





Center of Excellence on Neurodecenerative Diseases

BRVR

Il CEND incontra le Scuole Superiori in occasione della Settimana del CERVELLO

Lunedì 24 febbraio

Inaugurazione

ore 10 Saluto del Prorettore vicario Giorgio Metta ore 11.30 Diego Fornasari

<u>Le malattie Neurodegenerative</u> ore 15 Vincenzo Silani

Martedì 25 febbraio

IOLe nuove ricercheore IOStefano Gustincich

Aula Magna - Via Festa del Perdono, 7 Maria Daniela Candia Costruendo l'intelligenza per robot umanoidi Il circuito del piacere e la dipendenza da droghe

4

Aula 200 - via Celoria 20 - edificio 3 La neurodegenerazione e le sue malattie

Sala Levi - Via Valvassori Peroni 21 La genomica funzionale nello studio delle malattie neurodegenerative

ore 11:30	Piero Paolo Battaglini	Interfacce cervello-computer: principi di base e prospettive		
	<u>ll cervello</u>	Aula 200 - via Celoria 20 - edificio 3		
ore 14:30	Fabrizio Gardoni	Sinaptopatie: cosa succede quando si ammalano le sinapsi?		
1ercoledì 26 febbraio	<u>Il comportamento del cervello</u>	Aula Magna - Via Festa del Perdono, 7		
ore I0	Alice Mado Proverbio	l Neuroni specchio		
ore 11:30	Abutalebi Jubin	II bilinguismo		
ore 15	<u>La psichiatria</u> Raffaella Molteni	Aula 200 - via Celoria 20 - edificio 3 Ansia e stress: un equilibrio tra normalita' e patologia		
Giovedì 27 febbraio	<u>Chirurgia cerebrale e dipendenze</u>	Aula Magna - Via Festa del Perdono, 7		
ore I0	Lorenzo Bello	Chirurgia funzionale cerebrale coadiuvata da		
ore 11:30	Diego Fornasari	immagini, neurofisiologia e neuropsicologia Il circuito del piacere e la dipendenza da droghe		
ore I5	<u>La psichiatria</u> Daniela Tardito	Centro Congressi Corridoni –Via Corridoni, 16 I disturbi dell'umore: biologia e terapia		
/enerdì 28 febbraio ore 10 ore 11:30	<u>Ricerca e qualità della vita</u> Pietro Calissano Enzo Nisoli	Centro Congressi Corridoni – Via Corridoni, 16 Rita Levi Montalcini: vita e ricerca Alimentazione, stili di vita e malattie		
ore I5	<u>La biologia</u> Alessandra Colciago	Centro Congressi Corridoni – Via Corridoni, 16 Anche il cervello ha un sesso? Basi biologiche della differenziazione sessuale		
Con il patrocinio di: RegioneL	Sombardia Provincia di Milano	Milano per informazioni: Milano Komune Rossana Notarantonio Tel. 0250318290 Centro.eccellenza@unimi.it Milano Image: Comune Image: Comune Milano Image: Comune Image: Comune Milano Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune Image: Comune		



Allegato al Comunicato stampa

GIORNATA MONDIALE PER IL CUORE 2014 AL CENTRO CARDIOLOGICO MONZINO CON IL PATROCINIO DEL COMUNE DI MILANO - CONSIGLIO DI ZONA 4

PROGRAMMA DI SABATO 27 SETTEMBRE

Ore 10.00 "Allenati nel verde" costituisce un invito alla popolazione a partecipare ad un momento per lo svolgimento in gruppo di **attività fisica** presso il nuovo parco di Via Vittorini, in cui è stato allestito un percorso salute permanente con la consulenza degli esperti in prevenzione del Centro Cardiologico Monzino.

Con la guida di allenatori volontari del Centro Sportivo Italiano, i partecipanti potranno eseguire esercizi ritmici a corpo libero e imparare l'utilizzo appropriato dei diversi attrezzi sportivi che compongono il percorso salute del parco.

