of it concentration with or without plant hormones were used, Characteristics of the profile of VOCs developed of *in vitro* cultivated plants were determined by HS-SPME/GC-MS, and the results were compared with the wild plant.

## Results / Discussion / Conclusions

PCA was performed and the results are presented in Fig. 1 principal component analyses.




As a result, 50 VOCs were determined and they were identified by comparing their mass spectra with library data (match ≥ 90%), and by the determination of the respective Kovat´s retention index (KI). The major components were pulegone, menthone, isomenthone and cis-isopulegone in the most of the media studied. The results showed a wide variability in the VOCs compositions

depending on the growing conditions; hence, to interpret how the volatile profiles discriminated between the plants.

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# OC44- 2',3,4-trihydroxychalcone, phloretin and calomelanone from *Stevia lucida*. The first chalcones reported in *Stevia* Genus

Santiago Dugarte C.[1,2*], Chacón-Morales P.A.[1,2], Amaro-Luis J.M.[2]
*carolina.santiagod@ug.edu.ec

[1]*PhD/ Faculty of Chemical Sciences, University of Guayaquil, Ciudadela Universitaria Av. Delta y Av. Kennedy s/n, C. P. 090112, Guayaquil, Ecuador*
[2]*PhD/ Natural Products Laboratory, Department of Chemistry, Faculty of Science, University of Los Andes, Av. Alberto Carnevalli La Hechicera, C.P. 5101, Mérida, Venezuela*

## Introduction

Chalcones are widely distributed in plants, but they are particularly abundant in some genera of the families Asteraceae[1] and Moracea[2]. In the scientific literature there are numerous reports focusing on the wide range of biological activities exhibited by natural and synthetic chalcones. Consequently, this aspect makes these products very important from a biological and pharmacological point of view. There are several reports on the presence of flavones and flavonols in the genus *Stevia*[3], but interestingly, there are no reports on isolation of chalcones that precede this research.

## Method

The dry uncrushed leaves and stems were exhaustively extracted with ethanol in a sohxlet. The obtained solution was filtered and concentrated "in vacuo", to produce a crude extract, which was preadsorbed on silica gel and extracted successively with petroleum ether, acetone and methanol. Acetone-solution was concentrated under reduced pressure to dryness and a brown residue was obtained. The acetone extract was preadsorbed on silica gel and chromatographed (VLC), eluting with hexane and EtOAc in mixtures of increasing polarity. Fractions were collected and combined according to the TLC characteristics to afford twelve major fractions. Fractions 5 and 8 were purified by repeated flash chromatography and preparative TLC to furnish 2',3,4-trihydroxychalcone (**1**) and phloretin (**2**) respectively. Fraction 3 was treated with size-exclusion chromatography on Sephadex LH-20 and preparative TLC yielding calomelanone (**3**). These compounds were characterized on the basis of spectroscopic studies [UV, IR, LWSM and NMR (1D and 2D)] (see the procedures detailed in reference 4).

## Results/Discussion/Conclusion

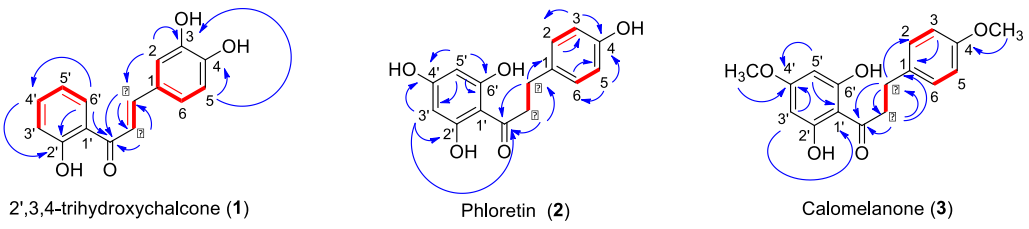


**Fig. 1**. $^{1}$H-$^{1}$H COSY and HMBC correlations of chalcones **1-3**.

This is the first report of chalcones and dihydrochalcones in the *Stevia* genus and the fourth report in the Eupatoriae tribe (see the chemotaxonomic significance in reference 4).

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# OC45- Biochemometrics-based identification of antifungal quinolizidines

Cely-Veloza W.[1], Coy-Barrera E.[1*]
*inquibio@unimilitar.edu.co

[1]*Laboratorio de Química Bioorgánica, Universidad Militar Nueva Granada, Cajicá, Colombia.*

## Introduction

Quinolizidine alkaloids are naturally-occurring compounds that are present in species of *Genisteae* tribe[1]. Wild species from this tribe has a wide distribution in our country, but they have been little studied at biochemical and phytochemical level. These plants can synthesize a large number of nitrogen-containing non-protein molecules intimately involved in N storage such as quinolizidines. Additionally, most of these compounds are produced as part of a defense strategy against the attack herbivores and pathogens, so their chemical characterization and variability is important to be evaluated for a potential use within pest and disease control strategies. Thus, as part of our research on bio and chemoprospecting of *Genistae* plants, several accessions (n>60) of different genera from various places in Bogotá plateu, were separately investigated through a comprehensive targeted GC-MS-based metabolomics approach from alkaloid-enriched extracts in order to observe the quinolizidine-based chemical variability between samples (ontogeny and environmental factors) and its implication on antifungal activity against *F. oxysporum*.

## Method

Alkaloidal extracts were obtained from leaves of Genistae tribe plants (n>60) by means of ultrasound-assisted acid-base extraction. Resulting extracts were separately tested against *F. oxysporum* through a micro-scale amended-medium method at 0.1-5.0 μg/μL. After 48 h, the resulting mycelial growth inhibition was observed. Extracts were also analyzed by GC-MS in order to achieve a peak annotation by mass spectra data. The variation of GC-MS profiles was examined by OPLS discriminant analysis and single-Y OPLS to correlate the chemical and biological activity as biochemometrics approach. Additionally, the binding mode of identified quinolizidines within the active site of different enzyme targets was studied through molecular docking using Autodock/Vina and molecular dynamics using Gromacs.

## Results / Discussion / Conclusion

MS-mediated annotation showed the occurrence of different cytisine, sparteine and lupanine-like tetracyclic quinolizidines. Phytomaterials also showed antifungal capacity at different levels ($2>IC_{50}(\mu g/mL)>55$). OPLS-DA-derived score plots indicated several differences between samples but clustered according characteristic chemical constituents and/or activity. Supervised analysis indicated the existence of three quinolizidine-related compounds to be responsible of the antifungal activity. *In silico* results were examined through Vina scores and ligand-residues interactions. Good Vina scores were obtained for docked structures at different levels. Most stable conformers of NMT enzyme and quinolizidine **1** was found to exhibit comparable docking energies to that of control. Quinolizidine-related compounds might be considered as good

candidates for structural optimization leading natural product-based design of anti-phytopathogens. The present targeted metabolomics exploration of these *Genistae* plants is an excellent approach for quinolizidine-based antifungal finding from nature as well as ontogeny and environmental indicators.

**Acknowledgement**

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# POSTER COMMUNICATIONS

# PS001- Antifungal effect of 2-allylphenol derivatives on phytopathogen *Phythopthora cinamomi.*

Carrasco H.[1]*, Diaz K.[2], Sedan C.[3], Thomas M.[3], Martínez R.[3]
*hector.carrasco@uautonoma.cl

[1]*Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, El Llano Subercaseaux 2801, San Miguel, Santiago, Chile. C.P 8900000..*
[2]*Departamento de Química, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso, Chile, C.P. 2340000.*
[3]*Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andrés Bello, Quillota 910, Viña del Mar.C.P 2520000.*

## Introduction

Phytopathogen *Phytophthora cinnamomi* is currently considered one of the most devastating in the world[1] and occurs with a high incidence and severity in clay soils, saturated for prolonged periods (Latorre, et al., 1998).[2] Due to its characteristics, it is associated with severe root rot of Pinus radiata D. Don and Avocado.[3] The serious effects caused by this fungus in the agricultural production of several countries, make the use of fungicides a key factor for the control of this disease.[4] However, the impact ecological and environmental caused by the commercial antifungals, has led to the rethinking of agriculture, encouraging the use of inputs with a high ecological potential. From this point of view, 2-Allylphenol a biomimetic synthetic fungicide that mimics the compound ginkgol found in gingko fruit (Gingko biloba L.), emerges as an interesting starting product that allows us to obtain synthetic derivatives with possible antifungal activity. In this work we report the synthesis of a series of 2-allylphenol derivatives in order to evaluate its effectiveness against *P. cinnamomi*.

## Method

The derivatives of 2-allylphenol, are obtained by nitration of acetylated allylphenol with a mixture of $HNO_3/H_2SO_4$, as well as direct treatment of allylphenol with $KHSO_4$, $NaNO_3$ in wet silica (50%). Acetylation of the hydroxyl group of allylphenol is accomplished by reaction with acetic anhydride in presence of DMAP as catalyst. The reduction of nitro group is realized by treatment of respective compound with zinc metal in hydrochloric acid. Pure compounds are obtained by column chromatography of the reaction mixture. The characterization was performed by $^{1}H$ NMR, $^{13}C$ NMR. The antifungal activity of the evaluated derivatives was obtained through the evaluation of the percentage of inhibition of mycelial growth of the pathogen by the radial growth test in Petri dish and comparing it with the positive control metalaxil.

## Results / Discussion / Conclusion

Our results shown that 2-allylphenol derivatives present EC50 values similar to the commercial antifungal metalaxyl, so in the future they could be used in the treatment of *P. cinnamomi*.

| **Percentage of Inhibition of mycelial growth of *P. cinnamomi* (%)** | | | |
|---|---|---|---|
| **Compound** | **50 ppm** | **150 ppm** | **250 ppm** |
| 2-allylphenol | 36±0 | 51±0 | 99±1 |
| 2-allyl-4,6-dinitrophenol | 100±0 | 100±0 | 100±0 |
| 2-allylphenylacetate | 26±3,4 | 36±0 | 51±4,9 |
| 2-allyl-6-nitrophenol | 0±0 | 54±2,7 | 66±2,3 |
| 2-allyl-6-nitrophenyl acetate | 100±0 | 100±0 | 100±0 |
| Metalaxil® | 100±0 | 100±0 | 100±0 |

**Acknowledgments:** We thank support to FONDECYT for Grant 1170706.

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# PS002- Anticancer activity of 2-allylphenol derivatives

Carrasco H.[1*], Sedan C.[2], Thomas M.[2], Martínez R.[2], Villena J.[3]
*hector.carrasco@uautonoma.cl

[1]*Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, El Llano Subercaseaux 2801, San Miguel, Santiago, Chile. C.P 8900000.*
[2]*Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andrés Bello, Quillota 910, Viña del Mar.C.P 2520000.*
[3] *Facultad de Medicina, Universidad de Valparaíso, Hontaneda 2664, Valparaíso, Chile, Chile. C.P 2340000.*

## Introduction

Cancer is a principal killer disease in the world in the actuality, since are attributed 7.6 million of death representing 13% of all causes of death worldwide. This pathology involved a series of transformations in the genome, leading to the modification of the expression of a number of factors associated with different processes, among which we would highlight the regulation of cell death and the regulation of DNA.[1,2] Then it is of interest to study the effect of naturally occurring chemical compounds or synthetic derivatives that may affect the process of cell death.
Previous studies showed that derivatives of eugenol, 5-allyl-3-nitrobenzene-1,2-diol and 4-allyl-2-methoxy-5-nitrophenyl acetate showed anticancer activity, with IC50 values in DU-145 cells of 19.02 and 21.5 μM , and in KB cells of 18.11 and 21.26 μM, respectively.[3] With this in mind and in order to study other analogous system and their effect on anticancer activity, we proceeded to the synthesis of a series of 2-allylphenol derivatives, where we introduced nitro groups on the aromatic ring, in order to increase the polarity of these compounds and therefore their solubility in water, which should increase their bioavailability.

## Method

HT-29 cells (colon cancer cell line), PC-3 (prostate cancer cell lines), MCF7 (breast cancer cell lines) and CCD 841 CoN (human colon epithelial cells) were obtained from the American Type Culture Collection (Rockville, MD, USA). Stock cells were incubated at 37 °C under humid atmosphere with 5% $CO_2$ for 24 h before the test. The cell suspension was set up at 3,000 cells per well of a 96-flat-bottomed 200 μL well microplate. Compounds were dissolved in dimethylsulfoxide (DMSO) at a concentration of 0,1M and diluted with the growth medium to the desired concentrations (0-100 μM). Negative control cultures were prepared by adding just 0.1% DMSO. All culture microplates were incubated at 37°C in a $CO_2$ incubator with humidified 5% $CO_2$ for 72 h. The obtained data were expressed as percentages of viability cells versus solvent control, whose viability was considered 100%. Values shown are the mean ±SD of three independent experiments in triplicate. The software used to calculate the IC50 values was Prism 6® version 6.0d.

## Results / Discussion / Conclusion

Our results shown that 2-allylphenol derivative 2-allyl-4,6-dinitrophenol significantly decreases cell viability of cancer cell lines, such as HT-29, PC-3, MCF7 with EC50 values which vary

between 49.7 to 59.1 μM. Furthermore, it can be seen that this derivate act specifically on tumor lines, not affecting non-tumor cell lines.

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# PS003- Bioactive lipids from endemic plant seed oils of Reunion island: a prospective study

Maillot M.L.[1], Reiss H.[1], Vellayoudom S.[1], Clerc P.[1], Petit T.[1,2], Dufossé L.[1], Giuffrida D.[3], Tranchida P.Q.[4], Giocastro B.[4], Aloisi I.[4], Murador D.[5], Mondello L.[4,6,7], Thomas H. [8], Grondin I.[1] Caro Y.[1,2*]

laurent.dufosse@univ-reunion.fr; *yanis.caro@univ-reunion.fr

[1]*Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNSA-EA 2212), Université de La Réunion, BP 373, 97455 Saint-Pierre Cedex, Réunion.*

[2]*IUT de La Réunion, Département HSE, 40 avenue de Soweto, BP 373, 97455 Saint-Pierre Cedex, Réunion.*

[3]*Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, University of Messina, Polo Annunziata, c/o Pan Lab, Viale Annunziata, 98168 Messina, Italy.*

[4]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, Polo Annunziata, Viale Annunziata, 98168, Messina, Italy.*

[5]*Departamento de Biosciências, Universidade Federal de São Paulo, Rua Silva Jardin 136, 11015-020, Santos, Brazil.*

[6]*Chromaleont s.r.l., c/o Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, 98168 Messina, Italy.*

[7]*Department of Medicine, University Campus Bio-Medico of Rome, via Alvaro del Portillo 21, 00128 Rome, Italy.*

[8]*Parc National de la Reunion, 258 Rue de La République, 97431 Plaine des Palmistes, Réunion.*

## Introduction

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man, animals and plants. Lipids consist of fatty acids classified as saturated (without double bonds), monounsaturated (with one double bond) and polyunsaturated fatty acids (PUFAs - with two or up to six double bonds); further, as Omega-3 or Omega-6 PUFAs depending on the position of the first double bond from the fatty acid methyl-end. Several groups of lipids have been shown to provide health benefits either through modification of tissue fatty acid composition or induction of cell signalling pathways. PUFAs are found mostly in plant seed oils and are important substrates for the biosynthesis of cellular hormones (eicosanoids) and other signalling compounds that modulate human health. Some particular plant seed oils are also rich in unsaponifiable matters which have varying effects: conservation and stability (e.g. tocopherols, tocotrienols), anti-inflammatory properties (triterpene alcohols, sterols), cholesterol-lowering and anti-cancer activities (sterols), and antioxidant properties (carotenoids, apocarotenoids).

## Method

After oil extraction by pressurized liquid extraction method under nitrogen with *n*-hexane as solvent, the characterizations of fatty acids and unsaponifiable matters of fifteen endemic plant seed oils of Reunion island, in Indian Ocean, are obtained by using gas chromatography (GC) and high-performance liquid chromatography combined with mass spectrometry (HPLC-MS).

**Results / Discussion / Conclusion**

Omega-3 PUFAs are found predominant in five species: *Myonima obovata* (34% w/w in total fatty acids), *Hyophorbe indica* (25%), *Cossinia pinnata* (12%), *Eugenia buxifolia* (5%) and *Pittosporum senacia subsp. senacia* (2%). Some of the seed oils are also rich in unsaponifiable matters: *Hyophorbe indica* (13g for 100g of seed oil), *Cassine orientalis* (9% w/w), *Mimusops maxima* (9% w/w), *Terminalia bentzoë subsp. bentzoë* (7% w/w), *Olea lancea* and *Olea europaea subsp. africana* (4% et 3%, w/w, respectively), and *Dictyosperma album* (3% w/w). Among the major pigments extracted from the plant seed oils, the results indicate the presence of particular carotenoids: phytoene and phytofluene (in *Hyophorbe indica*), apocarotenoids (in *Latania lontaroides*), and oxygenated compounds such as xanthophylls (beta-cryptoxanthin-5,6-epoxide in *Mimusops maxima*, all-trans-lutein in *Dictyosperma album* and *Cossinia pinnata,* etc.). The seeds of these plants contain a great number of valuable phytosterols, which have a potential high value as a functional ingredient in foods and for production of non-food products (pharmaceutics). Interestingly, the oil extracted from *Cossinia pinnata* presents a peculiarity: a strong content of a not yet identified plant sterol, and the presence of longer chain PUFAs. Further structural elucidation of the bioactive lipids by NMR is in progress.

# PS004- Natural pigments from Madagascar dyeing plants: from tradition to innovation for applications as functional ingredients for foods, cosmetics and pharmaceutics

Andriamanantena M.[1,2], Raonizafinimanana B.[2], Fawbush F.[2], Danthu P.[3], Dufossé L.[1], Petit T.[1,4] and Caro Y.[1,4]*
laurent.dufosse@univ-reunion.fr; *yanis.caro@univ-reunion.fr

[1]*Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNSA-EA 2212), Université de La Réunion, Saint-Denis, Réunion, France.*
[2]*IAA-ESSA, Ecole Supérieure des Sciences Agronomiques, Université d'Antananarivo, Madagascar.*
[3]*CIRAD, Unité HortSys, Montpellier, France.*
[4]*Département Hygiène Sécurité Environnement, IUT de La Réunion, Saint-Pierre, Réunion, France.*

## Introduction

The demand for natural colorants is increasing worldwide due to the increased public awareness about properties and health benefits. The renewed interest for natural colorants is also a consequence of bad perception for the synthetic ones that have been suspected to cause allergic reactions, neurological effects, potential carcinogenicity and environmental disasters. Even if "natural" does not mean harmless, this undoubtedly generates a strong pressure on the market and a limited number of synthetic dyes are still used in food today. The proliferation of products with labels that state they contain "No artificial colours" suggests that the future of synthetic dyes is strictly limited. Natural colorants are those derived from naturally occurring sources such as plants, insects and microorganisms. Among them, pigments generated from plants have often medicinal values and are mostly preferred. They are of growing interest for industries involved in the production of fabrics, foods and pharmaceutics. However, they are subject to application limitations and stability problems.

## Method / Collection of plants

Malagasy craftsmen have used natural dyeing for centuries, up to now mainly in textile working mainly on silk and raffia. We identified more than 237 plants from several families, either endemic or introduced to Madagascar, which could be used to obtain a panoply of colors. Red is the color that may come from the largest natural source (up to 32%) of dyeing plants from Madagascar, followed by yellow and black. Various plant parts can serve as colorant sources and some of them make excellent dyes. The exploitation of plant dyes is however dependent of good practices in collecting extracts and renewable usage of these plants. The stem bark (such as the one from *Harungana* sp.) or the leaves are the parts that create the least damage to the plant for intensive use, while the roots (as in the case of *Pentanisia* sp.) or wood need to be exploited in a more restricted way because it definitively destroys the potential endangered species.

## Results / Discussion / Conclusion

This communication emphasizes on pigments from some dyeing plants isolated from Madagascar, namely *Acridocarpus excelsus*, *Carphalea kirondron*, *Tectonia grandis*, *Harungana*

*madagascariensis*, *Indigofera arrecta*, *Rhizophora mucronata*, *Ceriops tagal*, *Woodfordia fruticosa*, *Xylocarpus granatum* and *Psiadia altissima* that could be used as prospective functional ingredient for food, cosmetic and pharmaceutical industries. Physicochemical and technological properties of a number of relevant natural dyes will be presented here as well as details about their basic chemical information.

# PS005- Compounds of *Copaifera* spp.: a new antibiofilm resource against *P. gingivalis* and *A. actinomycetemcomitans*

Martins C.H.G.[1]*, Abrão F.[2], Veneziani R.C.S.[3], Ambrósio S.R.[4], Bastos J.K.[5]
*carlos.martins@unifran.edu.br

*[1]PhD/ Research Laboratory of Applied Microbiology, University of Franca, São Paulo, Brazil, CEP 14404-600.*
*[2]PhD student/ Research Laboratory of Applied Microbiology, University of Franca, São Paulo, Brazil, CEP 14404-600.*
*[3, 4]PhD/ Nucleus of Research in Sciences and Technology, University of Franca, São Paulo, Brazil, CEP 14404-600.*
*5PhD/ School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil, CEP 14040-900.*

## Introduction

Among putative periodontal microorganisms, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* have been detected in deep periodontal pockets and have been implicated as etiological agents in periodontitis[1]. Species belonging to the genus *Copaifera* spp. play an important role against bacteria that cause periodontal diseases. *C. paupera*, *C. pubiflora*, and *C. reticulata* are well studied species among Brazilian medicinal plants[2]. This study aims to evaluate the antibiofilm properties of compounds obtained from *Copaifera* spp. against *P. gingivalis* and *A. actinomycetemcomitans*.

## Method

Hardwickiic acid, polyalthic acid, and kaurenoic acid were obtained from *C. pubiflora*, *C. reticulate*, and *C. paupera*, respectively. These compounds were evaluated at concentrations ranging from 0.78 to 1,600 µg/mL against strains (clinical isolates and standard) of *P. gingivalis* (CI 01 and CI 03) and *A. actinomycetemcomitans* (ATCC 43717 and CI 02, 03 and 04). The Minimum Inhibitory Concentration of Biofilm ($MICB_{50}$) was determined on the basis of the minimum concentration of antimicrobial agent that was able to inhibit biofilm formation by at least 50%[3]. Biofilm formation was quantified by staining with violet crystal 0.2%, and the number of microorganisms was counted ($\log_{10}$ CFU/mL). $MICB_{50}$ was assessed for multispecies and monospecies in the biofilm mode.

## Results / Discussion / Conclusion

On the basis of the low $MICB_{50}$ results, the assayed compounds can be employed in the search for new effective agents that act against oral pathogens involved in periodontal diseases.

| **Bacteria x Compound** | **$MICB_{50}$** |
|---|---|
| *P. gingivalis* CI 03 x Polyalthic acid | 25 µg/mL |
| *P. gingivalis* CI 01 x Kaurenoic acid | 100 µg/mL |
| *P. gingivalis* CI 03 x Hardwickiic acid | 400 µg/mL |
| *A. actinomycetemcomitans* CI 02 x Polyalthic acid | 100 µg/mL |
| *A. actinomycetemcomitans* CI 02 x Kaurenoic acid | 100 µg/mL |
| *A. actinomycetemcomitans* CI 03 x Hardwickiic acid | 100 µg/mL |
| *A. actinomycetemcomitans* CI 02 + *P. gingivalis* CI 03 x Polyalthic acid | 400 µg/mL |
| *A. actinomycetemcomitans* CI 03 + *P. gingivalis* CI 03 x Hardwickiic acid | 200 µg/mL |
| *A. actinomycetemcomitans* CI 03 + *P. gingivalis* CI 03 x Kaurenoic acid | 200 µg/mL |

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# PS006- Green Synthesis of (*E*)-2-(3-nitrobenzylidene)-1,1-diphenylhydrazine and evaluation on cancer cell lines

Aguirre Cabrera C. [1*], Cabrera-Vivas B.M. [2], Palillero-Cisneros A.[2], Castillo F., Zabdi A.[1], Meléndez-Balbuena L.[2], Ramírez-García J. C.[2]
*carla.aguirre27@gmail.com

[1]*Bachelor´s degree in Chemistry Student, Facultad de Ciencias Químicas, Edificio 9, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*
[2]*PhD/Facultad de Ciencias Químicas, Edificio 9, 18 sur y Av. San Claudio. BUAP, C. P. 72001, Col. Sn. Manuel, Puebla, México*

## Introduction

Cancer is a disease that affects millions of people around the globe although it is not a contagious disease, its treatment is complicated because the drug administered depends on the type of cancer and time of residence.[1] There are many types of cancer treatment. Surgery is the oldest one, which offers the greatest chance of cure for many types of cancer; however, it causes aesthetic and psycologycal effects as a consecuence losing a body limb, which is hard to cope with. Other tretments include: radiotherapy, where high energy waves are used; chemotherapy, which is the administration of drugs.[2]

It is important to look for new drugs that can help in the treatment of cancer. Different studies have shown that hydrazones own a versatile structure that help in the development of compound with antiproliferative activity against different cancerous cells, which is the purpose of this project: to synthetize hydrazones with structures different from the already reported in order to evaluate them in specific cancerous cell lines. In this project, HTB-38 (rectal colon), HTB-177 (lung), and MOLT-4 (Lymphoblastic Leukaemia) were examined with great results for the majority.

## Method

Diphenylhydrazine (0.95 mmol, 318.05 mg) was dissolved in a green solvent (EtOH), a chemical equivalent (0.95 mmol, 300 mg) of 3-Nitrobenzaldehyde which was previously dissolved in the same solvent and it was added drop by drop stirring constantly and which was developed with greener procedure[3]. The action mixture was kept at room temperature and was monitored by TLC, and then vacuum filtered. The hydrazones were recrystallized with cold methanol by a continuous and controlled process until yellow crystals with adequate size and purity were developed in order to obtain X-ray studies and characterization was performed by m. p., I.R., $^{1}$H NMR, $^{13}$C NMR, x-Ray, M. E.[4]

## Results / Discussion / Conclusion

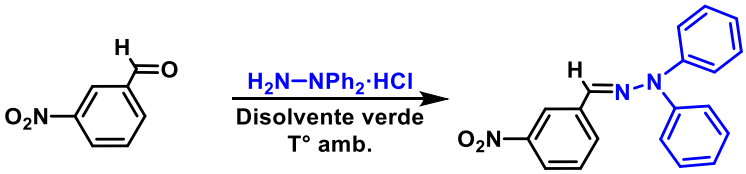


**Fig. 1.** Synthesis of (*E*)-2-(3-nitrobenzylidene)-1,1-diphenylhydrazine through Green Chemistry.

Yellow crystals; yield: 81% at 25 °C, mp 108-110 °C. Anal. calc. for $C_{19}H_{15}N_3O_2$ (MW = 317.35 g mol $^{-1}$). FT IR (film): (cm$^{-1}$): 3129 ν (Csp$^2$-H); 158 ν (C=N); 1236 (C-N); 881, 750 δ(Ph-C-H), 697 δ (C-H) Ph out of plane. RMN $^1$H (400 MHz, $(CD_3)_2CO$):8.44 (m, 1H, C2), 8.09 (td, 1H, C4), 8.04 (dd, 1H, C6), 7.62 (t, 1H, C5), 7.48 (t, 4H, C3'), 7.29 (s, 1H, C=N) 7.25 (m, 6H, C2', C4'). RMN $^{13}$C (400 MHz, $(CD_3)_2CO$): (δ/ppm): 148.76 (C3), 143.21 (C1´), 138.29 (C1), 132.54 (Ci), 131.81 (C6), 129.98 (C3'), 129.87(C5), 125.08 (C4'), 122.38 (C2´), 122.11 (C4), 120.22 (C2).

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# PS007- Concentration of serum vitamin C in pulmonary diseases

Begovic D.[1]*, Corkovic Lj.[1], Milosevic T.[2], Maksimovic T.[1]
*gagabeg@gmail.com

[1]*PhD /Clinical Health Center "Dr Dragisa Misovic- Dedinje", H. Milana Tepica 1, 11 000 Belgrade, Serbia*
[2]*MD/ Clinical Health Center "Dr Dragisa Misovic- Dedinje", H. Milana Tepica 1, 11 000 Belgrade, Serbia*

## Introduction

Complementary/ alternative medicine (CAM) is more popular than ever before. Patients with pulmonary diseases often seek so-called alternative treatment as supplementation but seldom as real alternative medicine. The lungs are constantly exposed to oxidants such as ozone and nitrogen dioxide that are inhaled or release from inflammatory leukocytes. In order to protect the lung from increased endogenous or exogenous oxidant burden, several antioxidant systems are available, including vitamin C. Recent study suggests that vitamin C, also known as ascorbic acid is important antioxidant and indicate that taking supplement such as vitamin C could benefit patients with pulmonary diseases. [1,2]

## Method

We examined the vitamin C status in patients with chronic obstructive pulmonary disease (COPD) and children with asthma. Diagnosis was established by clinical, roentgenographic, laboratory and lung function examinations. Vital capacity (VC), forced expiratory volume in one second ($FEV_1$) and the ratio 100 $FEV_1$/VC were determined. Laboratory analyses included blood leukocyte count, ESR and serum fibrinogen Serum ascorbate concentrations were determinated by spectrophotometric method (our reference values 30 - 110μmol/L) in patients with pulmonary diseases and in control group.

## Results/ Discussion/Conclusion

The levels of serum vitamin C were lower in COPD patients than in control group. In patients with COPD and respiratory failure serum vitamin C concentration is highly significantly decreased ($p<0.001$) than in patients with COPD and with normal arterial blood gases. Concentrations of serum vitamin C were not in correlation with clinical score of symptoms and/or spirometry in children with asthma. The obtained data indicate a relation between vitamin C status and COPD. Our findings are also compatible with observations that dietary vitamin C has a protective effect on pulmonary function. Higher blood levels of vitamin C may be ideal nutritional marker for overall health.

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# PS008- Pozol, a Mexican ancestral sacred beverage, has anxiolytic effects

Rejón-Orantes J.C.[1]*, Orantes-Castañeda C.V.[1], Pascacio-Pérez M.J.[1], Velasco-Jiménez E.A.[1], Pérez de la Mora M.[2]
rejonjose@hotmail.com

[1]*Experimental Laboratory of Pharmacobiology. Faculty of Human Medicine. Autonomous University of Chiapas.*
[2]*Division of Neurosciences. Institute of Cellular Physiology. National Autonomous University of Mexico.*

## Introduction

The custom of cacao beverage, among which is the pozol, it was sacred nature among the prehispanic Indians of Mesoamerica "beverage of Gods the nahuas said"[1]. With the arrival of Spanish it lose its scrality, and the consumtion is exposed to the common population. The pozol survived to the Spanish conquest and actually it is a food and a refreshing beverage of popular consumtion in the lunch on the states of Chiapas and Tabasco, Mexico. It is elaborated with cacao and maize, the importance of maize in the diet of diverse prehispanic towns it manifests at the level of myths and legends, even associated with the creation of the world and human genesis [2,3]. It is cooked for nixtamal which is ground with thick texture, mixed with toasted cocoa without peel and is also ground. Later, the paste is mixed with wather. In rural regions it is wrapped in banana leaf on short trips, in order to be consumed during the break of working hours, it is considered a good energizer [4]. In this work we study the possible anxiolytic effects of this beverage using models in mice to evaluate anxiety.

## Method

Pozol is made by cooking maize in an approximately 1% (w/v) lime solution, washing with water, grinding to make a dough known as nixtamal, mixed with grinding toasted cocoa in a 3:1 ratio respectively, shaping into paste balls. This is mixed with water. It was administered orally at doses of 0.1 ml / 10gr. One hour later of administration, the open field, hole board, dark light box and exploration cylinder tests were performed. Diazepam was used as a positive control (1 mg/kg;i.p.).

## Results

Our results show that in the open field test a dose of pozol did not modificate the locomotive horizontal nor vertical activity when it was compared between the negative control group. In the hole board test the treated group with pozol and the positive control group (diazepam) increased the explorations to the holes compared to the negative control group. Diazepam but no pozol increased the transitions in the light/dark box, nevertheless in the permanence time on the iluminated side, both diazepam and pozol increased the permanence compared against the control group. Finally, in the exploration cylinder test both the diazepam and the pozol group decreased the number of rearings on the cylinder wall when they were compared against the negative control group at 30 and 90 minutes.

## Discussion / Conclusion

Pozol is a refreshing non-alcoholic beverage, consumed in Southeastern Mexico since pre-hispanic times as an important part of the daily diet of diferent ethnic groups[5]. Our results indicate that this ancestral beverage has an anxiolytic effect in animal models and it is possible that this activity contributes to its pupular consumption. It is not yet clear whether the total activity of the pozol preparations is attributable solely to their cacao content.

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# PS009- Activity of *Excoecaria lucida* Sw upon *Trypanosoma cruzi*

Da Silva C. F.[1]*, Pacheco A.O.[2], Alves R. A.[1], Tavares J.F.[3], da Silva M.S.[3], Arranz J.C.E.[2] and Soeiro M.N.C. [1]
*franca@ioc.fiocruz.br

[1]*Laboratório de Biologia Celular, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil.*
[2]*Departamento de Farmacia. Universidad de Oriente, Santiago de Cuba, Cuba.*
[3]*Programa de Pos-graduação em Produtos Naturais e Sintéticos Bioativos, Universidade Federal de Paraíba, João Pessoa, Brazil*

## Introduction

American trypanosomiasis also known as Chagas disease (CD) is a neglected disease caused by the intracellular protozoan *Trypanosoma cruzi,* where about 6-7 million people are infected[1]. Natural products are an excellent source of active compounds[2]. The Euphorbiaceae family has been the subject of abundant phytochemical and pharmacological research because of its potential medical applications[3]. The aim of this work is investigate the in vitro the trypanocidal effects of a total extract, phases and isolated compounds of leaves of *Excoecaria lucida* with the goal of identifying novel candidates for possible future alternative therapies for CD

## Method

1.0 Total extract (TE) of E. lucida Sw. leaves was obtained by ethanol extract therefore fractionated sequentially with hexane, ethyl acetate and n-butanol, to obtain three phases: Hex, EA and But, respectively. Ellagic acid (EL1) was purified from both EA and But phases, while EL2; a 1:1 stigmasterol-3-O-β-D-glucopyranoside plus sitosterol-3-O-β-D-glucopyranoside mixture was obtained from the Hex phase.

2.0 As part of a multidisciplinary study to identify anti-*T. cruzi* novel candidates, bloodstream trypomastigotes (BTs – Y strain) were incubated at 37°C for 2 and 24 h in the presence of increasing doses (0-300 μg/mL)[4].

3.0 For the assay on intracellular forms, Tulahuen strains with β-galactosidase were employed using L929 cells[4].

4.0 Uninfected cultures (L929 cells) was submitted to compounds exposure and evaluated of colorimetric assays with Alamar blue for cytotoxicity[4].

## Results / Discussion / Conclusion

The EL1 and EL2 samples were more active against bloodstream trypomastigote forms with EC50 of 53.0±3.6 and 58.2±29.0 μg/mL, respectively; at 100 μg/mL. These samples also shown 70% of inhibition of L929 cells infection. Toxicity assays demonstrated that after 96 h of treatment only the fractions Hex and EA presented detectable cytotoxicity.

Ellagic acid, stigmasterol-3-O-β-D-glucopyranoside and sitosterol-3-O-β-D-glucopyranoside are reported for the first time in E. lucida Sw. leaves as well as additional screenings are needed aiming identify new agents that could be used to treat Chagas disease.

## Adknowledgment

FAPERJ, CAPES e FIOCRUZ.

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# PS010- Green synthesis, characterization and possible anticancer activity of (*E*)-1,2-diphenyl-2-(2-phenylhydrazineylidene)ethan-1-one

Cabrera-Vivas B.M.[1]*, Palillero-C. Á.[1], Ariza-R.J.[2]; Aguirre-C.C.[2], Meléndez-B.L.[1]; Ramírez-García, J. C.[1]
*bmcabreravivas@yahoo.com

[1]*PhD/Facultad de Ciencias Químicas, Edificio 9, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México*
[2]*Bachelor´s degree Student in Chemistry, F.C.Q., Edificio 9, 18 sur y Av. San Claudio. BUAP, C. P. 72001, Col. Sn Manuel, Puebla, México.*

## Introduction

Cancer is considered a genetic disease, because gens are responsible for controlling the function of cells, specially growth and cell division, it is considered one of the leading causes of mortality in the world.

Excluding skin cancer, colon-rectal cancer is the third cancer diagnosed more frequently in men and women in U.S. According to American Society for Cancer by the year 2018 rates for this cancer will increase: 95520 diagnosed of colon cancer and 39910 of rectal cancer[1a]. *Acute lymphocytic leukemia*, ALL is a type of cancer that begins in the limphocytes in the marrow[1b], and invade the blood fairly quickly. These type of cells propagate to other parts of the body such as lymphatic ganglion, liver, spleen and central nervous system. Research has shown that the derivitives hydrazones and hydracides[2] own interesting bioactivities, such as: antiinflamatory, antiplaquetary, antituberculosis and anticancer, among others**.** In this research paper the synthesis of a particular structure hydrazone is developed through the methodology takes place through Green Chemistry[3] because the reactants are used in equimolar quantities, Ethanol as a green solvent and no heating. Furthermore, since the products are crystals, their separation and purification is a simple recrystalizations.

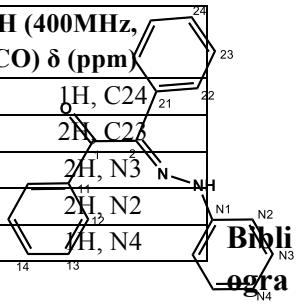
## Method

Phenylhydrazine (1.43 mmol, 263.16 mg) was dissolved in a solvent, a chemical equivalent (1.43 mmol, 300 mg) of benzyl which was previously dissolved in the same solvent and it was added drop by drop stirring constantly and which was developed with greener procedure[3]. The action mixture was kept at room temperature and was monitored by TLC, and then vacuum filtered. The hydrazone was recrystallized with cold methanol by a continuous and controlled process until light yellow powder obtain and characterization was performed by U.V, I.R. $^{1}$H NMR, $^{13}$C NMR.[4]

## Results / Discussion / Conclusion

Light yellow needle; yield: 82% at 25 $^{o}$C, mp. 100-102°C. (MW = 300.36 g mol $^{-1}$) UV $\lambda_{max}$ =210 nm. FT IR: (film): (cm$^{-1}$): 3310, 3283 ν(N-H); 3055 ν(Csp$^{2}$-H); 1637 ν(C=C-C=O), 1601, 1543 ν(C=N), 1249 ν(C-N); 891, 750 δ(Ph-C-H). $C_{20}H_{16}N_2O$.

| **RMN $^{1}$H (400MHz, $(CD_3)_2CO$) δ (ppm)** | | **RMN $^{1}$H (400MHz, $(CD_3)_2CO$) δ (ppm)** | |
|---|---|---|---|
| 9.45, s | 1H,N-H | 7.47, m | 1H, C24 |
| 8.03, m | 2H,C22 | 7.43, m | 2H, C23 |
| 7.59, m | 1H,C14 | 7.23, m | 2H, N3 |
| 7.53, m | 4H, C12, C13 | 7.16, m | 2H, N2 |
| | | 6.91, m | 1H, N4 |




| **RMN $^{13}$C (400 MHz, $(CD_3)_2CO$) δ (ppm)** | |
|---|---|
| 143.87 (C11) | 129.13 (C12) |
| 142.01 (C21) | 129.07 (C2) |
| 139.22 (N1) | 128.99 (C14) |
| 131.17 (C22) | 127.61(C24) |
| 130.97 (N4) | 121.83 (N3) |
| 129.55 (C23) | 114.34 (N2) |

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# PS011- Development and characterization of liquid crystal containing gemfibrozil

Herrera-Paredes M.E.[1], Alonso-Durán J.[2], Cañarte-Soledispa P.A.[1], Reyes-Baque A.D.[1], Murillo-Reyes L. C.[1], Kolenyak-Santos F.[1,3*]
*fernandakolenyak@outlook.com

[1] *MSc/Facultad de Ciencias Químicas, Av. Delta y Av. Kennedy, Universidad de Guayaquil, C. P. 471, Guayaquil, Ecuador.*
[2]*MSc/, Universidad Estadual Paulista, UNESP, Instituto de Química Departamento de Química Analítica. Rua Profesor Francisco Degni, Araraquara, São Paulo, Brasil, C.P. 14800-060.*
[3]*PhD/Universidad Estadual Paulista, UNESP, Programa de Nanotecnología Farmacéutica, Facultad de Ciencias Farmacéuticas, Departamento de Fármacos y Medicamentos Rodovia Araraquara-Jaú, Araraquara, São Paulo, C.P.14800-903.*

## Introduction

Gemfibrozil (GFZ) is an anti-lipid agent, derived from fibric acid, although widely used, gemfibrozil (GFZ)[1] has some characteristics that limit its use, such as poor solubility in water. Liquid crystals have intermitate properties between liquid and solid and can be structure in different mesophases, such as hexagonal, lamellar or cubic[2]. The aim of this work was developed and to characterize liquid crystals containing gemfibrozil (GFZ).

## Method

The phase diagram was developed with the mixture of oleic acid, tween 80® and water Milli Q®. All the sample the samples were maintained at 25 ± 0.1 °C for 24 h to complete system equilibration. The liquid crystals (LC31) were characterized by Polarized light microscopy, Texture profile analysis (TPA), Thermal Analysis Differential Scattering Calorimetry (DSC).

## Results / Discussion / Conclusion

**Fig. 1**. Polarized light microscopy.

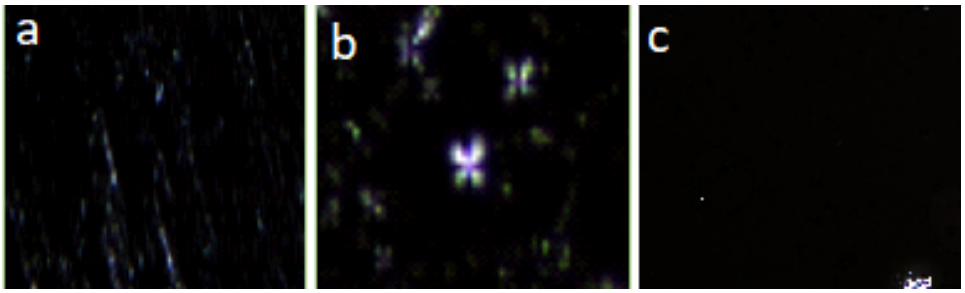


**Fig. 2.** Thermal Analysis Differential Scattering Calorimetry (DSC).

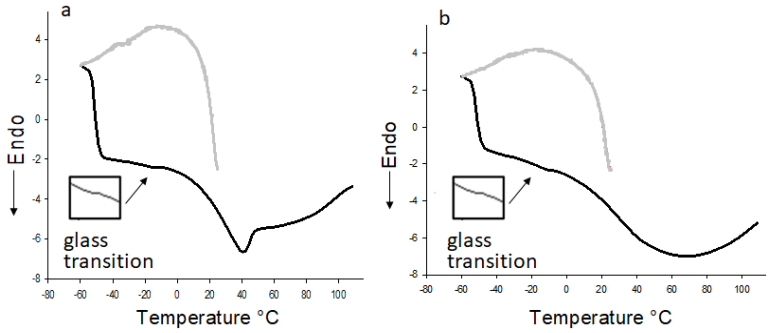


**Table 1**. Texture profile analysis (TPA)

| Sample | Hardness | Compressibility | Adhesiveness | Cohesiveness |
|---|---|---|---|---|
| LC 31 | 12,61967± 0,000702 | 126,1967± 9,776054 | 40,05667± 5,236892 | 99,47493± 0,010861 |
| LC-GFZ 31 | 7±0,0013 | 71,23±7,95 | 31,76±4.557 | 99,59±0,012 |

It was possible to obtaining hexagonal (Fig.1 a) lamellar (Fig. 1b) phases (LC31) incorporation of the GFZ (LC-GFZ 31) led to the presence of a black field (Fig. 1c), which can be indicative of microemulsions. DSC analysis showed an interaction of the drug with the liquid crystal (Fig 2a) due the emergence of a new fusion event and the drug fusion occurred in a distinct temperature and non-crystalline material (Fig 2b) due absence of peak. GFZ decreased the values of hardness and compressibility for all sample (Table 1) with can indicate greater fluidity. The results are confirmed by the values of adhesiveness with can indicate that the system does not need force to compress. The high values of cohesiveness may indicate that the sample does not break during the compression. These systems can be potential carriers for GFZ administration, providing potential advantages over conventional pharmaceutical forms.