Ore 12.00 Saluti delle Autorità

Nell'Aula Magna del Centro Cardiologico Monzino interverrà **l'Assessore alle Politiche sociali e Cultura della Salute** del Comune di Milano, **Pierfrancesco Majorino.**

A seguire:

"Pranzo Multietnico al Monzino": rappresenta un modo per integrare in forma attiva e concreta, attraverso la cucina e l'alimentazione sana, le differenti culture ed etnie esistenti nella realtà locale. Il menu, disegnato da una nutrizionista esperta in prevenzione cardiovascolare del Monzino, in collaborazione con cittadini di diversa nazionalità e cultura residenti nel Quartiere di Ponte Lambro, sarà offerto ai presenti gratuitamente.

Ore 14.00 "Un nuovo spazio per Te": apertura del Poliambulatorio di via Serrati Menotti 12, un progetto sociosanitario condiviso con il Comune di Milano. Oltre ad altre attività di prevenzione (odontoiatrica, psicologica, ecc...), al nuovo Poliambulatorio sarà avviato il **Programma di Salute Cardiovascolare** che intende coinvolgere la popolazione locale in iniziative di educazione alla salute e in attività di screening e controllo dei fattori di rischio cardiovascolare.

Le iniziative sono aperte a tutti i cittadini e gratuite Per organizzare la giornata al meglio è gradita la prenotazione: <u>eventi@ccfm.it</u> o 339 7275532



EC project number 245199

Pretoria Symposium raises the value of plant-based health products

On March 5th, 2014 took place in Pretoria, South Africa, the PlantLIBRA Symposium on: "Plant Foods and Food Supplements: Quality, Safety, and Regulation Across the Value Chain") organized by SISTE in collaboration with CSIR (Council for Scientific and Industrial Research), PlantLIBRA local partner. The event has been a good and interesting occasion of discussion on quality and safety use of botanical base products and their regulation across the value chain.

Participants of the symposium were from 16 local and international institutes and organisations. The symposium has been an important opportunity for disseminating the results obtained by the PlantLIBRA project in the field of quality and safety of plants and final products through the development and the optimization of new analytical methods capable of correctly identify the plant species or the presence of natural compounds of some concern, unintentional contaminants, or fraudulent adulteration with other plants and/or the addition of illegal substances.

The main topics of the seminar, that received the patronage of the Department of Science and Technology of the Republic of South Africa, had been: aspects concerning the quality of plant raw materials used in plant food supplements (PFS) and differences in the regulatory framework of health products in Europe and South Africa. Newsletter

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Website: www.plantlibra.eu

This project has received from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° [245199]

This report does not necessarily reflect the Commission views or its future policy on this areas.





DALU MANCAMA explained that CSIR's role in the project had been the development of in vitro-based assays to assess risks and benefits associated with priority plant-based botanicals used in food supplements, as well as identify the chemically fingerprinting of the compounds contained in such products. Key issues such as levels of pesticides found in the material have also been investigated. CSIR has also contributed to the

development of the ePlantLIBRA database, which represents the largest database containing validated scientific information on Plant Food Supplement bioactive compounds.

Patrizia RESTANI of the University of Milan, scientific coordinator of PlantLIBRA, and Johannes NOVAK of the University of Vienna, explained the important PlantLIBRA activity in developing new analytical DNA-based methods to identify plants in raw materials and PFS, and traditional or advanced analytical techniques (HPLC, HPTLC, mass spectrometry, ecc.) to detect contaminants and adulterations.



Dalu MANCAMA showed the results obtained by CSIR to develop and optimize chromatographic methods to characterize the phytochemical profile of some plants such as Harpagophytum procumbens (Burch.) DC. Ex Meisn., a plant native to South Africa, Namibia, and Botswana, Boswellia serrata Roxb. Ex Colebr and Valeriana officinalis L. Analytical methods such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry



The CSIR is one of the leading scientific and technology research, development and implementation organizations in Africa. Constituted by an Act of Parliament in 1945 as a science council, the CSIR in South Africa performs multidisciplinary research and technological innovation with the aim of contributing to industrial development and the quality of life of people of this country, and to improve national competitiveness in the global economy. Science and technology services and solutions are provided in support of various stakeholders, and opportunities are identified where new technologies can be further developed and exploited in the private and public sectors for commercial and social benefit.

The CSIR's shareholder is the South African Parliament, held in proxy by the Minister of Science and Technology.

(LC-MS/MS) have been set up by CSIR to detect residues of pesticides in plant raw materials. CSIR is working to develop in vitro assays to evaluate antimicrobial and antiviral effects of plant extracts.

In the afternoon session of the symposium Patrizia *RESTANI*, on behalf of Paul FINGLAS (Institute

of Food Research, UK) showed the development and the potential applications of the ePlantLIBRA Database for Plant Food Supplements.

The closing remarks were made by Marinella TROVATO (SISTE, Italy) and Alice SIGOBODHLA of the Department of Health Medicines Control Council (South Africa), who explained the main differences in the legislative framework of plant food products in Europe and South Africa.

The symposium provided several points of interest which gave rise, at the end of the day and during the breaks, to a lively debate among researchers, authorities and companies in the sector of the plant food products, on the various topics dealt.



















Pictures of PlantLIBRA Pretoria Symposium









NEWS FROM THE PROJECT

Data collection on the use of plant food supplements (PFS) contributes to the assessment of their risks and benefits



The survey fieldwork data collection for the estimation of PFS (Plant Food Supplements) has been completed and the data were finally published in *PLOS ONE* (eISSN-1932-6203), an important international, peer-reviewed, open-access, online publication. *PLOS ONE* features reports of original research from all disciplines within science and medicine. Below is the press release sent to scientific journalists associations and press agencies to communicate and broadcast the publication of the article.

PRESS RELEASE

Although PFS are popularly consumed products, currently there is still no specific Community legislation on their regulation, therefore, their safety and efficacy are still questioned

PFS consumption data from the consumers' perspective at European level were until now nonexistent. This is the first European survey on the use of PFS among consumers of these products

Barcelona, 19th March 2014 - The first European survey on consumption of plant food supplements (PFS) - led and coordinated by researchers at the Fundación para la Investigación Nutricional (FIN - Foundation for Nutritional Research) from the Parc Científic de Barcelona in Spain, and whose results have just been published in the journal PLOS ONE -, concludes that **the evaluation of the consumption of these products, especially at European level, is as complex as necessary.** Researchers recommend that **future national nutrition surveys in Europe collect data on the consumption of plant-derived products in order to facilitate the assessment of their risks and benefits in the short and long term.**

The survey was conducted as part of the project "PlantLIBRA" (acronym for "PLANT Food Supplements: Levels of Intake, Benefit and Risk Assessment" (www.plantlibra.eu)), co-financed by the European Commission within the European Union's 7th Framework Programme. PlantLIBRA is a 4-year project involving a Consortium of 25 partner centres from 4 continents and whose aim is "to promote the safe use of PFS". The survey was conducted by 6 partner centres from the 6 European countries in which it was conducted: Finland, Germany, Italy, Romania, Spain and the United Kingdom. Fieldwork lasted over 15 months, from May 2011 to August 2012. Data were collected from 2359 PFS consumers residing in 24 European cities (4 per country) through a questionnaire of 58 questions completed during personal interviews. Study results have provided data to assess the socio-demographic profile of PFS users, the usage patterns of these products, the actual products consumed and their botanical ingredients.

According to **Alicia García-Álvarez** - Researcher at FIN and coordinator of the survey -, "the survey was conducted using the same methodology in the 6 countries, which allows us to compare the data obtained and bring to light information about the consumption of these products that to date was nonexistent or not comparable. We now know for example, that in these countries a wide variety of PFS from a wide variety of manufacturers and distributors is consumed and that the 83.7% of respondents consume only 1 product, and 51.5% of products usually contain a single botanical ingredient".