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# PS012- Natural antioxidants in the recovery of silver from radiographic plates

Meléndez Balbuena L[1]*; Torres Mentado D. M[1].; Cabrera-Vivas, B. M[1].; Ramírez Monroy A[1].; Garate Morales J. L[1]; Arroyo Carranza S. L. M[1].
*lmbalbuena@hotmail.com; bmcabreravivas@yahoo.com

[1]*PhD/Facultad de Ciencias Químicas, Edificio 179, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72530, Col. San Manuel, Puebla, México*

## Introduction

This work shows the results obtained from the recovery of the silver contained in radiographic plates, using vegetable extracts that have antioxidant properties such as Parsley (Petroselinum sativum), Rosemary (Rosmarinus officinalis) , Pasiflora (Passiflora incarnata) and Hierbabuena (Mentha spicata) as reducing agents of the ionic silver to metallic silver[1]. This method offers vast possibilities taking into account the great diversity of plants with a wide range of metabolites that includes reducing and stabilizing agents in their composition, not only avoids the use of chemical reducing agents in many cases, expensive and toxic2, but it is also a more economical method and respectful with-it environment. The silver was recovered with different yields according to the extract used[3].

## Method

200 g of radiographs were treated with a dilution of 30% nitric acid, and then ammonium chloride was added for the formation of silver chloride, finally the silver chloride was treated with ammonium hydroxide in order to obtain the diaminplate (I) [4], to which each of the previously prepared aqueous plant extracts was added. The silver obtained was filtered from the supernatant, washed several times with distilled water and finally weighed. You get a clean silver with bright appearance in all four cases.

## Results / Discussion / Conclusion

Table 1 shows the amounts of silver recovered in each of the samples.

**Table 1**. Amount of silver recovered by 200 g of radiographs.

| Aqueous extracts | Recovered silver (grams) |
|---|---|
| Perejil (Petroselinum sativum) | 1.0996 |
| Hierbabuena (*Mentha spicata*) | 1.1135 |
| Romero (*Rosmarinus officinalis*) | 1.0429 |
| Pasiflora (*Passiflora incarnata*) | 1.1367 |

As expected, the amount of silver obtained in the four cases is not the same because the amount of silver depends on the components of each of the extracts used.
The obtained results indicate that, of the three extracts used in the recovery of silver. Showing the antioxidant capacity of plant extracts in all three cases.

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# PS013- Hierbabuena and epazote extracts as reducing agents in the synthesis of silver nanoparticles: antimicrobial activity

Meléndez Balbuena L[1]*.; Torres Mentado D. M[1].; Cabrera-Vivas, B. M[1].; Lobo Sánchez M[1].; Ramírez Monroy A[1].; Arroyo Carranza S.L.M[1].
*lmbalbuena@hotmail.com;bmcabreravivas@yahoo.com

[1]*PhD/Facultad de Ciencias Químicas, Edificio 9, 18 sur y Av. San Claudio. BUAP, C. P. 72001, Col. Sn. Manuel, Puebla, México. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*

## Introduction

This work focuses on obtaining silver nanoparticles using the aqueous extracts of Hierbabuena (*Mentha sativa*) and the natural extract obtained from epazote leaves (*Chenopodium ambrosioides*) as reducing agents, the effectiveness of the extracts is attributed to the antioxidant capacity of the polyphenols present[1]. Its reducing power was verified through the formation of silver nanoparticles, monitoring using UV-Vis spectra, identifying its presence by the appearance of surface plasmons located in the range of 430 to 455 $nm^{-1}$. characteristic in the metallic silver nanoparticles[2]. The antibacterial activity of the nanoparticles was evaluated in the presence of Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa isolated from the air in the Puebla city[3].

## Method

Synthesis of nanoparticles:

The extracts obtained from the leaves of epazote and mint plants were used to synthesize the nanoparticles starting from a 10-3M aqueous solution of $AgNO_3$. The synthesis was carried out by mixing 20 ml of the extract and 180 ml of the $AgNO_3$ solution in a vessel with constant agitation. Work was carried out at room temperature (26 ° C).

## Results / Discussion / Conclusion

The UV-Vis spectrophotometry used to characterize the Nps is a technique that has proved to be very useful for the rapid analysis of colloidal solutions of the Nps. Since the reduction of metallic ions produces solutions that in the case of silver have a yellowish color with an intense band between 400-450 nm, attributed to the collective excitation of the electrons on the surface of the particles (superficial plasmonic absorption)[2].

| Extracts | Wavelength |
|---|---|
| Epazote (*Chenopodium ambrosioides*) | 437 nm |
| Hierbabuena (*Mentha sativa*) | 445 nm |

The antibacterial activity of the nanoparticles was evaluated in the presence of Staphylococcus aureus, Staphylococcus epidermidis and Pseudomona aeruginosa isolated from the air in the Puebla city. The tests were carried out in triplicate with the Minimum Inhibitory Concentration

(MIC) and Minimum Bactericidal Concentration (CMB) by dilution in plate[4] methods. The results of this work show that the silver nanoparticles formation of is possible using a method based on green chemistry, with the same properties of antimicrobial activity as those that are prepared using chemical reducing agents[5].

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# PS014- Anti-cancer effects of wild mint's crude extract in adrenocortical tumor cell lines

Pezzani R.[1,2]*, Vitalini S.[3], Bertazza L.[1], Rubin B.[1], Redaelli M[4], Zorzan M.[1], Scaroni C.[1], Mian C.[1], Iacobone M.[5], Boscaro M.[1], Barollo S.[1], Armanini D.[1]
*raffaele.pezzani@unipd.it; sara.vitalini@unimi.it

[1] *Endocrinology Unit, Department of Medicine (DIMED), University of Padova, via Ospedale 105, Padova, 35128, Italy*
[2] *AIROB, Associazione Italiana per la Ricerca Oncologica di Base, Padova, Italy*
[3] *Department of Agricultural and Environmental Sciences, University of Milan, via G. Celoria 2, 20133 Milano, Italy*
[4] *Venetian Institute for Molecular Science and Experimental Technologies (VIMSET), pz. Milani, 30010, Campolongo Maggiore, Venezia, Italy*
[5] *Minimally Invasive Endocrine Surgery Unit, Department of Surgery, Oncology and Gastroenterology (DISCOG), University of Padova, Via Giustiniani 2, 35128, Padova, Italy*

## Introduction

Mint is an aromatic plant that belongs to Lamiaceae family. It is traditionally used as herbal tea in Europe, Australia and North Africa and shows numerous pharmacological effects, such as spasmolytic, antioxidant, antimicrobial and anti-hemolytic [1]. Recently its antiproliferative role has been suggested in a small number of tumor cell models, but no data are available on adrenocortical carcinoma, a malignancy with a survival rate at 5 years of 20-30% which frequently metastasize [2]. This work analyzed the effects of Mentha longifolia L. extract on adrenocortical tumor cell models.

## Method

Two cell lines were used: H295R and SW13 cells. Different cellular and in vitro experiments were performed to evaluate the crude methanolic extract of wild mountain mint harvested in Vermiglio (TN), Italy.

## Results / Discussion / Conclusion

Chemical composition of methanolic extract of wild mountain mint was assessed by gas-chromatography/mass spectrometry analysis. Cell viability and vitality were evaluated by MTT, SRB and trypan blue assays in H295R and SW13 cells. The anti-proliferative effects of mint were more evident in SW13 cells at 72h. Combination of the extract with mitotane (approved drug for adrenocortical carcinoma) reinforced the efficacy of the herb. As control, human fibroblasts were treated with mint, though no effect on cell viability was perceived. Brine shrimp lethality assay showed no alteration of mortality at lower mint doses. Wright staining demonstrated the presence of both necrotic and apoptotic cells, more evident with combined treatments (mint+mitotane). Other experiments are in progress to expand the possible effects of mint extract.
The crude extract of wild mint can decrease cell viability, vitality and survival of adrenocortical tumor cell models, in particular of SW13 cells. These data show the potential of mint extract, still more work is needed to corroborate these findings.

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# PS015- The seed oil from Sicilian *Opuntia ficus-indica* Sanguigna cultivar as a promising bioactive food ingredient

Loizzo M.R.[1]; Bruno M.[2,*], Pacetti D.;[3] Leporini M.[3]; Falco T.[3]; Tundis R.[1]
*maurizio.bruno@unipa.it

[1]*Prof., Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, University of Calabria, Arcavacata di Rende (CS), Italy.*
[2]*Prof., Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze, I-90128 Palermo, Italy.*
[3]*Prof., Department of Food Science, Politecnica delle Marche University, via Brecce Bianche, 60131 Ancona, Italy.*
[4]*PhD, Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, University of Calabria, Arcavacata di Rende (CS), Italy.*

## Introduction

During the last ten years, consumers more addressed their attention to high quality of foods (Akinya et al., 2014). *Opuntia ficus-indica* belongs to the Cactaceae family. Almost 300 species of the genus *Opuntia* are known but so far, only 12 species have been used in food industries. Italy is the second largest *O. ficus-indica* producer in the world. The yellow cultivar Surfarina is the main cultivar in Sicily, followed by the red cultivar Sanguigna (Butera et al., 2002). The aim of this study is investigate the radicals scavenging potential of Sanguigna seed oil in order to propose its use as food preserving agent.

## Methods

The red fruits of *Opuntia ficus-indica* Sanguigna cultivar were collected in Roccapalumba area (Palermo, Sicily, Italy) at an altitude of 550 m above sea level. Seeds powder was extracted by Soxhlet apparatus using *n*-hexane as solvent according to AOAC (2005). The radicals scavenging activity was assessed by using DPPH and ABTS test (Loizzo et al., 2013). The total carotenoids and γ-tocopherol content was also determined.

## Results / Discussion / Conclusion

Extraction procedure lead a yellow oil with a percentage of 9.3% and a total carotenoid content of 8.4 mg/kg. DPPH and $ABTS^{\cdot+}$ radicals have a different stereochemistry and a different training mechanism, and therefore, after reaction with antioxidants, they give a qualitatively different response to the inactivation of their radicals. For this reason, to assess the antioxidant capacity of a complex matrix more than one test should be applied. *O. ficus-indica* Sanguigna oil showed a promising radical scavenging activity with $IC_{50}$ values of 40.9 and 48.5 μg/mL in DPPH and ABTS tests, respectively. Significant content of vitamin E was also detected.
In conclusion, result confirm the potential use Sanguigna oil as promising source of healthy compounds useful not only as antioxidant to preserve lipid components in food preparation but also as functional ingredient. All these activities will result in a further increase in the commercial value of this oil.

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# PS016- Triterpenes present in *E. tereticornis* reduce their toxicity and improve their anti-inflammatory properties when they are in a plant extract

Balcazar N.[1]; Muñoz D.L.[2]; Castaño A.[4], Echeverri L.F.[3], Acín S[1]*.
*sergio.acin@udea.edu.co

*[1]PhD, [2]MSc/Grupo GENMOL. Sede de Investigación Universitaria (SIU), Universidad de Antioquia, Calle 62 # 52-59, Medellín, Colombia.*
*[1]PhD, [2]MSc/Dpto de Fisiología y Bioquímica, Facultad de Medicina, Universidad de Antioquia, Carrera 51D Nº 62-29, Medellín, Colombia*
*[3]PhD, [4]MSc student/ QOPN Group - School of Natural and Exacts Sciences. SIU, Universidad de Antioquia, Calle 62 # 52-59, Medellín, Colombia*

## Introduction

Obesity-associated inflammation is a risk factor associated with insulin resistance and it is linked with cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). New anti-inflammatory treatments are targeting this adipose tissue inflammation (Reilly SM. et al., 2017). Ursolic acid (UA) and Oleanolic acid (OA) are natural triterpene compounds with anti-inflammatory and anti-oxidant properties that suggest these molecules could have a promising role in the treatment of inflammatory disorders (Dae Yun Seo et al., 2018; Dharambir Kashyap et al. 2016). We have identified UA, OA and Ursolic acid lactone (UAL) as the main molecules (78%) in an *E. tereticornis* extract and shown the mixture of these three different triterpenes has a potent effect of reducing pro-inflammatory cytokine levels in adipose tissue and other immunometabolic abnormalities generated in adipose tissue cells (Guillén A., et al. 2015; Susana Ceballos, et al. 2018). Our aim is to compare the effects of the triterpenes mixture present in the natural extract and the molecules outside the extract on macrophage cell viability, oxidative stress and expression of pro-inflammatory genes.

## Method

A crude methanolic extract (OBE100) from leaves of Eu was partitioned with ethyl acetate, concentrated and purified by Sephadex LH-20. UA and OA were purchased from Sigma-Aldrich (St. Louis, MO) and UAL was purified by Sephadex column and preparative chromatography from OBE100. Murine macrophage cell line J774A.1, activated with lipopolysaccharide (LPS) and INF-γ was used to evaluate OBE100, mix of triterpenes with the concentration present in OBE100 (M1), UA, OA and UAL anti-inflammatory action. Cell line viability was determined using MTT assay and 7-AAD fluorometric assay, oxidative burst was quantified by flow cytometry using Dihydrorhodamine 123 (DHR) and Gene expression levels of TNF-α, IL-6 and IL-1β were detected by real-time RT-PCR.

## Results / Discussion / Conclusion

The treatment of macrophages with M1 mixture or UA is more toxic than the treatment of the cells with the same amount of triterpenes present in the OBE100 extract. Only the treatment with OBE100 reduced the oxidative burst in activated J774A.1 macrophages. The gene expression of

pro-inflammatory cytokines is reduced mainly by the treatment of the cells with M1, UA and specially OBE100 taking into account that we can use it in higher concentration because of its lower toxicity. *Conclusions:* These results suggest that triterpenes present in *E. tereticornis* extract have anti-inflammatory and anti-oxidant properties, as previously has been described, but the combination of these triterpenes with other minor molecules present in the vegetal extract have a synergistic effect that improves them, reducing the toxicity of the triterpenes.

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# PS017- Application of dispersive liquid–liquid microextraction for the determination of pyrrolizidine alkaloids in honey

Celano R.[1], Campone L.[1,2], Rastrelli L.[1], Russo M.[2], Piccinelli A.L.[1]

[1]*Department of Pharmacy, University of Salerno;*
[2]*Food Chemistry, Authentication, Safety and Sensoromic Laboratory – Università Mediterranea di Reggio Calabria*

## Introduction

Pyrrolizidine alkaloids (PA) are secondary plant metabolites with carcinogenic and genotoxic properties. Currently, more than 600 PA are known. They occur in plants of the families of Boraginaceae, Asteraceae and Fabaceae. The pollen of these plants are a potential source for pyrrolizidine alkaloids and may lead to a contamination of honey [1]. The aim of this research was to develop a multiresidue method for the determination of five PAs and four PANOs in honey samples.

## Methods

The determination and quantitative analysis were performed by UHPLC-MS/MS. Dispersive liquid–liquid microextraction (DLLME) was selected as extraction and clean-up technique considering its many advantages: simplicity, speed, efficiency and eco-sustainability.

## Results / Discussion / Conclusion

The experimental parameters affecting on the DLLME efficiency were carefully studied and optimized using a multilevel experimental design. The obtained results showed acceptable recovery (75-100%) for almost all analytes (Fig. 1) with a precision (expressed as relative standard deviation, RSD) of 0.4–4.2%. Zn reduction was employed to obtain an accurate quantification of total PAs levels. The developed method showed excellent limits of detection (LOD) and limits of quantification (LOQ). Finally, the whole analytical procedure was validated, applied to the analysis of 26 Italian honey samples and compared with the method most widely used in the analysis of PAs in honey (SPE with strong cation exchange sorbent).

To the best of our knowledge, this report describes the first application of DLLME to the determination of PAs in food matrices. The proposed method, compared with the most widely used method in the analysis of PA in honey, provided similar or higher extraction efficiency. The main advantages of developed method are the simplicity of operation, the rapidity to achieve a very high sample throughput and low cost.

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# PS018- Content of phytoestrogen coumestrol in alfalfa genotypes infected with a viral disease

Silva M.[1]*, Chiacchiera S.[2], Mamaní, E.[1], Manzur M.E.[3], Basigalup D.[1]
*silva.mercedes@inta.gob.ar

[1] *EEA – Manfredi. Ruta Nacional N° 9. km 636. CP 5988. Córdoba, Argentina.*
[2] *Departamento de Química. Facultad de Ciencias Exactas Física Química y Naturales. Universidad Nacional de Rio Cuarto.*
[3]*IIB-INTECH-UNSAM-CONICET, Av. 25 de Mayo y Francia CP 1650. San Martín, Prov. de Buenos Aires, Argentina.*

## Introduction

Crop plants are frequently exposed to different biotic and abiotic stress conditions[1]. In the last years, the production of alfalfa in Argentina has been affected by several viral diseases[2], particularly a co-infection of rhabdovirus and alfalfa mosaic virus[3]. Consequently, when the plant defense machinery is activated, the synthesis of several secondary metabolites can increase[1]. In alfalfa, there are some metabolites with important nutraceutical properties which are mainly localized in leaves. Two of them, coumestrans and isoflavonoids[4], rise dramatically when the plants are exposed to viral infection. The objective of this work was to compare coumestrol content in individual genotypes (clones) from two alfalfa varieties under two conditions: infected vs non-infected.

## Materials and Methods

Seven genotypes from *cv* Monarca SP INTA and six genotypes from *cv* Traful PV INTA were used in this study. Plants were cloned and divided into two groups: infected with viral complex (I) and free of infection (NI). Coumestrol content was measured in both conditions when plants reached 380° degree - day. For each genotype, plant homogenate was obtained adding 2 ml of 3.5M HCl and 8ml of ethanol to 0.5 grams of herbage dry sample. This mixture was heated at 83°C for 30 min. and cooled to room temperature. Then, the solution was centrifuged for 10 minutes at 10,000 rpm at 4°C. After filtration, 10 µl aliquot was injected into the HPLC (Agilent 1100 series) and Coumestrol was detected at 260 nm (Diode Array Detector, series G1315B) and identified using an external standard (Sigma-Aldrich®). All data were analyzed using the Linear Mix Model test, followed by a post-hoc comparison test DGC (Prueba de Di Rienzo, Guzmán y Casanoves, 2002).

## Results and Discussion

Differences in coumestrol content among genotypes and cultivars were detected (Table 1). Overall, there was a general trend to increase coumestrol production in response to viral infection, but only three genotypes (M18, M29 and T124) showed significant increases when infected with virus over the virus-free treatment.

**Table 1.** Content of coumestrol for 13genotypes from cultivars Monarca (M) and Traful (T). Genotypes were cloned and the clones were infected (I) or non-infected (NI) with a viral complex.

| M | NI | | I | | T | NI | | I | |
|---|---|---|---|---|---|---|---|---|---|
| M10 | 381,2 | ± 39,6 $^{F}$ | 337,7 | ± 41,3 $^{F}$ | T112 | nd | | nd | |
| M11 | 1107,9 | ± 40,2 $^{A}$ | 1125,5 | ± 40,2 $^{A}$ | **T124** | **727,6** | **± 40,2 $^{D}$ **** | **856,4** | **± 40,2 $^{C}$** |
| **M18** | **382,1** | **± 39,7 $^{F}$*** | **524,9** | **± 39,8 $^{E}$** | T129 | 941,4 | ± 39,7 $^{B}$ | 950,5 | ± 39,8 $^{B}$ |
| M23 | 784,0 | ± 39,7 $^{C}$ | 835,4 | ± 41,0 $^{C}$ | T140 | 650,1 | ± 39,6 $^{D}$ | 759,9 | ± 39,8 $^{D}$ |
| **M29** | **695,5** | **± 39,7 $^{D}$*** | **795,1** | **± 39,7 $^{C}$** | T141 | 728,6 | ± 31,2 $^{D}$ | 741,3 | ± 31,7 $^{D}$ |
| M36 | 444,3 | ± 39,7 $^{E}$ | 521,0 | ± 40,1 $^{E}$ | T152 | nd | | nd | |
| M37 | 448,8 | ± 40,2 $^{E}$ | 527,0 | ± 39,8 $^{E}$ | | | | | |

Indicated values are mean peak areas ± E.E. and are expressed in ppm/material dry weight. Values are mean of 18 plants ± EE. COU: coumestrol; NI: free of viral infection; I: infected with viral complex; Different letters indicate overall significant differences (P<0.05) between treatments and genotypes. Differences within genotypes (unpaired t-test) between NI and I treatments are indicated by * (P<0.05); ** (P<0.01); *** (P<0.001); and nd (not detected).

## Conclusions

Our results suggest that the viral complex stimulates coumestrol accumulation along alfalfa life cycle and that such viral induction may be heterogeneous among varieties. The individual genotypes might be considered as interesting alternative source of these metabolites.

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# PS019- Avaliação da citotoxicidade e genotoxicidade do extrato hidroalcoólico de *Inga marginata* W.

Maziero M.[1]*, Lovatto-Ortiz M.[1], Dornelles-Castro R.[2], Moraes G.G.[1], Sagrillo-Rorato M.[3], Manfron-Palermo M.[4]
*maiaramazziero@gmail.com; melaniapalermo@gmail.com

[1]*Mestrandas/Doutoranda - Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*
[2]*Doutoranda - Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*
[3]*Doutora - Universidade Franciscana, Santa Maria, RS, Brasil.*
[4]*Doutora – Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

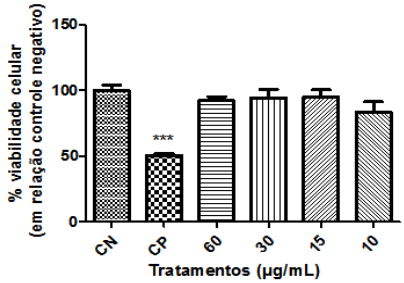
## Introdução

As plantas medicinais são usadas desde a antiguidade pelo homem para curar diversas enfermidades. Ainda hoje, são muito utilizadas pela população de forma paliativa, para a cura e também associadas aos medicamentos convencionais. *Inga marginata* W. pertence à família Fabaceae, gênero Inga, possui diversas nomenclaturas populares como ingá e ingá-mirim, é uma planta, com ampla distribuição na América Latina (LORENZI, 2002). *Inga marginata* possui propriedades antidiarreicas, antimicrobianas, antinociceptivas, anti-inflamatórias, dentre outras (POMPEU et al., 2012). Tendo em vista os diferentes usos desta planta, é necessário avaliar a toxicidade celular e em nível de DNA, podendo assim, utilizá-la de forma segura e eficaz.

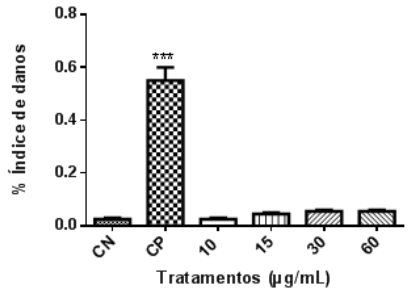
## Métodos

A citotoxicidade do extrato de *I. marginata* foi realizada por meio do ensaio de MTT, (FUKUI et al., 2010) modificado. Em uma placa de Elisa de 96 poços foram pipetados 200 µL de suspensão celular previamente preparada e 20 µL de MTT, incubando por 1h em estufa a 37 $^{0}$C. Após descartou-se 180 µL do sobrenadante e, adicionado 200 µL de DMSO, centrifugou-se, e retirados 100 µL da solução para leitura em espectrofotômetro. O ensaio Cometa,(SINGH et al., 1988) modificado foi utilizado para avaliar a genotoxicidade. Lâminas com agarose e suspensão celular, foram previamente preparadas e realizada lise celular e eletroforese. As etapas subsequentes foram neutralização, fixação, coloração e análise em microscópio.

## Resultados / Discussão / Conclusão




Ensaio MTT após 24h de incubação. CN: controle negativo, CP: controle positivo.




Ensaio Cometa após 24h de incubação. CN: controle negativo, CP: controle positivo.

Todos os resultados foram expressos em porcentagem do controle negativo (100%). Sendo CN o controle negativo:células em meio de cultura. Os dados foram expressos com média ± desvio padrão (DP). As análises foram realizadas por variância (ANOVA) de1 via, seguida por teste *post hoc* de Dunnet. Os valores com $p < 0,05$ foram considerados estatisticamente significativos. Sendo $^{*}p < 0,05$, $^{**}p < 0,01$ e $^{***}p < 0,001$.

Em relação aos resultados obtidos no ensaio do MTT, em nenhuma concentração testada do extrato de *I. marginata* houve morte celular portanto não apresentou toxicidade celular. Todas as concentrações testadas foram equivalentes ao controle negativo. No ensaio Cometa todas as concentrações do extrato testadas também não apresentaram dano significativo ao DNA. A concentração de 10 µg/mL apresentou resultado próximo do controle negativo, apresentando o menor dano quando comparado as demais concentrações do extrato.

Com base nesses resultados, pode-se concluir que o extrato de *I. marginata* não é citotóxico e genotóxico.

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# PS020- *Plinia peruviana:* atividade antioxidante, teor de fenois totais, flavonoides totais e citotoxicidade em glioblastoma (C6)

Moraes G.G.[1]*, Ortolan S.A[2*], Maziero M.[3], Lovatto M.O.[3], Dornelles R.C.[4], Silva C.M.[5], Manfron M.P.[6]

*greicegmoraes@yahoo.com.br; melaniapalermo@gmail.com

[1]*Doutoranda / Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

[2]*Acadêmico de Farmácia / Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brasil.*

[3]*Mestranda / Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil*

[4]*Doutoranda / Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

[5]*Doutora / Universidade de Santa Cruz do Sul, Santa Cruz do Sul, RS, Brasil.*

[6]*Doutora / Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

## Introdução

*Plinia peruviana* (Poir.) Govaerts (jaboticabeira), pertencente à família Myrtaceae, é uma árvore de porte médio com frutos comestíveis e ocorre do estado do Pará ao Rio Grande do Sul no Brasil[1,2]. Estudos farmacológicos indicam que os frutos desta espécie apresentam atividades biológicas como anti-inflamatória[3], antiproliferativa[3,4], antibacteriana[5] e antidiarreica[6], mas estudos utilizando suas folhas são escassos na literatura. Assim, o extrato hidroalcoólico liofilizado das folhas de *P. peruviana* foi avaliado quanto à capacidade antioxidante, a quantificação de fenois totais e de flavonoides totais e a atividade citotóxica frente à linhagem de célula tumoral C6 (glioblastoma).

## Métodos

A atividade antioxidante foi determinada pelo ensaio de capacidade de absorção de radicais de oxigênio (ORAC), conforme descrito por OU et al. (2001)[7]. Utilizou-se fluoresceína como sonda fluorescente e 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) como gerador de radicais peroxila. A quantificação de fenois[8] totais e flavonoides[9] totais foram realizadas através de técnicas espectrofotométricas. A atividade antiproliferativa foi realizada *in vitro* frente à linhagem de célula tumoral de glioblastoma (C6), segundo Monks (1991) [10], com algumas modificações, através do ensaio do corante proteico sulforrodamina B (SRB).

## Resultados / Discussão / Conclusão

A Tabela 1 apresenta os resultados obtidos para as determinações de atividade antioxidante (ORAC), polifenois totais e flavonoides totais. O extrato liofilizado de *P. peruviana* apresentou citotoxicidade frente à linhagem de célula tumoral de glioblastoma (C6). Considerando-se os valores de morte em 50%, determinou-se a concentração ($IC_{50}$) de 360 µg/mL do extrato, em 48 horas de tratamento sobre as células C6.

**Tabela 1** – Atividade antioxidante e quantificação dos conteúdos totais do extrato hidroalcoólico das folhas de *P. peruviana*

| Análises | Média[a] |
|---|---|
| ORAC (g extrato/g Trolox)[b] | 5,29±0,57 |
| Teor de polifenois (mg EAG/g)[c] | 61,885±0,0856 |
| Teor de flavonoides (mg ER/g)[d] | 146,594±0,0040 |

[a]Valores apresentados em média ± desvio padrão dos ensaios em triplicata; [b]Equivalente Trolox; [c]Total de polifenois expressos em gramas de ácido gálico equivalentes por grama de extrato (mg de GAE/g). [d]Flavonoides totais expressos em gramas de rutina equivalentes por grama de extrato (R/g).

Os resultados se mostram promissores para as futuras pesquisas com a planta medicinal *P. peruviana* sobre o potencial biológico, bem como estabelecer os possíveis mecanismos de ação.

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# PS021- Genotoxicidade e determinação de polifenois por UHPLC/MS em extratos brutos de *Richardia brasiliensis* Gomes

Dornelles R.D.[1]*, Moraes G.G.[2], Maziero M.[2], Lima R.[2], Vianna C.[3], Machado M.M.[4], Manfron M.P.[3]

*rafacdornelles@gmail.com; melaniapalermo@gmail.com

[1]*Doutoranda - Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

[2]*Mestranda/Doutoranda - Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

[3]*Doutora – Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.*

[4]*Doutor – Universidade Federal do Pampa, Uruguaiana, RS, Brasil.*

## Introdução

*Richardia brasiliensis* Gomes é uma espécie nativa da América do Sul, encontrada em ambientes com intensa atvidade agrícola[1,2]. Tem sido reportada como antiemética, antidiabética, anti-hemorroidal, vermífuga, antimicrobiana e antiproliferativa, além de terem sido identificadas substâncias ativas de interesse medicinal, como os flavonoides canferol e isorametina-3-O-rutinosideo, cumarinas como a cumarieletefina, escopoletina, cedrelopsina, norbraylina e braylina, alcaloides, esteroides, triterpenos como o ácido oleanólico, resinas e ácidos orgânicos como o ácido p-hidroxi-benzoico e ácido m-metoxi-p-hidroxi-benzóico[3,4]. Em vista disso, esta pesquisa identificou compostos polifenolicos e avaliou a genotoxicidade dos extratos hidroetanólicos das partes aéreas de *R. brasiliensis*, obtidos nas diferentes estações do ano.

## Métodos

Os extratos foram caracterizados por UHPLC-MS/MS com ionização por electrospray, através de metodologia desenvolvida e validada por Faccin *et al*. (2016)[5]. Os parâmetros genotóxicos foram avaliados em culturas de leucócitos[6], no qual foi avaliada a viabilidade celular[7], micronúcleo[8] e cometa alcalino[9]. Foram utilizados os quatro extratos brutos (EBV, EBO, EBI, EBP) nas concentrações de 10, 100 e 500 µg/mL, um controle negativo com o PBS pH 7,4 e um controle positivo com o peróxido de hidrogênio 100 µM. Os parâmetros foram avaliados em triplicata, após 72h de contato.

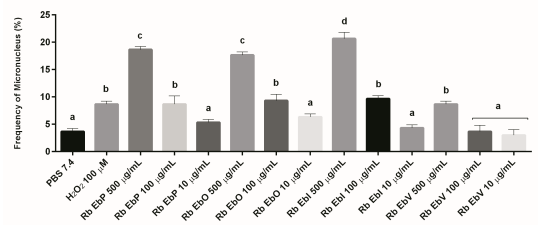
## Resultados / Discussão / Conclusões

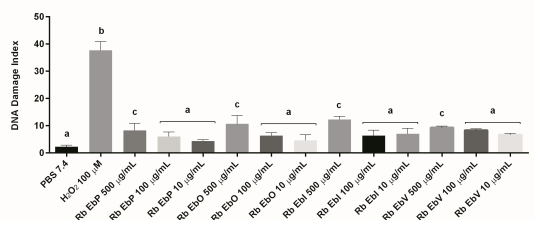
As substâncias identificadas nos extratos foram: ácido clorogênico, catequina, ácido vanílico, ácido cafeíco, ácido p-cumárico, ácido ferúlico, rutina, ácido rosmarínico, quercitrina, ácido trans-cinâmico, quercetina, luteolina, apigenina e canferol. Ácido clorogênico e rutina foram as substâncias com maiores concentrações principalmente no EBO. Nos ensaios de genotoxicidade, as três concentrações testadas demonstraram uma viabilidade maior que 85%. O gráfico 1 apresenta os resultados da frequência de micronúcleo, demonstrando que os EB de *R. brasiliensis* nas concentrações de 500 e 100 µg/mL (com exceção do EBV 100 µg/mL) podem ser mutagênicos, já o gráfico 2 apresenta os resultados do ensaio cometa, resultando em índice de

**Gráfico 1**: Frequência de micronúcleo dos diferentes EB de R. brasiliensis nas concentrações de 10, 100 e 500 µg/mL.

**Gráfico 2**: Ensaio do DNA cometa com ID dos diferentes EB de R. brasiliensis nas concentrações de 10, 100 e 500 µg/mL.

dano ao DNA (ID). É possível observar que os EB nas diferentes concentrações apresentaram baixos ID, diferindo estatisticamente do controle positivo.




Com base nos resultados obtidos, *R. brasiliensis* demonstrou ser uma espécie com potenciais atividades biológicas devido à importantes substâncias ativas identificadas em seus EB, porém merece estudos mais aprofundados em relação a genotoxicidade, pois foi mutagênica quando utilizada em concentrações mais elevadas.

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# PS022- New lignans from *Cedrela odorata* L. Stem Bark

Muñoz Camero C.[1]*, De Leo M.[1], D'Ambola M.[2], Gualtieri M.[3], Braca A.[1], De Tommasi N.[2]
*cesar.munoz@farm.unipi.it; cesare.mc1@gmail.com

[1]*Università di Pisa, Dipartimento di Farmacia. Via Bonnano Pisano 33, 56126 Pisa, Italy.*
[2] *Università di Salerno, Dipartimento di Farmacia. Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy.*
[3] *Department of Pharmacognosy and Organic Drug, University of Los Andes, Sector Campo de Oro, detras del IAHULA, 5101 Mérida, Venezuela.*

## Introduction

*Cedrela odorata* L., a plant member of the Meliaceae family, is a timber tree native to tropical regions of America, also introduced as a cultivated species in Africa and many tropical countries of Asia and Oceania. The infusion of *C. odorata* stem bark is used in South American folk medicine for the treatment of fever, hemorrhage, inflammation, and digestive diseases, including diarrhea, vomiting, and indigestion. The decoction of the bark is also used in Africa as a remedy for malaria and fever[1]. Meliaceae plants, particularly species of *Cedrela* genus, are distinguished by the occurrence of limonoids, alkaloids, and polyphenols such as lignans and proantocianydins[2,3]. Limonoids has been suggested to be the main responsible of *C. odorata* pest resistence, however, other biological studies have shown that polyphenols contained in several parts of the plant can have detrimental effects on insects[4]. This consideration prompted us to investigate *C. odorata* stem bark polar extracts.

## Method

The dried and powdered *C. odorata* stem bark (300 g), collected in Merida, Venezuela, was sequentially extracted with *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH. The $CHCl_3$-MeOH extract was subjected to silica gel chromatography, using $CHCl_3$ and increasing concentrations of MeOH in $CHCl_3$ (0-100%) as eluents, while the MeOH extract was separated on Sephadex LH-20 column eluting with MeOH as eluent. Subsequently, the fractions obtained from both silica gel and Sephadex LH-20 column chromatographies were submitted to RP-HPLC.

## Results / Discussion / Conclusion

The phytochemical study of *C. odorata* stem bark polar extracts led to the isolation of eight compounds including two new lignans (**1**–**2**) (**Fig. 1**), which structures are shown in **Fig.1**, and six known substances that were characterized as 4,5-dihydroblumenol A (**3**), 7-megastigmene-3α,6,9-triol (**4**), catechin (**5**), scopoletin (**6**), homovanillic alcohol (**7**), and 2-(3,4-dimethoxyphenyl)ethyl-*O*-β-d-glucopyranoside (**8**). The structural determination of the isolated secondary metabolites was performed by 1D- and 2D-NMR spectroscopic techniques, and by mass spectrometry analyses. All compounds obtained from this species are in accordance with the ones found in other Meliaceae plants.

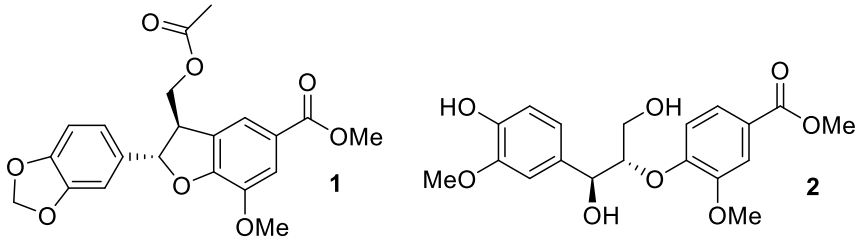


**Fig. 1** Structures of compounds **1**–**2**

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# PS023- Determination of the effect of BSS-4 cholestanic derivative on carrageenan-induced inflammatory process

Casimiro-Rosas M.[1]*, Fernández-Herrera M.A., Sandoval-Ramírez J.[2], Mendieta L., Luna F., Martínez I.[1]
*mary_cr25_05@hotmail.com

*[1]Departamento de Farmacia, [2]Laboratorio de Síntesis y Modificación de productos naturales Benemérita Universidad Autónoma de Puebla, 14 sur y Av. San Claudio, Col. San Manuel, Puebla C. P. 72592, México.*

## Introduction

Inflammatory diseases are a major cause of morbidity worldwide. At present, non-steroidal anti-inflammatory drugs and steroids are the most common drugs used to treat inflammation, however, the side effects of these limit their application. That is why the search for effective natural substances in the treatment of inflammatory diseases is increasing. Sapogenins are natural compounds of great importance due to their use in the preparation of some biologically active steroidal products[1]. They are substances that are widely distributed in an extensive variety of plants, many of them with medicinal properties. The triacetate of 22-oxocolest-5-en-3β, 16β, 26-triyl (BSS-4) is a cholestanic compound synthesized from diosgenin[2], which has been shown to have anti-inflammatory properties by having a similar structure to that of anti-inflammatory steroid drugs[3].

## Method

A baseline measurement of the posterior extremities of the subjects was obtained using a plethysmometer. The inflammation occurred in rats of the Wistar strain (250-300g) through with the subcutaneous administration of 50µl of carrageenan in the plantar area of the right paw of each subject. One hour later, the different treatments were administered: 0.04 ml of vehicle or isotonic saline solution, BSS-4 at doses of 0.5 and 1.0 mg / kg and dexamethasone 0.5 mg / kg; the inflammation was evaluated every 30 minutes for three hours after the treatments administration.
Finally, samples from the plantar tissue were obtained to evaluate the expression of two proinflammatory cytokines, TNF-alpha and IL-1beta by immunohistochemistry; also histological analysis was performed by hematoxylin and eosin staining of tissue.

## Results / Discussion / Conclusion

In this work was evaluated the anti-inflammatory effect of BSS-4, whose molecular structure is similar to that of steroids, widely used as a treatment against inflammation.
Our results showed that BSS-4 may be exerting an anti-inflammatory effect, observing that inflammation of the paw decreased after the administration of the treatments compared to those who did not receive any treatment.
On the other hand, it was observed that the expression of the cytokines TNF-α and IL-1β also decreased in the groups with treatment compared to the control groups.

In summary, the BSS-4 causes an anti-inflammatory effect by suppressing the expression of proinflammatory cytokines.

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# PS024- Antinociceptive and anti-inflammatory effect of extracts of *Salvia purpurea* Cav. (Lamiaceae)

Cuevas-Morales C.[1*], Zavala-Ocampo L.M.[1], Basurto-Peña F.[2], Carmona-Castro A.[3], Ramírez-Aguirre R.[1], Aguirre-Hernández E.[1]
*cristhiancuevas@ciencias.unam.mx

[1]*Laboratorio de Fitoquímica, Departamento de Ecología y Recursos Naturales. Facultad de Ciencias.*
[2]*Jardín Botánico, Instituto de Biología.*
[3]*Bioterio de la Facultad de Ciencias, Universidad Nacional Autónoma de México. Ciudad Universitaria Coyoacán, Av. Universidad 3000, Distrito Federal, C.P. 04510, CDMX.*

## Introduction

*Salvia purpurea* Cav (Lamiaceae) is a medicinal plant used as an anti-inflammatory and anti-diarrheal agent in the states of Chiapas and Veracruz[1], [2]. Likewise, an ethnobotanical study conducted in the town of Santiago Huauclilla, Oaxaca[3] showed that *S. purpurea*, known by the common name of "Salvia moradita", is widely used by the inhabitants of said community to relieve toothache[3]. In this sense and in order to corroborate its medicinal use for pain and inflammation, in this work the antinociceptive effect of *S. purpurea* was evaluated in two experimental models in rodents.

## Methods

The aerial part of the plant was dried at room temperature, then triturated and macerated with acetone and methanol, the solvents were evaporated by distillation under reduced pressure. For the aqueous extract, a decoction was made and lyophilized. The antinociceptive activity was evaluated in CD1 mice, using the formalin and "Writhing" tests. The identification of the secondary metabolites present in the active extracts was carried out by chromatographic techniques.

## Results / Discussion / Conclusion

All extracts produce a significant decrease in pain in the early (neurogenic) phase of the formalin test (Fig. 1A), however, only organic extracts significantly inhibit the behavioral response in the late phase of the test (inflammatory) (Fig. 1B). Likewise, both the organic and aquous extract of *S. purpurea* significantly inhibit the number of abdominal stretches caused by the administration of 1% acetic acid (Fig, 1C). The results of this study give evidence of the antinociceptive activity both neurogenic and anti-inflammatory of *S. purpurea*, where terpenoid and flavonoid constituents possibly participate as partial responsible for this activity.

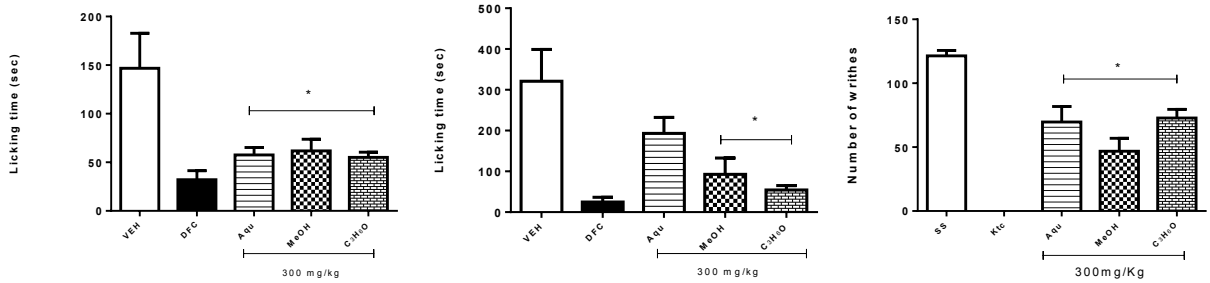


**Figure 1**. antinociceptive effect of extracts of *Salvia purpurea*. Licking time, in early phase of formalin test 0-10 min (A), Licking time, late phase in formalin test 10-30 min (B). Number of abdominal stretches in 30 min (C). Saline solution (VEH), Diclofenac (DFC), Ketorolac (KTC), Aquous extract (Aqu), Methanol extract (MeOH), Acetone extract ($C_3H_6O$). The bars represent the mean ± E.E.M. of six animals. * P <0.05, ANOVA followed by the Dunnett test.

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# PS025- Vascular interactions of the main flavonoid metabolites isolated from *Croton schiedeanus* "Almizclillo"

Ortiz Sánchez A. P.[1]*, Puebla P.[2], Guerrero Pabón M.F.[3]
*alportizsa@unal.edu.co1

[1]*MSc-Pharmacology student/ Facultad de Ciencias, Departamento de Farmacia - Edificio 450, Carrera 30 No. 45-03. Universidad Nacional de Colombia, C. P. 111321. Bogotá, Colombia.*
[2]*DSc/Departamento de Ciencias Farmacéuticas, Facultad de Farmacia, Campus Miguel de Unamuno. Universidad de Salamanca. P. 37007. Salamanca, España.*
[3]*DSc/Facultad de Ciencias, Departamento de Farmacia - Edificio 450, Carrera 30 No. 45-03. Universidad Nacional de Colombia, C. P. 111321. Bogotá, Colombia.*

## Introduction

Combination therapy is an effective therapeutic strategy in clinical practice when the agents improve the pharmacological response and allow to reduce the doses and the risk of potential adverse effects[1,2]. With this therapeutic approach, new therapeutic alternatives are researched to reduce the current notable impact of arterial hypertension on public health. Natural sources could provide active principles that could interact in a synergistic way and offer new pharmacological options of combination therapy[3]. *Croton schiedeanus*, (NV "Amizclillo"), a traditionally specie used in Colombia for antihypertensive purposes, has major metabolites such as 3,7-Di-O-methylquercetin (DMQ) and 3,7,4'-Tri-O-methylquercetin (ayanin), agents which vasorelaxant profile is linked at least in part to the nitric oxide/guanylate cyclase pathway[4,5], however their possible synergistic interaction has not been studied until the date.

## Method

The vasodilator effect obtained with ayanin ($10^{-8}$ to $6x10^{-5}$M) was examined in absence and presence of increasing concentrations of DMQ ($10^{-8}$ to $3x10^{-5}$M), in Wistar rat isolated aortic rings previously contracted with phenylephrine ($10^{-6}$M). Concentration – response curves were obtained, and data were analyzed throw sigmoidal fitting regression. Results were treated in *GraphPad* and *Combenefit*. The concentration - effect of these flavonoids was compared with the ethanolic extract from the aerial parts of *C. schiedeanus* ($10^{-7}$ to $3x10^{-4}$mg/mL), as well as their behavior in the presence of nitric oxide inhibitor, L-NAME ($10^{-4}$M) and guanylate cyclase inhibitor, methylene blue ($10^{-4}$M).

## Results / Discussion / Conclusion

Results showed that DMQ has a dual effect on the vasorelaxant response induced by ayanin: antagonist at lower concentrations ($10^{-8}$ to $3x10^{-7}$M), and synergistic at higher ($10^{-6}$ to $3x10^{-5}$M). Appropriate combinations of ayanin and DMQ overcomes in potency and efficacy the vasorelaxant effect elicited by the whole extract ($pEC_{50}$ 7.4 [7.2-7.5] and $E_{máx}$ 170.1±2.0 vs $pEC_{50}$: 4.1 [3.6-4.6] and $E_{máx}$: 101.6± 3.1, respectively). This response is attenuated but not

reverted when the nitric oxide/GMPc pathway is inhibited. Therefore, DMQ and ayanin combination could be useful for vasodilator therapeutic uses.

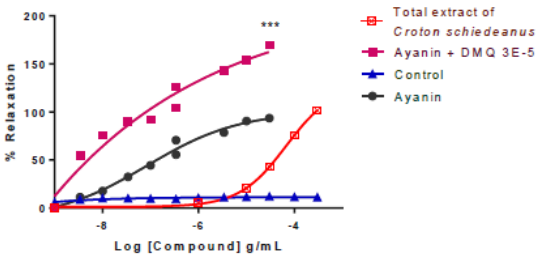


**Fig. 1**. Response of ayanin in the absence and presence of DMQ $3x10^{-5}$M, in precontracted aortic rings with phenylephrine $1x10^{-6}$M (n: 4-11). ***p <0.001 vs. Extract of *Croton schiedeanus*

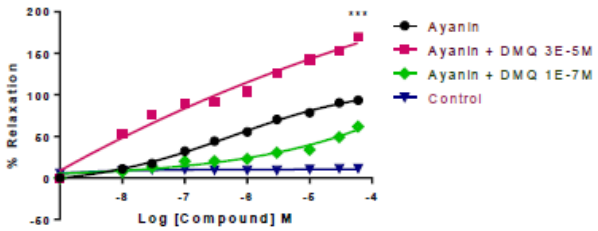


**Fig. 2.** Response of ayanin in the absence and presence of DMQ ($1x10^{-7}$ and $3x10^{-5}$M), in precontracted aortic rings with phenylephrine $1x10^{-6}$M (n: 4-11). ***p <0.001 vs. Ayanin.

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# PS026- Vasoconstrictor triterpenic saponins isolated from *Passiflora quadrangularis L.* leaves

Bareño-Ariza L[1]*, Puebla Ibáñez P.[2], San Feliciano A.[2], Guerrero Pabón M.[1]*,
*llbarenoa@unal.edu.co

*1MSc, PhD / Departamento de Farmacia, Edificio 450, Carrera 45 N° 26 - 85. Universidad Nacional de Colombia, C. P. 111321, Bogotá D.C, Colombia..*
*2PhD/Universidad de Salamanca, Facultad de Farmacia, Departamento de Ciencias Farmacéuticas. Campus Miguel de Unamuno, Salamanca, C.P. 37007.*

## Introduction

The extract of leaves from *Passiflora quadrangularis* L. are used in Colombian folk medicine for treatment of diabetes[1], anxiety[2] and hypertension[3]. This plant belongs to the *Passifloraceae* family. Colombia is particularly rich in this family with 167 species from *Ancistrothyrsus* (2), *Dilkea* (4) and *Passiflora* (162) genera[4]. *P. quadrangularis* L. main constituents are flavonoids [5,6,] β - carboline harmane alkaloids[7] and triterpenic saponins[5,6,8–10]. This work shows the isolation, structure elucidation and vascular activity of two saponins from *Passiflora quadrangularis* L. leaves, one of them reported for the first time in this plant. The compounds obtained were 3-*O*-*β*–D- glucopyranosyl oleanolic acid, 3-*O*-*β*–D-glucopyranosyl-(1-2)-*O*-*β*–D glucopyranosyl oleanolic acid[8].

## Method

The ethanolic extract of *Passiflora quadrangularis* L leaves was dissolved in methanol and storage at 18°C during 14 hours, until the wax precipitated. The extract without wax was separated in two fractions with dicloromethane and methanol in different proportions. It was obtained a soluble $CH_2Cl_2$ fraction and insoluble $CH_2Cl_2$ fraction. The soluble $CH_2Cl_2$ fraction was fractionated by flash column chromatography using as eluent $CH_2Cl_2$/MeOH (9:1, 8:2), isolating two compounds: 3-*O*-*β*–D-glucopyranosyl oleanolic acid (**1)** and 3-*O*-*β*–D-glucopyranosyl-(1-2)-*O*-*β*–D-glucopyranosyl oleanolic acid (**2**). The characterization of these compounds was performed by IR, $^{1}$H NMR, $^{13}$C NMR, HMBC, HSQC and HRMS. The triterpenic saponins isolated was evaluated in aortic rings with intact endothelium and removed endothelium of Wistar rats. A basal tension of 2 g was applied to each aortic ring with a stabilization period of 60–90 min, during which time the Krebs solution was changed every 5–10 min. Once equilibrium was reached, the aortic rings were exposed to $10^{-6}$ M phenylephrine until the contractile response reached a steady tension. $10^{-6}$ M acetylcholine (ACh) was then added to check presence or absence of the endothelium in the aortic rings. After this, they were washed with Krebs solution until reaching a basal tension again, then the compounds (**1**), (**2**) and ethanolic extract (**3**) were added cumulatively between $10^{-6}$ and $10^{-4}$ g/mL every 15 min using a logarithmic scale.

## Results / Discussion / Conclusion

| Compound 1 | | |
|---|---|---|
| RMN $^1H$ and $^{13}C$ (400 MHz, ($CD_3OD$) δ (ppm) | | |
| $^1H$ | | $^{13}C$ |
| 3.25 | 1H, m, H-3 | 90,8 (CH, C-3) |
| 5.23 | 1H, sa, H-12 | 123.6 (CH, C-12) |
| 4.30 | 1H, d, J=7.2 Hz, H1' | 145.2 (CH, C-13) |
| | | 180.8 (C, C-28) |
| | | 106.7 (CH, C-1') |

| Compound 2 | | |
|---|---|---|
| RMN $^1H$ and $^{13}C$ (400 MHz, ($CD_3OD$) δ (ppm) | | |
| $^1H$ | | $^{13}C$ |
| 3.20-3.40 | 1H, m, H-3 | 91.5 (CH, C-3) |
| 5.23 | 1H, sa, H-12 | 123.6 (CH. C-12) |
| 4.42 | 1H, d, J= 7.2Hz, H1' | 145.2 (CH, C-13) |
| | | 181.8 (C, C-28) |
| | | 106.4 (CH, C-1') |
| | | 105.4 (CH, C-1'') |

| Compound/fraction | pEC50 | Emax |
|---|---|---|
| Compound 1- E | - 4.15 [-4.18 to -4.11] | 109.80 ± 4.72 |
| Compound 1- WE | - 4.25 [-4.32 to -4.18] | 83.25 ± 9.68 |
| Compound 2 - E | - 4.19 [-4.22 to -4.16] | 109.40± 5.18 |
| Compound 2 - WE | - 4.30 [-4.33 to -4.25] | 130.50± 8.87 |
| Ethanolic extract -E | -4.17 [-4.21 to -4.12] | 67.83 ± 5.33 |
| Ethanolic extract - WE | -4.09 [-4.13 to -4.03] | 113.20 ± 10.74 |

Results showed that the triterpenic saponins 3-*O*-*β*–D- glucopyranosyl oleanolic acid and 3-*O*-*β*–D-glucopyranosyl- (1-2)-*O*-*β*–D-glucopyranosyl seem to be the main metabolites implicated in the vasoconstrictor response induced by *Passiflora quadrangularis* leaves extract. This response is greater in compound (**2**) and is not dependent of endothelium. Alpha adrenergic mechanisms could be implicated[2,3].

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# PS027- Antiviral activity on dengue virus type-2 and chemical constitution of *Eugenia brasiliensis* leaves

Santos P.F.P.[1,2], Siani A.C.[3]*, Gomes L.N.L.F.[2], Mazzei J.L.[3], Assunção-Miranda I.[4], Neris R.L.S.[4], Guimarães-Andrade I.P.[4], Valente L.M.M.[2]
*antonio.siani@far.fiocruz.br

[1]Centro Federal de Educação Tecnológica Celso Suckow da Fonseca, Campus Angra dos Reis, Rua do Areal 522, Parque Mambucaba, 23953-030, Angra dos Reis, Brazil
[2]Universidade Federal do Rio de Janeiro, Instituto de Química, Av. Athos da Silveira Ramos 149, Centro de Tecnologia, Bl. A, Cidade Universitária, 21941-909, Rio de Janeiro, RJ, Brazil
[3]Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos, R. Sizenando Nabuco 100, Manguinhos, 21041-250, Rio de Janeiro, Brazil
[4]Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Paulo Góes, Av. Carlos Chagas Filho 373, Centro de Ciências da Saúde, Bl. I, Cidade Universitária, 21941-902, Rio de Janeiro, RJ, Brazil

## Introduction

*Eugenia brasiliensis* Lam. (Myrtaceae), an endemic species to the Brazilian Atlantic forest is known as *ibaporoiti* (indigenous), *grumixama* or *brazilian-cherry*. Its edible fruit and leaves are popularly used to treat rheumatism and general inflammatory conditions[1]. Previous studies showed the potential antidepressant and anti-inflammatory activities of their leaves[2]. Mono-, sesquiterpenes and triterpenic acids have been found in its essential oil and flavonoids have been identified in their leaves[2]. Dengue is a neglected mosquito-borne viral infection whose incidence has dramatically increased in the last decades. In Brazil, it is placed among one of the most serious public health issues[3]. Currently there is no antiviral drug approved for the routine treatment of dengue patients. This work describes the antiviral activity on dengue virus type-2 (DENV-2) and the chemical study of the acetone-water leaf extract and its derived partition fractions of *E. brasiliensis*.

## Method

Dried and milled leaves of *Eugenia brasiliensis* were macerated with 7:3 acetone-water at r.t. to yield the crude extract. Part of this extract was sequentially partitioned with hexane, chloroform, ethyl acetate and butanol. The extract and derived fractions were assayed for *in vitro* cytotoxic and anti-DENV-2 effects in hepatocarcinoma cell lineage (HepG2). DENV-2 infected and uninfected HepG2 cells were treated with 50 µg/ml of the all samples. After 48 h of infection, the culture medium was collected for virus titration by plaque assay and cellular extracts were used to determine cell viability by MTT assay. Total phenolic content was determined by the Folin-Ciocalteu method and results were expressed as microgram of quercetin equivalents (QE)/g of dried extract/fraction. The chemical composition of the fractions was determined by GC-MS after trimethylsilylation with NSTFA and UFLC-DAD-ESI-qTOF-$MS^n$.

**Results / Discussion / Conclusion**

The extract and its derived fractions, except the hexane, showed non-cytotoxicity. The extract, the ethyl acetate and butanol fractions showed high cytoprotective effect in DENV-2 infected cells, while the chloroform fractions presented moderate cytoprotection. Quantification of DENV particles demonstrated a 100% reduction of the viral load when treated with the chloroform and ethyl acetate fractions and 76% with de butanol fraction, when compared with untreated condition. The bioactive chloroform fraction showed by GC-MS fatty acids, steroids, triterpenes and triterpenic acids. The total phenolic content of the crude extract attained 285 ± 25 mg QE/g and just the bioactive ethyl acetate and butanol fractions showed phenolic compounds (336 ± 24 and 459 ± 37 mg QE/g, respectively), which revealed by UFLC-DAD-ESI-qTOF-$MS^n$ to be mainly flavonoids.

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# PS028- Polyphenol contents, heavy metal analysis and *in vitro* antibacterial activity of extracts from *Cladanthus arabicus* and *Bubonium imbricatum* of Moroccan origin

Aghraz A.[1]*, Gervasi T.[2], Benameur Q.[4,5], Bua G.D.[2], Larhsini M.[1], Markouk M.[1], Albergamo A.[2], Cicero N.[2,3].
*abdellah.aghraz@gmail.com; tgervasunime.it

[1]*Laboratory of Biotechnology, Protection and Valorisation of Plant Ressources (URAC35 Association Unit), Cadi Ayyad University, Marrakesh, Morocco.*
[2]*Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy.*
[3]*Science4Life, Spin Off Company, University of Messina, Italy.*
[4]*Research Laboratory "Animal Health and Production", Higher National Veterinary School, Algiers, Algeria.*
[5]*Nursing Department, Faculty of Natural Sciences and Life, Abdelhamid Ibn Badis University, Mostaganem, Algeria.*

## Introduction

Antimicrobial resistance in bacterial pathogens is a challenge that is associated with high morbidity and mortality. Due to the anarchic, inadequate and abusive use of antibiotics in human and veterinary health, the emergence of multidrug-resistant bacteria is a growing public health problem. Multidrug resistance patterns bacteria are difficult to treat and may even be untreatable with conventional antibiotics. These resistances led to the search for new antimicrobial agents with greater efficacy than synthetic drugs, which can be well accepted by the organism. The aim of this study was to evaluate the polyphenols and heavy metals contents of extracts from *Cladanthus arabicus* and *Bubonium imbricatum* of Moroccan origin. Their *in vitro* antibacterial activity was also evaluated against multidrug resistant *Enterobacteriaceae*.

## Method

Ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) and inductively coupled plasma mass spectrometry (ICP-MS) were used to evaluate the polyphenols and heavy metals levels, respectively. The antibacterial activity of the two extracts against six multidrug resistant *Enterobacteriaceae* isolates (*E. coli* S33/16, *E. coli* S34/16, *Proteus mirabilis* S32/16, *Klebsiella pneumoniae* S12/16, *Enterobacter cloacae* S5/16, and *Salmonella* sp S12/14) was assessed using agar disk diffusion and micro-broth dilution methods.

## Results / Discussion / Conclusion

The two plant extracts showed strong *in vitro* antibacterial activity against the selected *Enterobacteriaceae* isolates, particularly *E. coli* S33/16 (MIC, 0.125 mg/mL). UHPLC-MS analysis allowed the identification of 13 phenolic compounds. Caffeic acid and procatechuic acid were revealed in higher amounts in both plants extracts. Whereas, ferulic acid was found abundant only in *B. imbricatum* extract. The studied plant extracts contain a tolerable quantity of heavy metals, such as Cd, As, and Pb, as they results within the safety ranges recommended by the World Health Organization (WHO). Overall, information on single polyphenols, and heavy metal contents, as well as *in vitro* antibacterial activity, for both Moroccan *Cladanthus arabicus* and

*Bubonium imbricatum* were provided. The present study could encourage further *in vivo* studies for clinical therapeutics.

# PS029- Polyphenolic changes in *Cucumis melo* during ripening

Vella F.M.[1], Noviello M.[2], Caliendo G.[2], Laratta B.[1]*
*bruna.laratta@cnr.it

[1]*Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biologia Agro-ambientale e Forestale (IBAF), Via P. Castellino 111, Napoli, Italia*
[2]*Enza Zaden Italia S.r.l.- S.S. Aurelia km 96.710, Tarquinia, Italia*

## Introduction

Cantaloupe (*Cucumis melo* L.) is one of the most consumed melon worldwide due to its sweetness, juicy taste, pleasing flavor, and nutritional value[1]. In 2016, about 1,9 million tons were harvested in the Mediterranean area, with Spain, Italy, and France the main European producers with 35%, 34%, and 13%, respectively. Recently, cantaloupe pulp has been demonstrated to possess useful medicinal properties such as anti-inflammatory, antioxidant, anticancer, antimicrobial, and antidiabetic[2]. Moreover, during melon consumption and industrial processing, a large quantity of waste materials such as peels is produced. This by-product is still rich in phytochemicals with healthy effect on human, above all defending the body cells against free radicals damage[1,2]. The present study provides a biochemical characterization of peel and pulp from an Italian cantaloupe variety, in particular the phenolic content as well as the antioxidant activity were evaluated during different ripening stages.

## Methods

Three melons were harvested and analyzed per each ripening stage, named S0, S1, S2, S3 and S4, ranging from the unripe to overripe condition. Cantaloupe peel and pulp were extracted using a 95% ethanol solution at 50°C for 6 h. Total polyphenols, *ortho*-diphenols, flavonoids, and tannins of extracts were investigated. The antioxidant properties were evaluated through DPPH radical-scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assays. The results were expressed as μg of bioactive compound per mg of extract.

## Results and Discussion

The phenolic composition and antioxidant activities of Italian cantaloupe pulp and peel are both studied in order to explore their beneficial properties as potential applications in industry. In particular, peel showed in S3 stage a polyphenolic content 2.7 folds higher than that recorded in pulp. Among polyphenols, *ortho*-diphenols are recognized as the most important in antioxidant activity and they showed the same tendency: in fact peel was 4.1 folds richer than pulp. Flavonoids, the most common and widely distributed group of plant phenolic compounds, are important in fruits for normal growth, development and defense from infection and injury. Cantaloupe peel showed in S3 a content 4.9 folds higher than pulp. Within polyphenols, tannin have been considered health-promoting components in plant derived foods and beverages, possessing antioxidant properties[3]. Tannins also showed the same trend of the other bioactive components, reaching a content 7.0 folds higher in peel than in pulp. Phenolic phytochemicals have the capacity to protect cellular components against free radicals. The antioxidant power,

measured by FRAP assay, was 2.6 higher in peel respect to pulp at S3 stage. The DPPH radical scavenging activity showed similar trend to FRAP: in fact in peel extract $EC_{50}$ was 10.57 mg/mL, meanwhile pulp showed a value of 17.94 mg/mL.

**Conclusion**

Overall, in all ripening stages (S0-S4) peel extracts were richer than their corresponding pulps, for both phenolic data and their antioxidant activities. In particular, S3 stage, corresponding to the commercial maturity condition that was about 40-45 days from the time of fruit setting, showed the highest amounts of phytochemicals.

The polyphenolic extracts from cantaloupe melon peel and pulp may be considered as potential sources of natural antioxidants to use as supplement in food, cosmetic, and nutraceutical applications.

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# PS030- Genetic variability within a *Capsicum annuum* collection

De Masi L.[1,2], Vella F.M.[1], Pignone D.[3], Laratta B.[1]*
*bruna.laratta@cnr.it

[1]*Consiglio Nazionale delle Ricerche (CNR) – Istituto di Biologia Agro-ambientale e Forestale (IBAF), Via P. Castellino 111, Napoli, Italy.*
[2]*Present address: CNR - Istituto di Bioscienze e BioRisorse (IBBR), via Università, 133 - 80055 Portici, Napoli, Italy.*
[3]*CNR - Istituto di Bioscienze e BioRisorse (IBBR), via G. Amendola, 165/A - 70126 Bari, Italy.*

## Introduction

Pepper belongs to the genus *Capsicum* (Solanaceae), which includes about 25 wild and five domesticated species. Besides being one of the most commonly used spices, condiments and vegetables, peppers have several versatile and innovative food and non-food uses, being natural source of capsaicinoids, alkaloids, antioxidants, vitamins, and carotenoids to use in therapeutic and wellness applications[1]. Among *Capsicum spp.*, *C. annuum* pungent and non-pungent (sweet) fruits are commercially important because cultivated worldwide. They originated in the Americas and spread rapidly through the globe after the voyage of Columbus. Routine species and cultivar identification of pepper is essentially based on phenotypic traits, which can be variable indicators of the specific pepper genotype since affected by environmental conditions[1,2]. Level of genomic diversity among and within species of the genus *Capsicum* can be evaluated by means of DNA-based molecular markers, a versatile and effective diagnostic tool. This study investigated the genetic diversity in a population of *C. annuum* using Random Amplified Polymorphic DNA (RAPD) markers, which are regions of genome amplified by polymerase chain reaction (PCR) using single primer of arbitrary sequence.

## Method

The 32 *C. annuum* individuals here analyzed represent a newly assembled hot pepper collection consisting of 14 accessions. Leaves for molecular analysis were harvested from one to three individuals per each accession. Genomic DNA extraction and RAPD-PCR followed previous protocols with minor modifications[1,2]. The three primers AE19: GACAGTCCCT, AG14: CTCTCGGCGA, and AN19: ACCACGCCTT were selected. Amplicons were detected after gel electrophoresis and reproducible bands were coded in a binary format by scoring for the presence or absence. A similarity matrix based on Nei and Li's coefficient was calculated for all genotypes and used to construct a tree plot (dendrogram) based on UPGMA algorithm.

## Results and Discussion

RAPD analysis identified 31 reliable alleles, with a mean of 10.33 alleles per primer on *C. annuum* collection. Twenty-eight loci were polymorphic (90%) and their variation allowed to properly identify the 14 *C. annuum* accessions and to assess their genetic relationships. Cluster analysis, according to their genetic polymorphism, confirmed the morphological classification with sub-clustering that reflect the differences at genetic level of the individuals of the same variety. These results were in agreement with previous studies[1,2] and confirmed the complex

nature of relationships within the species level. In this regard, further studies are ongoing on the species *C. chinense, C. baccatum*, and *C. frutescens*.

## Conclusion

Based on these outcomes, RAPD-based method could be used as molecular tool for distinguishing *Capsicum* species and varieties. Moreover, these findings strengthen the idea to use molecular markers to appropriately manage hot pepper collections so to perform innovative breeding programs.

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# PS031- A microethnographic and ethnobotanical approach to Llayta consumption among the Andes feeding practices

Gómez-Silva B.[1]*, Galetović A.[1], Rivera M.[2], Licuime R.[2]
*benito.gomez@uantof.cl

[1]*Laboratorio de Bioquímica, Depto. Biomédico, Facultad Ciencias de la Salud y Centre for Biotechnology and Bioengineering (CeBiB), Universidad de Antofagasta, Avenida Angamos N° 601, Antofagasta, Chile.*
[2] *Departamento de Educación, Facultad de Educación, Universidad de Antofagasta, Avenida Angamos N° 601, Antofagasta, Chile.*

## Introduction

Llayta are sun-dried colonies of a cyanobacterium (*Nostoc* genus) consumed by andean communities since pre-Columbian days[1,2]. Llayta is commercially available at food markets in southern Perú, and Arica and Iquique, in northern Chile. Llayta has a substantial content of essential amino acids closer to 60% of total amino acids and polyunsaturated fatty acids (30% total fatty acids); in addition, this microorganism does not accumulate hepatotoxic microcystin, reinforcing the notion that it is a safe and nutritious supplement[2]. Our objective was to provide the first anthropological perspective on Llayta as an ancestral Andean feeding practice.

## Methods

This study included interviews in order to collect social representations and drawing about Llayta[3]. Observations were carried out with a total of 21 participants from Putre (Chile) and Tacna (Perú). Participants were ethnographically registered and analyzed in order to explain their social worlds and their understanding of the natural, social and cultural environment that surround them. The ethnographic goal was to document the anthropological and cultural tensions found in these social representations on the knowledge the communities have on Andean algae and how they are valued. This study was focused on the description of Llayta pertinence and context, documentation of Llayta social representations and the analysis of the direct or indirect knowledge on Llayta.

## Results, Discussion and Conclusion

Our study registered anthropological knowledge on Llayta, in regions of northern Chile and southern Peru where Llayta is still consumed**.** Social representations and Llayta drawings confirmed direct or indirect knowledge on Llayta. Only 37% of the participants (mostly adults) have had direct experience of Llayta. The remaining participants (mostly children) did not have any knowledge about Llayta. The social representations to Llayta reflect anthropological and cultural tensions concerning the partial knowledge about Andean algae, places where to find Llayta, where it is commercialized, how it is cooked and on its nutritional quality. New anthropological and educational strategies are urgently needed to rescue this ancestral feeding tradition transmitted through generations in the rural Andean world of South America. (Grants SI-5305, Universidad de Antofagasta, Chile; CeBiB F-0001, CONICYT, Chile)

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# PS032- Microbiological and physicochemical characterization of amaranth "alegrías"

Cruz Peña S.B[1], Martínez-Hernández V.R.[1]., Cuenca-Mendoza F.[1], Colín Cruz M.A.[1]*
*macolinc@uaemex.mx

[1] *Universidad Autónoma del Estado de México, Facultad de Química. Paseo Colón S/N. Toluca, 50000, Estado de México. México.*

## Introduction

The amaranth (*Amaranthus hypondriaccus*) is cultivated since 5000-7000 years ago, in Mesoamerica.

Among the Aztecs, it was a ritual food widely used in religious ceremonies: the amaranth flour was mixed with maguey honey to form a dough, which was used to make figures and images of deities then, the figures were consumed by the faithful[1].

This rite disappeared at the arrival of the Spaniards, as well as amaranth farming [2]. Current significance of amaranth lies in its nutritional quality: it has a significant content of lysine (16.6%) and its protein content is similar to that of cow milk (16.5%) [3].

In Mexico 58.9% of the amaranth products are consumed in the form of "alegrías" (traditional snack), which are elaborated with the heated amaranth grain that bursts like a popcorn, added with honey, sugar and some other ingredients[4]. The objective of this study was to characterize the commercial "alegrias" from the physicochemical and microbiological point of view to verify its nutritional quality and safety.

## Method

Thirty samples of commercial "alegrias" were analyzed, 20 with label and 10 without label. Physicochemical analyzes were carried out: moisture, protein and ashes. The microbial flora was determined: mesophilic bacteria, fungi, yeasts and *Staphylococcus aureus*, as well as mineral content: magnesium, phosphorus, potassium, copper, calcium, iron, manganese and heavy metals (lead, arsenic, cadmium and mercury). The thirty samples of "alegrías" were aquired and analyzed twice.

## Results / Discussion / Conclusion

The chemical composition of the "alegrias" varies depending on the form of presentation: in the labeled ones, 6.96%, 1.24% and 1.65% of moisture, protein and ash, respectively, were found; while the "alegrias" without label contained 7.4%, 1.15% and 1.46% moisture, protein and ash, respectively. However, there were no significant differences between presentation forms ($p < 0.05$).

Microbial population was similar in both types of "alegrías": 100 CFU/g of mesophilic bacteria, 60 CFU/g of fungi and yeasts, and 10 CFU/g of *S. aureus*. Although some "alegrías" are sold in bulk and there is manipulation during processing, microbial populations are low. Low microbial populations can be attributed to the low moisture of "alegrías" (□7.0%) and honey and sugar

content that generate an osmotic environment. “Alegrías” contain magnesium, calcium, phosphate, iron, manganese, cupper, potassium in small proportions; no heavy metals were detected.

Results indicate that "alegrías" are a healthy snack and can be eaten with freedom since they have a very low microbial population and do not contain heavy metals.

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# PS033- Actividad antioxidante en extractos de plantas colombianas de la familia Melastomataceae

Castro-Ramirez A.P.[1], Jiménez-González F.J.[1], Veloza-Castiblanco L.A.[1]*, Sepúlveda-Arias J.C.[2]
*lveloza@utp.edu.co

[1]*Universidad Tecnológica de Pereira, Facultad de Tecnologías, Escuela de Química, Grupo Polifenoles. Cra. 27 #10-02 Alamos, Pereira, Risaralda, Colombia.*
[2]*Universidad Tecnológica de Pereira, Facultad de Ciencias de la Salud, Grupo Infección e Inmunidad, Cra. 27 #10-02 Alamos, Pereira, Risaralda, Colombia.*

## Introducción

En la actualidad se han realizado estudios enfocados al control del estrés oxidativo provocado por los radicales libres y las especies reactivas del oxígeno, debido a que dicho estrés está involucrado en la fisiopatología de diversas patologías como aterosclerosis, cáncer, enfermedades neurodegenerativas, así como en el proceso normal de envejecimiento[1]. Las plantas han sido usadas en medicina natural debido a que poseen diversas actividades biológicas como antimicrobianos, antiinflamatorios y antioxidantes, entre otras; las cuales son atribuidas a la presencia de compuestos de tipo fenólico[2], lo cual ha impulsado el uso de los productos naturales como una fuente de moléculas promisorias que pueden ser empleadas para neutralizar los efectos de los radicales libres. Por lo tanto, el presente estudio tiene como objetivo evaluar la actividad antioxidante de extractos de plantas que conforman la biodiversidad colombiana.

## Metodología

**Actividad antioxidante por el método DPPH.** 25 µL de los extractos y Quercetina, a diferentes concentraciones en metanol, y los respectivos controles, se agregaron a una placa transparente de 96 pozos seguidos de 100 µL de una solución de DPPH a 20 µg/mL. La placa con la mezcla se incubo en oscuridad a temperatura ambiente durante 30 minutos, y finalmente se midió la absorbancia a 517 nm. Se determinó el valor $IC_{50}$[3] por medio de una regresión no-lineal empleando el programa GraphPad Prism 6.

**Actividad antioxidante por el método ORAC.** 30 µL de los extractos y Quercetina, a diferentes concentraciones en solución buffer fosfato 75 mmol/L, y los respectivos controles, se adicionaron a una placa de 96 pozos seguidos de 180 µL de una solución de Fluoresceína a 120 nmol/L. La mezcla se incubo en oscuridad a 37°C durante 15 minutos, se adicionaron 30 µL de solución AAPH a 140 mmol/L y se procedió con la lectura de la fluorescencia [4] con λ de excitación de 485 nm y λ de emisión de 538 nm, durante 120 minutos en intervalos de 1 minuto. Se elaboró una curva de calibración con Trolox. La capacidad antioxidante se calculó como el área bajo la curva y se expresó como µmol equivalentes de Trolox/g de extracto.

## Resultados / Discusión / Conclusión

Se evaluaron cuatro diferentes especies de plantas de la familia Melastomataceae y los resultados obtenidos fueron comparados con los estándares de Quercetina y Trolox. Se observó que los extractos de *Tibouchina ciliaris*-Acetona 70%, *Miconia sp*–Acuoso y *Miconia sp*–Butanol

presentaron una mayor actividad antioxidante por el método de DPPH, de otro lado, para el ensayo ORAC *Tibouchina ciliaris*-Acetona 70% presentó la mayor actividad respecto a los demás extractos evaluados. Adicionalmente, la determinación de los núcleos fotoquímicos de las especies con mayor actividad antioxidante mostró la presencia de compuestos fenólicos, flavonoides y taninos hidrolizables y condensados, compuestos a los que se les puede atribuir la actividad evaluada.

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# PS034- Evaluation of aflatoxin M1 impact on the metabolism of a human hepatoma cell line

Marchese S. [1, 2], Ariano A.[1]*, Sorice A.[2], Florio S.[1], Budillon A.[2], Costantini S.[2], Severino L.[1]
*andrea.ariano@unina.it

[1]*Unità di Farmacologia e Tossicologia—Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II", 80138 Napoli, Italy*
[2]*Unità di Farmacologia Sperimentale, Istituto Nazionale Tumori - IRCCS - "Fondazione G. Pascale", 80131 Napoli, Italy*

## Introduction

Aflatoxin M1 (AFM1) is the principal active metabolite of aflatoxin B1 (AFB1), a mycotoxin produced by *Aspergillus flavus* and *A. parasiticus* and indicated as a Group 1 hepatocarcinogen by IARC[1]. Involvement of AFM1 in carcinogenesis has been recently reviewed by our group[2], but still few *in vitro* studies can be found in literature. Since this metabolite is commonly found in milk of mammals fed upon contaminated feedstuff, including lactating women[3], a more extensive assessment of its effect on cancer development should be carried out. Considering that the liver is the main target of AFB1, the effects of AFM1 on a human hepatoma cell line have been preliminarily tested.

## Method

Cell viability was assessed by colorimetric assay with sulforhodamine B (SRB, Sigma Aldrich) on human hepatoma cell line, HepG2, treated with different concentrations of AFM1 ranging from 0.1 to 50 mM for 48h. Apoptosis and cell cycle were assessed at $IC_{50}$ using the Annexin V and Dead Cell Assay kit and the Cell Cycle Assay Kit (Merck Millipore, Darmstadt, Germany), respectively. Moreover, metabolomic analysis by $^1$H-NMR was performed. In detail, after 48 h HepG2 cells were detached and $2\times10^6$ cells were counted, centrifuged and stored at −80°C. Cellular pellets were extracted using a mixture of water, methanol and chloroform (0.7:1:1) to separate the polar and apolar components that were separately collected and evaporated. Then, polar samples were dissolved in PBS-$D_2O$ using sodium salt of 3-(trimethylsilyl)-1-propanesulfonic acid (1% in $D_2O$) as the internal standard, whereas the apolar fractions were dissolved in $CDCl_3$. $^1$H-NMR spectra were acquired on a 600-MHz Bruker Avance spectrometer at 300K. Some statistical analyses like PCA and Loading Plot were used to identify the differently expressed metabolites between the untreated and treated cells.

## Results / Discussion / Conclusion

Our studies have showed that AFM1 was able to block the cellular proliferation of HepG2 cells reaching $IC_{50}$ at concentration of 9 mM. AFM1 did not induce activation of apoptosis but was able to increase the percentage of cells in G0/G1 phase of the cell cycle in accordance with the results of SRB assay. Principal component analysis (PCA) plot indicated that $^1$H-NMR spectra obtained in triplicates for the apolar and polar fractions of untreated and treated HepG2 cells clustered in different groups, suggesting the presence of some metabolites modulated by AFM1. In fact, the

levels of acyl groups of fatty acids as well as those of lactate, choline, citrulline and some amino acids resulted to be increased in HepG2 cells treated with AFM1 compared to untreated cells. On the other hand, the levels of formate and ornithine were decreased after AFM1 treatment. Therefore, the metabolomic analysis evidenced that AFM1 induces a modulation of the lipidic, glycolytic and amino acid metabolism. Considering that high levels of some metabolites like lactate, glycine, and choline have been reported to correlate with liver cancer development[4], we can conclude that AFM1 induces metabolic changes involved in liver carcinogenesis. Further studies will be aimed to the evaluation of the metabolic effects induced by AFB1 to understand if these two aflatoxins act on the same or different metabolic pathways.

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# PS035- *In vitro* antioxidant, anti-inflammatory, antidiabetic activitiy of an endemic plant in Turkey-named *Aethionema dumanii*

Yılmaz B.S.[1]*, Ağca A.C.[2], Sarıaltın S.Y.[3], Yazgan A.N.[1], İşcan G.S.[1], Çoban T.[3]
*sever@pharmacy.ankara.edu.tr

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey*
[2] *Turkish Medicines and Medical Devices Agency, 06100 Ankara, Turkey*
[3]*Department of Toxicology, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey*

## Introduction

Herbal drugs have been popular in healthcare system globally day by day. With increasing use of medicinal plants, pharmaceutical industry focuses on new natural sources. *Aethionema dumanii* belongs to Brassicaceae family and endemic in Turkey. 43 taxa has been listed under genus *Aethionema* on Turkish Plants Data Service. In this study, we aimed to investigate the antioxidant, antinflammatory and antidiabetic activities of the ethanol and aqueous extracts of *Aethionema dumanii*.

## Method

ABTS and DPPH free radical scavenging activities were performed Blois and Re et al's method with minor modifications, respectively.[1,2] Membrane stabilizing activity of the extracts were assessed using heat-induced human erythrocyte hemolysis method.[3] α-glucosidase inhibitory activities of the extracts were determined according to the method of Liu et al. with slight modifications.[4]

## Results / Discussion / Conclusion

The antioxidant potentials of the extracts were evaluated by DPPH and ABTS radical scavenging activities. Human red blood cell membrane stabilization was measured as a mechanism of anti-inflammatory activity. α-glucosidase inhibitory activity of extract was carried out as a mechanism of antidiabetic activity. Ethanol extracts showed higher DPPH and ABTS free radical scavenging activity than aqueous extracts. However, aqueous extracts inhibited heat induced hemolysis of the HRBC as a mechanism of the anti-inflammatory activity more than ethanol extracts. Similar to the results of anti-inflammatory activity, aqueous extracts showed stronger in-vitro α-glucosidase inhibitory activity than ethanol extracts. According to our results, *Aethonema dumanii* could be good candidate as herbal drug but of course, further sudies are needed for the isolation and identification of the effective compounds from the extracts of this endemic plant.

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# PS036- Extraction and characterization of bioactive compounds from agro-industrial by-products in an environmental sustainability context

Cavazza A.[1]*, Carà D.[1], Grimaldi M.[1], Ornaghi P. [2], Corradini C.[1]
*antonella.cavazza@unipr.it

[1]*Università di Parma, Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Parco Area delle Scienze 17/A, 43124, Parma, Italy.*
[2]*Velp Scientifica, via Stazione 15, Usmate (MB), Italy*

## Introduction

Agro-industrial by-products represent enormous amounts of wastes with a high environmental impact, and their disposal requires significant costs. On the other hand, they are often still rich of bioactive molecules. In the late years, Europe politic is moving towards a more sustainable economy, implementing the ambitious Circular Economy plan with the intent of reducing waste and reuse by-products. The main goal would be to introduce waste in the productive cycle again to obtain new resources that can be used as new raw materials, providing an additional economic value.

## Method

Waste material from onion, artichoke, asparagus, cardoons and grapes were submitted to different extraction procedures. The obtained extracts were analysed in order to characterize their composition. Carbohydrate fractions were analysed by high performance anionic exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD), while phenolic compounds by reversed phase chromatography equipped with UV/DAD and mass spectrometry. Folin-Ciocalteau assay was also used for total phenolic content assessment, and Oxitest reactor allowed the evaluation of the antioxidant power by measuring the oxidative stability of a model matrix enriched with the extracts.

## Results and Discussion

The first part of the work was aimed at setting up the optimum conditions to obtain rich extracts. The use of microwave assisted extraction was revealed as the best technique.

Analysis of extracts demonstrated the presence of valuable bioactive substances in all waste materials considered. Artichoke leafs and stems were found to be rich in polyphenolic compounds[1], as well as grapes residues and onion wastes. The main molecules occurring were chlorogenic acid and quercetin[1,2].

The antioxidant power of the extracts were evaluated by Oxitest, measuring the increment in the oxidative stability of vegetable oil when enriched with even small proportion of extracts.

Extracts from residues of asparagus, cardoons, onions and artichokes also contained fructooligosaccharides and inulins, molecules with prebiotic activity[3], and characterized by important technological features for their properties to form gel when mixed with water. This phenomenon can be exploited to obtain bulk effect and to improve texture of food preparations such as ice-creams, yogurt, sweets etc..

Agro-industrial by-products can be, therefore considered as a valuable source of nutraceutical ingredients[4] that may be used as a potential material for the production of functional foods, or in the cosmetic field.

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# PS037- Carbohydrate profile assessment during biosynthesis of gold and silver nanoparticles from alga *Ulva lactuca*

Grimaldi M.[1]*, Bigi F.[1], Carà D.[1], Cavazza A.[1], Corradini C.[1],González-Ballesteros N.[2], Rodríguez-Argüelles M.C.[2]
*maria.grimaldi@unipr.it

[1] *Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze 17/A, 43124, Parma, Italy.*
[2]*Departamento de Química Inorgánica, Centro de Investigaciones Biomédicas (CINBIO) Universidade de Vigo, 36310 Vigo, Spain*

## Introduction

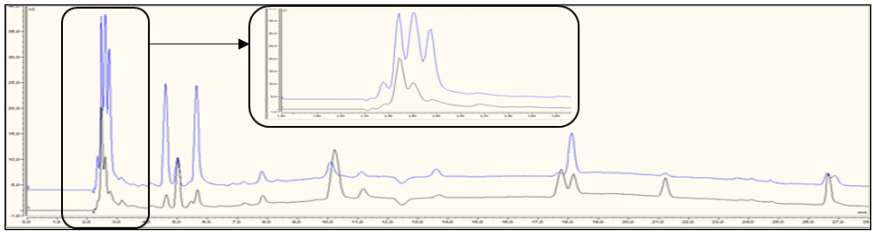
Marine bio-resources are being widely studied for being an invaluable source of compounds with therapeutic utility. In particular, macroalgae contain a wide variety of bioactive compounds with different structures and promising biological applications[1]. *Ulva lactuca L.* has been proposed for the synthesis of gold and silver nanoparticles (Au@UL and Ag@UL respectively)[2,3]. In this work, we explored the composition of carbohydrates of UL extract and the changes observed after nanoparticles synthesis, in order to investigate their possible role in the biosynthetic process.

## Methods

Au@UL were prepared from the extract obtained by boiling algae in water at the concentration of 1 g/mL and a final gold concentration of 0.4 mM, at room temperature. By a similar procedure, Ag@UL were obtained using an extract concentration of 0.5 g/mL, with a final silver concentration of 0.17 mM, at 100 ºC.
The analysis of carbohydrates was performed before and after nanoparticle formation. The nanoparticles were centrifuged and the supernatant and the extract were analyzed by high performance anionic exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) using a Dionex CarboPac$^{TM}$ SA10 column with an opportune gradient system of eluents (ultrapure water, NaOH and sodium acetate).

## Results and Discussion

The analysis of the extract provided a rich chromatogram, characterized by several peaks belonging to different classes of compounds: alditols, eluted in the first part, then mono and di-saccharides, followed by oligosaccharides. Many changes from both qualitative and quantitative points of view in the entire carbohydrate profile, after nanoparticles formation, are shown in the Figure. Some new peaks belonging to all types of carbohydrates appeared in both the extracts obtained after nanoparticles formation, while some other peaks disappeared. No sensible differences can be observed between the extracts from Au@UL and Ag@UL.



Carbohydrates profile of *Ulva Lactuca* extract before (below) and after Au nanoparticles biosynthesis (above).

The obtained results give new information about the possible role of sugars in nanoparticles formation, allowing to raise the hypothesis of a direct involvement of the carbohydrate fraction in their biosynthesis[4].

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# PS038- Etnobotany in Praia do Sossego, Niterói, Rio de Janeiro, Brasil

Boscolo O.H. [1]*, Ribeiro R.S.S.[1]
*odaraboscolo@hotmail.com

[1*]*Profa. Dra./Universidade Federal Fluminense, Faculdade de Ciências Biológicas, Departamento de Biologia Geral, Setor Botânica, Laboratório de Botânica Econômica e Etnobotânica (LABOTEE). Campus do Valonguinho, Rua Outeiro São João Batista, s/n, C.P. 24020-141, Niterói, Rio de Janeiro, Brasil.*

## Introduction

The traditional community of Praia do Sossego is located in the Municipality of Niterói[1], which has lived there for about 40 years, currently living in artisanal fishing and sustainable extractivism of vegetable species. The lack of historical and cultural knowledge of this community entails the need to register and value these knowledge. The objectives of this work were to performing the ethnobotanical survey and identify the species used by the residents.

## Method

Interviews[2] were conducted with the selected residents. The collection of the botanical material and the information about the uses were made through the "walking in the woods"[3] approach. The collected material was identified and deposited in the Herbarium of the Federal Fluminense University.

## Results / Discussion / Conclusion

50 ethnospecies were cited by the informants, referring to 25 botanical species. Four cultivated species were registered, 7 naturalized and 13 native. Of the native plants, three are endemic to Brazil, two of which are exclusive to the Atlantic Forest. 20 botanical families were found, being the most representative: Myrtaceae (4 species) and Anacardiaceae (3 species).

The tradicional community of Praia do Sossego has great contact with the native flora, through the sustainable extraction of medicinal and food plants. In addition, the community presents great cultural dynamism evidenced by the incorporation of new knowledge and the disuse of others. However the demolition of housing has an effect on the use of plant species. There is a need for greater appreciation and recognition of this group as a holder of local ethnobotanical knowledge by public agencies.