In addition, **Lluís Serra-Majem** - Chairman of the FIN, Professor at the IUIBS-ULPGC and CIBER OBN, and responsible for the survey -, explains that "through this survey, 491 different botanical ingredients contained in the PFS consumed have been identified. Global Survey raw data show that the 10 botanical ingredients most frequently consumed in PFS (i.e. by more than 100 consumers) are in descending order: *Ginkgo biloba* (ginkgo), *Oenothera biennis* (evening primrose), *Cynara scolymus* (artichoke), *Panax ginseng* (ginseng), *Aloe vera* (aloe), *Foeniculum vulgare* (fennel), *Valeriana officinalis* (valerian), *Glycine max* (soybean), *Melissa officinalis* (lemon balm), *Echinacea purpurea* (echinacea) and *Vaccinium myrtillus* (bilberry), this order changes if we analyze each country separately, for example, in Spain the most consumed PFS botanical ingredient is *Cynara scolymus* (artichoke).

Other main results include:

- A total of 1288 different products were reported across the six countries, with the highest proportion of different PFS being used in Italy and Spain. In the United Kingdom, the number of different products was approximately half that of the other countries
- 22.2% of participants said that they use PFS when experiencing a 'flare up or worsening of a condition'
- The most common dose forms were capsules and pills/tablets/lozenges

Moreover, **Monique Raats**– Director of the Food, Consumer Behaviour and Health Research Centre at the University of Surrey in the United Kingdom, one of the survey's partner centres-, points outs that "this survey provides us with a much clearer understanding of consumer behaviour, giving us data on the numbers of products/ingredients being consumed, details of forms of supplements consumers are taking".

From a more global perspective, **Patrizia Restani** - from the Dipartimento di Scienze Farmacologiche e Biomolecolari (Department of Pharmacological and Biomolecular Sciences) at the University of Milan in Italy, Coordinator of the project PlantLIBRA and partner centre of the survey -, adds that "having consumption data for these products (i.e. what products are consumed, how and by whom) will help assess the risks and benefits of PFS and their botanical ingredients, which in turn will facilitate the decision-making by regulators and the industry. Our work can be used by other groups who want to contribute to the pool of knowledge in this field".

Over a decade ago, there were problems with the European legislation regulating these products, problems that were linked to the lack of full harmonization of Directive 2002/46/EU (particularly for the plants that can be used in these products, since current legislation varies across Europe, with significant differences in the botanical species permitted in PFS), and the lack of adequate methodologies to assess their safety and efficacy. PFS fall under the general classification of "food supplements" and, as such, may have a physiological effect on the human body for maintaining wellness and normal functionality. However, there are too many questions about their safety, efficacy and consumption, hence the need for a project such as PlantLIBRA and an initiative such as the "PFS PlantLIBRA Consumer Survey".

"Usage of Plant Food Supplements across Six European Countries: Findings from the PlantLIBRA Consumer Survey". **Authors:** Alicia Garcia-Alvarez, Bernadette Egan, Simone de Klein, Lorena Dima, Franco M Maggi, MerjaIsoniemi, Lourdes Ribas-Barba, Monique M Raats, Eva Melanie Meissner, Mihaela Badea, Flavia Bruno, Maija Salmenhaara, Raimon Milà-Villarroel, Viktoria Knaze, Charo Hodgkins, Angela Marculescu, Liisa Uusitalo, Patrizia Restani, Lluís Serra-Majem. PLoS ONE 9(3): e92265. doi:10.1371/journal.pone.0092265

Available at PLOS ONE: http://dx.plos.org/10.1371/journal.pone.0092265

Analytical control of contaminants and residues - heavy metals, mycotoxins and pesticides

Hazardous chemical contaminants or undesirable substances in food and feed are often unavoidable. The topic is of major interest for EFSA and especially for **the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel)** that carries out risk assessment on



contaminants in food and feed. CONTAM Panel relies on scientific information that is in the public domain. EFSA often launches calls for data on occurrence of contaminants in food and feedstuffs in order to update the knowledge in this field and to identify some possible gaps to be solved by scientific researchers.