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# PS039- Medicinal plants selection method for research, developing and innovation (RD&I) within Brazilian biodiversity

Abreu B.L.F.[1]*, Galvão M.N.[2], Boscolo O.H.[3]
*barbara.abreu@far.fiocruz.br

[1]*Biologist/Instituto de Tecnologia em Fármacos- FIOCRUZ/Plataforma Agroecológica de Fitomedicamentos. Av. Comt. Guaranys 447, Rio de janeiro, RJ, Brazil, CEP. 22775903*
[2]*PhD/ Instituto de Tecnologia em Fármacos - FIOCRUZ/Plataforma Agroecológica de Fitomedicamentos. Av. Comt. Guaranys 447, Rio de janeiro, RJ, Brazil, CEP. 22775903*
[3]*PhD/ Universidade Federal Fluminense/ Laboratório de Botânica Econômica e Etnobotânica (LABOTEE). R. Mario Santos Braga, s/n -, Niterói, RJ, Brazil, CEP. 24020-141*

## Introduction

Brazil is one of the 17-megadiverse countries in the world presenting 20% of the living species known [1]. The existence of endemic species and high levels of destruction of their habitat makes it a biodiversity hotspot [2]. Anthropic actions threaten plant species, including medicinal plants and the knowledge that traditional populations have about them [3]. In this scenario, Brazil has developed two policies (PNPMF and PNPIC) that seek to encourage actions aimed at Research, Development and Innovation (RD & I) of medicinal plants and phytotherapics, as well as their use in the Unified Health System (UHS) [4]. However, most official medicinal plant lists made by Brazil's government do not value the country's biodiversity; the criteria used on the lists are more political than technical [5]. Aligned within the scope of PNPMF and PNPIC, there is a foundation, FIOCRUZ, focused on producing, disseminating and sharing knowledge and technologies aimed at SUS consolidation [6]. FIOCRUZ belongs to Brazil's Ministry of Health. In FIOCRUZ, there is the Agroecological Platform of Phytomedicines (APP), which performs services and studies focused on innovation in the medicinal plants and phytotherapics field of knowledge[7]. This work represents an effort by APP to create a methodology for medicinal plant species selection that will be able to represent Brazilian biodiversity. Hopefully this method can serve as a basis for RD & I studies in the field of phytotherapics and an incentive for the use of medicinal plants in SUS.

## Method

A subjective allocation quantitative method used in ethnobotany studies was adapted to create a medicinal plant selection that can be indicated to RD & I in the phytotherapics field of knowledge. The Cultural Significance Index proposed by Turner [8] was adapted and applied in a list of species from the "Campus Fiocruz Mata Atlântica" flora, in order to test its viability. This Campus is located in an environmental preservation area - The State Park of Pedra Branca. The new index denominated as Medicinal Significance Index (MSI) is given by the formula: MSI = (*LxBxI*).*Fc.* Where: (L) Lists: For each species were assigned values (1 to 5) inversely proportional to the presence in the main official lists of medicinal plants offered by the government. (B) Biodiversity: The value 1 was assigned for exotic species and 2 for native species. In order to differentiate native species from exotic species, the database of the Flora of Brazil 2020 Program was used.

The values of 1 to 3 were assigned according to the threat level of each species. To evaluate the degree of threat, the National Plant Conservation Center (CNC Flora) database was used. These values were multiplied to generate the final value of "B". (I) Intensity of use: It is represented by the number of scientific papers that quoted certain traditional medicinal use for a given species. A bibliographical survey of the last 10 years was carried out on each species studied to generate the value of "I". (Fc) Correction factor: It is given by the relation between number of articles found for each species and the number of articles of the most cited species.

**Results / Discussion / Conclusion**

After applying the MSI in the list of 83 species from the flora of Campus Fiocruz Mata Atlântica, 12 species were elected as the most important: *Schinus terebintifolia* Raddi (MSI = 120); *Costus spiralis* (Jacq.) Roscoe. (MSI = 54); *Mangífera indica* L. (MSI = 48); *Bixa orellana* L. (MSI = 24); *Bauhinia forficata* Link. (MSI = 24); *Borreria verticillata* (L.) G.Mey. (MSI = 6); *Trema micrantha* (L.) Blume (MSI = 2.67); *Anadenanthera peregrina* (L.) Speg. (MSI = 2.67); *Indigofera suffruticosa* Mill. (MSI = 2.67); *Luehea divaricata* Mart. & Zucc (MSI = 2.67) and *Virola bicuhyba* (Schott ex Spreng.) Warb (MSI = 2).

The method selected 10 native species, 9 species unpublished in the official lists and among them a species with high degree of threat (*Virola bicuhyba)*. It is believed that the presence of 3 species that are at least in one oficial list (but no more than two) and 2 exotic ones is due to the large number of publications reffering to them ,therefore impacting the value of "I". However, the percentage of species that attend to the objective of the method in the final list is 75%.

The MSI could have shown even better results if applied in larger databases, with a more diverse listing of species and associated with a wider bibliographic survey. It is believed that MSI fulfills its role in selecting species that serve as a subsidy for pharmacological research, product development and use of medicinal plants in policies in Brazil, valuing the native flora.

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# PS040- HPLC-PDA green chromatographic method for the standardization of *Serjania marginata* extracts

Zanatta A.C.[1]*, Borges M.S.[1,2], Rinaldo D.[2], Vilegas W.[3]
*anaczanatta@gmail.com

[1]*UNESP - São Paulo State University, Institute of Chemistry. Prof. Francisco Degni, 55, 14800-060, Araraquara, São Paulo, Brazil.*
[2]*UNESP - São Paulo State University, School of Sciences, Chemistry Department. Engenheiro Luiz Edmundo Carrijo Coube Ave., 14-01, 17033-360, Vargem Limpa, Bauru, São Paulo, Brazil*
[3]*UNESP - São Paulo State University, Institute of Biosciences. Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, São Paulo, Brazil.*

## Introduction

A large part of the world's population uses "green medicine" to treat a wide variety of diseases. The use of chromatographic techniques such as High Performance Liquid Chromatography (HPLC) coupled to the Photo Diiode Array (PDA) detector enables qualitative and quantitative analysis of plant extracts in a fast and efficient way[1]. Unfortunately, most chemical studies make use of toxic solvents generating a large number of wastes[2]. Therefore, in order to minimize the production of toxic residues and to enable sustainable alternatives[3,4], this work intends to develop green chromatographic methods to standardize *S. marginata* extract, a medicinal species used in folk medicine to treat stomach ache.

## Method

Leaves of *S. marginata* (Sapindaceae) were collected in Dourados-MS, Brazil (22º08'05 "S and 55º08'17"W). They were dried at 40°C and pulverized using a grinder. 1 g of powder was extracted with 10 mL of Ethanol 70% (v/v) in an ultrasound bath, three times for 20 min. The extract (50 mg) was solubilized in EtOH/$H_2O$ 1:9, loaded to a C-18 cartridge (500 mg) and eluted sequentially with 3 mL of EtOH/$H_2O$ 1:9, EtOH/$H_2O$ 1:1 and EtOH 100%. The EtOH/$H_2O$ 1:1 fraction was redissolved to a concentration of 10 mg.mL$^{-1}$ and analyzed by HPLC-PDA. A green chromatographic method was developed using aA fractional experimental design $2v^{7-2}$ (screening variables) accordingly to Doehlert design, which considered the variation of seven chromatographic factors (% initial of ethanol, % final of ethanol, temperature, % acetic acid in $H_2O$, flow rate, run time and column).

## Results / Discussion / Conclusion

The mathematical model that expresses the best response (maximum number of peaks, MNP) for the green chromatographic method developed was

$$Y = 42.22 + 2.25X_5 + 4.91X_6 + 7.66X_3^2 - 6.93X_5X_6 - 7.34X_5X_3.$$

where X5 (flow rate, 1 mL.min$^{-1}$), X6 (run time, 30 min) and X3 (temperature, 25 °C) were the most relevant factors. Under these conditions, MNP was 57. The temperature was found to be a

relevant factor in the response probably due to the presence of procyanidins that are heat sensitive compounds, besides flavonoids and saponins.

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# PS041- Exfoliating composition with oils of vegetable origin (*Calendula officinalis*)

Ramírez-Hernández J.[1]*, Bárcena-Vicuña G.G.[1], Castelán-Aparicio B.[1], Hernández-Páez E.[2]
*yetzka0027@yahoo.com

[1]*PhD/Ingeniería Bioquímica, Edificio K, Prolongación Heliotropo No.1201 Colonia Vista Hermosa, Atlixco, Puebla. Instituto Tecnológico Superior de Atlixco, C. P. 74210, Atlixco, Puebla, México.*
[2]*M.I.Q/Ingeniería Electromecánica, Edificio L, Prolongación Heliotropo No.1201 Colonia Vista Hermosa, Atlixco, Puebla. Instituto Tecnológico Superior de Atlixco, C. P. 74210, Atlixco, Puebla, México.*

## Introduction

*Calendula officinalis* is an annual plant that is cultivated throughout the world and its flowers are used both from the ornamental point of view and for the preparation of finished products in the pharmaceutical and cosmetic industries[1]. *Calendula officinalis*, also called marigold or simply calendula, is one of the 29 most commonly used plants along with chamomile, sage and rosemary. It is known that the active principles of this plant are carotenoids (0.8%) and flavonoids (0.4%)[4]. Among the pharmacological properties of the calendula are tissue regeneration, antiseptic activity and anti-inflammatory activity[1]. In the area of cosmetology, its use is much smaller and reduced, an example is the treatment of non-serious acne, the prevention of dermatitis and recent investigations mention the reduction of spots related to exposure to UV rays[3]. On the other hand, clay is a product widely used in the cosmetic industry as an exfoliating agent due to its composition rich in silicon oxide, silicates and aluminum in addition to magnesium carbonate and calcium which help to give light to the skin. Specifically, white clay or *kaolin* is formed by several minerals (hydrated alumina silicates). The white clay has anti-inflammatory and cell regeneration properties, softens the skin, helps it to be much smoother and eliminates the flaccidity that occurs over time[5].

According to all these characteristics, an exfoliating cream based on *calendula officinalis* oil, *kaolin* (micronized dry kaolin with a very high degree of whiteness and fineness) and *lavender officinalis* extract was developed, which removes and regenerates dead cells, creates tension effect that helps to control flaccidity and reduces the stains caused by age and UV rays by up to 75%.

## Method

6 chemical equivalents of kaolinite were mixed with continuous stirring was added 1.0 chemical equivalent of calendula oil drop by drop. The reaction mixture was kept at room temperature. At the end was added with continuous stirring 0.02 chemical equivalents of lavender extract. The product was stirring for 10 more minutes at room temperature. The product obtained has a dark brown coloration. Finally the microbiological and irritability analyzes were carried out.

## Results / Discussion / Conclusion

According to the results, each of the products analyzed does not exceed the maximum permissible limits under NOM089-SSA1-1994 and NOM039-SSA1-1994.

| Sample | Mesophilic aerobes | Mushrooms | Yeasts | Observations |
|---|---|---|---|---|
| Calendula (*Calendula officinalis*) 02-04-18 | -10 | -10 | -10 | Does not exceed |
| Lavander (*Lavanda officinalis*) 09-04-18 | 1 | -10 | -10 | Does not exceed |
| Exfoliating composition 24-04-18 | -10 | -10 | -10 | Does not exceed |

| Product | Hemorrhage | Vasoconstriction | Coagulation | Index Irritation (II) |
|---|---|---|---|---|
| Exfoliating composition 11-06-18 | 0 | 0 | 0 | 0 |
| SDS control 11-06-18 | 6 | 4 | 4 | 14 |

Evaluation of the ophthalmic irritability of cosmetic creams by an in vitro method in substitution of the test in rabbits. According to the irritation index obtained, the exfoliating composition analyzed was classified as non-irritating in the test model used. According to NOM039-SSA1-1994.

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# PS042- Antimicrobial activity of *Escherichia coli* and *Staphylococcus aureus* with SMO solution

Hernández-Paez E.[1]*, Ramírez-Hernández J.[2], Bárcena-Vicuña G. G.[2], Castelán-Aparicio B.[2]
*hepe7410@hotmail.com; baviga@yahoo.com.mx

[1]*M.I.Q./Ingeniería Electromecánica, Edificio L, Prolongación Heliotropo No.1201 Colonia Vista Hermosa, Atlixco, Puebla. Instituto Tecnológico Superior de Atlixco, C. P. 74210, Atlixco, Puebla, México.*
[2]*PhD/Ingeniería Bioquímica, Edificio K, Prolongación Heliotropo No.1201 Colonia Vista Hermosa, Atlixco, Puebla. Instituto Tecnológico Superior de Atlixco, C. P. 74210, Atlixco, Puebla, México.*

## Introduction

Water is fundamental to perpetuate life on the planet and this is demanded by all living beings, hence the importance of a good quality in it at a low economic cost and with no risks to human health.

The Moringa belongs to the Moringáceas family, a small group of plants within the immense order Brassicales that includes the family of cabbage and radish, together with the family of cress and capers. Moringaceae comprises only one genus, Moringa.These pathogenic microorganisms of fecal-oral transmission may be present in non-potable water, or in rainwater and rivers, such as Salmonella, Shigella and *Vibrio cholerae*, which represent major public health problems in communities of extreme poverty (Ahmed, W., Sritharan, T., Palmer, A., Sidhu, 2013) [3] Moringa seed (SMO) has high bactericidal activity and fungicides, especially affecting *Escherichia coli* (E. coli), Shigella, *Bacillus cereus* and *Salmonella typhi*, common pathogens of turbid water. (Ahmed, W., Hodgers, L., Sidhu, 2012)[2]. Despite the antimicrobial properties of Moringa, *Moringa oleifera* has been described as a plant with antibacterial activity against some human pathogens, such as Shigella, Pseudomonas, *Staphylococcus aureus, Bacillus cereus* and Streptococcus (Ahmed, W., Goonetilleke, A., 2010)[1].

## Method

The Kirby-Bauer method (Bauer et al., 1966) (method of diffusion in Agar) is used to determine the sensitivity of a microbial agent to an antibiotic, the method comprises what is called an antibiogram or bacterial susceptibility test against to specific drugs.

## Application form

A standardized amount of bacteria is inoculated onto the surface of a Müller-Hinton Agar plate, planted uniformly to obtain a bacterial "turf". Immediately, filter paper discs impregnated with known concentrations of the different antibiotics are placed. The antibiotic will diffuse from the filter paper to the agar radially. The plate is incubated for 18-24 hours at 37°C (respect this parameter, because lower temperatures can slow down the growth of the germ and the diffusion of the antibiotic, giving irregular halos difficult to measure), and then measure the halos of inhibition of development, interpreted according to tables previously made.

## Results / Discussion / Conclusion

The results of the Agar samples that correspond to the antibiograms of *E. coli* strains are shown below. corresponding to table 1; and strains of *Staphylococcus aureus* (S. aureus) corresponding to table 2.

**Table 1** Measurement of Halo diameters without inhibition in mm *of antibiograms in E. coli strains*

| No Antibiogram | Halo diameter mm | Average mm |
|---|---|---|
| **1** | 13, 13, 13 | 13 |
| **2** | 11, 13, 11 | 11.6 |
| **3** | 14, 14, 15 | 14.6 |
| **4** | 7, 10, 8 | 8.3 |
| **5** | 7, 11, 10.5 | 9.5 |
| | Total | 11.4 |

**Table 2** Measurement of Halo diameters without inhibition in mm of antibiograms in strains S. aureus

| No Antibiogram | Halo diameter mm. | Average mm |
|---|---|---|
| **1** | 12, 11, 12 | 11.6 |
| **2** | 11, 13, 11 | 11.6 |
| **3** | 12, 12, 12 | 12 |
| | Total | 11.7 |

The antimicrobial activity tests showed the generation of inhibition halos in antibiograms of strains of *E. coli* with diameters of 11.4 mm and for *Staphylococcus aureus*, diameters of 11.7 mm respectively, with which it was concluded that moringa presents a sensitive microbial activity.

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# PS043- Evaluation of the antifungal efficiency of *Justicia spicigera* extract in aflatoxin-producing fungi

Bárcena-Vicuña G.G.[1]*, Ramírez -Hernández J.[1], Delgado-López M.G[2], Castelán-Aparicio B.[1], Hernández-Páez E.[1] Casiano-Reyes L.A.[1]
*baviga@yahoo.com.mx

[1]*M.T.A. Ingeniería Bioquímica, Edificio K, Prolongación Heliotropo 102. Instituto Tecnológico Superior de Atlixco, C. P. 74210, Col. Vista Hermosa, Atlixco, Puebla; México.*
[2]*PhD/Centro de Investigación Biomédica de Oriente, Laboratorio de Biología Celular,* HGZ No. 5 Km 4.5 Carretera Federal Atlixco- Metepec C.P. 74360 Metepec, Puebla, México.

## Introduction

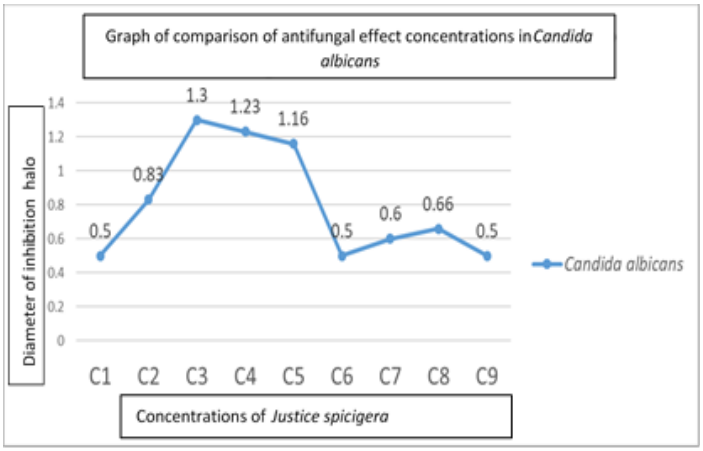
Fungi are a mystery, so great that it is the kingdom that houses different genres and little known so far. More than 200 species of fungi have been described that may be pathogenic to humans. The most common are the different species of *Candida and Aspergillus* that can produce both localized and invasive infections[1]; among other diseases which may end in some type of cancer.
In recent decades there has been a notable increase in infections caused by fungi, which may be attributable to factors such as: low bioavailability of the drug and drug resistance. This situation has led to the search of antifungals of natural origin that have ethnopharmacological background that could be used as an alternative in this type of infections, pharmacologically to the extract of *Justicia spicigera* has been proven antibacterial, antiprotozoal and antifungal activity, therefore we consider the importance to continue with the study of *Justicia spicigera* to determine its activity against different species of fungi of medical importance.

## Method

The fungi were inoculated on the dextrose and potato agar, with the Methods standardized by the Institute of Clinical and Laboratory Standards (CLSI), planting by dispersion with isopo, planting by extension in plate; for the study of sensitivity to antifungal agents (documents M27-A3, M38-A and M44-A).

## Results / Discussion / Conclusion

The extract of the *Justicia spicigera* plant (muicle) in the concentrations 25 µg / ml, 20 µg / ml, 19 µg / ml, 15 µg / ml, is promising for the inhibition of the growth of the strain *Candida albicans*.
The concentrations were classified as: C1 (30 µg / ml): moderately sensitive C6 -C9 (10, 9, 5, 4 µg / ml): moderately sensitive C2-C5 (25, 20, 19, 15 µg / ml): sensitive The MIC of *Justice spicigera*, for *Candida albicans* is 4 µg / mL.



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# PS044- Comparative study of phenolic profile, antioxidant and antimicrobial properties of leaves and flower buds of *Inula viscosa* (L.) Aiton (Asteraceae)

Mohti H.[1,2]*, Taviano M.F.[3*], Cacciola F.[4], Dugo P.[3], Mondello L.[3], Marino A.[3], Crisafi G.[3], Benameur Q.[5], Zaid A.[1,2], Miceli N.[3]
*hicham.mohti@gmail.com, *mtaviano@unime.it

[1]*PhD, Prof/Department of Biology, Faculty of Sciences, Moulay Ismail University, BP 11201 Zitoune, Meknes, Morocco.*
[2]*PhD, Prof/CUI-UMI-UHasselt program (Morocco-Belgium).*
[3]*PhD, Prof/Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy.*
[4]*PhD, Prof/Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, 98125 Messina, Italy.*
[5]*PhD/Nursing Department, Faculty of Natural Sciences and Life, Abdelhamid Ibn Badis University, 27000 Mostaganem, Algeria.*

## Introduction

*Inula viscosa* (L.) Aiton (Asteraceae) is widely utilized in folk medicine for the treatment of several ailments, including inflammations, infections and skin diseases[1]. The aim of the present work was to compare the antioxidant potential of leaves and flower buds extracts of *I. viscosa* growing in Morocco obtained by different extraction techniques and solvents. Besides, the antimicrobial properties, the toxicity and the phenolic profile of the best antioxidant extracts were investigated.

## Method

*I. viscosa* dried leaves and flower buds were extracted by different methods and solvents: hot extraction (methanol and water extracts, hot-MeOH and hot-$H_2O$, and Soxhlet ethanol extract, Sox-EtOH) and maceration (methanol and water extracts, mac-MeOH and mac-$H_2O$). Three *in vitro* assays were used to screen the antioxidant potential of the ten extracts: DPPH test and reducing power to evaluate the primary antioxidant effectiveness, ferrous ions chelating activity assay for the secondary antioxidant properties[2,3]. The Sox-EtOH extracts of both leaves and flower buds displayed the strongest activity in the DPPH test, thus they have been selected for further investigations. The antimicrobial efficacy of the Sox-EtOH extracts against ATCC and food isolates strains was assayed by standard methods[3]. The toxicity was evaluated by using *Artemia salina* lethality bioassay[3]. The phenolic profile was characterized by HPLC-PDA-ESI-MS analysis.

## Results / Discussion / Conclusion

The results of antioxidant tests showed that *I. viscosa* extracts displayed the strongest effect on scavenging free radicals in the DPPH test, with $IC_{50}$ values ranging from 54.24 ± 0.21 µg/mL (Sox-EtOH) to 148.79 ± 0.11 µg/mL (mac-MeOH) for the leaves and from 39.77 ± 0.23 µg/mL (Sox-EtOH) to 86.06 ± 0.25 µg/mL (mac-MeOH) for flower buds. *I. viscosa* Sox-EtOH extracts exhibited antimicrobial activity; the leaves extract was found to be more active than flower buds

extract, displaying the best efficacy against *Candida albicans* ATCC 10231 (MIC= 125 µg/mL); among bacteria *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* S20/16 food isolate were the most sensitive strains. In the *A. salina* lethality bioassay, the extracts did not show any toxicity against brine shrimps ($LC_{50}$ > 1000 µg/mL). By HPLC-PDA/ESI-MS analysis of the Sox-EtOH extracts of leaves and flower buds a total of 29 and 26 phenolic compounds, respectively, were successfully separated and identified, being flavonoids the most abundant components. It's well known that flavonoids are effective antioxidant and antimicrobial agents, so it can be hypothesized that the observed activities could depend, almost in part, on the presence of these compounds. Our findings contribute to an increase in knowledge about *Inula viscosa,* as well as demonstrating that Sox-EtOH extracts of both leaves and flower buds are a good and safe source of natural antioxidant and antimicrobial compounds.

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# PS045- CHAYA (espinaca de árbol): importante fuente de β-caroteno y luteína

Murillo E.[1], Molinar-Toribio E.M.[1,2], Jiménez-Montero M.[2]*
*eice7@hotmail.com; emurillo29@hotmail.com

[1] *Departamento de Bioquímica, Facultad de Ciencias Naturales Exactas y Tecnologías, Universidad de Panamá, Cuidad de Panamá, Panamá.*
[2] *Investigador Nacional I del Sistema Nacional de Investigación de Panamá.*

## Introducción

La Chaya (espinaca de árbol) recibe el nombre científico de *Cnidoscolus aconitifolius* (Milles)[1], esta especie presenta dos subespecies: *Cnidoscolus aconitifolius* subsp. *aconitifolius* y *Cnidoscolus aconitifolius* subsp. *Polyanthus*[2]. En Panamá la subespecie típica es *Cnidoscolus aconitifolius* subsp. *aconitifolius*, en la cual se puede distinguir dos variedades cultivadas (cv), *Cnidoscolus aconitifolius* subsp. *aconitifolius* cv. "Picuda" y *Cnidoscolus aconitifolius* subsp. *aconitifolius* cv. "Estrella". Diversos estudios indican que las hojas de Chaya pueden revertir los daños por diabetes, estrés oxidativo o cáncer[3,4]. El objetivo de este estudio fue determinar en las diferentes vc Chaya, en Panamá los polifenoles totales, carotenoides totales y perfil de carotenoides.

## Método

Cuatro vc de Chaya, Berro y Espinaca se evaluaron en hojas frescas en este estudio. Las muestras de Espinaca (*Spinacia oleracea*) y Berro (*Nasturtium. officinale)* se obtuvieron del mercado Metropolitano en la ciudad de Panamá y las muestras de chaya fueron colectadas en los corregimientos de Arraijan y La Chorrera, en Panamá Oeste. Evaluamos la cantidad total de compuestos fenólicos y carotenoides totales usando un espectrofotómetro a 765 nm y 450 nm, respectivamente. La determinación e identificación de los diferentes carotenoides se analizó con cromatografía líquida de alta resolución (HPLC) con detector de matriz de fotodiodos UV/Visible.

## Resultados

En el contenido total de polifenoles no se observaron diferencias significativas entre la Espinaca, el Berro y las variedades de *Cnidoscolus aconitifolius* subsp. *aconitifolius*. (variedades picuda, estrella-1 y 2). En el contenido de carotenoides totales, β-caroteno y luteína se observaron diferencias significativas $^{***}P<0.0001$ con respecto a la Espinaca (**cuadro 1)**.

**Cuadro 1.** Determinación de polifenoles y carotenoides en hojas de Espinaca, Berro y dos variedades de Chaya (n=6).

| Especies de plantas | Polifenoles totales ( mg EAG/g ) | Carotenoides totales (μg/g) | β-caroteno (μg/g) | Luteína (μg/g) |
|---|---|---|---|---|
| | Media ± SE | Media ± SE | Media ± SE | Media ± SE |
| *S. oleracea*. "espinaca" | 40.76±3.5 | 110.8±5.9 | 26.7±0.8 | 46.4±3.2 |
| *N. officinale.* "berro" | 38.0±2.9 | 178.6±17.7$^{*}$ | 39.4±4.1$^{*}$ | 75.5±9.0$^{*}$ |
| *C. aconitifolius*. "picuda" | 45.3±9.1 | 439.3±56.7$^{***}$ | 117.8±24.4$^{***}$ | 184.3±28.1$^{***}$ |
| *C. aconitifolius*. "estrella-1" | 51.4±13.4 | 511.8±69.7$^{***}$ | 136.7±30.5$^{***}$ | 227.8±40.5$^{***}$ |
| *C. aconitifolius*. "estrella-2" | 41.8±9.1 | 411.6±56.9$^{***}$ | 116.0±20.0$^{***}$ | 181.6±24.5$^{***}$ |
| *C. aconitifolius*. "estrella-3" | 43.0±9.3 | 532.7±72.8$^{***}$ | 143.9±30.0$^{***}$ | 218.3±47.7$^{***}$ |

Todos los resultados son expresados en peso de hoja fresca. Los valores están expresados como Media±SE (error estándar). Valores de la media significativamente diferentes de Espinaca: $^{*}$P<0.05 y $^{***}$P<0.0001.

## Conclusión

Este estudio mostró que las variedades de Chaya son importantes fuentes de β-caroteno y luteína y el contenido de estos carotenoides es mayor que el de hojas comestibles tradicionales.

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# PS046- Encapsulation in phosphatidyl choline increases the antimalarial activity of a-mangosteen

Upegui Y.A.[1], Echeverri F.[2], Velasquez J.P.[3], Gil J.F.[4], Quiñones W.[5], Archbold R.[6], Torres F.[7], Escobar G.[8], Robledo S.M.[9]*
*sara.robledo@udea.edu.co

[1]*PhDc/PECET, Facultad de Medicina, Universidad de Antioquia, Calle 70 # 52-71, Medellín, Colombia. C. P.050010*
[2,3,4,5,6,7]*PhD/Instituto de Química, Universidad de Antioquia, Calle 70 # 52-71, Medellín, Colombia. C. P. 050010*
[9]*PhD/ Facultad de Medicina, Universidad de Antioquia, Calle 70 # 52-71, Medellín, Colombia. C. P. 050010*

## Introduction

Xanthones isolated from the husk (pericarp) and latex of *Garcinia mangostana* Linn. GML (mangosteen, fam. Clusiaceae) possess a wide spectrum of pharmacological properties, including antioxidant, antitumor, antiallergic, antiinflammatory, antibacterial, antifungal, antiviral and antiplasmodial activity. Previously, crude extract and a-mangosteen showed high antiplasmodial activity *in vitro* [1], but a marginal response in the murine model disease. Thus, these results could be affected by the bioavailability of these type of compounds, due to their high polarity. Here we report a pharmaceutical approach to improve the antimalarial activity.

## Method

The α-mangostin xanthone was isolated from *G. mangostana* husk through column chromatography in Sephaadex LH20. Pure compound was encapsulated in phosphatidyl choline (soy lecithin) for oral administration. The antiplasmodial therapeutic response of pure and encapsulated compounds was evaluated in the *Plasmodium berghei* murine model for malaria according to the Rane´s test[2,3].

## Results / Discussion / Conclusion

Encapsulation of compounds in soy lecithin improved the bioavailability and therefore the therapeutic response of the α-mangostin xhantone. Thus, treatment of infected mice with a daily dose of 100 mg/kg for 7 days of free α-mangostin reduced parasitemia by approximately 30% while the treatment with the encapsulated compound at the same dose and frequency reduced the parasitemia by 80%. Noticeable toxicological effects were not observed. These results suggest that this formulation could be an alternative for malaria therapy.

## Acknowledgments.

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# PS047- *In vitro* α-glucosidase inhibition by Brazilian plant extracts characterized by mass spectrometry

Lianza M.[1]*, Poli F.[1], Simas R.C.[2], Leitão S.G.[2]
*mariacaterina.lianz2@studio.unibo.it, sgleitao@pharma.ufrj.br

[1]*Department of Pharmacy and Biotechnologies, University of Bologna, via Irnerio, 42, 40126, Bologna, Italy*
[2]*Faculdade de Farmácia, Departamento de Produtos Naturais e Alimentos, Universidade Federal do Rio de Janeiro, CCS, Bl. A, Ilha do Fundão, 21.941-902, Rio de Janeiro, Brazil.*

## Introduction

Inhibition of α-glucosidase, an enzyme involved in carbohydrates digestion, represent a strategy to treat type 2 diabetes mellitus. Drugs such as acarbose, miglitol and voglibose act as α-glucosidases competitive inhibitors, decreasing both postprandial hyperglycaemia and hyperinsulinaemia [1]. Agents that reduce postprandial hyperglycaemia have a key role in the treatment of type 2 diabetes and pre-diabetic states such us obesity and hyperglycemia [2] [3]. Amylin is a hormone co-secreted with insulin in response to the high concentrations of nutrients in the blood. When excess amylin is produced, its precipitation forms amyloid plaques, which sediment in pancreas β-cells causing their damage. It has been hypothesized that this phenomenon causes type 2 diabetes mellitus [4]. Brazil is one of the richest countries in biodiversity, and encompasses 2 of the world´s 25 "Hotspots". These regions, that together sum only 1.4% of the world´s surface, concentrate more than 60% of the world´s biodiversity. The Atlantic Forest is within the 5 most important Hotspots. In this work, 15 extracts prepared from Brazilian medicinal plants of Atlantic Forest, which were previously tested positive in the inhibition of amylin aggregation (unpublished results), were tested *in vitro* against α -glucosidase. For complex samples such as plant extracts, it is necessary to use robust, sensitive instrumentation with good resolution, capable of generating reliable chemical information. Mass analysis has been successfully used in the qualification of components in mixtures of plant extracts, providing data on weight and molecular formula, enabling the identification characteristic fragments of a molecule [5]. Confirmation of the presence of chemical compounds is possible due to the information obtained by the equipment, such as the accuracy of mass / load ratio measurements (*m/z*), isotope ratio and fragmentation of molecules. Thus, Mass Spectrometry (MS) was elected to the previous screening of the species under study.

## Method

α-Glucosidase inhibition assay was performed according to Venditti et al. [6] with slight modifications. α-Glucosidase from *Saccharomyces cerevisiae* (E.C. 3.2.1.20, Sigma Aldrich) was used. Organic plant extracts from our laboratory collection were assayed in DMSO (final concentration in the assay was 12.5% v/v). Extraction procedures were based on different solvents in order to obtain extracts without tannins. Negative control was performed in the same conditions. One mU of enzyme prepared in 0.1 M phosphate buffer pH 6.8 were incubated for 5 min with test samples. The synthetic substrate p-nitrophenyl-α-D-glucopyranoside (p-NPG), prepared in buffer, was added to a final concentration of 2 mM, to start the reaction with a final

volume of 200 µL. The percentage of inhibition of enzyme activity was calculated by the following formula: % Inhibition = {[1-(ΔAbs/min sample)]/ΔAbs/min neg. contr}.The $IC_{50}$ value was calculated by constructing a logarithmic curve showing sample concentrations on x-axes and the percentage inhibition on y-axes. Acarbose, showing an $IC_{50}$ of 105 µM, was used as positive control. All extracts (1% w/v) were analyzed by direct injection in the range of *m/*z 100-1100. The mass spectrometer (LCQ Feet, ThermoFisher Scientific), equipped with Electrospray source (ESI), was operated in negative and positive ion mode.

**Results / Discussion / Conclusion**

All extracts were first screened at a fixed concentration of 100 µg/mL and those showing inhibition higher than 30% were considered active. Four extracts out of the 15 tested had their $IC_{50}$ were calculated. The selected plants were *Hyptis monticola*, *Lantana trifolia* and *Lippia origanoides* from two different localities. The $IC_{50}$ ranges were 15-115 µg/mL, being the most active that of *Hyptis monticola*. Our proposal involves the investigation of polar and apolar organic extracts. Samples from different fractions of extracts were then analyzed by tandem mass spectrometry coupled to a trap ion with electrospray ionization interface using direct sample insertion mode (FIA-ESI-IT-MS). The $MS^2$ studies are still being done with the aim of confirming the structures based on the fragmentation patterns already described in the literature for all compounds. This strategy allowed to know the secondary metabolites of plants belonging to the different genera identifying flavonoids, catechins, isoprenoids, phenolic acids among other molecules. Thus, our proposal led to a rapid and efficient chemical characterization of secondary plant metabolites with activity by α-glucosidase inhibition. These results are useful for a better understanding of the pharmacological activities of different Brazilian plants. The use of qualitative mass spectrometry in the dereplication of plant extracts is an important tool for the chemical substances characterization and has contributed greatly to advances in the studies of the vegetal area.

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# PS048- Identification of saponins from bark and wood of *Ampelozizyphus amazonicus* Ducke by MS

Mendonça S.C.[1], Simas R.C.[2], Leitão G.G.[1], Leitão S.G.[2]*
*sgleitao@gmail.com

[1]*Instituto de Pesquisas de Produtos Naturais (IPPN), CCS, Bloco H, Ilha do Fundão, Universidade Federal do Rio de Janeiro, 21941-902, Rio de Janeiro, RJ, Brasil;*
[2]*Departamento de Produtos Naturais e Alimentos (DPNA), Faculdade de Farmácia, CCS, Bloco A 2° andar, Ilha do Fundão, Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, Brasil.*

## Introduction

Preparations of *Ampelozizyphus amazonicus* (AA) are known for their high content of saponins, especially those of dammarane-type triterpenoid aglycone[1,2]. In the Amazon region, roots and barks are widely used in the treatment and prevention of malaria, and the wood is discarded in the preparation of the drink by *quilombolas*[3]. The extract prepared from the woods is also saponin-rich, but nothing is known about its chemical composition. In this work, we aimed at the dereplication studies of the aqueous and ethanol extracts of barks and wood of this plant, focusing on the saponins, by the use of LC-MS/MS, in order to verify the possibility of using the wood as raw material.

## Method

Four extracts were prepared (1%, w/v) being: ethanol extract from barks and Wood, and aqueous extract from barks and wood, which were named EB, EW, AB and AW, respectively. These extracts were analyzed by UHPLC-MS/MS and HRMS equipped with APCI source in negative ion mode. The data were treated in MZmine software in order to enable comparative viewing of data across the samples.

## Results / Discussion / Conclusion

The Base Peak Chromatograms (BP) showed a complex saponin profile with deprotonated molecular ions $[M-H]^-$ in the range of *m/z* 600–1300. Analysis of $[M-H]^-$ ions implied that the compounds belong to the class of dammarane-type triterpenoid saponins. The extracts of bark and wood showed a great chemical similarity and in all, 96 saponins were identified within wood and bark, including 68 new ones, not yet described in the literature for this species. From this total, 29% have previously been described for AA, 24% have been described for other species of the Rhamnaceae family, but for the first time in AA, and 47% have been first proposed in AA, based on fragmentation patterns and precise mass measurements. All the saponins already known in AA were identified in both bark and wood. Regarding the extracting solvent, the ethanol extracts showed to be slightly richer in these substances than the aqueous extracts in about 12.5% for wood and 3.5% for bark. In relation to the studied plant part, the wood presented about 9% more saponins than the bark in the ethanolic extracts. In aqueous extracts the percentage is the same when compared bark and wood. Taken together, these results revealed that the developed method was useful to rapidly and simultaneously identify the constituents in AA extracts. It also allowed

to demonstrate that bark and wood samples exhibited a very similar chemical profile. These are considered promising to provide larger use of plant material and conservation of the species.

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# PS049- Inhibitory activities of some flavonoids on collagenase, elastase and hyaluronidase enzymes

Bahadır Acıkara Ö.,[1]* Küpeli Akkol E.,[2] Kurtul E.,[1] Ilhan M.,[2] Saltan İşcan G.,[1] Güragac F.T., [2] Šmejkal K.,[3] Cvačka J.,[4] Buděšínský M.[5]
*obahadir@ankara.edu.tr

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Tandoğan, 06100 Ankara, Turkey*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler, 06330 Ankara, Turkey*
[3]*Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1946/1, 61242 Brno, Czech Republic*
[4]*Mass Spectrometry Group, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague, Czech Republic*
[5]*NMR group, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague, Czech Republic*

## Introduction

*Scorzonera latifolia* (Fisch. & Mey.) DC. belongs to the Asteraceae family widely distributed in Eastern Anatolia, northeastern Iran, and Caucasus[1]. *S. latifolia* roots, are used in Turkish folk medicine for their analgesic activity against headache, externally to cure infertility in women, and internally as an antihelmintic[2]. Antinociceptive, anti-inflammatory, wound-healing, antioxidant, and antimicrobial activities have previously been reported for *S. latifolia*[3-4]. In current study flavonoids isolated from methanolic extract of the *Scorzonera latifolia* aerial parts were tested for their inhibitory activities on collagenase, elastase and hyaluronidase enzymes.

## Method

*Scorzonera latifolia* Fisch. & Mey. was collected in the district of Kars-Arpaçay, Eastern Anatolia, Turkey. (AEF no 23830). The dried and powdered aerial parts of the plant (1.5 kg) were extracted at room temperature using methanol. Methanolic extract was filtered, and the solvent was evaporated at 40–50 °C under reduced pressure to obtain the crude extract (231 g). This crude extract was dissolved in water and then subjected to liquid-liquid extraction, using petroleum ether, chloroform, and ethyl acetate successively. Including the residual water solution, four fractions were obtained from the methanol extract of *S. latifolia*. The ethyl acetate fraction (21.5 g) was selected for further separation by column chromatography on silicagel and eluted with an EtOAc:MeOH:water (100:13.5:10, *v*/*v*/*v*). Four flavonoid compounds were isolated and their structures were identified using spectroscopic methods (U.V, I.R., M.S., $^{1}$H NMR, $^{13}$C NMR). Their inhibitory activities on collagenase, elastase and hyaluronidase enzymes were measured by in vitro test systems.

## Results / Discussion / Conclusion

Chromatographic separation of the ethyl acetate extract yielded an isolation of four flavonoids and their structures were established as quercetin-3-O-β-apiofuranosyl-(1´´´→2´´)-β-D-glucopyranoside (1), quercetin-3-O-α-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (2), isoorientin (3), and 7-methylisoorientin (4). 7-methylisoorientin (4) exerted inhibitory activity on

collagenase and elastase, while quercetin-3-O-β-apiofuranosyl-(1´´´→2´´)-β–glucopyranoside (1) inhibited only collagenase. None of the isolated compounds showed any inhibitory effect on hyaluronidase.

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# PS050- Antibacterial activity of cinnamaldehyde against Shiga toxin- and extended-spectrum β-lactamase-producing *E. coli*

Benameur Q.[1,*], Gervasi T.[2], La Camera E.[3], Crisafi G..[3], Dugo G. [2,4], Cicero N.[2,4], Marino A.[3]
*qada.benameur@univ-mosta.dz

[1]*Faculty of Natural Sciences and life, University of Abdelhamid Ibn Badis of Mostaganem, 27000 Mostaganem, Algeria.*
[2]*Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy.*
[3]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy*
[4]*Science4Life, Spin Off Company, University of Messina, Italy.*

## Introduction

Infections with antibiotic-resistant bacteria result in increased mortality, morbidity, and social and economic costs.[1–4] By 2050, an estimated 10 million deaths per year globally will be attributable to antimicrobial resistance, with a cumulative economic cost of US$100 trillion.[5] Shiga toxin-producing *Escherichia coli* (STEC) bacteria, including the O157 serogroup, are well described human pathogens associated with bloody diarrhea, hemolytic-uremic syndrome, and death. The aim of this study was to evaluate the sensitivity of antimicrobial-resistant Enterobacteriaceae isolates, including Shiga toxin (ST) and ESBL producing *E. coli*, to cinnamaldehyde.

## Method

In the current study, the effect of cinnamaldehyde was studied on eight multidrug resistant Enterobacteriaceae strains (five *E. coli*, one *Klebsiella pneumoniae*, one *Enterobacter cloacae* and one *Salmonella* Sp.), including some ST and ESBL producers, which were previously characterized. Antibacterial activity of cinnamaldehyde against the Enterobacteriaceae isolates was investigated by disc diffusion method and minimum inhibitory concentration (MIC).

## Results / Discussion / Conclusion

Cinnamaldehyde showed a significant antibacterial activity against all the studied multidrug resistant Enterobacteriaceae isolates with MIC values in the range of 155 and 310 µg/ml.

| Bacterial species | Virulence Genes | Antimicrobial resistance pattern | MIC (µg/ml) |
|---|---|---|---|
| *E. coli (ESBL and STEC)* | *stx2* | NA, AML, AUG, CTX, SXT, TE, N | 310 |
| *E. coli (STEC O157:H7)* | *rfbE, fliC, eaeA, stx2* | / | 310 |
| *E. coli (S43/16)* | / | AML, AMC, N | 310 |
| *E. coli (ATCC25922)* | / | / | 310 |
| *E. coli (ESBLEC)* | / | NA, CIP, AML, AUG, CTX, SXT, TE, C, N | 310 |
| *K. pneumoniae (ESBL)* | / | NA,CIP, AML, AUG, SXT, TE, C, N | 310 |
| *Salmonella* Sp. *(S13b/16)* | / | AML | 155 |
| *E. cloacae (ESBL)* | / | NA, CIP, AML, AUG, CTX, SXT, TE, N | 310 |

Cinnamaldehyde was very effective against all the selected antimicrobial resistant Enterobacteriaceae isolates, including ST and ESBL producers. Therefore, it could be suggested as an antibacterial agent in the future.

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# PS051- Antibacterial activity of *Origanum vulgar* essential oil against *bla*$_{ESBL}$ producing *E. coli*

Benameur Q.[1,*], Gervasi T.[2], Pellizzeri V.[2], Pľuchtová M.[3], Gruľová D.[3], Dugo G.[2,4], Marino A.[5], Cicero N.[2,4]

*qada.benameur@univ-mosta.dz

[1]*Faculty of Natural Sciences and Life, University of Abdelhamid Ibn Badis of Mostaganem, Mostaganem, Algeria.*
[2]*Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy.*
[3]*Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Slovakia.*
[4]*Science4Life, Spin Off Company, University of Messina, Italy.*
[5]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.*

## Introduction

Antibiotic resistance is on the rise worldwide and is associated with severe morbidity, mortality and increased healthcare-related costs[1]. The frequency of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae has been increasing steadily worldwide since the early1980s.[2,3] While antimicrobial resistance is rapidly spreading, research and development for new antimicrobial agents are languishing. The aim of this study was to evaluate the antibacterial effects of Slovakian origin *Origanum vulgar* essential oil (OVEO) both alone and combined with cefotaxime against ESBL producing *Enterobacteriaceae* isolates.

## Methods

Gas chromatograph-mass spectrometer (GC/MS) was used for the determination of OVEO composition. In the current study, the effect of OVEO was studied on eight multidrug resistant *Enterobacteriaceae* isolates, including *bla*$_{ESBL}$ (*bla*$_{CTX-M-1}$ and *bla*$_{SHV-12}$) producing isolates, which were previously characterized. Antibacterial activity of OVEO against the *Enterobacteriaceae* isolates was investigated by disc diffusion assay and minimum inhibitory concentration (MIC). The synergistic interaction between OVEO and cefotaxime was determined by checkerboard test and fractional inhibitory concentrations index was used to describe drug interaction.

## Results / Discussion / Conclusion

GC/MS analysis identified thymol (78.21%), m-cymene (4.35%) and γ-Terpinene (3.19%) as the major components present in the OVEO. OVEO showed an unexpected high antibacterial activity against all the studied multidrug resistant *Enterobacteriaceae* isolates, including *bla*$_{ESBL}$ producing isolates. OVEO showed variation in the synergistic effects in combination with cefotaxime on *bla*$_{ESBL}$ producing isolates.

These results confirmed the potential of Slovakian origin OVEO to be used alone against ESBL- and non ESBL-producing multidrug resistant *Enterobacteriaceae* isolates, and in combination with cefotaxime against *bla*$_{SHV-12}$ producing *Enterobacteriaceae* isolates.

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# PS052- *Psidium guajava* e *Eugenia uniflora* at the Health Sole System: an evaluation of cost-effectiveness

Wadt M.[1], Cavalini F.[2],Palomo B.R.[3] , Trindade B.L.[4], Batista E.R.N[4], Wadt N.S.Y[1*]
marcelowadt@gmail.com, *nilwadt@gmail.com.

*1 PhD/ University Paulista (UNIP) -Health Sciences Institute- Farmacy- Av. Armando Giassetti, 577 - Jundiaí - SP –*
*2 Esp/ Municipal Health Secretary/ Valinhos Prefecture*
*3 Student/ University Paulista (UNIP) -Health Sciences Institute- Farmacy*
*4 Doc/ Municipal Health Secretary / Valinhos Prefecture*

## Introduction

The treatment of wounds, specially those resulting of venous insufficiency and the diabetic foot are a public health issue, and the Health Sole System (HSS) deals with the expenses arising from these complications, which are peculiar to these lesions, being that the same interfere in health quality of their bearers. Finding efficient and low-cost alternatives is a challenge for the researchers. The objective of the present study was to evaluate the cost-effectiveness when using tea (decoction) of guava tree and pitanga tree leaves for treatment of wounds in patients of SELVEN (Neuropathic and Vascular Injury Specialized Service – SELVEN), in Valinhos (SP), Brazil.

## Method

The project was approved by the in Humans Ethics Committee (CAAE: 60579916.7.0000.5512) being 2 groups with 25 patients each (control and decoction). Ten leaves of guava tree and pitanga tree were boiled in one liter of water, and this decoction was used as bath or compress for 30 minutes directly in the wound. After applying the decoction, was applied the bandage of coating standardized by SELVEN. The study is retrospective and evaluated the time of treatment and cost of products and medicine of local use utilized in the bandages until medical release. The wound were classified in three degrees of tissue commitment: (1) epidermis; (2) hypodermic; (3) muscular.

## Results / Discussion / Conclusion

Patients that used the guava tree and pitanga tree leaves decoction presented significant diminishment in total treatment time, and cost of material used. The averages of treatment time and cost per patient were of 128 days and US$ 164, respectively. The use of decoction resulted in an average reduction of 37,3% in days of treatment, and of 53,2% in cost of material used. In subgroups with larger tissue commitment (Degree 3 – muscular), were observed the highest diminishment, of 67,9% in average treatment time and 64,9% in average cost.

There was no incremental cost, due to the decoction used being made of native plants that are abundant in Brazil, therefore not representing an increase in cost. There was also no alteration in the protocols for wound treatment used at SELVEN.

It was possible to conclude that the introduction of decoction is clinically and economically advantageous, and with a null or negligible investment. However, wider cost-effectiveness, and preferably, prospective, analysis should be performed to hone results.

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# PS053- Intestinal, vesical and uterine antispasmodic effects of the patagonic plant *Chiliotrichum diffussum* (Asteraceae)

Consolini A.E.[1]*, Matera S. [1], Colareda G.A.[1,2], Bayley M.[1], Alcalde Bahamonde S.M. [3], Flores M.L.[4], Córdoba O.L.[5]
*dinamia@biol.unlp.edu.ar

[1]*PhD, Farmacología, Dpto Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calles 47 y 115, La Plata, Buenos Aires, Argentina.*
[2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.*
[3]*PhD, Farmacología I,* [4]*PhD, Farmacognosia y* [5]*PhD, Química Biológica II; Facultad de Ciencias Naturales y Ciencias de la Salud, GQBMRNP y AAI – CRIDECIT, Universidad Nacional de la Patagonia San Juan Bosco, Km 4, s/N°, 9000, Comodoro Rivadavia, Chubut, Argentina.*

## Introduction

*Chiliotrichum diffussum* (G. Forst.) Kuntze (Asteraceae) grows in the lands of Patagonia, from Argentina and Chile. It is popularly known as "mata negra"; their leaves and flowers were traditionally used by onas community "*to clarify the sight*" and by mapuches people to alleviate uterine and intestinal spasms[1]. Some pharmacological activities have been previously reported, such as antibacterial, antiinflammatory, antioxidant and hypotensor[2]. In the phytochemical profile there were detected chlorogenic acid and flavonoids such as quercetin, hiperoside, isoquercitrin, kaempferol and vitexin. The aim of this work was to evaluate wheter the ethanolic extract of *Chiliotrichum diffussum* leaves have antispasmodic effects in *ex vivo* experiments on isolated thin intestine, bladder and uterus of rat. The mechanism of action evaluated through concentration-response curves was the non-competitive inhibition of both, cholinergic contraction and $Ca^{2+}$ influx[3,4].

## Methods

Aerial parts of the plant were collected in the summer of 2014 in 28 de Noviembre locality (Santa Cruz, Patagonia Argentina) and dried at air. The ethanolic extract was prepared by maceration in ethanol 96°, 48 h, and dried. The day of the experiment the extract was dissolved in ethanol 96° at 3 mg/ml and then dilluted in water to obtain a concentration series (1, 0.3, 0.1, 0.03 and 0.01) mg/ml, which then were added to the organ´s solution in dillution 1/100 saline solution the day of the experiment. After rat euthanasia, the organs were isolated and submerged respectively in organ chambers containing solutions of Tyrode´s (1.8 mM Ca) for intestines, Tyrode´s (2.5 mM Ca) for bladder, and Jalon´s (0.5 mM Ca) for uterus. Several contractile concentration-response curves (CRC) of carbachol (Cbl, cholinergic agonist) were done in each isolated rat tissue, in the absence and the presence of unique and growing concentrations of the ethanolic extract of *Chiliotrichum diffussum* (Chd). Contractility was measured by force transducers and acquisted on a computer. The mechanism of action was evaluated by adding Chd on CRC of calcium ($Ca^{2+}$) done in a depolarizing high [$K^+$] media[3,4].

**Results**

Chd induced a non-competitive inhibition of the Cbl-CRC in intestine, because they reduced the maximal effect of the agonist with $IC_{50}$ of 9.5 ± 4.2 µg/ml (n= 6). In bladder, Chd also non-competitively inhibited the Cbl-CRC with $IC_{50}$ of 51.8 ± 3.5 µg/ml (n= 6). The same behavior had Chd in uterus, with $IC_{25}$ of 49.2 ± 1.6 µg/ml (n= 5). When studied the mechanism of action in isolated thin intestines, Chd non-competitively blocked the $Ca^{2+}$ CRC with $IC_{50}$ of 16.4 ± 5.5 µg/ml (n= 6) in a pattern similar to that of verapamil ($IC_{50}$: 240 ± 40 µg/ml, $pIC_{50}$: 6.28).

**Conclusion**

The ethanolic extract of *Chiliotrichum diffussum* leaves showed antispasmodic effects in *ex vivo* experiments in intestine, bladder and uterus. The extract was about 5 times more potent in intestines than in bladder or uterus. The mechanism was a non-competitive inhibition of contraction and of $Ca^{2+}$ influx to smooth muscle, as well as verapamil showed, but with about 15 times more potency than it in intestine. These activity is related to the phytochemical composition, fundamentally phenolic acid and flavonoids.

**Acknowledgements:** grants from Universidad Nacional de La Plata (UNLP 11X-642) and UNPSJB (PI 1201).

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# PS054- Study of biological activityof *Croton linearis* leaf essential oil on *Trypanosoma cruzi* and *Leishmania sp. in vitro*

Batista D.G.J. [1*], Díaz J.G.[2], Arranz J.C. E. [2], Fidalgo L.M. [3], Acosta J.V. [4], Macedo M.B. [5], Cos P.[5], Soeiro M.N.C. [1]
*denisegama@ioc.fiocruz.br

[1]*Laboratory of Cellular Biology, Oswaldo Cruz Institute/FIOCRUZ, RJ, Brazil.*
[2]*Pharmacy Department, Faculty of Natural and Exact Sciences, University of Oriente, Santiago de Cuba, Cuba.*
[3]*Department of Parasitology, Institute of Tropical Medicine "Pedro Kourí", La Habana, Cuba.*
[4]*Medical Toxicology Centre (TOXIMED), Medical Sciences University, Santiago de Cuba, Cuba.*
[5]*Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Belgium*

## Introduction

Neglected tropical diseases such as Chagas disease and Leishmaniasis that are caused by family protozoa Tripanosomatides: *Trypanosoma cruzi* and *Leishmania spp*, respectivily, affects millions of people in endemic areas of Latin America (Who, 2018). The treatment of these diseases has side effects and limited efficacy. Therefore, it is necessary to study the activity of new natural or synthetic compounds. The *in vitro* antimicrobial activity of essential oils has been well archived against a wide range of microorganisms, in particular, bacteria, fungi and parasites (Doman & Deans, 2000). The objective of the work was to determine the activity of *Croton linearis* leaf essential oil on two different parasites *Leishmania amazonensis* and *Trypanosoma cruzi.*

## Method

Leaves of *C. linearis* were collected from Siboney-Juticí Ecological Reserve, Santiago de Cuba. Fresh leaves from *C. linearis* were subjected to hydrodistillation-cohobation,,described in Diaz e cols., 2018. Biological activityof *Croton linearis* leaf essential oil on *Trypanosoma cruzi* and *Leishmania sp*. *in vitro* were performed as described in Diaz e cols., 2018. In the cytotoxicity tests, we used peritoneal macrophages and L929 cell and we evaluated by MTT and Alamar Blue® for the possible toxicity of essential oil.

## Results / Discussion / Conclusion

Our results showed a remarkable activity against *L. amazonensis* ($EC_{50}$ promastigote: 20.0±4.9 µg/mL; $EC_{50}$ amastigote: 13.8±4.3 µg/mL) and moderate activity against *T. cruzi* (Y strain) showed values of $EC_{50}$ (trypomastigote) = 197.26±8.7 µg/mL. When we treated the L929 cell infected with cepa Tulahuen to evaluate activity on intracellular forms with a single concentration (10 µg/ML), showed a percentage of Inhibition infection 13.32±4.7%. Cytotoxicity values were $CC_{50}$=89.1±3.4 µg/mL for macrophages BALB/c and $CC_{50}$= 306± 110 µg/mL for L929 cells. Regarding the selectivity index (SI), to *L. amazonensis* was determinated using two different cell types: peritoneal macrophages from BALB/c mice and murine fibroblasts L929 cells. Colorimetric methods (MTT) as well as direct counting in optical microscopes were performed and we results

demonstred values os SI= 4 (promastigote) and SI=6 (amastigote). The cytotoxicity studies were performed with cardiac cells (primary culture), that are the target of infection of the parasite, showed values SI=1.55. Finally, the *Croton linearis* Jacq. leaf essential oil, could be a potential candidate for future investigations regarding Leishmaniasis and Chagas diseases treatment.

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# PS055- Gastroprotective activity and toxicological evaluation of the flowers aqueous extract from *Chiliotrichum diffusum* (G. Forst.) Kuntze (Asteraceae)

Bucciarelli A.[1], Alcalde Bahamonde S.M.[2,3], Uhrich A.V.[3], Flores M.L.[4], Córdoba O.L.[5]*
*okylola@gmail.com

[1]*Farmacognosia, Depto. de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina.*
[2]*Farmacología I,* [3]*Farmacología II,* [4]*Farmacognosia and* [5]*Química Biológica II; GQBMRNP y AAI - CRIDECIT, Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco, Km 4, s/N°, 9000, Comodoro Rivadavia, Chubut, Argentina.*

## Introduction

Diseases of the digestive system generate an important impact on community health since these are usually difficult to treat and prevent, being gastric ulcer as a relevant pathology. Medicinal plants produce active compounds for such diseases. Chiliotrichum diffusum is a native medicinal Asteraceae from Patagonia with anti-inflammatory activity, among others[1]. Flowers biosynthesize flavonoids, phenolic acids and terpenes, including kaempferol-3-*O*-glycoside; apigenin-7-*O*-glycoside; quercitrin, ferulic, caffeic and chlorogenic acids, bisabolol, spatulenol, azulene, farmesano[1,2]. Various flavonoids have protective capacity of the intestinal epithelium, preventing lesions by eliminating free radicals[3]; these modulate the activity of enzymes involved in the carbohydrates absorption, the immune system of the gastrointestinal tract, these have a potential activity against colorectal cancer and to model microbiota composition and function[4]. In this context, the objective of the present work was evaluate gastroprotective activity and acute oral toxicity in mice, and antioxidant activity by bioautography, of the of the flowers aqueous extract from C. diffusum.

## Methods

Flowers were collected in the 28 de Noviembre town (Santa Cruz, Argentina). The extraction was carried out from the dried material with distilled water (2 g/40 ml), at boiling for 20 min[5]. Aqueous extract was lyophilized and weighed. The gastroprotective activity was determined against a gastric ulcer model induced by ethanol, omeprazole was reference antiulcer drug and extract doses were 100, 500 and 1000 mg/kg of body weight (0.05% Tween 80 : 0.5% Carboxymethylcellulose in distilled water, 1:1). Groups of 5 female CF1 albino mice (8 week old) weighing between 25 and 30 g were used. In the acute toxicity study, 2 groups of 8 animals each were used and the extract was administered orally in a dose of 2,000 mg/kg of weight/day. Behavioral and functional parameters of the nervous system were evaluated (neuromuscular, sensory and autonomic activity, and locomotor activity)[6]. Presence of antioxidant compounds was analyzed by bioautography on TLC using the free radical 2, 2 diphenyl-1-picrylhydracil (DPPH)[2].

## Results

The yield of the extract was 36% (w/w). The results showed that aqueous extract from C. diffusum has gastroprotective activity against gastric damage induced by ethanol in mice,

reaching gastroprotection percentages of 61, 67 and 75 % (100, 500 and 1000 mg/kg of body weight, respectively), while omeprazole reached an inhibition of 75.9%. The extract administered orally did not show toxicity by acute exposure in mice; the histopathological study did not show alterations in the analyzed animal organs (control and treated animal). Presence of flavonoids and phenolic acids with antioxidant activity was observed by bioautography, mainly kaemferol-3-*O*-glycoside, apigenin-7-*O*-glycoside, chlorogenic acid, ferulic acid and caffeic acid.

## Conclusion

The aqueous extract from C. diffusum showed gastroprotective activity dose dependent against gastric damage induced by ethanol in mice, reaching an inhibition of 75%. The gastroprotective activity is related to the main phenols present in the extract, which also have antioxidant capacity. These results, added to the absence of acute toxicity and to the anti-inflammatory activity previously determined, demonstrate the pharmacological potential of C. diffusum flowers.

## Acknowledgements

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# PS056- Heavy metals levels in *Mytilus galloprovincialis* of south Mediterranean Sea: correlation with the expression of metallothioneins

Cammilleri G.[1]*, Montana G. [2], Lampiasi N. [2], Vazzana M.[3], Lo Dico G.M.[2], Collura R. [1], Pulvirenti A.[4], Buscemi D.M. [1], Graci S.[1], Giangrosso G.[1], Vella A.[1], Macaluso A.[1], Ferrantelli V.[1]
*gaetano.cammilleri86@gmail.com

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", via Gino Marinuzzi 3, Palermo, Italy. C.P. 90129*
[2]*IBIM, Consiglio Nazionale delle Ricerche, via Ugo La Malfa 153, Palermo, Italy, C.P. 90146*
[3]*Dipartimento STEBICEF, Università degli studi di Palermo, Via Archirafi 18, Palermo, Italy, C.P. 90123*
[4]*Dipartimento di Scienze della Vita, Università degli studi di Modena e Reggio Emilia, Via Università 4, Modena, Italy, C.P. 41121*

## Introduction

Different studies confirmed how heavy metals can compromise the health of various organisms, including humans because of their interaction with a wide variety of intracellular molecules carrying out crucial functions. Technological progress has determined an uncontrolled industrial development and in turn, a substantial risk of marine pollution over time. Marine organisms have evolved biochemical adaptations to response to the presence of these pollutants. Among the heavy metals detoxification mechanisms, the most studied is certainly the use of intracellular ligands. The metallothioneins are 60-68 aa polypeptides having low molecular weight, cysteine residues rich (in 20 Cys mammals they are able to bind the equivalent of 7 divalent metals)[1]. The neosynthesis of these compounds represents a specific response of organisms to heavy metal pollution. *Mytilus galloprovincialis* is a mollusc belonging to the Mytilidae family, often used as a sentinel organism to monitor the biological effects associated with the presence of heavy metals. They are sessile and filtering organisms widely distributed in coastal areas, with the ability to accumulate different classes of pollutants. However, currently studies concerning the correlations between the quantities of heavy metals present at sea and the amount of metallothioneins synthesized by these organisms remain inconsistent. The present work aimed at establishing a relationship between the presence of heavy metals in *Mytilus galloprovincialis* of the Sicilian coasts and the expression of metallothioneins.

## Method

The assessment of heavy metals (V, Cr, Mn, As, Cd, Sn, Sb, Pb and Hg) in samples of mussels belonging to different areas of Sicily was carried out through analytical chemistry methods based on ICP-MS; the determination of metallothioneins expression was carried out by a q-PCR with the use of primers that amplify for Mt2 and Mt1.

## Results / Discussion / Conclusion

Low levels of heavy metals were verified in the mussels' samples examined, in contrast to what was reported on other works belonging to the Mediterranean Sea[2,3,4]. The highest values of heavy metals were found in samples from Gela, due to the intense industrial activity of the area. Important levels of Hg were found in samples of mussels from Catania, with an average value of

0.014 ± 0.005 mg/kg, probably due to the volcanic activity of the Etna volcano. Nevertheless, significant differences were found in the heavy metal content only for vanadium and lead ($p < 0.05$). Molecular analysis have verified a basal expression of the Mt1 gene and the absence of over-expression of the Mt2 gene due to the low concentrations of heavy metals found in the same samples. No significant differences in the expression of Mt1 and Mt2 between the mussel samples was even found between the different areas.

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# PS057- Sicilian medicinal plants: quali-quantitative analysis of pesticide and heavy metals residues

Lo Cascio G.[1]*, Cammilleri G.[1], Randisi B.,[1], Pantano L.,[1], Lo Dico G. M.[1], Collura R. [1], Pulvirenti A.[2], Giaccone V. [1], Buscemi D. M. [1], Cicero A. [1], Giangrosso G.[1], Vella A.[1], Macaluso A.[1], Ferrantelli V. [1]
*Giovanni.locascio71@gmail.com

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", via Gino Marinuzzi 3, Palermo, Italy. C.P. 90129*
[2]*Dipartimento di Scienze della Vita, Università degli studi di Modena e Reggio Emilia, Via Università 4, Modena, Italy, C.P. 41121*

## Introduction

Medicinal plants have properties and healthy actions involved in the balance of the physiological functions of the human organism. To this group belong the medicinal plants, aromatic plants and perfume plants, used for the preparation of teas, infusions, decoctions or as extracts in ingredients for the production of food, supplements, cosmetics, medicines and medical devices[1]. Due to its strategic position in the Mediterranean, the Sicilian territory presents a vast patrimony of medicinal plants, cultivated and exploited in various fields. However, a sanitary characterization of this supply chain is necessary in order to target the studies on the territorial plants, analyzing the potential benefits of these plants. The present work aimed at assess the residues of heavy metals and pesticides in different typologies of medicinal plants cultivated in Sicily, in order to have a detailed exposure risk assessment.

## Method

The monitoring was done during 2015 in 7 sites belonging to the Agrigento territory. A total of 45 samples of *Origano vulgare*, 14 of *Salvia officinalis* and 18 of *Rosmarino officinalis* were sampled and analysed for pesticide and heavy metals residues assessment. The pesticide detection was done after extraction by QuEChERS tubes, with GC-MS and HPLC-MS/MS methods. The assessment of As, Cd and Pb was carried out by an ICP-MS method after microwave-assisted digestion.

## Results / Discussion / Conclusion

A consistence presence of antifungal pesticides was found in oregano samples. In fact, molds and fungi can infect officinal herbs, mainly affecting the aesthetics of the product and their purchase. Traces of organochlorine and organophosphorus pesticides banned by EC Reg. 1107/2009 have also been found. The highest pesticides amounts were found in the zone A, comprising Agrigento, Favara and Naro. Our results confirmed that the highest amounts were found in intensive cultivation areas. Pb and As concentrations were found in all the samples examined. Only 10% of the samples showed Cd levels above the LOD of the method. A significant difference on heavy metals levels was found between dry and fresh oregano ($p < 0.05$) probably due to the chemical

properties of the heavy metals examined. However, all the samples examined showed heavy metals concentrations inder the limits imposed by the EC Reg. 1881/2006.

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# PS058- Effect of the hydroalcoholic extract of *Ganoderma lucidum* in rats inoculated with Pristane

Bach E.E.[1]*, Hi E.M.B..[2], Santos A.C.A.[3], Wadt N.S.Y.[4]
*ernabach@gmail.com

[1] *PhD/UNINOVE (Universidade Nove de Julho), Health Department, Biomedical Sciences, SP-Brasil,*
[2] *Ms/UNILUS (Universidade Lusiadas), Experimental Biochemistry Academic Nucleum (NABEX), Santos, SP-Brasil*
*3 Ms/USP (Universidade São Paulo), Toxicological and Clinical Analisys Department, SP-Brasil*
[4] *PhD/UNIP (Universidade Paulista), Health Sciences Institute, SP-Brasil*

## Introduction

*Ganoderma lucidum* (Leyss Ex Fr) Karst is a basidiomycete mushroom used for several years as a food and medicinal supplement, mainly in China and Asia. The fruiting body enjoys great popularity in Asian countries due to its multiple biological activities, among them the immunostimulatory, antitumor, antimicrobial, anti-inflammatory, antihypertensive, antihyperglycaemic, hypocholesterolemic, renal and hepatic protector, antioxidant and antiviral effects[1-3]. Such therapeutic effects are provided due to the presence of polysaccharides, triterpenoids and some proteins as structural components of this fungus[4-6]. The objective of the present study was to evaluate the antitumor and protective activity in Wistar rats inoculated with the carcinogenic agent Pristane (2,6,10,14-tetramethylpentadecane) and to determine markers of amino acids present in the blood.

## Method

The hydroalcoholic extract was made and the concentration of polysaccharides, proteins, phenols and ganodermic acid were evaluated. The animals (Ethics Committee AN 37/2014) were separated into groups as control receiving water (C), control receiving extract(G), inoculated with pristane and treated with extract (G+P), and another group with pristane only (P). After 30 days of treatment, the animals were euthanized and serum concentrations of albumin, total proteins, urea, kreatinine, aminotransferases (ALT and AST) were evaluated; serum was precipitated with ammonium sulfate, followed by dialysis, and HPLC analysis was performed, and in addition histopathological analyzes of the kidneys affected by pristane were made.

## Results / Discussion / Conclusion

The hydroalcoholic extract presented beta-glucan, proteins and phenols. Through biochemical analysis it was possible to observe protection in the kidneys in animals treated with extract and submitted to Pristane when compared to pristane only animals, with confirmation by histological analysis. As for the markers in HPLC, it was possible to identify a peak in Retention Time (Rt) of 1.51min in G, G+P and C samples in descending order of intensity, without identification of which amino acid. Tryptophan appeared in Rt=2.18min in descending order for C, G and G+P. kynurenine marker (Kyn) (degradation of Trp) with Rt=2.09min appeared only in Pristane. Methionine appeared in Rt=3min present in descending order for C, G and G+P.

In conclusion, the results demonstrated that the drug pristane is a carcinogenic agent, besides evidencing that the extract of *Ganoderma lucidum* has protective activity and possible immunomodulatory activity, without producing toxic effect in the animals studied. The mechanism can be explained that in the animal with pristane Trp depletion and Kyn formation occurred in addition to the maintenance of Trp and other unidentified amino acids in the treated animal. Acknowledgments: Financial Support from CNPq 474681 / 2013-0.

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**PS059-**

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**PS060-**

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# REMOVED - DUPLICATE OF PS083

# PS061- 165 pesticides in citrus fruits using LC-MS/MS. A study of the pesticides distribution from the peel to the pulp

Calvaruso E.[1], Cammilleri G.[1], Lo Cascio G. [1]*, Randisi B.[1], Pantano L.[1], Lo Dico G. M.[1], Cusimano M. [1], Collura R. [1], Pulvirenti A.[2], Giaccone V. [1], Buscemi D. M. [1], Cicero A. [1], Giangrosso G.[1], Vella A.[1], Macaluso A.[1], Ferrantelli V. [1]
*giovanni.locascio71@gmail.com

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", via Gino Marinuzzi 3, Palermo, Italy. C.P. 90129*
[2]*Dipartimento di Scienze della Vita, Università degli studi di Modena e Reggio Emilia, Via Università 4, Modena, Italy, C.P. 41121*

## Introduction

Pesticides are part of a large group of chemical compounds that present extremely diverse physico-chemical properties and are widely used in the control or prevention in the food of plant origin crop diseases. Plant protection products (more commonly known as pesticides) are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops[1]. In Europe, European Union Council Directive describes the regulatory framework for which MRLs are set. The MRLs list for a wide variety of commodities and pesticides is updated from time to time and is part of the EU Plant Protection Products Directive (2005/396/EEC) (European Commission, 2005) and (2009/1107/ EEC) (European Commission, 2009), which is the update of the former directive (91/414/EEC) (European Commission, 1991). Typical MRL is in the range of 0.01 and 5 mg/kg, depending on the pesticide. Pesticide-residue analysis has been routinely performed for more years in numerous laboratories around the world because of the need for strict control of these compounds in food. Despite the vast experience and many papers published, further research is still needed, because of the most-restrictive regulations, the continuous appearance of new active ingredients on the market, and the increasing complexity of residue definitions, including more metabolites and pesticide-related compounds.
This work focused on development and validation of an analytical methodology for the simultaneous determination of a wide range of pesticides in the food of plant origin. The foods considered are with: high water content ( tomatoes, melons, lettuce, apricots, cherries, apple, etc..), high acid content and high water content (citrus fruit, small fruit and berries), and high sugar and low water content(honey, dried fruits) using LC- MS/MS.

## Method

A sensitive multi-residue pesticide method was developed for the determination of 165 multi-class pesticides, using an LC–MS/MS system with an electrospray interface, operated in positive ionization mode. The method was applied on one vegetable matrix (tomato) represent high water, high sugar, and high acidic content commodities. Validation of the method has been carried out by the document SANTE/11945/2015.
Moreover, the assessment of pesticides in the layers (peel, albedo, and pulp) of citrus from all over Sicily was carried out.

**Results / Discussion / Conclusion**

An economical and reliable analytical method for the determination of multiclass pesticides using QuEChERS and LC-ESI-MS-MS was developed and validated to analyze 165 selected pesticides in the food of plant origin sample. The coupling of the chromatographic techniques with QqQ MS provided an efficient and reliable method for the multi-classes of pesticide residue analysis in food of plant origin, especially for the pesticide with polarity, thermal
lability or low volatility. LC-ESI-MS-MS showed high sensitivity with detection limits below 5.0 ng L -1 for all compounds. The method presented acceptable trueness, precision, and linearity with an LOQ set at 5 µg/kg. The LOQs were much lower than the MRLs established by the EU legislations. Instrument LOD values varied from 0.13 to 2.32 µg L -1 . Good linearity of the calibration curves was obtained over the range from 2.5 of each compound to 40 µg L -1 , with r 2 > 0.99. Recovery percent of each analyte was calculated as the concentration found divided by the concentration added multiplied by 100. In general, the accuracy of the method (recovery percent) and its precision (standard deviation between replicates) was acceptable. The analysis of the samples showed that the pesticides present in major concentration belong to the class of fungicides. In particular, a major concentration of fungicides is detected in the peel of an orange, lemon, and mandarins. A significant difference of concentration is present in the layers of citrus fruits ($p < 0.05$). Especially, the peel and albedo present the highest concentration of pesticides, while the pulp showed very low concentration. The major pesticides found in the citrus sample belong to the fungicides categories as imazalil and fenhexamide. In particular, Imazalil is a post-harvest fungicide. Indeed, post-harvest treatments, including dipping and treatment with a water-emulsion wax containing a fungicide, are extensively used for preventing moisture loss during storage, shipment, and marketing[2].

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# PS062- Concentrations of essential and non essential elements in edible insects: preliminary results

Ariano A.[1]*, Loponte R.[1], Zaccaroni A[2]., Avallone L. [1], Bovera F. [1], Severino L.[1]
*andrea.ariano@unina.it

[1] *Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via Delpino 1,*
[2]*Department of Veterinary Sciences, University of Bologna, Italy*

## Introduction

In order to satisfy the growing demand of raw materials due to the demographic increase of world population, described by FAO estimates, the researchers have identified the edible insects as a suitable protein source in human food and animal feeding. There are different studies in literature on the chemical-nutritional characteristics and the benefits of their use, but further studies are needed to identify any toxicological risks related to their use. As a consequence, accurate monitoring of essential and non-essential element concentrations, in edible insects is necessary from the point of view of both nutrition and contamination. In light of this, the aims of the present study were to quantify the total concentrations of essential (Cu, Se, Cr, Fe, Mn, Ni) and non-essential (Hg, Cd, Pb and As) elements in two species of edible insects and to assess the health risk related to human and animal consumption of edible insects.

## Method

Samples (n=9) of two species of edible insects (larvae of *Tenebrio molitor* and larvae of *Hermetia illucens*), were purchased from different European companies. For each sample of edible insects, individuals were pooled and homogenized with a blender. Then, they where freeze-dried and stored at -20 °C and kept at the same temperature until further analyses. Each sample was digested in ultrapure 65% $HNO_3$ and $H_2O_2$ in a microwave digestion system. Concentrations of trace elements were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) technique using a Perkin Elmer Optima 2100 DV instrument coupled with a CETAC U5000AT.

## Results / Discussion / Conclusion

Mean concentrations of essential and non-essential elements in edible insects are summarized in Table 1.

Results showed higher concentration of Mn and Fe in *H. illucens* than *T. molitor* ($P < 0.05$) according to other studies on edible insects[2]. Copper amount was lower than those reported in literature[1,2]; on the contrary, Cr levels were approximately comparable to data reported by other authors[1]. Low concentrations of Co, Ni and Se were found in edible insects and were lower than the conventional food. There are no differences in Cr, Cu, Se, Co, and Ni levels between the two species of insects examined. Concentrations of Cd, Pb and Hg were negligible in all analyzed samples. Total As concentrations were higher than those reported in literature[1]. Compared to maximum levels of heavy metals in foodstuffs set by the European Commission Cd, Pb, and Hg levels in insect samples were always lower than maximum values for all types of foods. Overall,

our results indicate that the risk of exposure to heavy metals from consumption of edible insects is relatively low and in compliance with EU regulations.

Although further studies on metals and other pollutants, on a greater number of samples, are needed, these preliminary results support the possibility to consume these insect species with no additional hazards in comparison to more commonly consumed animal products.

**Table 1.** Mean concentration (mg/kg w.w.) of trace elements in edible insects and their standard deviations.

| **Sample** | **As** | **Cr** | **Cd** | **Pb** | **Hg** | **Co** | **Cu** | **Mn** | **Fe** | **Ni** | **Se** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Hermetia illucens* (n.5) | 0.30 ±0.112 | 0.28 ±0.124 | 0.04 ±0.006 | 0.02 ±0.009 | 0.08 ±0.005 | 0.01 ±0.002 | 3.68 ±0.958 | 46.23 ±7.516 | 16.6 ±4.582 | 0.2 ±0.15 | 0.08 ±0.04 |
| *Tenebrio molitor* (n.4) | 0.53 ±0.155 | 0.15 ±0.083 | 0.03 ±0.005 | 0.02 ±0.015 | 0.06 ±0.001 | 0.01 ±0.003 | 7.88 ±4.129 | 2.68 ±0.597 | 6.12 ±1.349 | 0.15 ±0.094 | 0.06 ±0.007 |

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# PS063- Comparison of the effectiveness of modeling massage for localized fat reduction applied with neutral cream or cream with *Citrus aurantium* extract

Wadt M.[1] Wadt N.S.Y.[1], Assis T.R.[2], Maciel L.T.S[2], Marques S.A.[3], Soares V.C.G.[2]*
*vcgsoares@gmail.com

[1]*PhD/Instituto de Ciências da Sáude (ICS), Curso de Farmácia, Av. Armando Giassetti, 577 - Vila Hortolândia - Trevo Itu/Itatiba, Jundiaí -SP, 13214-525*
[2]*PhD/Instituto de Ciências da Sáude (ICS), Curso Estética e Cosmética, Av. Armando Giassetti, 577 - Vila Hortolândia - Trevo Itu/Itatiba, Jundiaí - SP, 13214-525*
[3]*PhD/UNIANCHIETA, Faculdade de Farmácia, Rua Bom Jesus de Pirapora, 100/140, Centro, Jundiaí-SP, 13.207-270, Brasil.*

## Introduction

Man has used massage as a therapeutic resource since prehistoric times. The actions, of the massage in the tissue alteration are: aid in the penetration of products with specific active decrease of the resistance of the skin to the chains and increase of the tissue malleability. Perfection begins with a smooth functioning of the body healthy habits and practices have a tremendous weight in the consequences of bodily and emotional balance. Therefore, for the complete functioning of aesthetics, it is fundamental to integrate sensorial modalities and psychic processes that are in constant interchange with the body[1]. The techniques used in modeling massage can promote increased blood and lymphatic circulation, increased tissue nutrition, removal of catabolites stimulate visceral functions, aid inactive penetration, mobilization and fluidification of fat, and local cutaneous hyperemia[2,3].

## Method

The study was conducted in a sample of 10 individuals, female, with an average age of 25 years. Approved by the Human Ethics Committee: No. 2,464,970. Participants were randomly divided into two groups of 5 individuals. Thus, 5 participants were part of group I (control), submitted to modeling massage with cream without dermatological assets and group II, submitted to modeling massage with cream containing Citrus aurantium extract incorporated.

## Results / Discussion / Conclusion

After performing the massages, there was an average reduction of 1cm of the body measurements of the volunteers, which was superior to that found in the control group, which is satisfactory for the research, since the chosen asset is known for its systemic properties.

| Control | Weight | | Abdomen | | Waist | | Hip | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| 1 | 68,9 | 67,1 | 92,0 | 89,0 | 98,0 | 94,0 | 102,0 | 98,0 |
| 2 | 60,6 | 60,8 | 83,5 | 81,0 | 89,0 | 90,0 | 93,0 | 94,0 |
| 3 | 52,2 | 52,2 | 69,0 | 66,0 | 74,0 | 72,0 | 90,0 | 89,0 |
| 4 | 61,3 | 61,3 | 68,0 | 67,0 | 68,0 | 74,0 | 92,0 | 93,0 |
| 5 | 71,2 | 77,6 | 79,0 | 83,0 | 87,0 | 93,0 | 98,0 | 105,0 |
| Average | 62,8 | 63,8 | 78,3 | 77,2 | 83,2 | 84,6 | 95,0 | 95,8 |
| Stand. Desv. | 6,7 | 8,4 | 9,0 | 9,1 | 10,8 | 9,6 | 4,4 | 5,4 |

| Citrus a. | Weight | | Abdomen | | Waist | | Hip | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| 6 | 58,6 | 59,1 | 81,0 | 81,0 | 87,0 | 84,0 | 91,0 | 94,0 |
| 7 | 55,0 | 55,0 | 71,0 | 70,0 | 71,0 | 71,0 | 92,0 | 87,0 |
| 8 | 65,0 | 65,0 | 70,0 | 68,0 | 72,0 | 71,0 | 94,0 | 92,0 |
| 9 | 70,0 | 68,0 | 70,0 | 65,0 | 70,0 | 64,0 | 100,0 | 99,0 |
| 10 | 67,0 | 65,0 | 71,0 | 68,0 | 72,0 | 70,0 | 101,0 | 100,0 |
| Average | 63,1 | 62,4 | 72,6 | 70,4 | 74,4 | 72,0 | 95,6 | 94,4 |
| Stand. Desv. | 5,5 | 4,7 | 4,2 | 5,5 | 6,3 | 6,5 | 4,1 | 4,8 |

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# PS064- Profile bioactive compounds: guava pulp and microencapsulate guava pulp

Porcu O.M.[1]*, Xavier D.[1]
*ornella@utfpr.edu.br; ornellamporcu@gmail.com

[1]*Departament of Chemistry, Federal University of Technology – Paraná (UTFPR), Campus Medianeira /PR, Brazil. Post Graduation Program of Chemical and Biochemical Process (PPGTP), Federal University of Technology - Paraná (UTFPR), Campus Pato Branco/PR, Via do Conhecimento, Km 1,CEP 85503-390, Pato Branco/PR,Brazil.*

## Introduction

Native to Brazil the guava fruit (*Psidium guajava,* Paluma cultivar) is widely marketed fresh and processed. In the guava fruit we found bioactive compounds[1] (carotenoids, flavonoids, phenolic and vitamin C), which contribute to the reduction of cardiovascular disease risk and increase the stimulation to the immune system. These properties are important for health-promoting foods challenges the food industry. The use of functional ingredients has been an useful alternative for the nutritional and functional enrichment of new food products. In this context, the aim of this study was to develop, characterize and compare the bioactive composition of a guava pulp (G) and its derivative microencapsulated with dextrine (MG).

## Method

Guava pulp (cv. Paluma), was purchased from local food industry, in Santa Catarina state, Brazil. Dextrin was added to the guava pulp in a proportion of 1:1, homogenised in a colloid mill, until the complete dissolution. For guava pulp (G) it was necessary to add water according to the carrier agent concentration. The spray dryer operates concurrently and has a spray nozzle with an orifice of 1.0 mm in diameter. The flow of the drying air was about 0.54 L/h and the temperature ranged from 40-60 °C. The powder (MG) produced were stored in desiccators, containing silica gel. The physicochemical parameters followed the methods described by Analytical Standards Methods of Institute Adolfo Lutz[2]. All analyzes were performed in triplicate.

## Results / Discussion / Conclusion

The results showed that the composition of (G) and (MG) presented scores no similar to each other. A higher bioactive content is evident in (MG), which demonstrated that the lyophilization process did not influence drastically on these nutrients.

| Bioactive Compound Content | Guava Pulp (G) | Microencapsulate Guava Pulp (MG) |
|---|---|---|
| Vitamin C (mg ac. Ascórbico.100g $^{-1}$) | 51.09 $^{a}$ ± 2.52 | 153.62 $^{b}$ ± 2.68 |
| Total carotenoids (µg.g $^{-1}$) | 36.81 $^{a}$ ± 5.22 | 27.56 $^{b}$ ± 1.74 |
| Total flavonoids (mg quercetina.g $^{-1}$) | 0.72 $^{a}$ ± 0.03 | 1.81 $^{b}$ ± 0.04 |
| Total phenolics (mg GAE.g $^{-1}$) | 1.34 $^{a}$ ± 0.05 | 3.29 $^{b}$ ± 0.08 |

The bioactive compound characterization showed good nutritional and functional quality of guava pulp and microencapsulate guava pulp so it can be used as ingredients that add value to a food product.

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# PS065- Phytochemical study of the rainforest cactus *Rhipsalis teres* Gärtner

Kamikawachi R.C.[1]*, Almeida O.J.G.[2], Vilegas W.[2]
*renan_kami@hotmail.com; vilegasw@iq.unesp.br

[1]*Master/Universidade Estadual Paulista, Faculdade de Ciências Farmacêuticas de Araraquara, Rodovia Araraquara Jaú, Km 01 - s/n, Araraquara, São Paulo – Brasil.*
[2]*PhD/Universidade Estadual Paulista, Instituto de Biociências – Campus do Litoral Paulista, Praça Infante Dom Henrique s/nº, São Vicente, São Paulo – Brasil.*

## Introduction

The genus *Rhipsalis* (Cactaceae) stands out from the other cacti by absence of spines, being epiphytes and inhabiting humid forests. This genus has a great focus of bio-diversity in the Atlantic Forest and has a high number of endemism in this biome[1]. Among the cacti of this genus, the species *Rhipsalis teres* calls attention to its popular use as a medicine for coronary diseases and to treat pneumonia[2]. However, there are no studies investigating the chemical composition of this species neither its biological activity. The present study aimed to evaluate the chemical composition of *Rhipsalis teres*.

## Method

The plant material was collected and dried in a circulating air oven, and then the dried material was powdered in a mill. The powder (122g) was defatted with hexane and extracted with 70% ethanol by percolation resulting in the 70% EtOH extract (32g). An aliquot (2g) of the 70% EtOH extract was fractionated by medium performance liquid chromatography (MPLC). The MPLC fractions of interest were purified using a high efficiency liquid chromatograph coupled with a refractive index detector (HPLC-RI). The isolated compounds were characterized by ultraviolet (UV), mass spectrometry and Nuclear Magnetic Resonance (NMR).

## Results / Discussion / Conclusion

Five compounds were elucidated from the EtOH 70% extract of *R. teres*, 2 flavonoids and 3 triterpene saponins derived from the oleanolic acid (trigucosilated, tetraglucosilated and pentaglucosilated)

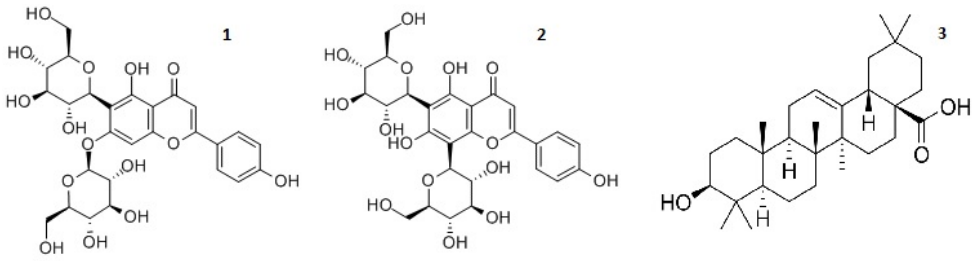


**Figure 1:** Structure o saponarin (1), vicenin 2 (2) and the sapogenin oleanolic acid (3)

The sugar moieties of the saponins as well as the full composition of the extract are under investigation. Based on these results, we can infer that the traditional use of *Rhipsalis teres* as a medicine for heart disease may be linked to the cholesterol-complexing property of saponins while the treatment of pneumonia could result from the anti-inflammatory activity of flavonoids and saponins[5,6].