The possibility to have reliable detection methods for contaminants and residues is one of the major aim and priority for the research community working in this field.

One of the tasks of the PlantLIBRA project has as its principal aim the development and testing of methods for contaminants and residues determination and production of new data. The partnership divided the work, based on their available methods and expertise on these fields. Some of the planned activities have been already finalised and some others are still on-going. Based on findings of previous literature data search, priority compounds with missing data and methods were selected. During the project implementation, known and novel methods are developed and tested, establishing performance criteria. An approach to screening on raw material will be also proposed. The list of elaborated analytical methods has been reported and will be included in the project database.

Scientific literature indicates several analytical methods for detection of contaminants and residues from different biological matrices as: HPLC, ELISA, enzymatic methods, AAS, sensors, biosensors, Surphace Plasmon Resonance (SPR). The multidisciplinary team from UTBV contributed to apply

already known methods and to develop new (bio)sensor and SPR models for detection of heavy metals, pesticides and mycotoxins. It is important to mention the involvement of a research group from UMIL (coordinated by Prof Patrizia Restani) and other former collaborators from Perpignan (France) and Bucharest (Romania). The analytical device requests the presence of recognizing sensing layer being in



an intimately contact with the sample (analyte) and with a transducer that converts the physicochemical changes occurred after the specific interaction into a measurable signal. Biorecognition use the presence of enzymes, antibodies, aptamers - single-stranded nucleic acid RNA and DNA molecules (ca.30 to 100 nucleotides) that recognize specific and with high affinity several ligands, molecular imprinted polymers (MIPs). The biomolecules are immobilized on the surphace of the working electrodes or, for some other examples, are part of the columns used for cleaning procedures of several extracts before to perform the analytical method. Accuracy, sensitivity, specificity, stability, speed of response, the possibility to be reused (sometime after reactivation), low cost and the possibility to be used on the labs or directly on the field (telemonitoring) are some of the advantages for using (bio)sensors in different analysis. For complex samples there are recommended multiple, simultaneous and specific detections, followed by a specific mathematic calculation using artificial neural network

Square-Wave Anodic Stripping Voltammetry (SWASV) methods, using Bismuth Film modified carbon screen-printed electrodes (BiF-SPE) were developed for cadmium and lead detection.

Acethylcholinesterase-based biosensors were characterized for detection of heavy metals and organophosphorus pesticides. There were used different types of enzyme (commercial from Electric eel or mutants of AChE –Drosophila melanogaster-B394), different immobilization methods, different pH of analysis, different incubation times. Some of the studies are still on-going.

Other new point is to the use of Surphace Plasmon Resonance (SPR) as possibility of mycotoxin



detection, having also some important advantages as: measurement of interactions on a surface, realtime measurement of biological system, no special labeling required, non-invasive optical measuring technique, high quality data, simple and flexible assay design, robust and reproducible. The sensor and experimental conditions need to be optimized for stable and sensitive detection of ochratoxin A.

An amplification strategy has to be developed in order to improve the sensitivity of detection and to be able mitigate the solvent effect when real samples.

Part of the studies and obtained results were published on scientific journals (or are in press) and were communicated in the frame of international conferences/congress/symposium. Some of the final results will be communicated in the frame of Central and Eastern Europe Conference on Health and the Environment (CEECHE), Cluj-Napoca, Romania (25th-30th May, 2014) and New Trends on Sensing- Monitoring-Telediagnosis for Life Sciences, Brasov, Romania (24th-26th July, 2014).

This article is a brief summary of the work of UTBV, Universitatea Transilvania din Brasov (Romania) for PlantLIBRA project and of the results presented by Prof. Mihaela Badea at the training course on "How to monitor the quality of plants for food supplements" in Milan, last 11th February 2014.

News from the ePlantLIBRA database

The ePlantLIBRA database is progressing well in its task of becoming a sustainable, reliable, flexible and fit-for-purpose internet-deployed database, which will provide a unique comprehensive resource on PFS for researchers, health professionals, health educators, the food industry and policy makers.

The types and quantities of information included in the database are highlighted below:

- BENEFICIAL BIOEFFECTS DATA covers 33 plants/PFS, a systematic literature search provided us with 220 references which have been evaluated and entered into the database covering the period 1970 2010 prioritising the following important health areas:
 - Cardiovascular health
 - Post-menopausal bone health
 - Menopausal symptoms
 - Gastrointestinal health
 - Inflammation

ADVERSE EFFECTS DATA has been included following a literature search covering 67 plants, ranked for frequency of adverse effect, we have included papers with the highest causality assessment according to the WHO guidelines: certain and probable. This has led to 210 case studies on adverse effects from 41 plants being entered into ePlantLIBRA.

- COMPOSITION DATA quality assessed data on 80 plants/PFS has been included from over 250 peer reviewed publications producing over 5000 data points, on 300 individual compounds.
- CONTAMINANTS DATA a system have been set up for transfer of MoniQA contaminants data, with an additional output developed from the HorizonScan system to deliver examples of contaminant, residue or other issues being found globally in traded food plant sources used to derive PFS.
- ADDITIONAL INFORMATION includes general information on PFS, including claimed effects, target groups, contraindications and legislation. New information on plant names, synonyms and pictures for botanical ingredients of PFS. To increase the value of the database to users, links and information on grey literature, books and related databases have been included.

Users of the database are wide ranging including Food Authorities, Industry, Policy makers, Researchers and Risk Managers. Examples of use could include:

- **safety assessment of botanicals** for use in PFS
- **estimating exposure to bioactive compounds** from PFS in population groups

The ePlantLIBRA database has recently been presented and demonstrated at a number of conferences and will be presented at the PlantLIBRA International PlantLibra Conference, 12-14 May 2014, Vienna, Austria.

Sustainability plans for ePlantLIBRA are being discussed and finalised to ensure we:

- Meeting users' needs and requirements
- Involvement of experts and continuous updates on new data
- Appropriate dissemination and promotion including launch
- Develop membership model, pay-per-access & income
- Agree on continual access to include MoniQA data

Acknowledgements to Jenny Plumb (IFR) for this contribution. For information on access to ePlantLIBRA contact: **fdnc@ifr.ac.uk** and/or **secretariat@eurofir.org**.

NEWS FROM EFSA

EFSA – Nomination of the next Executive Director

The European Food Safety Authority's Management Board has nominated Dr **Bernhard Url** as EFSA's next Executive Director.

Dr Url, up to now EFSA's acting Executive Director, was one of the candidates shortlisted by the European Commission after an open competition, and who were interviewed by EFSA's

Management Board for the post. As part of the appointment procedure, Dr Url has made a statement before members of the European Parliament's Environment, Public Health and Food Safety (ENVI) Committee on 14 April, 2014 in Strasbourg, France.



The European Parliament will subsequently submit its opinion on the appointment to the European Commissioner for Health and Consumer Policy, Tonio Borg, and to the chair of EFSA's Management Board, Sue Davies, which is taken into consideration before the Board makes its final decision on the appointment.

LATEST EVENTS

PlantLIBRA Policy Advisory Board (PAB) meeting



On 18-19 March, the fourth meeting of the PlantLIBRA Policy Advisory Board (PAB) took place in Brussels. The meeting was organised by Work Package 10 and hosted by the Belgian Ministry of Health (Federal Public Service). It was attended by 20 representatives from the EU Member States, participants from the Chinese and Argentinean authorities, 13 PlantLIBRA partners and 5 invited speakers, including EFSA.

The meeting focused on the achievements of the PlantLIBRA project with presentation by the various work packages on the latest results and discussions on the policy implications already identified. Invited speakers covered aspects of EFSA work on botanicals, tradition of use, the development of botanical monographs by ESCOP and various aspects of quality. PAB members contributed with updates on regulatory developments in their respective countries both on national and international level.