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# PS066- Transfer of major and trace elements along the "farm-to-fork" chain of different whole grain products

Albergamo A.[1,2]*, Bua G.D.[1,2], Rotondo A.[2], Bartolomeo G.[1,2], Ferrantelli V.[3], Colombo A.[4], Barrega L.[5], Leonardi M.[2], Costa R.[2], Dugo G.[1,2,6]
*aalbergamo@unime.it

[1]*Science4Life S.r.l, a spin-off of the University of Messina, Messina, Italy.*
[2]*Dipartimento di Scienze Biomediche, Odontoiatriche, e delle Immagini Morfologiche e Funzionali (BIOMORF), Università di Messina, Viale Annunziata, 98168 Messina, Italy.*
[3]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via G. Marinuzzi 3, I-90129 Palermo, Italy.*
[4]*Azienda Sanitaria Provinciale (ASP) Messina, Via Giuseppe La Farina, 263, Messina, Italy.*
[5]*Libero Professionist, Messina ( Italy)*
[6]*Co.Ri.Bi.A., Consorzio di Ricerca sul Rischio Biologico in Agricoltura, Palermo, Italy.*

## Introduction

The present work aims to tracking the evolution of the elemental profile in the "farm-to-fork" chain of whole grain (WG) KAMUT® and common buckwheat, considering the respective grains, flours and pasta. A conventional WG durum wheat chain was also investigated for comparative purposes. Specifically durum wheat (cultivar Simeto) from Sicily was considered. A set of 18 major and trace elements was determined in the selected matrices during milling, pasta making, and cooking processes, to assess which elements are more susceptible to the considered steps, as well as which step plays a central role in affecting their levels.

## Methods

An ICP-MS validated method was employed for monitoring major and trace elements along the "farm-to-fork" chains of WG KAMUT®, common buckwheat and durum wheat, considering the different steps of pasta production, and the cooking procedure as well. A PCA analysis identified the elements responsible for sample discrimination, thus providing potential markers of food quality and safety.

## Results / Discussion / Conclusion

Stone milling of grains was responsible for maintaining high mineral contents in the downstream products of all the chains, thus defining their WG nature (mean losses in the range of 1.08–5.52% were tracked). Pasta making affected to a greater extent the elemental profile of the different types of pasta, probably due to bronze extruders and long-time drying processes (mean enrichments between 4.00% and 90.08% were monitored). Pasta cooking induced the most severe elemental enrichments (22–225%) and losses (7.70–84.90%) in the "ready-to-eat-product", as a consequence of complex chemical transformations underlying moisture gain and leaching events. Overall, WG common buckwheat grains, flour and pasta resulted the major source of valuable minerals (*e.g.* Mg, Ca, P, Mn, Fe, Cu and Zn) and the minor source of contaminants (*e.g.* Ni, Cd, and Pb), when compared to the WG durum wheat and KAMUT® counterparts. Nevertheless, the Sicilian durum wheat (cultivar Simeto) chain was marked by a precious content of Se.

# PS067- Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by supervised and unsupervised chemometric methods

Albergamo A.[1,2], Mottese A.F.[1,2], Bua G.D:[1,2], Caridi F.[3], Sabatino G.[4], Barrega L.[1], Costa R.[2], Caratozzolo M.[1], Dugo G.[1,2,5].
*aalbergamo@unime.it

[1]*Science4Life S.r.l, a spin-off of the University of Messina, Messina, Italy.*
[2]*Dipartimento di Scienze Biomediche, Odontoiatriche, e delle Immagini Morfologiche e Funzionali (BIOMORF), Università di Messina, Viale Annunziata, 98168 Messina, Italy.*
[3]*Agenzia Regionale Protezione Ambiente della Calabria (ARPACal), Via Troncovito SNC, 89135, Reggio Calabria, Italy.*
[4]*Dipartimento di Scienze Matematiche e Informatiche, Scienze fisiche e della Terra (MIFT), Università di Messina, Viale F. Stagno d'Alcontres, 31 98166 Messina – Italy*
[5]*Co.Ri.Bi.A., Consorzio di Ricerca sul Rischio Biologico in Agricoltura, Palermo, Italy*

## Introduction

Different multivariate techniques were tested in an attempt to build up a statistical model for predicting the origin of prickly pears (*Opuntia ficus-indica* L., cv. Muscaredda) from several localities within the Sicilian region. Specifically, two areas known for producing fruits marked respectively by TAP (traditional agri-food product) and PDO (protected designation of origin) brands, and three sites producing non-branded fruits, were considered.

## Methods

A validated inductively coupled plasma mass spectrometry (ICP-MS) method allowed to obtain elemental fingerprints of prickly pears, which were subsequently elaborated by unsupervised tools, such as hierarchical clustering analysis (HCA) and principal component analysis (PCA), and supervised techniques, such as canonical discriminant analysis (CDA).

## Results / Discussion / Conclusion

Once again, elemental fingerprints demonstrated to be effective for verifying food provenance[1].
With the exception of HCA, which was not enough powerful to correctly cluster all selected samples, PCA and CDA successfully investigated the effect of subregional provenance on prickly pears, thus, discriminating labeled products from the non-labeled counterpart. Also, CDA revealed a restricted pool of traceability markers (K, Ca, and Mg) useful for building up a model able to correctly classify 100% of fruits on the basis of the production areas. This statistical model, including unsupervised and supervised techniques, may guarantee the provenance of prickly pears protected by quality labels and safeguard producers and consumers.

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# PS068- Sustainable management of forests for atmospheric $CO_2$ depletion

Mottese A.F.[1,2], Vadalà R.[1,2], Albergamo A.[1,2]*, Giacomo Dugo[1,2]
*aalbergamo@unime.it

[1]*Science4Life S.r.l, a spin-off of the University of Messina, Messina, Italy.*
[2]*Dipartimento di Scienze Biomediche, Odontoiatriche, e delle Immagini Morfologiche e Funzionali (BIOMORF), Università di Messina, Viale Annunziata, 98168 Messina, Italy.*

## Introduction

As the concentration of $CO_2$ in the atmosphere has increased by 1-2 ppm per year in the last few decades[1], the topic of forest sector carbon balance in connection with climate changes currently has both great scientific and political importance for ecological sustainability on a global scale. Forests play an important role in the global carbon cycle. Their temporal carbon dynamics are characterized by long periods of gradual build-up of biomass (sink), alternated with short periods of massive biomass loss (source). Forests thus switch between being a source or a sink of carbon, depending on the specific disturbance or management regime, and activities as well.

Current forest management standards are mainly oriented to conservation. Nevertheless, optimization of forest management specifically purposed to carbon storage may further enhance the role of Italian forest sector for atmospheric $CO_2$ depletion

Within this context, the present study examines the actual and potential role of forest management to deplete atmospheric $CO_2$ concentration, with specific reference to the Italian situation as a case study. Additionally, practical issues (*e.g.* amelioration of existing forest stands; adjustment of harvesting yield to the actual production capacity of forest stands; afforestation and tree cropping by means of changes in land use) are addressed within a sustainability framework. It may be concluded that with the right incentives and investments, a significant contribution can be expected from the sustainable management of Italian forests. These forests may not only reduce emissions, enhance sinks, and store carbon, but also provide a continuous stream of ecosystem services, including sustainable wood products, energy and biodiversity conservation.

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# PS069- Evaluation of fatty acids and inorganic elements by chemometrics for the traceability of the Sicilian *Capparis spinosa* L.

Mottese AF [1,2], Albergamo A.[1,2], Bartolomeo[1,2], Bua G.D.[1,2],
Rando R.[1], De Pasquale P.[1,2], Saija E.[1,2], Donato D.[1], Dugo[1,2].
*aalbergamo@unime.it

[1]*Science4Life S.r.l, a spin-off of the University of Messina, Messina, Italy.*
[2]*Dipartimento di Scienze Biomediche, Odontoiatriche, e delle Immagini Morfologiche e Funzionali (BIOMORF), Università di Messina, Viale Annunziata, 98168 Messina, Italy.*

## Introduction

Capers and caper berries (*Capparis spinosa* L.) from different Sicilian localities and marked by Protected Geographic Indication (PGI) and Protected Designation of Origin (PDO), along with corresponding Italian commercial samples (unknown area), were subjected to several analytical and multivariate techniques in an attempt to construct a statistical model for predicting the provenance of *Capparis spinosa* L. within the Sicilian region, and to discriminate Sicilian products from the non Sicilian counterpart.

## Methods

Samples were screened in terms of FA composition by GC-FID and GC-MS, and elemental signature by ICP-MS. Then, principal component analysis (PCA) and canonical discriminant analysis (CDA) were run to test the capacity of both chemical classes of exploring and classifying samples according to the provenance. Validity of PCA/CDA models was checked by a leave-one-out cross validation procedure.

## Results / Discussion / Conclusion

Although FA composition is reasonably employed to differentiate foods by geographical origin[1], in this case study, inorganic elements demonstrated to be more effective for verifying food provenance[2].

Considering elemental fingerprints, the combination of PCA and CDA led to a successful differentiation and classification of selected samples according to the geographical origin. Also, specific variables of classification, namely, Mg, K, and Ca, were revealed by CDA.

Conversely, FA compositions showed a lower differentiating and classification power, as the multivariate statistical approach failed to discriminate samples coming from a specific Sicilian area (Pantelleria, AG) from the unknown Italian area.

Coherently, the leave-one-out cross validation based on elemental data predicted the provenance of 100% samples; whereas, a slightly lower prediction rate (99%) was provided by FA compositions.

Consisting of the elaboration of elemental data by PCA plus CDA, the validated statistical model proposed in this study may be used to authenticate commercial Sicilian capers and caper berries

marked by important quality logos, such as PGI and PDO, and may help to counteract the frequent frauds affecting them[3,4].

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# PS070- Organic contaminant levels and mineral components in honey from Tunisia: preliminary results

Di Bella G.[1]*, Lo Turco V.[1], Potortì A.G.[1], Mobilia M. A[2]. Bua D.[1], Rahmouni R.[3], Saja E.[1], Bartolomeo G.[1], Ben Mansour H.[3], Dugo G.[1]
*gdibella@unime.it

[1] *BioMorf Department, University of Messina, Messina, Italy.*
[2]*Freelance Medical Doctor, Messina, Italy*
[3] *Research Unit of Analysis and Process Applied to Environmental –APAE UR17ES32 Higher Institute of Applied Sciences and Technology Mahdia, University of Monastir, Monastir, Tunisia.*

## Introduction

Many honey uses are related to their therapeutic properties. The health benefits of honey are about digestive, respiratory and circulatory apparatus. It also shows antibacterial, antioxidant, antitumor, antimutagenic and anti-inflammatory properties[1-2]. Unfortunately, several forms of contamination could affect this food. Some studies highlighted the presence in honey samples of organic residues[3-4], whereas other researches are focused on the mineral content[5-6]. Besides which, the literature is poor in contamination of honey from Tunisia.
Because the possibility to find in honey various types of contaminants could decrease its quality, this research is carried out in order to describe the Tunisian honey status related to levels of 13 Polycyclic Aromatic Hydrocarbons (PAH), 26 Organphosphorus pesticides (OPP), 21 Organochlorine pesticides (OCP), 24 Fungicides (F), 12 Herbicides (H), 11 Carbamates (CAR), 9 Pyrethroids (PYR), 2 Insect Growth Regulators (IGR) and 1 Synergist (SYN), and related to its content of 14 mineral elements (Na, Mg, Ca, K, Zn, Fe, Mn, Cr, As, Cu, Cd, Ni, Se and Pb).

## Method

Ten honey samples of different botanical origin (wildflower, eucalyptus, prickly pears, lemon-blossom, thyme, almond, rosemary and jujube) collected in 2017-2018 years from Tunisia were analyzed in this work. The potential content of 119 organic contaminants was simultaneously evaluated using a Thermo Scientific Trace GC Ultra coupled with a triple quadrupole mass spectrometer TSQ Quantum XLS. The mineral profile was attained by Agilent 7500cx (Agilent Technologies, Santa Clara, CA) ICP-MS spectrometer.

## Results / Discussion / Conclusion

PAH, OPP, CAR, IGR and SYN residues were lower than their LOQ in all analyzed samples. Among the others classes were determined merely 1 OCP (60% of samples were contaminated by Alachlor), 1 F (50% of samples were contaminated by Metalaxyl-M), 1 H (40% of samples were contaminated by Cyromazine) and 5 PYR (10% of samples were contaminated by Cyhalothrin, Cypermethrin isomer III, Cypermethrin isomer I, Cypermethrin isomer II and Deltamethrin). The almond honey was found free of all evaluated organic contaminants, whereas lemon-blossom honey showed residues of the aforesaid 5 PYR and 1H. Sadly, each determined residue is exceed

the maximum residue limits (MRL) fixed by Regulation (EC) n. 396/2005 and subsequent amendments (Regulations n. 149/2008, n. 459/2010, n. 520/2011 and n. 899/2012).
As regard mineral components, the obtained results showed that generally the mineral content were in line with data reported in samples from various European areas: the most abundant minerals were K, Ca, Na and Mg; Pb, As and Cd contents were very low, and were below their quantification limits in 30%, 60% and 60% of samples, respectively. So, the intake of minerals by assumption of 2g/day of honey excluded any toxicological risk.

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# PS071- Physico-chemical parameters and mineral content of honey samples from Sicilian black honeybee (Apis mellifera ssp. *sicula*)

Rando R.[1], Di Bella G.[1]*, Bua G. D.[1], Bartolomeo G.[1], Saija E.[1], Cicero N.[1], Colombo A.[2], Caratozzolo M. Dugo G.[1]
*gdibella@unime.it

[1]*BioMorf Department, University of Messina, Messina, Italy.*
[2] *ASP, Messina, Italy*

## Introduction

The Sicilian black honeybee (*Apis mellifera* ssp. *sicula*), an African subspecies of *Apis mellifera*, had the benefit of adapting in hot and hard lands including the regions of the Mediterranean area like Sicily (Italy). It differs from the more common *Apis mellifera* ssp. *ligustica* for both morphologic and physiologic characteristics, such as color and wing dimensions, better resistance to high temperature, higher ability of impollination, and higher physical and immunological resistance[1]. Recently the polyphenolic contents, antioxidant and antimicrobial properties of some Sicilian honeys produced by *Apis mellifera* ssp. *sicula* have been reported[2,3]. The aim of this preliminary study was to investigated some physico-chemical parameters and mineral content of Sicilian honey samples produced by the local black honeybee. Furthermore, Recommended Dietary Allowance (RDA) values and benchmark levels were employed to assess the honey quality and safety.

## Method

*A. mellifera* ssp. *sicula* honey samples of different botanical origin (orange, summer wildflower, chestnut, asphodel, honeydew) produced in Sant'Agata di Militello (Messina, Sicily, Italy) in 2017-2018 were analyzed. The evaluation of some physico-chemical parameters (free, combined and total acidity, pH value, electrical conductivity, moisture content) was carried out according to the methods reported by Official Journal of the Italian Republic (2003/185 of 11 August 2003). The concentrations of K, Ca, Mg, Na, Zn, Fe, Mn, Cu, Cr, Ni, Se, Pb, Cd and As were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS); each sample was previously mineralized using a closed-vessel microwave digestion system[4].

## Results / Discussion / Conclusion

The values of investigated physico-chemical parameters were within the limits established by Italian Legislative Decree 2004/179 of 21 May 2004 for honey trading and were in line with the different botanical typology of analyzed samples.

Regarding the mineral content, K, Ca, Mg and Na were the most abundant minerals, followed by Fe and Zn, which ranged between 1.26–6.56 and 0.84–2.68 mg $kg^{-1}$, respectively. Cu and Mn spanned in the same range. Se was found only in chestnut and asphodel honeys with a mean value of 0.013 and 0.016 mg $kg^{-1}$ respectively. Pb, As, Cd, Cr and Ni showed concentrations similar to those found in other types of honey. Maximum residue levels of toxic elements in honey have not

been established, except for Pb: the analyzed honeys were below the maximum level of 0.10 mg $kg^{-1}$, established by Commission Regulation (EU) 2015/1005 of 25 June 2015.
It can be concluded that consumption of 2 g/day of honey from Sicilian black honeybee can guarantee a small intake of mineral elements, but in line with an aliment such honey, and exclude any toxicological risk.

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# PS072- Organic pollutants in Italian and Tunisian herbs and spices

Di Bella G.[1]*, Potortì A.G.[1], Lo Turco V. [1], Naccari C.[1], Ben Tekaia A.[2] , Sajia E. [1], Bartolomeo G. [1], Ben Mansour H. [2], Beltifa A., Dugo G. [1]
*gdibella@unime.it

[1] *BioMorf Department, University of Messina, Messina, Italy.*
[2] *Research Unit of Analysis and Process Applied to Environmental –APAE UR17ES32 Higher Institute of Applied Sciences and Technology Mahdia, University of Monastir, Monastir, Tunisia.*

## Introduction

Herbs and spices of various types have been used, since the ancient times, as additives in culinary, medicinal, cosmetic and other compositions[1] and actually they play an important rule in the economy of the producing, importing and exporting countries[2]. Similar to other agricultural products, spices and herbs may be subjected to chemical contaminations due to agricultural practices, soil treatment, cultivation in contaminated soil and environmental contamination. The aim of this work was to analyze simultaneously 140 organic contaminants belonging to different classes, as polychlorobiphenyls, polycyclic aromatic hydrocarbons, organochlorine pesticides, organophosphorous pesticides, pyrethroid insecticides, fungicides, herbicides, synergists, carbamates, acaricides and insect growth regulators, by GC-MS/MS in 118 Italian and Tunisian culinary herbs and spices. Further, the observed levels of contaminants found in all samples were compared to the maximum residue limits (MRLs) setted by the EU Regulations.

## Method

A simplified method without cleanup step for extracting of pesticides[3] has been adapted in spices and herbs purchased from local markets of Tunisia's eastern Mediterranean coast and of Sicily (Italy). They were locally produced in these areas and were commercially available dried, not packed and cut into small pieces. Analyses were carried out using a Thermo Scientific Trace GC Ultra coupled with a triple quadrupole mass spectrometer TSQ Quantum. In order to evaluate the procedure for the analytical protocol, method was validating according to the European Union Guidelines[4].

## Results / Discussion / Conclusion

Among Tunisian spices, only the samples of origan were determined to be free of pesticides residues, conversely all coriander samples are contaminated. Among the herbs, samples of rosemary contain a notably high variety and number of pollutants, while among the samples of mint only one is contaminated. According to the EU legislation only the Tunisian rosemary samples overtake the MRLs: fenthion sulfone, ethion and methabenzthiazuron residues exceed the limits reported by the Regulation (EU) 310/2011; alachlor and phosalone levels exceed the limits of the Regulation (EU) 899/2012 and quinalphos of Regulation (EU) 868/2015. The results obtained by the Italian samples suggest a very different situation. In fact, in the spice samples no residues were found. Among the herbs, in the samples of rosemary and oregano some occasional residues can be observed. Only in the laurel samples there are some contaminants, but always

lower than the MRLs set by the subsequent regulations. Considering that In Tunisia, important areas are covered by rosemary and 90% of spices exported are destined for the European and the rest mainly to the US market, it would be desirable for non-EU countries to comply with European regulations.

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# PS073- Tracking of morphological and production parameters of olive tree (*Olea europaea* L. cv. Chemlali) irrigated with treated dairy wastewater

Sdiri W.[1, 2]*, Ben Mansour H.[1], Di Bella G.[3]
*wiemsdiri@yahoo.com

[1] *Research Unit of Analysis and Process Applied to Environmental –APAE UR17ES32 Higher Institute of Applied Sciences and Technology Mahdia - University of Monastir, Tunisia.*
[2]*Faculté des Sciences Mathématiques, Physiques et Naturelles Université Tunis El Manar, Tunisia.*
[3] *BioMorf Department, University of Messina, Messina, Italy.*

## Introduction

In arid and semi-arid environments, the reuse of treated wastewater has been widely practiced as an alternative water source for irrigation to supplement limited fresh water resources[1]. This study intended to reuse of treated dairy wastewater in agriculture to reward water scarcity in Tunisia.

## Method

The incidence of treated wastewater, which was collected from dairy industry located in Mahdia-city in Center of Tunisia, on growth and production characteristics of young 'Chemlali' olive trees, was investigated. Two irrigation systems such as surface drip irrigation and manual irrigation were applied. Experiments were carried out during 12 months and different parameters were followed monthly[2]. The parameters were: trunk diameter (cm), plant height (cm), shoots number/plant, nodes number/trunk, internodes length (cm), leaf area ($cm^2$), aerial part fresh weight (g), aerial part dry weight (g), roots fresh weight (g), roots dry weight (g), roots length (cm) and fruits number/plant.

## Results / Discussion / Conclusion

Results indicated that trunk diameter, nodes number and roots length show similar rates whatever the irrigation conditions. However, the leaf area and biomass parameters were not affected significantly by irrigation system using treated wastewater. In term of fruits number, only manual irrigation leads to fructification independently of water type. It has been inferred in this work that, manual irrigation system can be adopted using treated wastewater for yield obtainment in case of olive oil production.

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# PS074- Carotenoids from South Italy Sea-lake sponges: isolation, diversity and discovery of a new pigment

Salvo A.*, Giuffrida D., Rotondo A., La Torre G.L., De Pasquale P., Vadalà R., Mangano V., Saitta M. Dugo G.
*asalvo@unime.it

*University of Messina, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, 98168 Messina (Italy)*

## Introduction

Marine sponges, one of the oldest multicellular animals[1], are typically benthic and symbiotic animals living on the seabed that are usually associated to some algae and bacteia[2]. Their body shape, size and colour are extremely variable, with colours also bright (yellow, blue, purple, orange, red ...) and measures ranging from a few millimetres to even a couple of meters in length. Over the past decades, study on the chemistry, distribution and analysis of the most relevant pigments of marine origin focused that the marine sponge colour are due not only to symbiotic algae but also to peculiar aryl carotenoids like isorenieratene, renieratene and renierapurpurin[3]. The chance to identify novel interesting carotenoid molecules urged us to drive a complete pigment analysis of two sponge's species *Raspaciona aculeata* and *Dictyonella marsilii*, living in Ganzirri Lake (Messina), which have not been previously analysed.

## Method

Sponge samples were grinded, homogenized and later cooled at −80°C overnight. Afterword samples were poured into glass beakers and lyophilized until the total water loss. To the lyophilized sample, *n*-hexane was added and undergone to five cycles lasting ten minutes each at the controlled temperature of 25°C. The resulting mixture was centrifuged; the surnatant was recovered and dried. The dry matter was dissolved in ethyl acetate to run the same procedure described for the *n*-hexane again. After the second evaporation, samples were dissolved into methanol and methyl-terz-butyl-ether (MeOH/MTBE 1:1) used as the mobile phase. The carotenoids analyses were conducted by HPLC-MS on a reversed phase $C_{30}$ column. Pigments in the sample were identified by comparison with available standards, elution order, UV/vis spectra, APCI-MS spectra, recorded both in positive and negative ionization modes, and where available, by literature information.

## Results / Discussion / Conclusion

The HPLC-PDA chromatogram highlighted the presence of five principal peaks and the simultaneous coupling with the mass spectrometer allowed the easy identification of four pigments: α-carotene, β-carotene, renieratene and alloxanthine. The identified compounds were also confirmed by comparing the spectral data of authentic standards, when available, or by literature records. Renieratene, α-carotene and β-carotene were readily characterized in both sponge samples. The obtained data show that the two examined sponges have shared some

common characteristic since the concentration of a-carotene and β-carotene resulted comparable. Although renieratene was found in the two species of sea sponges, however it was prevalent in *R. aculeata* while it resulted the compound detected in lower amount in *D. marsilii*.
Unlike these carotenoids, the first main peak in the chromatogram of *D. marsilii* was not detected in *R. aculeata* samples; this peak was identified as alloxanthine. On the other hand, the first main peak of the *R. aculeata* chromatogram is an unidentified compound which certainly is not present in *D. marsilii*. Albeit it is not possible the specific quantification (as it is an unknown species), in *R. aculeata*, this compound is clearly very well represented within the pigments of this sponge and shows an UV/vis profile that is consistent with a carotenoid. More specific spectroscopic determination coupled with the MS and UV/vis data will allow us to unambiguously attribute the chemical structure of this unidentified compound.

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# PS075- Ultrasound assisted dispersive liquid-liquid microextraction for fast and accurate analysis of chloramphenicol in honey

Campone L.[1,2]*, Celano R.[1], Piccinelli A.L.[1], Pagano I.[1], Cicero N.[3], Di Sanzo R.[2], Carabbetta S.[2], Russo M.[2], Rastrelli L.[1].

[1] *Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 84084 Fisciano (SA), Italy.*
[2] *Department of Agriculture, Food Chemistry, Safety and Sensoromic Laboratory (FoCuSS Lab) University Mediterranea of Reggio Calabria, Via Melissari, 89124 Reggio Calabria (RC) Italy.*
[3] *Department of Biomorf, Pan Lab Università di Messina, Via Messina Italy.*

Honey is a food produced from honey bee widely used for the sweetening power and for its biological properties. In order to prevent the infection of the hive, different xenobiotics (antibiotics, pesticide) were frequently employed. One of this substances is the chloramphenicol, that given its chemical stability could often found in food. Chloramphenicol have several side effects in humans after their ingestion and for this reason its intake must be avoid. The aim of this study, was developed an ultrasound-assisted dispersive liquid-liquid microextraction method coupled with UHPLC MS/MS determination, for fast and accurate analysis of chloramphenicol in honey. The parameters affecting on extraction efficiency were carefully optimized using an experimental design in order to maximized the recovery reducing matrix effects. After the optimization the method was validated and successfully applied to 66 honey samples.

# PS076- Aislamiento biodirigido de las hojas de *Mimosa caesapiniifolia*

Silva M.J.D.[1]*, Simonet A.M.[2], Vilegas W.[1], Macías F.A.[2]
*marcelo_farma05@yahoo.com.br

[1] *Unesp - Univ. Estadual Paulista, Biosciences Institute, Coastal Campus of São Vicente, Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, São Paulo, Brazil.*
[2] *Facultad de Ciencias, Universidad de Cádiz, Grupo de Alelopatía, Departamento de Química Orgánica, Instituto de Biomoléculas (INBIO). C/República Saharaui, 9, 11510-Puerto Real (Cádiz), Spain.*

## Introducción

Las especies de la familia Mimosaceae se encuentran distribuidas por todo el mundo, y en gran abundancia en la región Neotropical en el Centro de Brasil, considerado uno de los mayores núcleos de diversidad[1]. Dentro de esta familia, *Mimosa caesalpiniifolia,* es una especie muy agresiva que predomina en su entorno, abriendo así la posibilidad de que se deba a efectos alelopáticos. Existen estudios previos del potencial alelopático del extracto acuoso de dicha especie[2]. El objetivo del trabajo es el aislamiento biodirigido, utilizando el bioensayo de coleoptilos etiolados de trigo[3] de los componentes activos del extracto acuoso de las hojas de *M. caesalpiniifolia.*

## Método

Se extrayeron hojas *M. caesalpiniifolia* procedentes del estado de Minas Gerais (Brasil) con agua mediante lixiviación y realizó la extracción líquido-líquido con distintos disolventes orgánicos (diclorometano, acetato de etilo, *n*-butanol). Se realizó el aislamiento biodirigido de los extractos que mostraron actividad, diclorometano y acetato de etilo, utilizando técnicas cromatográficas (cromatografía de gel filtración con Sephadex LH-20 y CC y TLC en fase normal y reversa). La estructura de los compuestos aislados fue elucidada mediante técnicas de Resonancia Magnética Nuclear (RMN) de protón y carbono (tanto monodimensional como bidimensional) y espectrometría de masas.

## Discusión de resultados

Los extractos de diclorometano y acetato de etilo provenientes de la extracción líquido-líquido del extracto acuoso de las hojas de *M. caesalpiniifolia* presentaron una inhibición significativa en el crecimiento de los coleoptilos etiolados de trigo. El aislamiento de dichos extracto, nos ofrece como productos mayoritarios, el pinitol, el blumenol A y la epicatequina. De estos compuestos, el blumenol A ha mostrado previamente una clara actividad en el bioensayo de coleoptilos etiolados de trigo[4].

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# PS077- Mycotoxins in spices and culinary herbs from Italy and Tunisia

Lo Turco V.[1*], Tropea A.[1], Potortì A.G.[1], Pellizzeri V.[1], Rando R.[1], Bua G.D.[1], Barrega L.[2], Dugo G.[1]
*vloturco@unime.it

[1]*BioMorf Department, University of Messina, Messina, Italy.*
[2]*Science4life srl, spin off company, University of Messina*

## Introduction

Spices and herbs are widely used for improving aroma and taste, and for seasoning, colouring and flavouring food or beverages[1]. Because of their preharvest, postharvest, and storage conditions, they can be contaminated with mycotoxins, natural secondary chemical metabolites produced by different toxigenic fungi strains such as *Aspergillus* and *Fusarium*[2-3]. They are a significant threat to both human and animal health because they have carcinogenic, mutagenic, teratogenic and immunosuppressive activity[4]. The purpose of this study was to evaluate the contamination level of mycotoxins in common herbs and spices and to highlight their risk assessment.

## Method

The study was carried out on 112 samples of 7 culinary herbs, fennel (*Foeniculum vulgare* L.), laurel (*Laurus nobilis* L.), mint (*Mentha piperita* L.), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and verbena (*Verbena officinalis* L.) and 2 spices, caraway (*Carum carvi* L.), coriander (*Coriandrum sativum* L.). Samples were purchased in 2017 from local markets of Tunisia's eastern Mediterranean coast and of Sicily (Italy). Samples were analyzed for G1, G2, B1, B2 aflatoxins, T-2 toxin, HT-2 toxin and B1 fumosine using uHPLC-MS/MS Shimadzu TQ8040 equipped with a C18 column (2.2 µm, 50 mm x 2.1 mm) operated in positive electrospray ionization mode (ESI).

## Results / Discussion / Conclusion

7% of analyzed samples were contaminated by AFB2 (67% of Tunisian rosemary and 80% of Italian rosemary samples), 2% by AFG1 (22% of Tunisian laurel samples), 12% by AFG2 (83% of Tunisian rosemary, 44% of Italian laurel and 80% of Italian rosemary samples), 89% by T2 (60% of Tunisian coriander, 80% of Tunisian verbena and 43% of Italian mint samples), 1% by HT2 (20% of Tunisian verbena samples) and none of the samples contained AFB1 and FB1. Thus, mycotoxins were occasionally detected in these spices and culinary herbs under analysis, despite both Tunisian and Italian rosemary showed a more important contamination. Therefore mycotoxin contamination of these products may occur both in the field and/or during storage.

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# PS078- *In vivo* anthelmintic activity of gelatinous capsules containing dry ethanolic extract of *Tagetes patula* (Asteraceae) against multiresistant isolate of *Haemonchus contortus*

Politi F.A.S.[1]*, Fantatto R.R.[2], Rodero C.[2], Chorilli M.[2], Pietro R.C.L.R.[2], Chagas A.C.S.[3], Furlan M.[1]
*flaviopoliti@hotmail.com

[1]*Instituto de Química, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Rua Professor Francisco Degni 55, Bairro Quitandinha, CEP 14800-060, Araraquara (SP), Brasil.*
[2]*Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Rodovia Araraquara-Jaú Km 01, Bairro Campos Ville, CEP 14800-903, Araraquara (SP), Brasil.*
[3]*Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Pecuária Sudeste, Laboratório de Sanidade Animal, Rodovia Washinton Luiz Km 234, Fazenda Canchim, CEP 13560-970, São Carlos (SP), Brasil.*

## Introduction

The sheep farming has suffered great economic losses due to parasitism by gastrointestinal nematodes. Among the parasites, *Haemonchus contortus* is very important due to its distribution and high prevalence in flocks in tropical countries. These helminths cause in infected animals from acute hemorrhagic anemia to the formation of submandibular edemas, damage to gastric functions and hypoproteinemia[1]. The main form of control is through the use of synthetic anthelmintics, but its indiscriminate use has resulted in the selection of resistant parasites. The aim of this work was to evaluate the potential to control *H. contortus* by oral administration of gelatinous capsules containing ethanolic extract of *Tagetes patula*, a plant species widely referenced in the literature for its biocidal properties.

## Method

For the *in vivo* test, performed twice at different times, Morada Nova sheep naturally infected with *H. contortus* (85%), *Oesophagostomum sp*. (8%) and *Trichostrongylus sp.* (7%) were used, distributed among three test groups with 5 animals each: *a)* capsules ($Tp_{EtOH}$); *b)* positive control (closantel 10 $mg.Kg^{-1}$ or monepantel 2.5 $mg.Kg^{-1}$); *c)* negative control (no treatment). The administered dose of $Tp_{EtOH}$ (12.8 $mg.mL^{-1}$) was determined from the $LC_{50}$ value obtained in the egg hatchability test (EHT) previously performed, and preliminary acute oral toxicity and cytotoxicity tests ensured that the sample did not present a risk of intoxication to the animals. After administration of the products, periodic egg counts per gram of faeces (EPG)[2] were made twice a week over 4 weeks.

## Results/Discussion/Conclusion

At the end of 14 days, the most evident action was that of the monepantel, which reduced the parasitic load to 6.21% of initial. Comparatively, diantel reduced to 34.97% of initial. The capsules was not effective. After administration on day 0, in both repetitions, there was a substantial increase in the egg count at the initial EPGs. It is suggested that the opening of the capsules in the digestive compartment of the sheep constitutes an action that unleash a defense

response of the parasites, which precipitate the oviposition in an accelerated way, in order to guarantee the survival of the species in the host. In polygastric animals, oral treatments must pass intact through the rumen, however, the microbiota present in the gastrointestinal tract may accelerate the degradation of the capsules, causing its opening in compartments prior to the abomasum, justifying the low effectiveness of the product. The animals of the control group maintained a constant load of eggs throughout the experiments. The presented results are preliminary findings and pharmacokinetic studies as well as the possibility of administering more than one dose per animal are parameters that should be investigated in further work.

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# PS079- PAHs concentrations in wart crab (*Eriphia verrucosa*) from the coastal areas of Campania region, Italy

Ariano A.[1]*, Serpe F.P.[2], Sanso N.[1], Velotto S.[1], Severino L.[1], Esposito M.[2]
*andrea.ariano@unina.it

[1] *Department of Veterinary Medicine and Animal Production, Division of Toxicology, University of Naples Federico II, Via Delpino 1, 80137 Napoli, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy*

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants of the marine environment resulting from petroleum spills, marine transport, and discharge of industrial sewage. Several of them have also mutagenic and carcinogenic effects both in human and animals. Due to hydrofobic nature, PAHs tend to accumulates in acquatic organisms, sediments and therefore enter in the food chain. The UE Regulation 1881/2006 establishes the maximun levels of these contaminants in certain food taking into consideration the known toxicity of 4 PAHs (benzo[a]pyrene, benzo[a]antharacene, benzo[b]fluoranthene and chrysene). Within fish products, maximum levels were fixed only for mollusks and smoked food. The warty crab (*Eriphia verrucosa)* is a bentonic species showing a preferential uptake of pollutants from sediments. The aim of this study was to evaluate the content of six PAHs in edible muscle of warty crab from various coastal areas of Campania region, including also two PAHs not included in Reg. 1881/2006 such as benzo[k]fluorantene (BkF) and dibenzo[a,h]anthracene (DahA), having similar toxicity (IARC, 2015).

## Method

Male samples (n=32) of warty crab (*Eriphia verrucosa*) were caught from various locations along the northern coast of the Campania Region between May and July 2014. After capture, the crabs were stored in polyethylene bags, frozen at -<20°C and kept at the same temperature until delivery to the laboratory. The mean weights and carapace lengths and widths were 93.8±27.6 g, 4.61±0.81 cm, and 6.03±0.80 cm, respectively. In each sample, the muscle from claws and appendages were separated and homogenized. Aliquots of samples (2.0 g) were saponified with 10 mL of a ethanolic solution of potassium hydroxide in a water bath at 80°C for 2 h and the extraction was performed with cyclohexane. The extract was cleaned up through a silica gel column to remove lipids and interferences compounds. The PAHs were determined using an HPLC system (Alliance e2695, Waters) equipped with a fluorescence detector (FLD 2475, Waters).

## Results and Discussion

PAH concentrations in muscle of *Eriphia verrucosa* are summarized in Table 1. Results were approximately comparable to data reported by literature for other species of crab[1,2]. The results also showed that BkF and DahA (classified by the IARC as possible and probable carcinogenic to humans, respectively) were detected at levels comparable to PAHs included in Regulation 1881/2006. Compared to maximum levels of PAHs in smoked foodstuff set by the European

Commission, PAH concentration in warty crab were always lower than maximum values for all types of foods. Therefore such contaminants should be monitored in aquatic ecosystems and crabs, such as bivalves, could be good indicators of environmental pollution.

| PAHs | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| **BaA** | 0,31 | 0,16 | 0,38 | 0,101 | 0,47 |
| **Cry** | 0,90 | 0,40 | 0,90 | 0,13 | 1,83 |
| **BbF** | 1,56 | 0,85 | 1,52 | 0,22 | 3,35 |
| **BkF** | 0,21 | 0,17 | 0,20 | 0,003 | 0,43 |
| **BaP** | 0,45 | 0,11 | 0,46 | 0,111 | 0,54 |
| **DahA** | 0,23 | 0,18 | 0,23 | 0,031 | 0,41 |
| **Total PAHs** | 1,60 | 1,53 | 1,52 | 0 | 4,89 |

**Table 1.** Mean concentration (µg/kg w.w.) of PAHs in muscle of *Eriphia verrucosa* (n=32) with associated standard deviations, median, minimum and maximum.

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# PS080- Antiproliferative activity and induction of apoptosis in human melanoma cells by *Drymis winteri* Forst extract and its active components

Pereira-Torres D.[1], Graziano A.[2], Gonçalves A.T.[3], Ulloa V.[1], Martínez R.[4], Carrasco H.[5], Olea A.F.[5], Espinoza L.[6], Gallardo-Escarate C.[3], Astuya A.[1], Avola R.[2], Cardile V.[2], Russo A.[7]*
*alrusso@unict.it

[1]*Laboratory of Cell Culture and Marine Genomics, Department of Oceanography and COPAS Sur-Austral, University of Concepcion, Casilla 160-C, Concepcion, Chile;*
[2]*Department of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Via S. Sofia, 89, 95123 Catania, Italy;*
[3]*Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepcion, Casilla 160-C, Concepcion, Chile;*
[4]*Departamento de Química, Facultad de Ciencias Exactas, Universidad Andres Bello, Quillota 910, Vina del Mar, Chile;*
[5]*Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autonoma de Chile, Llano Subercaseaux 2801, San Miguel, Santiago, Chile;*
[6]*Departamento de Química, Universidad Tecnica Federico Santa María, Av. Espana 1680, Valparaíso, Chile;*
[7]*Department of Drug Sciences, University of Catania, Via S. Sofia 64, 95125 Catania, Italy*

## Introduction

Melanoma is a highly invasive cancer that resists most conventional treatments. Therefore, there is an urgent need to identify alternative anticancer agents able to affect new molecular targets[1]. *Drimys winteri* (Winteraceae) is a medicinal plant, employed in Brazil and many countries, in folk medicine against a variety of ailments, especially for the treatment of fevers, ulcers, pains, affections of respiratory tract and cancers[2]. Previous phytochemical studies have isolated and identified the presence of diverse classes of secondary metabolites in this plant such as flavonoids, sesquiterpenoids terpenoids[2]. In an ongoing to identify new natural anticancer compounds for the treatment and/or prevention of melanoma, we study the effects of *Drimys winteri* bark ethyl acetate extract and its components polygodial, drimenol, nordrimenone, isonordrimenone on two melanoma cells A2058 and A375.

## Method

*D. winteri* bark collection, extract preparation and compounds purification were performed as described previously[2]. The *D. winteri* bark used in this study originated from the Valdivia and Malleco regions of southern Chile at 100 and 600 m altitude respectively and were collected during summer season. Briefly, *D. winteri* bark was air dried and macerated in ethyl acetate and the first organic extract obtained during the process was fractioned under reduced pressure (0.015 mm Hg). The obtained fractions were analyzed by thin-layer chromatography, and polygodial, drimenol, nordrimenone, isonordrimenone were identified. Pure metabolites were obtained through column chromatography and characterized by nuclear magnetic resonance (1H NMR and 13C NMR). The cell viability was measured by 3(4,5-dimethyl-thiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) test and lactate dehydrogenase (LDH) release was used to quantify necrosis cell death[3]. Genomic DNA, caspase-3 activity, expression of cleaved caspase-9, B-cell

lymphoma 2 (Bcl-2) and Bcl-2 associated X (Bax), cleaved caspase-9 and Hsp70 proteins were evaluated in order to study the apoptotic process[3]. Generation of reactive oxygen species (ROS) was measured by using a fluorescent probe[3].

**Results / Discussion / Conclusion**

In A375 and A2058 cells extract, polygodial, drimenol and isordrimenone showed a clear dose-dependent response. In A375 cells polygodial showed the highest inhibitory growth activity. In addition, we demonstrated an apoptotic response after treatment of cancer cells with polygodial, drimenol and isordrimenone at 6.25-25 μM concentrations that probably involves the reduction of Hsp70 expression and reactive oxygen species production. Alternatively, the inhibition of the caspase cascade at higher concentrations (50 μM), correlated with additional reactive oxygen species increase, probably switched the mode of metabolite active-induced cell death from apoptosis to necrosis. Therefore, this evidence suggests that these natural molecules can be considered potential candidates to be tested also in *in vivo* models, alone or in combination with chemotherapy agents, to provide a scientific support for the anticancer employ of *Drimys winteri* in traditional medicinal and to hypothesize a possible use, in association with chemotherapy, for the management of melanoma.

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# PS081- Antifungal alkaloids from several Colombian Fabaceae species

Cely-Veloza W.[1], Coy-Barrera E.[1]*
*inquibio@unimilitar.edu.co

[1]*Laboratorio de Química Bioorgánica, Universidad Militar Nueva Granada, Cajicá, Colombia.*

## Introduction

Fabaceae family comprises 745 genera and more than 19,500 species which represent the third largest family of plants worldwide[1]; because of that, its enormous diversity of secondary metabolites is not surprising. In addition, Fabaceae plants can fix atmospheric nitrogen and they can be therefore adapted in any kind of soil (even in those with poor nutritional content) as well as produce nitrogen-containing metabolites such as quinolizidine alkaloids[2]. Thus, as part of our research on the Fabaceae chemistry, a phytochemical study on various Fabaceae plants (from genus *Genista, Lupinus, Ulex*) was then performed focusing on separation, identification and structural elucidation of this kind of compounds.

## Method

Alkaloidal extracts were obtained from seeds and leaves of Fabaceae plants through ultrasound-assisted acid-base extraction. A portion of crude alkaloidal extracts was separately fractioned and depurated by successive column chromatography (CC) on $SiO_2$ processes (under Dragendorff's reagent monitoring) to yield compounds **1-16**, following a detailed workflow. Isolated alkaloids were structurally elucidated using spectrometric/spectroscopic techniques such as MS, $^{1}H$ and $^{13}C$ NMR (1D and 2D). Resulting extracts and pure compounds were separately tested against *F. oxysporum* through a micro-scale amended-medium method at 0.1-5.0 μg/μL. the resulting mycelial growth inhibition was observed

## Results / Discussion / Conclusion

Ultrasound-assisted acid-base extraction protocol over samples resulted in a yield within 2-5% range of alkaloid-enriched extracts, indicating good alkaloid content in evaluated samples, highlighting the content from seeds of *Lupinus mutabilis*. CC procedures let to the isolation of sixteen quinolizidine-like alkaloids. MS analyses showed the typical diagnostic peaks for this type of compounds and the retention indexes were in agreement to those of reported ones. $^{1}H$ NMR and $^{13}C$ NMR exhibited the typical signals for quinolizidines and indicated the presence of α-pyridone-like moiety as common structural feature of some isolated compounds. The present work constitutes the first report of isolated alkaloids from these plants. All compounds exhibited antifungal activity against *F. oxysporum* at different levels above 60% inhibition of mycelial growth. Alkaloid 5 showed the best antifungal activity (<1.5 μM).

## Acknowledgement

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# PS082- Evaluation of tea and tincture of guava tree leaves at controlo f bacterial plaque in children at the E.M.E.B. Cecília Meireles school, Valinhos - SP

Wadt N.S.Y.[1]*, Mello J.A.[2], Almeida M.O.[2], Bach E.E.[3],Roncaglia P.L.F.F.[4], Anzai L.N.I.[4], Scabar L.F.[5]
*nilwadt@gmail.com

[1] *PhD/ University Paulista (UNIP) -Health Sciences Institute- Pharmacy - Av. Marquês de São Vicente, 3001, São Paulo,SP.*
[2] *Scientific research// University Paulista (UNIP) -Health Sciences Institute- Pharmacy*
[3] *PhD/UNINOVE (Universidade Nove de Julho), Health Department, Biomedical Sciences,*
[4] *Dentist/CEO- Municipal Health Secretary – Valinhos Prefecture*
[5] *PhD/ University Paulista (UNIP) -Health Sciences Institute - odontology*

## Introduction

Bacterial plaque is a damage that affects people in every age group and can be defined as a community of micro-organisms, found at the surface of teeth forming a microbial biofilm that may lead to cavities. The prevention of these is the best strategy in public health1. Guava tree leaves have a proved effectivity at the microbial growth inhibition in the oral mucosa2. The objective of the present work was to certify the efficiency of mouthwash with decoction and tincture of guava tree leaves for plaque control, and consequently, the decrease of dental cavities in students of the municipality of Valinhos.

## Method

The project is being performed in children of primary school age (2ºyear) with approval by the In Humans Ethics Committee (CAAE: 81079919.9.0000.5512), was performed evaluation of dental plaque at the beginning, and after 30 and 60 days; divided in 4 groups being: control (water), solvent, tea (decoction of guava tree leaves) and tincture. Guava tree leaves were agrochemicals free, being that the tea was prepared boiling 10g of dried leaves in 1L of water. The tincture of guava tree leaves was made by maceration (1:5) using glycerin/ethanol 70% (1:1) as solvent and diluted in 1 mL of the tincture for every 150mL of water. The mouthwashes were performed in school days after the lunch break. Protein quantification was executed by Lowry method, equivalents of bovine serum albumin (BSA) and phenols by Swain & Hillis method, equivalents in chlorogenc acid.

## Results / Discussion / Conclusion:

There was no significative improvement at prevention of bacterial plaque and cavities in the groups tested, tea (decoction) and tincture of guava tree leaves, when compared with control group, however it was possible to observe that the water control group at the end of 60 days presented a higher quantity of plaques when compared to the treated. In treated groups, including the solvent group, the teachers reported an improvement in halitosis.

**Table 1:** Quantity of protein and phenol

| Sample | mg/mL protein | mg/mL phenol |
|---|---|---|
| Guava tree leaves decoction | 0,54 | 0,21 |
| Guava tree leaves tincture | 20,35 | 6,25 |

It is possible to conclude that there is a decrease of halitosis, and tendency for diminishment of bacterial plaques.

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# PS083- Aqueous extract from Urucum (norbixin) (*Bixa orellana* L.): antimicrobial, antioxidants and healing activity

Wadt NSY.[1]*, Zamboni A.[1]; Cardoso V.O.[2], Franklin V.A.[3], Bach E.E.[4]
*nilwadt@gmail.com

[1] *PhD/UNIP (Universidade Paulista), Health Sciences Institute, SP-Brasil Av.Marquês de São Vicente, 3001, São Paulo,SP -.*
[2] *Ms/UNINOVE (Universidade Nove de Julho), Health Department, Pharmacy Sciences, SP-Brasil*
[3] *UNINOVE (Universidade Nove de Julho), Health Department, IC Biomedical Sciences, SP-Brasil*
[4] *PhD/UNINOVE (Universidade Nove de Julho), Health Department, Biomedical Sciences, SP-Brasil,*

## Introduction

The urucum (*Bixa Orellana* L.) is a bush and from its seed is extracted orange colored pigments due to the presence of the bixin (fat soluble) and norbixin (water soluble) carotenoids[1]. South American natives use urucum as a burn dressing, antipyretic, measles treatment, anti-diarrheic and anti-asthmatic agent[2,3,4] . It's also used as condiment and dye. Galindo-Cuspinera et al. identified that some urucum extract possesses antimicrobial activity. The objective of the present study was to identify the antimicrobial and antioxidant activity of the extract containing norbixin, and its skin healing potential in exposed cutaneous lesions treated with gel containing the extract.

## Method

One kilogram of seeds was harvested in Ibiúna, washed with 70% alcohol, died and crushed. 8 grams of dust was homogenized in 100mL of water and filtered. Norbixin was quantified in UV-Visible specter (453nm). For the antimicrobial assay was used *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) strains. The MIC was determined using 4mL of norbixin solution with 150, 75, 37.5 and 18.75 mg, being added 1mL of the inoculum ($15x10^8$ UFC/mL) in thee tubes. After incubation, the tubes that did no present growth indicated inhibition. It was also performed the disc halo inhibition with the same concentrations. The antioxidant capability was executed by DPPH (2,2-difenil-1-procrilhidrazide)[6] method, Trolox as standard, and results expressed in TEAC. For skin healing of wound in rats (Ethics Committee AN 37/2014), it was used 20 adults male Wistar rats (UNINOVE Vivarium), divided in three groups. Animals in group 1 were treated with 1mL of gel at 10% norbixin; group 2 with 1mL of distilled aqueous gel and group 3 with fibrinase. Was performed daily application over the wound of total $4cm^2$ area in dorsal region of each animal. The wound evaluation was made macroscopically in 0, 7, and 14 days, and the wound evolution, and skin healing retraction measures evaluated by digital planimetry.

## Results / Discussion / Conclusion

The total amount of norbixin extracted was 250mg, with 125mg being used in the test. There was inhibition at 3 concentrations (150, 75 and 37.5 mg) for *Staphylococcus aureus*, and in the assay of disc halo inhibition the diameters were: 18mm for 150mg, 9mm for 75mg and 8mm for physiological solution. For *Escherichia coli* the inhibitory effect was present with 150mg and

75mg (diameters were 27mm and 21mm respectively), but no inhibition was present with 37.5mg. As antioxidant, norbixin presented $IC_{50}$ = 904uM of Trolox. After 14 days, the wounds in animals treated with norbixin were healed corresponding to 94.98% of control group, while animals with fibrinase corresponded to 56,67% of control group. This demonstrates that fibrinase was not as efficient as norbixin and when compared to control group. It is possible to conclude that the gel with norbixin is effective at skin healing in rats, being used as a phytoterapic, besides possessing antioxidant and antimicrobial activity.

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# PS084- An in-depth study on the volatile variability of Chinotto (*Citrus myrtifolia* Raf.) induced by the extraction procedure

Arrigo S.©, Albergamo A.[1,2], Salvo A.[1,2], Rotondo A.[1], Costa R.[1]*, Saitta M. Caratozzolo M.[1], Dugo G.[1,2]

*costar@unime.it

[1]*Dipartimento di Scienze Biomediche, Odontoiatriche, e delle Immagini Morfologiche e Funzionali (Biomorf), University of Messina, Viale Annunziata, 98168 Messina, Italy*

[2]*Science4Life s.r.l., a spin-off of the University of Messina, Messina, Italy*

## Introduction

*Citrus myrtifolia* Rafinesque is native to southern China, although its origin hasn't been exactly ascertained; probably, it is a mutation of sour orange that eventually evolved into the species known today. As a matter of fact, the plant has been imported in 1500 to Savona (Italy), which became the place of choice for its cultivation. However, the plant can be easily found also in Calabria and Sicily.

In Italy, from the fruit's extract a commercial carbonated soft drink, with a bitter taste and digestive properties, is produced, which is simply known as "chinotto". To a smaller extent, *Citrus myrtifolia* fruits are used in the Italian confectionery industry, while its essential oil in perfumery.

The scientific literature reports a series of studies focusing on the characterization of the juice and/or pulp of *C. myrtifolia* fruits[1-3], but it seems underestimated the importance of the essential oil. In view of this lack of information, scope of the present work was to study the volatile composition of chinotto fruits harvested in Sicily, with a particular focus on the influence exerted by the extraction methodology used. To this aim, hand-squeezing, distillation, direct and post-mashing solvent extraction, were applied in order to find correlations between volatile isolation strategy and olfactory properties of the oil.

## Method

Fruits of *Citrus myrtifolia* were harvested in June 2017 in Milazzo (Messina), Sicily. Different extraction methodologies have been applied to the fruits of chinotto for the isolation of the essential oil, namely hydrodistillation, solid-liquid extraction (SLE), hand squeezing, blending + SLE, vegetable strainer + SLE, vegetable strainer + funnel.

Multidimensional gas chromatography and sensory analyses were applied for the characterization of the volatile fingerprints and the assessment of the olfactory attributes. PCA statistical analysis was performed for data handling and interpretation.

## Results / Discussion / Conclusion

The volatile fingerprints of the various samples differed significantly depending upon the extraction procedure. Some compounds were selectively extracted by blending plus addition of solvents. (E)-β-ocimene and nootkatone were considerably expressed in hand squeezed and solvent extracted samples, respectively. Conversely, linalyl acetate was the most abundant compound in solvent extracted samples, along with nootkatone. With respect to linalyl acetate, the

least effective methodologies of isolation proved to be distillation, and mashing followed by solid-liquid extraction. Sensory analysis showed that the flowery and the citrus notes were perceived in all samples by the majority of panelists; conversely, the minty attribute was the one least smelled in five of the six essential oils.

In conclusion, it can be safely stated that some of the extraction methodologies applied were more effective in terms of yields, some others in terms of selectivity. Sensory analyses confirmed the quality of the olfactory properties of chinotto essential oil and in particular integrated the information given by chemistry.

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# PS085- Analysis of hardness and frontier orbitals of the Diels-Alder adducts

Cabrera-Vivas B.M., Aguirre C., Palillero A., Pineda F., Ramírez-García Juan C.*
*Juan.ramirez@correo.buap.mx

*PhD/Facultad de Ciencias Químicas, Edificio FCQ9, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*

## Introduction

The Diels-Alder reaction has been studied applying theoretical methods[1,2], analyzing atomic contributions of frontier molecular orbitals and its stereochemistry to verify the preferred mechanism on adducts formation. The hardness values of the reactants were analyzed to relate them with their stability[3]. In this work the reactions between different dienes with two dienophiles are presented to give an interpretation to those reactions using hardness from DFT theory.

## Method

Five dienes and two dienophiles were modeled and studied theoretically. The energy and structural optimization of the structures were performed using Hartree-Fock (HF) scheme 6-31G* basis set. A single point option, with HF/6-31G* basis set including "d" functions for heavy atoms, which enhances the description of the atomic coefficients of frontier orbitals. The energy of the frontier orbital was calculated to determine the hardness[4], used as a molecular reactivity index.

## Results / Discussion / Conclusion

This information suggests a relationship between the orbital and the hardness value, which in a competition reaction between different structures it indicates the feasibility to obtain products. The atomic contributions help us understand the way the reaction proceeds and the hardness allows to understand which adduct is more stable[5].

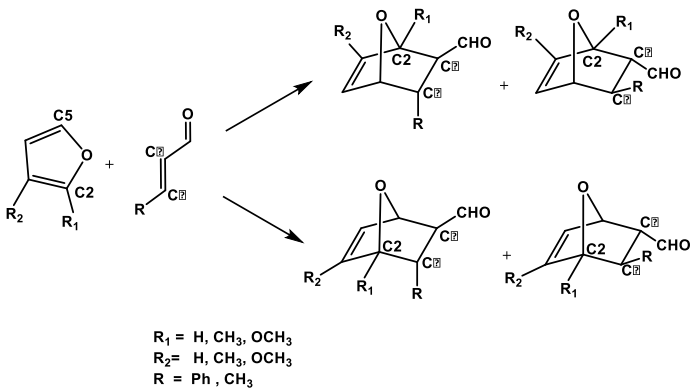


**Figure 1.** General scheme of Diels–Alder reaction. Cycled structure is diene, chain structure is the dienophile.

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# PS086- Theoretical study of anti-viral agents in HAART therapy

Cabrera-Vivas B. M., Aguirre-Cabrera C., Ramírez-García Juan C.*
*Juan.ramirez@correo.buap.mx

*PhD/Facultad de Ciencias Químicas, Edificio 179, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*

## Introduction

Theoretical electronic properties applied to chemistry are important in anti-viral drug development[1,2]. The HAART therapy is a combination of several antiretroviral drugs used to diminish the rate at which HIV makes copy of itself in the human body[3]. A combination of three or more antiretroviral drugs is more effective than the use of only one of them[4]. Tenofovir Alafenamide and Tenofovir Disoproxil Fumarate[5] are the most commoly used drugs for HIV therapy. The electronic structure of these two molecules are reported and analyzed in this work.

## Method

Energetic, geometric and electronic structures were calculated for the modeled molecules, as well as molecular orbitals, molecular electrostatic potential, hardness values and atomic charges. These calculations were done using ab initio RHF/6-31G* method[5]. With the theoretical information, a comparison of theoretical and pharmaceutical properties was performed in order to find any similarity.

## Results / Discussion / Conclusion

The molecular properties obtained of Tenofovir Disoproxil Fumarate (TDF), Tenofovir Alafenamide Fumarate (TAF), Tenofovir Bi-Alafenamide (TAB), are reported. Discussion of the implications on pharmaceutical activity of these values is made.

| **Molecule** | ***Energy*** | $\varepsilon_{HOMO}$ | $\varepsilon_{LUMO}$ | $\mu$ |
|---|---|---|---|---|
| TDF | -2072.19405 | -6.65 | 5.82 | 1.37 |
| TAF | -1830.90073 | -6.60 | 5.85 | 1.29 |
| TAB | -1962.60902 | -6.39 | 5.99 | 2.57 |

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# PS087- Quantification of trimethylamine (TMA) and trimethylamine oxide (TMA) for diagnostic and targeted diet purposes

Anchesi I.[1,2]*,Sidoti A.[2], La Torre G.L.[1], Salvo A.[1], Scimone C.[2], Donato L.[2], Rinaldi C.[2], D'Angelo R.[2], Dugo G.[1], Rotondo A.[1]
*ivan.anchesi@hotmail.com

[1]*Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali (BIOMORF. Università degli Studi di Messina, Sez. SASTAS Polo Annunziata, Cittadella Universitaria Viale Annunziata, 98158 Messina*
[2]*Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali (BIOMORF). Università degli Studi di Messina, Torre Biologica Policlinico "G.Martino", Via Consolare Valeria, 98125 Messina*

## Introduction

The Trimethylaminuria (TMAU) is a genetic pathology related to the mutation of the FMO3 gene in the chromosome 1. The gene is responsible for the synthesis of the corresponding FMO3 enzyme which, is suited to oxidize exogenous and endogens substrates. The mutations of this gene generate deficit of the physiological conversion of the trimethylamine (TMA) in trimethylamine oxide (TMAO), leading to an accumulation of TMA in the organism. In these conditions, the body will emanate a strong smell of rotten fish.[1] The detection and quantification of TMA and TMAO in biological fluids will be the starting-point toward two scientific purposes: 1) the selection the primary sources of TMA contained in foodstuff, tuning suited diets in order to limit TMA accumulation especially for people with specific burdens; 2) A direct diagnosis of the TMAU.

## Method

Blood and urine samples were mixed in a 1:9 volumetric ratio with a phosphate buffer at pH= 7.3. The resulting solution was placed inside specific 5mm NMR tubes. The obtained samples are analyzed at the same temperature (T = 25 °C) with a NMR Avance III 500 MHz spectrometer equipped with a reverse probe with gradients (SMARTprobe). The used pulse sequence was a pre-saturation followed by the noesy sequence (noesypresat). $^{1}$H-NMR noesypresat experiments were performed with a spectral width of 12 ppm, with 64 scans.

## Results / Discussion / Conclusion

In this work our team has focused on the quantitative analysis of TMA and TMAO in urines, this bio-fluid is chosen because it is easily available and can be readily analyzable via NMR. Other techniques used by our team are HPLC and GC.[2,3] The accurate and precise NMR quantification of TMA and TMAO (LOD <0.2 mM) is validated by adding the internal standard and integrating the related signals (Fig.1a); it was also possible to detect the increase in TMAO values in urine samples collected by subjects after a specific diet (Fig 1b, loads are based on more than 200g of fish).

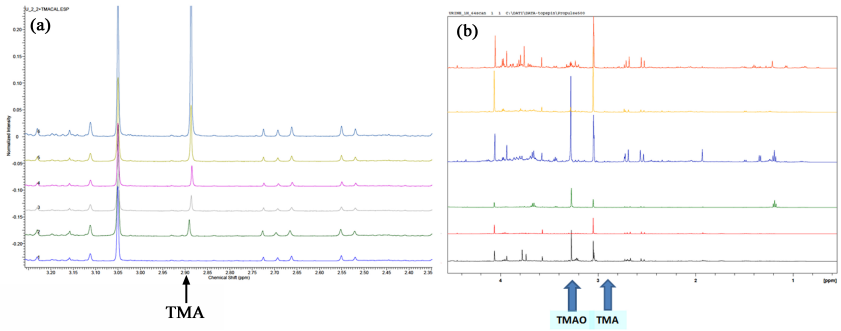


**Fig.1**.(a) 1H-NMR of urine with TMA standard addition, (b) urine loading tests on patient and control people.

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Journal of Chromatography B, 723 281–285

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# PS089- Treatment of cooling as a cultural disease in the Otomi temazcal of the state of Mexico

Villalobos-Contreras G. [1]*, Aguilera-Gómez L.I. [2], Romero-Contreras A.T. [3], Villalobos Aguilera P.H..
*genlobos@yahoo.com.mx

[1]*Facultad de Ciencias. Universidad Nacional Autónoma de México,*
[2]*Facultad de Ciencias, Universidad Autónoma del Estado de México,*
[3]*Centro de Investigación en Recursos del Agua, Universidad Autónoma del Estado de México. Facultad de Diseño. UAEMEX.*

The Temazcal or Mesoamerican steam bath, has a long history in the tradition of the cultures that settled in this part of the world. As an area of high biodiversity, Mesoamerica also has a wide range of ethnic groups in which, with various variants and according to the ecological zone and culture, there are records of use of this old bathroom. The word Temazcal or Temazcalli is of Nahuatl origin: Temax (Bath) and calli (house). This research was worked using the Observation-participant method , botanical tours, open or partially open interviews, botanical collections, identification and matching of the material were made. The historical method was also applied, in an evolutionary way. Pre-Hispanic, colonial and Twentieth century times were studied and field work in three Otomi communities performed. The information was processed and discharged to tokens for best handling.

The temazcal bath is not only used for personal hygiene but is used both in prevention and treatment of various ailments and natural diseases and cultural affiliation sicknesses. In some cases, it is used to cure the disease while in others it serves as a palliative. One of the most common uses among the Otomi, is to treat the "cooling", whose symptoms consist of the sensation of cold in various parts of the body or it is said that this occurs after childbirth, in the mother and in the newborn, after an abortion and it is presented after surgery. However, cooling is considered a precursor of other diseases and it is believed that if it is treated in time, they can be avoided.

In relation to the treatment into temazcal, it were identified 56 species of medicinal plants, of which 43 are used to treat "cooling"; of them, 74% are native plants of Mexico and 26% are introduced. The families Asteraceae and Lamiaceae are among the best represented. The preparation of plants is mainly based on the infusion of the fresh plant, for its ingestion or its external application. However, some species are macerated in alcohol, others are crushed and applied to the skin before bathing, while others are prepared in poultice. The therapeutic is varied, it can consist of approaching the steam with palms (palmearse), massages, time of permanence in the bath, to adopt several positions, to make prayer, to exercise, to apply water in the head, ingestion of plants infusions, rinse with the plant infusion, application of poultices of plants, etc. It is concluded that it is important for these communities to prevent diseases rather than cure them.

# PS090- Antioxidant activities of *Solanum nigrum* L. extracts

Campisi A.[1], Acquaviva R.[1], Nicolosi S N.A.[1], Duro A.[2], Rizzo M.[1]*, Santagati N.A.[1]

*milena.rizzo@unict.it

[1] *PhD/Department of Drug Sciences, University of Catania, Catania, Italy*

[2] *PhD/Department of Botany, University of Catania, Catania, Italy*

SNL1 and SNL2 extracts were able to quench the DPPH-radical in a dose-dependent manner and SNL1 showed a more potent capacity than SNL2. In addition, their effect appeared similar to Trolox (30 μM), a soluble analogous of vitamin E used as a standard. This set of experiments demonstrated that both extracts possess antioxidant properties.

## Introduction

*Solanum nigrum* Linn (SNL) is an herbal plant indigenous to Southeast Asia and commonly used in oriental medicine [1]. The leaves were found to be rich in polyphenols [2-4]. Herein, we assessed the total phenolic content, the concentration of phenolic acids and flavones compounds of two SNL polar extracts and their antioxidant activities in *in vitro* cellular free system and *in vitro* cellular system.

## Method

SN leaves were collected (Catania, Italy) and two extracts, SNL1 (MeOH/$H_2O$, 80:20) and SNL2 ($H_2O$ 100%) were prepared. The amount of total phenolic was determined and, spectrophotometric and HPLC methods were applied to determine phenolic acids and flavones. A DPPH radical was used to determine free radical-scavenging activity of the extracts SNL1 and SNL2 (0.5-1-2 mg/ml) were used to restore the oxidative status modified by glutamate in primary cultures of astrocytes. GSH levels and intracellular ROS production were evaluated.

## Results/Discussion/Conclusion

The total phenolic content was 92.2± 4.8 and 40.0 ± 6.9 mg/g of extract, for SNL1 and SNL2, respectively, as reported in Table 1.

**Table 1**: Contents of phenolic acid and flavones (mg/g of dry weight) in SNL1 and SNL2 leaves extracts (n=3).

| Extract | Gallic | Protocatechuic | Chlorogenic | Gentisic | Caffeic | Luteolin | Apigenina |
|---|---|---|---|---|---|---|---|
| SNL1 | 0.09 ± 0.02 | 0.24 ± 0.91 | 2.77 ± 0.45 | 1.50 ± 0.66 | 0.64 ± 0.87 | 0.98 ± 0.33 | 0.16 ± 0.74 |
| SNL2 | 0.04 ± 0.05 | 0.19 ± 1.11 | 2.01 ± 0.98 | 1,81 ± 0.75 | 0.42 ± 0.54 | 0.87 ± 0.62 | 0.12 ± 1.02 |

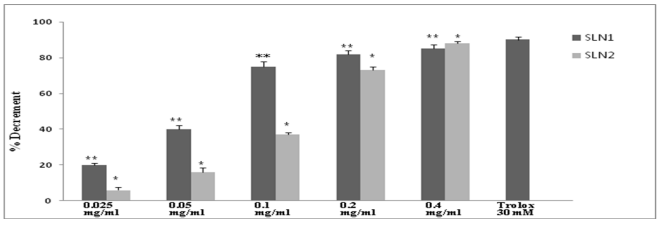


**Fig.1:** Quenching of DPPH of extracts of plant leaves from SN at different concentrations

Glutamate produced a significant decrease in the intracellular GSH levels and a significant increase of ROS levels, when compared to the untreated control ones. The pre-incubation of the cultures with both extracts (1-2 mg/ml) was able to restore, in a dose-dependent manner, GSH and ROS levels. In particular, experiment performed with 2 mg/ml of both extracts showed values similar to untreated control ones.

We demonstrated that methanolic/water (SNL1) and water (SNL2) extracts of *Solanum nigrum* L. leaves quenching radical in an *in vitro* free cellular system and restoring the oxidative status in *in vitro* primary rat astroglial cell cultures exposed to glutamate, possess notable antioxidant properties and are neuroprotective. These extracts are able to prevent the increase in glutamate uptake and to inhibit glutamate excitotoxicity which lead to cell damage.

Therefore, the SNL1 and SNL2 leaves polar extracts may represent a new natural therapeutic strategy in the neuropathological conditions associated to excitotoxicity.

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# PS091- Determination and quantification of PAHs, PCBs, heavy metals and plasticizers in *Hexanchus griseus* from the Straits of Messina (Italy).

Salvo A.*[a], La Torre G.L.[a], Saija E.[a], Bartolomeo G.[a], Mangano V.[a], Vadalà R.[a], Gervasi T.[a], Gervasi C.[a], Marino F.[b], Iaria C.[b], Ferracane A.[a] and Dugo G.[a].
*asalvo@unime.it

[a] *Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy.*

[b] *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.*

## Introduction

The shark (Hexanchus griseus) is a globally distributed deep-water shark species. It inhabits tropical and temperate waters throughout the world, including the Mediterranean Sea. Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution[1].

The aim of this study is to assess the accumulation of PAHs and PCBs (DL and NDL) heavy metals in *Hexanchus griseus* and to elucidate the suitability of this species as a bioindicator for monitoring contaminations of these compounds in the marine ecosystems of the Straits of Messina. In this study was evaluated POPs-induced ectopic lipid accumulation in liver of sexually mature shark from the Straits of Messina. This investigation was conducted on liver oil samples of one shark specimen collected during April 2018. Quantitative determination of PAHs and PCBs (DL and NDL)[2] in the various samples examined has been carried out using and HRGC-MS/MS; the determination of heavy metals was carried out using ICP-MS[3] .

## Method

All samples (1–2 g) were dried at 50°C and transferred into teflon containers for mineralization; then were added with 7mL of 65% $HNO_3$, 1mL of 30% $H_2O_2$ and 1mL of internal standard. Mineralization was carried out using a microwave oven (Milestone ETHOS 1, at 200°C 1000W for 20 min) in the operation manual of microwave ETHOS 1 for fish. After mineralization, the samples were cooled at room temperature and diluted to 25 mL with 2% $HNO_3$. Metal analysis was carried out using an Agilent 7500CX ICP-MS with octapolo reaction system, reaction/collision cell, pressurized with He.

For the determination of plasticizers, about 2mL of shark liver oil were subjected to liquid-liquid extraction two times with 1mL of acetonitrile. For each extraction, the supernatant was recovered and the collected solutions were spin-dried (5 min at 5000 rpm) to remove any traces of oil. Then, the solution was dried under vacuum and re-dissolved in 1 mL of DBP-$d_4$ and DEHP-$d_4$ at the concentration of 1mg*$L^{-1}$ (Internal Standard). The analyses were carried out using an HRGC-MS (GCMS-QP 2010 Plus Shimadzu) system equipped with a capillary column ZB-5MS (5% biphenyl–95% methyl polysiloxane) (30 m×0.25 mm; 0.25 μm film thickness). Helium was used as carrier gas (constant flow 0.90 mL $min^{-1}$) and the injections were performed at 250°C with a 60

s splittless. The identification was made by comparing retention time and mass spectra of each compound with those of the respective standard.
For the determination of PAHs and PCBs, the sample purification was carried out on a glass chromatographic column, packed with silica gel (8 g, 25% deactivate) previously conditioned with an eluent mixture consisting of *n*-hexane/ethyl acetate (9: 1). Then, about 2mL of shark liver oil were loaded on the top of the conditioned chromatographic column and eluted with 110 mL of the eluent solution used for conditioning. The eluate was collected, dried under vacuum, and re-dissolved in 1 mL of bromophos ethyl (Internal Standard). The analyses were carried out with an HRGC-MS (GCMS-TQ 8030 Shimadzu) system equipped with a capillary column ZB-5MS (5% biphenyl–95% methyl polysiloxane) (30 m×0.25 mm; 0.25 μm film thickness). Helium was used as carrier gas (constant flow 0.68 mL $min^{-1}$) and the injections were performed at 250°C with a 60 s splittless. The identification was made by comparing retention time and specific fragmentation patterns.

**Results / Discussion / Conclusion**

Elevated levels of PCB-Not Dioxin Like (congeners n. 153, 138, 180) were found in shark liver oil. The organochlorine pesticides DDT and their metabolites were found in the shark. The presence of this metabolite could be correlated to a previous use of DDT in agricultural activity, to different climatic conditions, to marine currents, to different migratory habits of aquatic organisms and to different feeding habits. The residual levels of metabolites of DDT in the shark liver are low, according to Decree dated 27 August 2004 (GU No. 292 of 14-12-2004- Ordinary Supplement n.179), on the contrary, the levels of metals and plasticizers were below legal limits.

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# PS092- Secondary metabolites from *Abies nebrodensis* (Lojac.) Mattei

Schicchi R.[1], Geraci A.[2], Maggio A.[2], Fontana G.[2], Bruno M.[2], Rosselli S.[1]*
*sergio.rosselli@unipa.it

[1] *Dipartimento di Scienze Agrarie, Alimentari e Forestali (SAAF), Università degli Studi di Palermo, Viale delle Scienze, ed. 4, I-90128 Palermo, Italy*
[2] *Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Viale delle Scienze, ed. 17, I-90128 Palermo, Italy.*

## Introduction

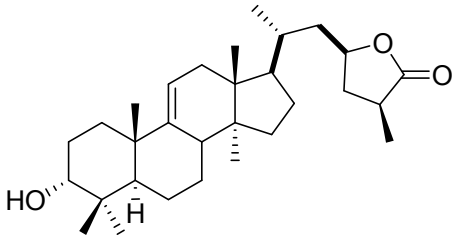
*Abies nebrodensis* (Lojac.) Mattei (Pinaceae) is a species occurring in a very small population only in a restricted area of Sicily. Its taxonomic classification as different species has been object of discussion. Recentely[1], the chemical composition of the essential oil from the leaves showed peculiar characteristics respect to the essential oil of other *Abies* species. A chemical investigation on the dichloromethane extract was carried out in order to assess the secondary metabolites content.

## Method

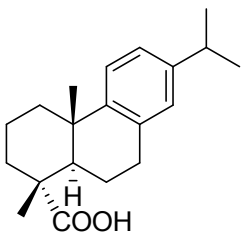
From 0.4 Kg of *Abies nebrodensis* leaves have been extracted using 0.5 L of dichloromethane as solvent, 11 g (2.7 %) of residue was obtained. After repeated column chromatographies of the residue four pure compound have been isolated.

## Results / Discussion / Conclusion

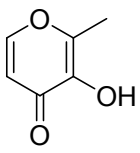
From *Abies nebrodensis* leaves a steroidal lactone, precisely a 3α-hydroxylanost-9(11)-en-26,23-olide (**1**), has been obtained. It was previously isolated only from *Abies alba* but stereochemistry and spectroscopic data were not completely assigned[2]. Herein we report the resolution of the structure by a 2D NMR full assignement. Dehydroabietic acid (**2**) was also isolated along with maltol (**3**) and rheosmin (**4**).



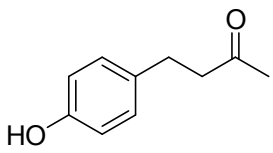

**1**




**2**




**3**




**4**

These compounds have showed interesting biological properties, for istance compound **2** was shown to have antimicrobial, anti-inflammatory or anti-cancer activity[3], wherease compound **3** exerts antitumor efficacy inducing apoptosis in hepatocellular carcinoma[4] and compound **4** exhibits anti-inflammatory property[5] by inhibiting production of iNOS and COX-2.

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# PS093- *In vitro* protective effects of an anthocyanin extract against palmitic acid-induced inflammation and insulin resistance in 3T3-L1 murine adipocytes

Muscarà C.[1,2]*, Molonia M.S.[1], Bashllari R.[1], Cristani M.[1], Occhiuto C.[1], Saija A.[1], Speciale A.[1] , Cimino F.[1]
*cmuscara@unime.it

[1]*Dept. of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy;*
*2Foundation Antonio Imbesi Messina, Italy; Email:*

## Introduction

The metabolic syndrome is characterized by various risk factors as hyperglycemia, dyslipidemia, obesity and hypertension. Excessive storage of fatty acid and dysfunction of adipose tissue in obese subjects produce an inflammatory state which is the main cause of development of obesity-correlated pathologies (Guilherme et al., 2008; Hotamisligil, 2006).Pro-inflammatory adipokines and free fatty acid (FFA) activate kinases triggering inflammatory pathway and insulin-resistance. Anthocyanins, a subclass belonging to the flavonoid family widely present in diet, have been shown to have several healthy effects. Indeed, it has been reported that dietary intake of anthocyanins produces anti-inflammatory effects and improve insulin-resistance (Jennings et al., 2014). However, the molecular mechanisms involved in their effects are not fully known. The present study was designed to investigate the in vitro protective effect of an anthocyanin-rich extract from bilberries and black currants on inflammation and insulin-resistance induced by high concentrations of palmitic acid (PA), the main circulating fatty acid, in murine adipocytes.

## Method

Fully differentiated 3T3-L1 adipocytes were pretreated with anthocyanins (ACN) extract for 24 h before they were exposed to PA 1mM for 24 h. For investigation of insulin signaling pathway, cells were also stimulated with insulin (100nM) for 15 minutes. After, NF-kB proinflammatory pathway and PI3K/Akt insulin signaling were evaluated.

## Results / Discussion / Conclusion

NF-kB is the most important transcriptional factor that plays a pitoval role in the inflammatory processes and the kinase IKK is its main activator. Thus, toevaluate the effects of ACN extract on adipocytes inflammation NF-kB pathway was studied. Results demonstrated that ACN extract was able to inhibit, in a dose-dependent way, PA-induced nuclear translocation of NF-kB, as observed by reduced nuclear proteinof p65 (NF-kB) and IKK phosphorylation. Moreover, ACN pretreatment prevented PA-induced IL-6 gene expression. In order to examine the effect of ACN extract on insulin-resistance, PI3K, pAkt and GLUT-1 protein levels were evaluated. Exposure of adipocytes to PA decreases PI3K and pAkt levels and transmembrane translocation of GLUT-1 carrier so demonstrating a reduced insulin-sensitivity. Conversely, the pretreatment with ACN extract improved insulin sensitivity, increasing all these markers, in PA exposed adipocytes.

These dataclarify the mechanisms that underline the protective effect of anthocyanins, suggesting that the assumption of this ACN extract could reduce the development of inflammation and insulin resistance induced by PA on adipose tissue.

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# PS094- Antiprotozoal activity of *Chromolaena perglabra* (B. L. Robinson) King & H. Rob.

Pombo-Ospina L.M.[1]*, Teherán-Valderrama A.A.[2], Ramírez-González J.D.[3], Patiño L.H.[3], Cruz-Saavedra L.[3], Rodríguez-Aguirre O.E.[4]
*miguel.pombo@juanncorpas.edu.co; rodriguezoscare@unbosque.edu.co

[1] *MSc/ Centro de Investigación. Carrera 111 No. 159 A 61 (Av. Corpas Km. 3 Suba). Universidad Juan N. Corpas, Bogotá, Colombia.*
[2] *MD/ Centro de Investigación. Carrera 111 No. 159 A 61 (Av. Corpas Km. 3 Suba). Universidad Juan N. Corpas, Bogotá, Colombia.*
[3] *Ph.D/ Facultad de Ciencias Naturales y Matemáticas. Calle 12C Nº 6-25. Universidad del Rosario, Bogotá, Colombia.*
[4] *Ph.D/Facultad de ingeniería programa ingeniería ambiental. Av. Carrera 9 No. 131 A-02. Universidad El Bosque, Bogotá-Colombia*

## Introduction

Chagas disease (CD) or American trypanosomiasis is one of the most prevalent parasitic diseases in humans. Its etiologic agent is *Trypanosoma cruzi*[1]. The WHO estimates that globally there are approximately 6 million people infected with *T.cruzi*, and more than 70 million people are living in areas with high risk of CD transmission[2]. It was estimated that more than 80% of the people in the world affected by CD did not have access to diagnosis and specific treatment[2]. On the other hand, Leishmaniasis are diseases with high incidence and wide geographic distribution in the Americas, it is caused by a protozoan parasite of the genus *Leishmania*. The highest percentage of cutaneous cases is found in Brazil, Colombia, Peru and Nicaragua (74.3% for 2016). For species of the genus *Chromolaena*, antimicrobial, antiparasitic and cytotoxic activities have been reported[3-4]. Considering the side effects of the treatments currently available for these diseases, the aim of this study was to evaluate the antiprotozoal activity of extracts and fractions of *Chromolaena perglabra* leaves against *Trypanosoma cruzi* epimastigotes and promastigotes of *Leishmania braziliensis*.

## Method

The extracts of *Chromolaena perglabra* leaves were obtained by Soxhlet with solvents of increasing polarity: petroleum ether, dichloromethane, ethyl acetate and ethanol. These extracts were subjected to solid-liquid fractionation with silica gel 60 support with increasing polarity solvents: petroleum ether, dichloromethane, ethyl acetate and ethanol, to obtain a total of 4 extracts and 16 fractions. The viability of the parasites was measured according to the formation of formazan crystals after adding 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) (MTT). The crystals formation was determined in an Elisa plate reader at a wavelength of 570 nm.

## Results / Discussion / Conclusion

Extracts and fractions with antiprotozoal potential with IC50 less than 1.8 ppm are presented in the table. The petroleum ether extract, one of the most active, by GC-MS presents the following

major compounds in order of abundance: Tridecane 12.0%, Heptacosane 6.1%, 3-Methyl-3,4-divinyl-1-cyclohexene 5.8%, Ethyl linolenate 3.5%, Heptacosane 2.6%, Lupanine 1.9%, Humulene epoxide II 1.2%, (-) - α -santalene 1.2%, Ethyl palmitate 0.9%.

| ***Chromolaena perglabra*** | ***Epimastigotes de Trypanosoma cruzi*** | ***Promastigotes de Leishmania sp*** |
|---|---|---|
| | $IC_{50}$ (ppm) | $IC_{50}$ (ppm) |
| **Ex. Petrol** | 1,47 | 0,585 |
| **Ex. Petrol - Fr Petrol** | 0,85 | 0,384 |
| **Ex. $CH_2CL_2$ -Fr Petrol** | 0,7845 | <0,12 |
| **Ex. $CH_2CL_2$ -Fr $CH_2CL_2$** | 1,8 | 0,674 |

The low polarity fractions obtained from the leaves of *Chromolaena perglabra*, showed the highest antiprotozoal activity against *Trypanosoma cruzi* epimastigotes and promastigotes of *Leishmania braziliensis*, activity that may be related to terpenoid compounds.

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# PS095- Antioxidant capacity and antimicrobial activity of *Chromolaena scabra* (L.f.) RM. King & H. Rob

Giraldo-Villamil P.A[1], Andrade-Burbano A.C.[1], Pombo-Ospina L.M.[2],
Rodríguez-Aguirre O.E.[2, 3]*
*rodriguezoscare@unbosque.edu.co

[1]*QF/Universidad de Ciencias Aplicadas y Ambientales. Calle 222 No. 55-37. UDCA, Bogotá, Colombia.*
[2]*MSc/ Centro de Investigación. Carrera 111 No. 159 A 61 (Av. Corpas Km. 3 Suba). Universidad Juan N. Corpas, Bogotá, Colombia.*
[3]*Ph.D/Facultad de ingeniería programa ingeniería ambiental. Av. Carrera 9 No. 131 A-02. Universidad El Bosque, Bogotá, Colombia.*

## Introduction

For decades, the human being in the process of adaptation has sought solutions in plants to improve their welfare, hence the importance of the search for medicinal plants throughout history as a phytotherapeutic alternative[1]. Colombia has a large variety of native species that have not been studied to date, such as some of the asteraceae family[2]; *Chromolaena scabra* (L.f.) RM. King & H. Rob., which has few studies of its biological activity, belongs to this family, other studied species of the genus have high antioxidant potential, antimicrobial, pesticide and antiprotozoal activities, among others[3]. The aim of the study was to evaluate the antioxidant capacity by the DPPH* and ABTS*$^{+}$ methods of the total extracts of petroleum ether (Ex. Petrol), dichloromethane (Ex. $CH_2Cl_2$) and ethanolic (Ex. EtOH), and determine their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* and antifungal against *Penicillium digitatum* and *Aspergillus niger.*

## Method

Extracts of leaves and flowers were obtained with solvents of increasing polarity: Petroleum ether, Dichloromethane and Ethanol. The antioxidant capacity was evaluated by the DPPH* and ABTS*$^{+}$ methods and the antimicrobial activity by the plate diffusion agar technique.

## Results / Discussion / Conclusion

By the DPPH* method the Ex. EtOH of the leaves showed a relative antioxidant capacity with respect to the ascorbic acid of 9.59 and Ex. $CH_2Cl_2$ of 10.73; by the method ABTS*$^{+}$ Ex. EtOH showed a relative antioxidant capacity with respect to ascorbic acid of 41.52 and Ex. $CH_2Cl_2$ of 48.73. By the DPPH* method, the Ex. EtOH showed a relative antioxidant capacity with respect to the Rutin of 3.57 and the Ex. $CH_2Cl_2$ of 3.99; by the method ABTS*$^{+}$ Ex. EtOH had a relative antioxidant capacity with respect to the Rutin of 17.74 and Ex. $CH_2Cl_2$ of 20.81. The other extracts showed no activity. For the antimicrobial activity of leaf and flower extracts against *Staphylococcus aureus* using Rifampicin as reference standard, it was found that for each mg of this antibiotic, 1.42 mg of Ex $CH_2Cl_2$-Leaves are required to obtain the same sensitivity; 1.44 mg of Ex. Petrol-Leaves and 2.04 mg Ex EtOH-Leaves, the extracts of the flowers presented lower sensitivity; with respect to *Escherichia coli*, the extracts and their fractions showed no activity. For the antifungal activity of the extracts, it was found that for each mg of fluconazole, 94.1 mg of

Ex. Petrol-Flowers against *Penicillium digitatum* and 195.4 mg of Ex. Petrol-Leaves against *Aspergillus niger* are required to obtain an equivalent sensitivity. Using ketoconazole as the standard for each mg of this, 87.85 mg of Ex. Petrol-Flowers against *Penicillium digitatum* are required; the other extracts showed no activity.

The extracts of medium and high polarity of the leaves of *Chromolaena scabra (L.f.) RM. King & H. Rob.,* showed higher antioxidant capacity, while the medium polarity extracts showed better antibacterial activity against Gram-positive bacteria; the antifungal activity was low.

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# PS096- Antioxidant capacity and antimicrobial activity of *Lourteigia stoechadifolia* (L.f.) RM. King & H. Rob

Rodríguez-Aguirre O.E.[1,2]*, Andrade-Barreiro W.A.[1], Zuluaga-Ortiz C.A.[2], Pombo-Ospina L. M.[2]
*rodriguezoscare@unbosque.edu.co

[1]*Ph.D-MSc/Facultad de ingeniería, programa de ingeniería ambiental. Av. Carrera 9 No. 131 A-02, Edificio Fundadores, tercer piso. Universidad El Bosque, Bogotá Colombia.*
[2]*MSc/ Centro de Investigación. Carrera 111 No. 159 A 61 (Av. Corpas Km. 3 Suba). Universidad Juan N. Corpas, Bogotá -Colombia.*

## Introduction

In many countries, medicines are obtained from plants[1], WHO estimates a high percentage of the world's inhabitants who depend on medicinal plants to meet their primary health care needs, and this has generated programs of study in search of active ingredients to improve health in the population. The above aims to positively impact the prevention of a large number of diseases through the use of alternative phytomedicines obtained from plants, which are low cost and highly available[2]. In Colombia a great variety of species belonging to the asteracea family, grow like weeds, being one of them *Lourteigia stoechadifolia* (L.f.) R.M.King & H.Rob.; native of South America, genus conformed by eleven restricted species to the high zones of the North end of the Andes, reported by holm oak of the 2500 meters of altitude; seven prosper in the paramos and subparamos of Colombia. *Lourteigia stoechadifolia* (L.f.) R.M.King & H.Rob. is a shrub or sub-shrubs of small to medium size, sometimes procumbent, with few to many branches; stem cylindrical, striated, puberulous or densely tomentose, white hairs, opposite leaves, petiolate, sometimes very short petioles; leaf blade ovate to narrowly elliptic, margin serrated to crenulate, lower surface often provided with white tomentum[3]. Terminal inflorescence, densely corymbose, short pedicels; filarias 20-30, weakly to moderately sub-assembled, arranged in 3-4 unequal series[4]. The aim of the study was to evaluate the antioxidant capacity of extracts and fractions of the leaves of *Lourteigia stoechadifolia*, by the DPPH* and ABTS*$^{+}$ methods and their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Penicillium digitatum*, *Aspergillus niger* and *Rhizopus nigrican*[4].

## Method

The dichloromethane ($CH_2Cl_2$) and ethanolic (EtOH) extracts of the leaves were obtained by using the Soxhlet equipment; subsequently, solid/liquid fractionation was carried out with solvents of increasing polarity: Hexane, Chloroform and Methanol for each one. The evaluation of the antioxidant capacity was carried out by the methods of DPPH* and ABTS *$^{+}$ and the antimicrobial activity was done by the diffusion technique in agar with perforation in plate.

**Results / Discussion / Conclusion**

By the DPPH* method, the $CH_2Cl_2$ extract showed a relative antioxidant capacity with respect to ascorbic acid of 88.2 and its methanolic fraction of 67.3, the EtOH extract of 28.6 and its methanolic fraction of 31.0; the extract $CH_2Cl_2$ with respect to the Rutine of 32.8 and its methanolic fraction of 25.1 and the EtOH extract of 10.6 and its methanolic fraction of 11.9. By the ABTS*$^{+}$ method, the $CH_2Cl_2$ extract showed a relative antioxidant capacity with respect to ascorbic acid of 96.9 and its methanolic fraction of 106.6 and the ethanolic extract of 58.5 and its methanolic fraction of 49.8; and with respect to Rutine, the $CH_2Cl_2$ extract showed an antioxidant capacity of 41.4 and its methanolic fraction of 45.6 and the ethanolic extract of 25.0 and its methanolic fraction of 21.2. The antimicrobial activity of the extracts and fractions against *Staphylococcus aureus* was determined using Rifampicin as reference standard, where for each mg of this antibiotic, 1.4 mg of Ex $CH_2Cl_2$ - Fr. Petrol are required to obtain the same sensitivity; 2.7 mg of Ex. EtOH - Fr. Petrol and 3.8 mg Ex $CH_2Cl_2$ - Fr. $CH_2Cl_2$, the other fractions presented lower sensitivity; with respect to *Escherichia coli*, the extracts and their fractions showed no activity. The antifungal activity of the fractions was determined, where for each mg of fluconazole, 1.69 mg of Ex. $CH_2Cl_2$-Fr. $CH_2Cl_2$, against *Penicillium digitatum,* 4.0 mg Ex. EtOH - Fr. Petrol against *Aspergillus niger* and 1.28 mg Ex. EtOH -Fr. MeOH against *Rhizopus nigricans*, were required to obtain an equivalent sensitivity. Using ketoconazole as a standard for each mg of this, 18.19 mg of Ex. $CH_2Cl_2$-Fr. $CH_2Cl_2$, against *Penicillium digitatum;* 4.54 mg of Ex. EtOH - Fr. Petrol against *Aspergillus niger* and 1.83 mg Ex. EtOH -Fr. MeOH against *Rhizopus nigricans*, were required to obtain an equivalent sensitivity.

The ethanolic extract and its methanolic fraction showed better antioxidant capacity, being potential to be used as free radical scavengers in marketable products. The fractions Ex. $CH_2Cl_2$-Fr. $CH_2Cl_2$ and Ex. EtOH -Fr. Petrol, showed the highest antibacterial and antifungal activities, which will allow to have a therapeutic alternative in the management of associated infectious diseases.

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# PS097- Antioxidant capacity and antifungal activity of *Teloschistes exilis* (Michaux) Vain.

Beltrán-Hernández M. F[1], Rodríguez-Romero M.L[1], Moncada-Cárdenas B.[1], Pombo-Ospina L. M[2], Rodríguez-Aguirre O. E[2, 3*]
*rodriguezoscare@unbosque.edu.co

[1]*Lic B-Ph.D/ Universidad Distrital Francisco José de Calda. Carrera 3 # 26A - 40 / Carrera 1 Este # 33 - 54 Piso 2. Bogotá, Colombia.*
[2]*MSc/ Centro de Investigación. Carrera 111 No. 159 A 61 (Av. Corpas Km. 3 Suba). Universidad Juan N. Corpas, Bogotá, Colombia.*
[3]*Ph.D/Facultad de ingeniería programa ingeniería ambiental. Av. Carrera 9 No. 131 A-02, Edificio Fundadores, tercer piso. Universidad El Bosque, Bogotá-Colombia.*

## Introduction

Colombia has a high diversity of lichens, this is due to the geographical location it has and the variety of climates it presents[1]. For the year 2015 Rangel-Ch reported 1700 species of these organisms, but it is estimated that there could be a number greater than or equal to 3,600 species, in fact, Colombia is currently one of the most diverse countries in these organisms, but only until now, its vast diversity and scientific potential is explored[1]. In the last decades there has been a growing interest in the study of lichen biodiversity as a source of new natural products, since these produce potential secondary metabolites for pharmacological and industrial uses, especially for their antioxidant and antifungal properties, properties already known in traditional medicine that have not been validated scientifically[2-4]. The aim of the study was to evaluate the antioxidant capacity by the ABTS*$^{+}$ method and to evaluate the antifungal activity against *Penicillium digitatum* and *Aspergillus niger*, of the total ethanolic extract and fractions of different polarity.

## Method

The total ethanolic extract was obtained in a Soxhlet equipment. Subsequently, a liquid/liquid fractionation was carried out with solvents of increasing polarity: Hexane, Dichloromethane and Ethyl Acetate. The evaluation of the antioxidant capacity was carried out by the ABTS*$^{+}$ technique and the evaluation of the antifungal activity by the agar diffusion method (Kirby-Bauer).

## Results / Discussion / Conclusion

Sing the ABTS*$^{+}$ method the Ex. EtOH- Fr. MeOH showed a relative antioxidant activity with respect to the ascorbic acid of 37.58 and the Ex. EtOH - Fr. AcOEt of 47.06, the other fractions showed no activity. The evaluation of the antifungal activity of the total ethanolic extract and its fractions, showed that for each mg of fluconazole, was required 228.3 mg of Ex. EtOH-Fr. Petrol against *Penicillium digitatum* and 174.6 mg of Ex. EtOH -Fr. Petrol against *Aspergillus niger*, to obtain an equivalent sensitivity. Using ketoconazole as a standard, it was found that for each mg of this compound, was required 213.4 mg of Ex. EtOH-Fr. Petrol, against *Penicillium digitatum* and 465.6 mg of Ex. EtOH -Fr. Petrol versus *Aspergillus niger*.

The fractions of medium and high polarity showed higher antioxidant activity, while the fractions of low polarity showed higher antifungal activity.

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# PS098- Effect of the drying method on the determination of color coordinates in agroindustrial pomace from grape juice production

Leder P.J.S.[1]*, De Lima V.A.[1], Pereira E.A.[1], Porcu O.M.[1*]
*ornella@utfpr.edu.br; ornellamporcu@gmail.com

[1]*Department of Chemistry, Federal University of Technology – Paraná (UTFPR), Campus Medianeira /PR, Brazil. Post Graduation Program of Chemical and Biochemical Process (PPGTP), Federal University of Technology– Paraná (UTFPR), Campus Pato Branco/PR, Via do Conhecimento, Km 1, CEP 85503-390, Pato Branco/PR, Brazil.*

## Introduction

The color of grapes used in the production of juices and red wines, either its intensity or its tonality, reflects an important quality aspect of the fruit. The phenolic compounds from grapes may provide sensorial aspects such as color, flavor, aroma and astringency, which are characteristic of purple grape products. This study aims to evaluate the influence of different drying methods on the color of grape pomace (peel and seeds) of *BRS Violeta* cultivar from juice production.

## Method

Grape pomace was dried using two different methods: (1) drying oven with air circulation for 5 days at 40º C, the whole pomace (RIS) and ground pomace (|RMS) were dried; (2) lyophilization process (RIML) for 30 horas. Two batches (1L and 2L) of both pomace were dried and assessed. Color analyses of samples were performed in triplicate, using Konica Minolta portable colorimeter (CR/400) (iluminante D 65). The *in natura* grape pomace sample (RIN) was used as standard.

## Results / Discussion / Conclusion

This study showed that all samples oven dried had a reduction in luminosity parameter (L*) when compared to standard (RIN) which presented L* = 20.73±0.57. The L* values for samples RIS 2L and RIS 1L were 17.77±0.71 and 16.22±0.53, respectively; and the values for samples RMS 1L and RMS 2L were 17.18±0.03 and 15.49±0.52. On the other hand, samples dried at low temperatures showed luminosity increasing. The L* value for RIML 1L was 16.33±0.24b, and 16.92±0.03 for RIML 2L. It was highlighted that higher temperatures favor the darkening process of grape pomace of *BRS Violeta* cultivar.

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# PS099- Technical quality of grape pomace of *BRS Violeta* cultivar

Leder P.J.S.[1], De Lima V.A.[1] , Pereira E.A.[1], Porcu O.M.[1]*
*ornella@utfpr.edu.br; ornellamporcu@gmail.com

[1]*Department of Chemistry, Federal University of Technology – Paraná (UTFPR), Campus Medianeira /PR, Brazil. Post Graduation Program of Chemical and Biochemical Process (PPGTP), Federal University of Technology– Paraná (UTFPR), Campus Pato Branco/PR, Via do Conhecimento, Km 1, CEP 85503-390, Pato Branco/PR, Brazil.*

## Introduction

BRS Violeta cultivar was presented in 2006 by EMBRAPA and it has been largely used to produce grape juice with other common cultivars in order to add greater color, flavor and improve the quality of these products. Regarding the potential reuse of grape pomace from BRS Violeta cultivar processing, this study aimed to determine the technical quality of this material.

## Method

The technical quality of grape pomace (peel and seeds) was assessed according to the following parameters and analyses: water activity (Aw) (Aqualab 4TEV); pH (Instituto Adolfo Lutz, 2008); total ash (method n. 8-12, AACC, 2000); and protein content (Kjeldahl). All the analyses were performed in triplicate. Grape pomace samples were separated in four conditions: *in natura* (RIN); whole dried pomace (RIS) and ground dried pomace (RMS), both dried in oven with air circulation for 5 days at 40º C; and lyophilized pomace (RIML). In this last process, samples were stored at -45 ºC for 5 hours in a lyophilizer (Terroni Fauvel, LH 0400/2L model), followed by vacuum application for 30 hours.

## Results / Discussion / Conclusion

This study showed that the dehydration processes caused a drastic reduction of Aw in the samples, which values were 0.38±0.00 for RIML and RIS; and 0.44±0.00 for RMS. The Aw value for RIN was 0.95±0.04, very close to 1, which is the maximum value for Aw. Ash content ranged from 0.66±0.04 to 2.70±0.40 (%), which is in accordance with the estimated content for plant foods (0.3 e 3.0 %). The pH values of samples varied from 4.09±0.00 to 4.35±0.23, higher than the pH of *in natura* fruit (3.70 and 3.80). The protein analyses indicated that the different drying processes did not influence the protein content of the samples, which were: 5.48±0.41 mg/100g (RIS), 5.92±0.59 mg/100g (RMS) and 5.91±0.11 mg/100g (RIML); these values were higher than protein content found in RIN (4.48±0.61 mg/100g).

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# PS100- Antibacterial potential of donkey milk against foodborne bacteria

Conte F.[1]*, Voslarova E.[2], Mangano V.[3],Cicero N.[3]
*fconte@unime.it

[1] *University of Messina, Department of Veterinary Sciences, Messina-Italy*
[2] *University of Veterinary and Pharmaceutical Sciences Brno, Department of Animal Protection, Welfare and Behaviour,*
*Faculty of Veterinary Hygiene and Ecology, Brno-Czech Republic*
[3] *University of Messina, Department of B.I.O.M.O.R.F, Messina-Italy*

## Introduction

Potential health benefits of donkey milk (DM) increased considerably over the last decade. DM is rcognized as *pharmafood* for its nutritional, nutraceutical, and functional properties [1]. Some reports show a strong antimicrobial properties of DM against pathogen or spoilage organisms[2,3,4]. The high lysozyme (LZ) content is mainly responsible of this activity[4]; other substances (lactoperoxydase, lactoferrin, immunoglobulins and free fatty acids) can also contribute to the antimicrobial effects[5]. The objective of this study was to preliminarly assess the natural antibacterial potential of DM against foodborne bacterial strains. To the best of our knowledge, this is the first study on the topic.

## Method

DM samples were collected from Ragusana breed donkey at three stage of lactation (A:early; B:middle; B:late). The antimicrobial activity of DM was investigated against 12 Gram negative bacterial foodborne strains taken from the Laboratory of Food Microbiology, Food Inspection Unit, University of Messina. Bacterial strains were: *Escherichia coli* (poultry and bovine fresh meat), *Salmonella* spp (ewe cheese, bovine meat), *Proteus* spp (poultry and bovine meat), *Enterobacter* spp (poultry meat), *Citrobacter* spp (ewe cheese, bovine meat). The Agar Diffusion Assay (ADA) was used to test the antimicrobial activity. It was further investigated for 1 strain of *E.coli* (Ec) (poultry meat) and 1 strain of *Salmonella* spp (S) (bovine meat) via *in situ* inhibition test; a bulk DM sample was used. In this case antibacterial assay was performed as described in the literature[6] with some modifications. Artificial contaminated milk samples (approximately to 7 $\log_{10}$/CFU/mL) were incubated for 96 hours (h) at 15 °C and 9°C for Ec and S, respectively, on the basis of the literature data[6]. Data were were analyzed by one way ANOVA; the level of significance was set at $p < 0.001$. LZ activity was assessed by EnzChek Lysozyme Kit (Invitrogen, Carlsbad CA, USA).

## Results / Discussion / Conclusion

The ADA showed that samples A, B and C had no inhibitory capability to tested strains. LZ activity was 6438, 6488 and 6533 U/mL in samples A, B and C respectively. LZ activity of bulk milk sample was 6486 U/mL. Ec count significantly decreased after 48 h (6.53 $\log_{10}$ CFU/mL) and 72 h (5.74 $\log_{10}$ CFU/mL) ($p<0.001$); at 96 h the reduction was significant ($p>0.05$). S count significantly decreased during 96 h ($p<0.001$). Both strains showed a sensible growth reduction (3

$log_{10}$ CFU/mL) after 96 h, in spite of their different incubation temperatures. The antibacterial potential of DM, over 96 h, might not have been influenced by high inocula employed in the experiments; in this case a steady residual activity of milk has been hypothesised.These results were different in comparison to data reported by other authors [5,6]. In such a complex medium as milk, the inhibition system might interact to give a specific pattern of resistance for different strains[5]. The natural antimicrobial activity in DM was mainly ascribed to the high concentration of LZ. The ADA was not considered as a useful test to assess the antibacterial activity against Gram negative bacteria in DM samples. Further trials should be carried out to assess the inhibitory potential of pure LZ from DM against foodborne organisms. In the future LZ could be recommended for use as a natural additive to minimize the risks related to microbiological contamination along food chains.

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# PS101- Effects of *Citrus sinensis* on diet-induced obese zebrafish

Mania M.[1]*, Guerrera M.C.[2], Navarra M.[3], Montalbano G.[2], Germanà A.[4]
*mmania@unime.it, manuela.mania87@gmail.com

[1] *PhD/University of Messina, Department of Veterinary Sciences, Polo Universitario SS: Annunziata, 98168 Messina, Italy*
[2] *Associate Professor/University of Messina, Department of Veterinary Sciences, Polo Universitario SS: Annunziata, 98168 Messina, Italy.*
[3] *Associate Professor/ University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Polo Universitario SS: Annunziata, 98168 Messina, Italy.*
[4] *Full Professor/ University of Messina, Department of Veterinary Sciences, Polo Universitario SS: Annunziata, 98168 Messina, Italy*

## Introduction

Obesity is a pathological condition, due to an imbalance between energy intake and consumption, that has reached epidemic proportions[1]. Therefore, worldwide scientists are making considerable effort to find novel strategies and treatments to prevent and cure obesity[2]. In recent years, increasing researchers' interests focused on plant derivatives, because of their potential health-promoting properties, including anti-obesity activity[3]. *Citrus sinensis* orange juice (OJ) can exert many beneficial effects on humans, playing also a role in weight management and obesity[4]. We designed the present study to evaluate the effect of a flavonoid-rich extract of OJ (OJe) from *C. sinensis* on a diet-induced obese zebrafish.

## Method

Adult zebrafish were divided into four diet groups and received a diet containing 20 mg (control group) or 60 mg (overfed group) *Artemia* with or without orange juice extract (OJe, 5ml/l in fish water) for 4 weeks. The extract has been characterized previously[5]. Body Mass Index (BMI) and body weight were examined weekly; at the end of the experiment, samples were routinely processed for light microscopy, RNA extraction and qRT-PCR[2, 6, 7].

## Results / Discussion

Overfed zebrafish exhibited increased body weight and body mass index (BMI), coupled to a variation in gene expression (leptin, ghrelin, orexin, POMC, NPY), both centrally and peripherally, compared to control group. Notably, the daily addition of OJe to overfed zebrafish significantly reduced BMI compared to matched controls and restored the expression of appetite-regulating genes.

## Conclusion

This study adds new insights into the anti-obesity and/or protective property of *Citrus* flavonoids also in a diet-induced obese zebrafish model, restoring a healthier phenotype (through weight loss and stabilization of weight gain) and confirms zebrafish as a useful model to study obesity.

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# PS102- Antibiotic susceptibility in strains isolated from raptors in Sicily

Gambino D.[1], Caracappa G.[2], Giardina S.[2], Persichetti M.F.[1*], Gentile A.[1], Currò V. [1], Crucitti D.[1], Vicari D.[1], Caracappa S.[1]*
*santo.caracappa@izssicilia.it

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via Gino Marinuzzi 3, Palermo (PA) 90129, Italy.*
[2] *Centro Recupero Fauna Selvatica Bosco di Ficuzza, Via del Bosco 1, Ficuzza di Corleone (PA) 90034, Italy.*

## Introduction

The last few decades have seen the inexorable proliferation of antibiotic-resistant bacteria (AMR) in some geographical areas, often with multiple resistances. These have caused the failure of antibiotic therapies in both human and veterinary medicine. Bacteria may acquire resistance following direct exposure to antibiotics or by exploiting multiple biochemical mechanisms or and extraordinary genetic flexibility. Several studies have shown that resistance genes are worldwide spread (pathogens, commensals and environmental microorganisms) [1-3]. In the wild, birds are considered *reservoirs* and potential diffusers of antibiotic resistance as well as indicators of the spread of this phenomenon. For this reason, microorganisms isolated from wild environments should be monitored [2,3]. The purpose of this work is to deepen the knowledge on the antibiotic-resistance present in strains isolated from raptors living in Sicily.

## Method

From January to July 2018, 16 samples of feces, 11 swabs (ocular, buccal, cutaneous) and organs taken from 26 carcasses (lung, heart, kidney, liver, intestine, spleen, brain) collected from wild birds living in Sicily were analyzed at the bacteriological laboratories of the *Istituto Zooprofilattico Sperimentale della Sicilia*. In details, most of the samples came from bird of prey belonging to the species: Allocco (*Strix aluco)*, Assiolo (*Otus scops*), Falco pellegrino (*Falco peregrinus*), Gheppio (*Falco tinnunculus*), Poiana (*Buteo buteo*), Barbagianni (*Tyto alba*), Falco pecchiaiolo (*Pernis apivorus*). For the isolation of zoonotic bacteria, selective and differential solid media, such as Mannitol salt agar (MSA), McConkey agar (MC), Xylose-Lysine-Desoxycholate agar (XLD) and Brilliant Green Agar (BGA) were used. Antibiotic susceptibility of the isolated strains were tested by the agar disc diffusion methods and the diameter of the inhibition zones was compared with CLSI standards [4]. At the moment, it was assessed the sensitivity of the strains to 8 antibiotics: ampicillin (10 µg), ceftiofur (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), sulfisoxazole (300 µg), sulfamethoxazole / trimethoprim (1,25 µg + 23,75 µg), tetracycline (30 µg), however, further analyzes are still ongoing.

## Results / Discussion / Conclusion

During this preliminary step of the study, 44 strains belonging to different bacterial species were isolated. Specifically, from January to July 2018, 13 *E. coli,* 1 *Klebsiella* spp*.,* 2 *Enterobacter* spp., 5 *Aeromonas hydrophila*, 10 *Clostridium* spp., 3 *Streptococcus* spp. and 10 *Staphylococcus* spp. were isolated. At present, 24 out of 44 isolates were investigated for antibiotic susceptibility.

The antibiograms showed the presence of multiple resistances for 16% of the strains tested, and mainly for *E. coli* strains. The *E. coli* is one of the microorganisms that can acquire and transfer resistance genes and it is also considered as an indicator of the antibiotic resistance evolution in wild animals (3,5). The isolation of these multi-resistant strains in wild birds samples analyzed in this study could be related to the transmission of resistance genes. To this end, further studies will be conducted on the 44 isolates and on those that will be isolated during the project; different antibiotic molecules will be tested in order to evaluate the sensitivity to the main classes of antibiotics and to assess the presence of resistance genes.

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# PS103- Monitoring of loggerhead sea turtles stranding on Sicilian coast during the last 4 years

Persichetti M.F.[1], Gentile A.[1], Gambino D.[2], Schirò G. [1], Caracappa G.[2], De Maria C. [2], Marino F.[2], Currò V. [1], Vicari D.[2]*, Caracappa S.[1]
*domenico.vicari@izssicilia.it

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Centro di Referenza Nazionale per il Benessere, Monitoraggio e Diagnostica delle Malattie delle Tartarughe Marine (C.Re.Ta.M.), Via Gino Marinuzzi 3, Palermo 90129, Italy.*
[2]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via Gino Marinuzzi 3, Palermo (PA) 90129, Italy.*

## Introduction

Often sea turtles are spotted in the Mediterranean basin and furthermore this territory is a nesting area for *Caretta caretta* species. The loggerhead sea turtle is included in the IUNC red list as a vulnerable specie, however, frequently they are victims of human activities that result in injury and death[1-5]. For these reasons, sea turtles can be considered as a bioindicator of the Mediterranean sea status[3-4]. The aims of this study were to report data collected from the Centro di Referenza Nazionale per il Benessere, Monitoraggio e Diagnostica delle Malattie delle Tartarughe Marine (C.Re.Ta.M.) during the last 4 years, to confirm the most common species of sea turtles in the Mediterranean sea and to describe the main cause of stranding in that animals.

## Method

Between March 2014 and March 2018, the C.Re.Ta.M. located at the *Istituto Zooprofilattico Sperimentale delle Sicilia* (Palermo, Italy) monitored Sicilian coastal areas to detect stranded sea turtles. Each rescued turtle was registered, identified by its morphological traits, sexed, measured and weighed. Physical and X-rays examinations were conducted to evaluate the health condition of the animal and to identify the presence of ectoparasites, external lesions and/or ingested fishing gears. Following techniques previously described[1-3], surgical procedures to remove accidentally ingested hooks and monofilament lines were carried out after few days of rehydration therapy. Necropsy were performed to identify the cause of death when conditions of conservation were good.

## Results / Discussion /

A total of 638 loggerhead sea turtles were rescued during 4 years of activity at the C.Re.Ta.M., about 2/3 of which were already died at the time of reporting or died in the first hours of hospitalization, including 128 missed recoveries for poor conditions of conservation of the carcass or failure to find the recovery. All turtles were identified as belonging to the *Caretta caretta* species excepted for 3 specimens (2 *Dermochelys coriacea* and 1 *Chelonia mydas*). Clinical examination findings suggested surgical approach (mainly esophagotomies and/or enterotomies, other techniques) under anesthesia in 141 cases to remove ingested fishing gears. Turtles with compromised intestinal and coelomic conditions, such as intestinal intussusception, volvulus, severe intestinal congestion, intestinal perforations and severe celomitis ensued from a therapeutic

failure. Clinical and post-mortem examinations found ingested foreign bodies (about half of animals with fishing gears followed by plastic debris) as the main cause of stranding of sea turtles on Sicilian coasts. Of the 2 *D. coriacea* recovered already died, a large amounts of various kinds of plastic debris were found in the gastro intestinal tract that led to intestinal lumen occlusion probably for their eating habits.

## Conclusion

Albeit medical treatments and surgical techniques for sea turtles are now very successful, therapeutic success depends on the health condition of the animals during the rescued. The present study shows that these animals were victims of human activities and of environmental contamination and that the poor conditions of the patients at recovery are associated with poor prognosis. Therefore, monitoring the cause of sea turtles standings as a sentinel of the sea status is of great importance both to protect the threatened species and to supervise the environmental contamination.

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# PS104- Potential use of anthocyanins from the red oranges of Sicily

Dugo G.[1]*, Calvagno R.[2], Di Benedetto G.[2], Bernardini R.[2], Cantarella G.[2]
*dugog@unime.it

[1] *Professor of food chemistry, University of Messina, Italy. President of Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), Palermo, Italy.*
[2] *University of Catania Medical School Catania, Italy*

## Introduction

Orange (Citrus sinensis (L.) is a fruit tree belonging to the Rutaceae family. These fruits can be considered a highly healthy food thanks to the presence of anthocyanins, flavonoids, hydroxycinamic acids and vitamin C. The aim of this work was to test the effects of these substances in in vitro and in vivo models of neurodegeneration, by investigating interactions of these compounds with oxido-reductive processes contributing to the pathology of neurodegenerative disorders.

## Method

The polyphenol content was determined using the Folin-Ciocalteu and HPLC methods. For the determination of antioxidant capacity, DPPH and ABTS assays were used. We characterized the molecular action of anthocyanins in neural and/or glial cells in vitro, as well as in rodent models of kindling.

## Discussion

The content of the total polyphenols in the analyzed oranges is included in the 171.9 mg/L range of the Tarocco Gallo and 223 mg/L GAE Tarocco Nucellare. The most represented polyphenols in the 4 varieties were chrysin, pinociambrine, galangine. Moreover, it is also the concentration of Glycoside-Trans-Resveratrol in the Tarocco Nucellare (16.21 ng/ul) and of the Kaempferol in the Tarocco Comune (3.92 ug/ul in the juice). To verify whether the ROC was able to reduce neuronal damage and also that it was involved in the repair processes, we evaluated the expression of nestin, cyclin D1, p53 and p21. The expression of these molecules increased as a result of treatment with ROC at 20 and 40 days, demonstrating that ROC reduces the effects detrimental factors on the nervous tissue, and that, in parallel, it stimulates neuronal repair processes.

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# PS105- Study of total poliphenols, TAA, nutraceutical substances and genetic profiles of Sicilian *Prunus* varieties (cherries and plums)

Dugo G.[1]*, Gottuso V.[2], Oliveri F.[2], Marino F.[2]
*dugog@unime.it

[1] *Professor of food chemistry, University of Messina, Italy. President of Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), Palermo, Italy.*
[2] *Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), Palermo, Italy.*

## Introduction

In this work, samples of *Prunus avium, Prunus salicina and Prunus domestica* were studied. Molecular, biochemical and chemical studies have been carried out to better deepen the knowledge of the Sicilian autochthonous varietal panorama and compare them with varieties of different origins. Antioxidant properties, polyphenols content and genetic profiles were investigated.

## Method

The content of total polyphenols (expressed in mgGAE/100g fresh weight) was determined by HPLC; the total antioxidant activity (TAA/100g) by the method of the ABTS (2,2'-azinobis-3-ethylbenzothiazolin-6-sulphonate) and the method of DPPH (2,2-diphenyl-1-picrylhydrazyl); the content of stilbenes and flavonoids was determined by HPLC-DAD / FLD system and the substances detected by the chromatograms of the sample extracts were recovered. To determine the genetic profiles, the "fingerprinting" technique has been used.

## Results

The results obtained from the analysis of *P. avium* (Graphic 1 and 2) show a higher total polyphenol content in the Sicilian cultivars Napoleona precoce, Dura and Napoleona verifica from Catania and in the Cappuccia from Chiusa Sclafani compared to the reference cultivars Ferrovia and Lapins, also taken in Sicily in the area of Altofonte (Palermo). The molecular profiles obtained from genomic studies were compared to each other in order to verify the diversity and uniqueness of the Sicilian autochthonous varieties. Each primer pair outlines an allelic profile. The comparison of the profiles obtained by the combination of primers, allows the discrimination of the analyzed cultivars

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# PS106- Qualitative analysis of catechin in spray dried green tea (*Camellia sinensis* L.) extract

Aslan Erdem S.[1]*, Isik S. [2]
*sinemaslanerdem@yahoo.com

[1]*PhD/ Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey.*
[2]*PhD/ Department of Analytical Chemistry , Faculty of Pharmacy, Near East University, 99138 Nicosia, Turkish Republic of Northern Cyprus.*

## Introduction

Tea is a pleasant, popular, socially accepted, economical and safe drink that is initially as medicine, later as beverage and well as future potential of becoming an important industrial and pharmaceutical raw material. Tea is the second most commonly drank liquid on earth after water (1). The chemical components of tea leaves include polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), essential oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), inorganic elements (e.g. aluminum, fluorine and manganese), etc. (2). Pharmacological properties of tea are due primarily to its alkaloids (caffeine) and catechins, which are divided into four primary compounds; epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and four secondary compounds, catechin (C), catechin gallate (CG), gallocatechin (GC), and gallocatechin gallate (GCG). EGCG is the predominant catechin present in green tea leaves (48–55% of total polyphenols) (3).

## Method

Samples were dust chamber residues and furnace fibers which obtained from ÇAY-KUR company. 9 gr sample dissolved in 250 ml distilled water and extracted with Soxhlet for 3 hours. After extraction, solvent was evaporated until total volume become 50 ml with rotary evaporator. Standardized green tea extract was obtained from Eastsign Bio-Tech Ltd. company (>90% polyphenol UV, >65% catechin HPLC, >35% EGCGwas used as standard material. Samples were analyzed with Agilent Technologies 1200 series HPLC and separated with Eclipse XDB-C18 column (150 mmx4.6 mm,5µm). For the solvent system; (A): 40Mm formic acid in water and (B): Acetonitrile used at a flow rate of 1 $mL.min^{-1}$. The gradient was initially 90%A; 70% for 18 min; 55%A for 7 min; 80% for 9 min and finally 90%A for 1 min. Analysis time was 35min. and detection wavelength was set at 254 nm. The injection volume was 10 µL for each sample and standard solution. Spray drying process was performed by using BÜCHI Mini Spray Dryer B-290. Different parameters were experimented on spray dryer and the best yield % obtained from 85-90$^{o}$C temp. and 9 mL $min^{-1}$ conditions.

## Results / Discussion / Conclusion

As a result of the spray dryer experiments, % 11.5 + 0.3 yield was obtained. It proves that green tea extract raw material can be produced by using these spray dryer conditions. Also the chromatograms which obtained from HPLC analysis verify that green tea dust chamber residues

and furnace fibers have the catechin varieties. Generally, these green tea residues considered as a waste and it is suggested that these green tea wastes can be evaluated as a raw material.

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# PS107- The use of citrus pulp (*pastazzo agrumario*) in the animal feed: nutritional values and statement

Caracappa G.[1]*, Persichetti M.F. [2], Gambino D. [2], Ristuccia M.E..[1], Gottuso V. [1], Oliveri F. [1,] Dugo G.[3]
*giuliacaracappa@gmail.com

*1 Science4Life ,Via Leonardo Sciascia Coop. Fede Pal.B, 98168 - Messina, Italy.*
*[2] Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via Gino Marinuzzi 3, Palermo (PA) 90129, Italy.*
*3 Co.Ri.Bi.A. Via G. Marinuzzi, 3 - 90129 Palermo - (Presso I.Z.S.), Italy.*

## Introduction

Orange, tangerine, lemon, bergamot, cedar and grapefruit, are citrus fruits belonging to the plant of the genus Citrus, angiosperms of the family Rutaceae. From the transformation of citrus products it's possible to obtain three main products: juice (35 - 45%), essential oil (0,2 - 0,5%) and citrus pulp (55 - 65%). Juice and essential oil are appreciated on the market, instead the citrus pulp is considered a low-value product. The citrus pulp, called *pastazzo agrumario* is made from peel (60-65%), part of pulp (30-35%) and seeds (1). Mainly the *pastazzo* is used in the animal feed. It can be consumed fresh by the animals and after the deposition on the pastures, therefore it can be used in the breeding places near the industries only during the working season. The dried product has many advantages such as an easy and economic way to transport the product over long distances, but also a better taste for the animals (2)(3). Thanks to its characteristics it can be administered in the diet of dairy cattle or beef cattle but also in the feeding of pigs without exceeding 20-25% of the diet (4). It is characterized by a high content of digestible carbohydrates and fibers (5) but with a moderate content of low digestible protein and low energy value (6), for this reason it is necessary a supplementation of protein, vitamin and mineral.

## Method / Results / Discussion

The chemical composition of the *pastazzo* is influenced by various factors such as climatic conditions, different way to cultivate fruits, stage of maturation and variety of the products (7).It consists of insoluble carbohydrates (cellulose, pectin), sugars (glucose, fructose, sucrose), acids (citric and malic acid), lipids (oleic, linoleic, linolenic, glycerol, phytosterol), mineral elements (nitrogen, calcium, potassium), volatile constituents (alcohols, aldehydes, ketones, esters ), flavonoids, limonoids, essential oils, enzymes, pigments and vitamins. (8) (3).
Parameters of orange's *pastazzo*:
organic carbon (% s.s.) 45,4
total N (% s.s.) 1,2
P (% s.s.) 0,13
K (% s.s.) 0,91
Ca (% s.s.) 0,77
Mg (% s.s.) 0,09
Na (% s.s.) 0,13
Cu (mg/kg) 9,3

Fe (mg/kg) 80,0
Zn (mg/kg) 8,4
Mn (mg/kg) 5,6
pH (on a wet basis) 3,70 Wet (% on a wet basis) 83,6
Every year the citrus industry produces over 700 thousand tons of citrus waste, 340 thousand only in Sicily. Such a big value rappresents a big problem in terms of disposal, at least until 2013. The *pastazzo* is today defined as a by-product of the citrus fruit industry obtained in compliance with the provisions of art. 12, paragraph 1, let. a) of Legislative Decree 205/2010. Citrus peels are clearly and explicitly defined in terms of both agricultural products and zootechnical products (Legislative Decree 360/99).
However, in the past years according to Legislative Decree 22/97 (Ronchi Decree in Implementation of Waste Directives 91/156 / EEC, 91/689 / EEC on hazardous waste and 94/62 / EC on packaging and packaging waste) *pastazzo* was considered a waste and it required a special disposal under conditions of safety and traceability, causing considerable increases in costs and bureaucratic procedures. In the past years, the use of *pastazzo* in agriculture, assimilating this product to a refusal, was condemned, since it was considered an abusive recovery of a special waste that, instead, it should have been dispose in the way it is. Legislative Decree 152/2006, amended by Legislative Decree 4/2008, made a distinction between "waste" and "by-products" compared to the previous one. The Sicilian Region, with the note nr. 14843 of 01.03.2012 of the Assesorato Regionale delle Risorse Agricole e Alimentari, gave specific instructions using the by-product as animal feed and agricultural use. Law 98/2013, art. 41-quarter, declared the *pastazzo* as a by-product of the processing of citrus fruits for agricultural and animal feed, removing it definitively from the waste discipline.

## Conclusion

It is now well established that the *pastazzo* is not a waste because it is defined as a byproduct of citrus fruit and it can be used without any issue. Considering the multiplicity of use of the *pastazzo*, it would be desirable that, especially in areas with a high citrus fruit vocation, the operations of collection and conferment to the processing and / or utilization plants, will be facilitated. It would be desirable too that the implementing decrees foreseen by art. 41-quater of the Law 98/2013 will be issued, which should contain precise provisions allowing the production, marketing and use of *pastazzo* as a by-product of the processing of citrus fruits for agricultural and zootechnical use. In this way can be definitively subtracting it from the waste discipline, as well as establishing qualitative and quantitative criteria for the use of substances produced during citrus processing.

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# PS108- Characterization of eight chicken meat preparations by sensory evaluation

Leonardi M.*; Potortì A.G.; Rando R.; Albergamo A; Lo Turco V.; Gervasi T.; Bartolomeo G.; Dugo G.
*leonardim@unime.it

*Biomorf Department, University of Messina, Messina, Italy.*

## Introduction

Chicken meat preparations include a large range of ready-to-cook products, which typically consist of whole meat, meat reduced to fragments or minced, stuffed with a variety of vegetables, or flavored with spices and/or aromatic herbs as well. For the chicken meat industry, a reliable sensory evaluation of these preparations is very important to define and guarantee their shelf-life, and eventually to extend it by improving some aspects of the manufacturing process [1-3]. Therefore, the aim of the present work was to describe the sensory characteristics of eight chicken meat preparations (namely, traditional chicken roulade, traditional chicken small rolled, rustic skewer, chicken sausage, classic chicken burger, chicken burger with spinach, chicken roll with ham and classic chicken roast) by a panel of trained assessors in order to (1) improve the shelf-life of each product by reporting possible sensory defects; (2) evaluate the evolution of sensory descriptors during the pre-established shelf-life; (3) verify the repeatability of the manufacturing process by performing the sensory analysis every 15 days.

## Method

According to the ISO guidelines[4], eleven assessors (6 females and 5 males; aged between 33 and 55), recruited among the staff of the University of Messina were involved in the study. All assessors were familiar with sensory evaluation procedures[5]. Before to proceed with the sensory analysis, a panel discussion allowed to generate nine different sensory attributes. For each descriptor, a continuous scale with scores ranging from 0 to 8 was considered. Then, the selected raw products were assessed by a starting visual and odor inspection, and right after they were cooked according to the label indications, to be evaluated according to the predetermined descriptors.

Such procedure was applied two times, namely at the production date and at the expiry date (after eight days). In both cases, the same lot was considered for each product.

All sensory analyses were performed on products characterized by intact packages and previously at 4°C. The study period lasted 10 months, and a total of 120 sensory evaluations were carried out.

## Results / Discussion / Conclusion

Results from sensory analyses highlighted differences among the eight chicken meat preparations tested, but, overall, all products have obtained good remarks. After 8 days of storage, the appreciation was lower. All products were improved from a manufacturing point of view according to the panelist judgments and suggestions, so that product development and innovation

was achieved. Also, this study could provide insights into the recent consumer preferences concerning chicken meat preparations.

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# PS109- Unravelling metabolic plasticity of *Glycyrrhiza glabra* leaves DOP calabrese by chemical profiling

Celano R.[1], Docimo T.[2], Piccinelli A.L.[1], Cannistrà C.[3], Massarotto A.[4], Fuda S.[5], Rastrelli L.[1], Russo M.[5]

*1Department of Pharmacy, University of Salerno;*
*2 L'incanto Sarl, Catanzaro;*
*3 Hôpital Bichat - Claude-Bernard, Paris, Francia;*
*4 Naturemed srl, Cosenza;*
*5 Food Chemistry, Authentication, Safety and Sensoromic Laboratory – Università Mediterranea di Reggio Calabria*

## Introduction

Quality, aroma and flavor of Calabrian licorice roots are worldwide recognized, although it's worth noting that aerial part of this plant , usually treated as waste product, represent a surprisingly source of bioactive metabolites. Indeed distribution of metabolites depicts a peculiar biosynthetic and developmental plasticity. In this work we report the chemical profiling of surface and inner leaf content.

## Methods

Licorice aerial parts were harvested in pre flowering and flowering time. Leaves, panicles and flowers in flowering periods were collected dried and were ultrasound extracted with EtOH /H2O 70% three times. Vacuum dried samples were reconstituted in MeOH/H20 1:1 for further qualitative analysis by UHPLC system. Exudates from outer licorice leaves was performed by ETOH brief immersion for 10 sec and vacuum dried , samples were analized as above.

## Results / Discussion / Conclusion

UHPLC-ESI-HRMS analyses were performed in negative and positive ionization mode to obtain complementary information useful for characterizing the extracts of leaves, exudates, panicles and flowers in flowering periods. Metabolite assignments were made comparing UV/Vis, HRMS and MS/MS spectra with reference standards when available, or with chemo-taxonomic data reported in the literature and databases. The UHPLC-DAD-HRMSn analysis has allowed to identify 46 compounds belonging to two major classes of secondary metabolites: dihydrostilbenes and flavonoids. The dihydrostilbenes have the same central scaffold with different functional groups on the two aromatic rings: hydroxyl, methoxy and prenyl units. The flavonoids are glycosilated derivatives of quercetin, kaempferol, myricetin and naringenin. All extracts showed UHPLC-UV profiles qualitatively comparable except for exudate, which showed dihydrostilbenes exclusively.
Licorice aerial parts are a very promising reservoir of bioactive molecules for plant itself and humans From an ecological perspective prenilated flavonoids in the outer leaves part represent a more efficient plant protection against insects. These molecules along with inner leaf content of glycosilated flavonoids augment flavonoid bioactivity by increasing membrane affinity and improving the interaction with target proteins.

# PS110- Tropea Onion skin: from a waste product to new valuable matrix for bioactivity

Celano R.[1], Docimo T.[2], Piccinelli A.L.[1], Campone L.[1,4],Cannistrà C.[3], Di Sanzo R..[4], Carabetta S.[4], Rastrelli L.[1], Russo M.[4]

[1]*Department of Pharmacy, University of Salerno;*
[2]*L'incanto Sarl, Catanzaro;*
[3] *Hôpital Bichat - Claude-Bernard, Paris, Francia;*
[4] *Food Chemistry, Authentication, Safety and Sensoromic Laboratory – Università Mediterranea di Reggio Calabria*

## Introduction

The red onion from Tropea is known for its sweetly taste**,** which is mainly due to the flavonoids and the alk(en)yl cysteine sulphoxides (ACSOs) compounds. The unique red onion envelope usually considered a waste product, represent a valuable source of biomolecules therefore accurate chemical characterization of this matrix is a fundamental prerequisite for a more sustainable and productive use in pharmaceutical, cosmetical and nutraceutical formulations.

## Methods

The outer layers of red skin onion bulbs were collected, dried and finely powdered. Exhaustive extraction of dried materials was performed by ultrasound-assisted extraction (UAE) with aqueous ethanol (70% v/v). A UHPLC-HRMS/MS method was developed and then applied to analyze the onion-skin extract.

## Results / Discussion / Conclusion

The chemical composition of onion-skin extract was determined by UHPLC-DAD-HRMS analysis. Metabolite assignments were made comparing UV/Vis, HRMS and MS/MS spectra with reference standards when available, or with chemo-taxonomic data reported in the literature and databases. The main compounds detected in the extract belonging to two major classes of secondary metabolites: anthocyanins and flavonols. The main flavonols found in this onion type were quercetin and quercetin glucosides, isorhamnetin glucosides, kaempferol glucoside, and, among anthocyanins, cyanidin glucosides. This extract has shown a high antioxidant activity. The activity antioxidant was measured by DPPH, ABTS and ORAC assays.
The obtained results have shown that the onion-skin are a promising and cheap source of bioactive compounds with high antioxidant activity. Furthermore, their use as a substitute for synthetic antioxidants, in addition to increasing food security, would contribute greatly to increase their added value.

# PS111- Application of innovative analytical methods for ensuring the authenticity of organic horticultural crops

Fabroni S., Timpanaro N., Amenta M., Romeo F.V., Ballistreri G., Rapisarda P.*
*paolo.rapisarda@crea.gov.it

*Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria - Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura*

## Introduction

In recent years there has been a growing demand for organic products as consumers consider them as safer and healthier than conventional products. Various researches have been carried out over the years to investigate nutraceutical quality and authenticity of products obtained using organic cultivation methods. Some studies focused on the content of ascorbic acid, phenolics, total sugars and antioxidant compounds of organic products [(1).] Other have been directed to identification of new quality "markers" that allow to differentiate, from field to fork, the organic product respect to the conventional one [(2)]. In particular, the monitoring of some chemical components, deriving from primary and/or secondary metabolism of organic and conventional products, has highlighted the diversity induced by the two production techniques. It is now well known that the difference in fertilization practices of the two cultivation methods influences the isotopic distribution of some elements present in fruits and vegetables, with particular reference to nitrogen. The possibility of using the $^{15}N/^{14}N$ ($\delta^{15}N$) isotopic ratio as a screening tool for the differentiation of biological products from conventional ones is based on the fact that $\delta^{15}N$ values of synthetic fertilizers are close to 0 ‰, thus determining higher $\delta^{15}N$ values of nitrogen metabolites of the organic crops respect to those of crops obtained by conventional agronomic practices.

## Method

The INNOVABIO ('Application of innovative methods for the traceability of organic farming products') research project (Italian Ministry of Agricultural, Food and Forestry Policies n. 93173/12/22/2017) aims to improve the understanding of the factors influencing the food quality of organic horticultural products, with particular reference to their differentiation respect to conventional ones and to the traceability from field to fork. The aim of the project is the implementation, for biological horticultural crops [tomato, fennel and cauliflower in three different production areas, i.e. Vittoria (RG), Metaponto (MT) and Monsampolo del Tronto (AP), of a chemometric system that allows, through the acquisition of isotopic data and nutraceutical parameters, to discriminate between productions obtained with synthetic fertilizers, usually employed in conventional agriculture and not allowed in organic farming, and productions obtained using the organic cultivation method which involves only the use of organic fertilizers and the application of agronomic methods for soil fertility such as crop rotations and the introduction of agroecological service crops and legume species.

**Results / Discussion / Conclusion**

Horticulture has the advantage, compared to fruit growing, of not having pluri-annual influences; for this reason, this research will give more immediate results than pluri-annual cultivation systems. Multivariate analysis of isotopic data ($\delta^{15}N$) and total nitrogen, inorganic nitrogen ($NO_3^-$, $NH_4^+$, combined with nutraceutical quality parameters (macro and micro elements, ascorbic acid, total polyphenols and antioxidant activity) will give the possibility to discriminate on the basis of the employed nitrogenous source.

**Bibliographic references**

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