Key aspects raised in the discussions included:

- The complexity and specificity of the area and the different regulatory approaches observed between countries and regions of the world.
- The shortage of expertise globally on which regulators can rely for the technical and scientific aspects involved.
- The strong request for harmonisation and if it would not be possible to agree on negative or positive lists on the basis of the safety data available and the work undertaken by Member States. During discussions, the positive list drafted within the BELFRIT project (an initiative of Belgium, France and Italy) was mentioned as good start in this direction.



 Concern relating to quality of botanical preparations and in particular the sales of unregulated products via internet. The main policy implications identified by WP10 were highlighted:

- The need for more investments of time, energy and resources in product quality, with the strengthening of the network of research and commercial laboratories.
- Clinical research in botanicals would ideally be needed but due to expense it is unrealistic that the volume of work could be funded and carried out.
- For many plants there is a lack of data. The PlantLIBRA list of plants could be a starting point for policy makers to identify priorities for risk assessment.
- There is a need to build on the experience of those Member States that have started implementing nutri-vigilance and eventually move to a EU-wide system.

Dieter Brigitta, the European Commission PlantLIBRA project officer, concluded that the area of botanicals is a complex, complicated and multidisciplinary subject in terms of health benefits, safety and quality and expressed that the efforts in this area from the project demonstrate that the Commission took the right decision in validating the call for the project five years ago. He emphasised that the results show that resources were well-spent and encouraged all participants to continue using the time and resources during the remaining months wisely.

A session on quality will be part of the final PlantLIBRA meeting in Vienna.

Acknowledgements to Patrick Coppens (EBF) for this contribution

PlantLIBRA Milan Training course: the quality of plant raw materials for Food Supplements.



On February 11th 2014, it was held in Milan (Italy) the PlantLIBRA Training course on the subject: "*How to monitor the quality of plant food supplements*". Organized by SISTE and University of Milan in the context of the dissemination activities of the PlantLIBRA project n. 245199 and hosted in the splendid Sala Napoleonica (Palazzo Greppi) of the University of Milan, the course received the patronage of Milan EXPO 2015 for the universities from the municipality of Milan.

This training course is part of a series of events organized by PlantLIBRA following the success and interest shown by the participants of the Vienna conference on the quality of plants for food supplements, on July 11th 2013. Milano was chosen as a venue for economic and organisational reasons and because Italy is the European country with the largest number of companies that produce food supplements or that sell herbs intended to use in this kind of products, and for the large number of SMEs. The next event will be in Buenos Aires (Argentina) on April 24th 2014.



The conference was successfull considering the large number of participants (80), who attended the seminar; among them representatives of Italian companies, especially small and medium-sized

enterprises (SMEs), universities of North Italy (professors, researchers, and students), Health authorities, and consultants.

The conference focused on the different aspects concerning the quality of plant raw materials used in plant food supplements (PFS). After an overview of the main project objectives and the outcomes reached by the consortium in relation to the European PFS consumption survey and the ePlantLIBRA Database, made by the scientific coordinator, *Patrizia RESTANI* (University of Milan), the morning session was dedicated to the description of the analytical methods optimized or developed *ex novo* by various research groups involved in a specific area of the project aiming to provide experimental data and methods with regard to identification of:

- plant raw material;
- bioactive constituents with beneficial physiological roles or of possible concern for consumers;
- environmental contaminants or residues from agricultural practice;
- biological markers useful to evaluate exposure, physiological activity, adverse effects and/or misidentification of plants.

These data will be useful to assure the quality of plants and final products.

Professor *Chlodwig FRANZ* of the University of Vienna (Austria), in his presentation with the title "*Plants for Food Supplements: identification, characterization and quality of herbal materials*", described the main analytical methods (multiplex PCR or high resolution melting curve analysis – HRM) set up or optimized by his research group to discriminate different plant species of the same genus, and the DNA-based methods to properly identify plants or to detect adulterations.





Hartwig SIEVERS of PhytoLab, a German company and GMP certified laboratory for botanical products, in his presentation on "*Phytochemical identity of herbal raw materials and preparations*", showed examples of the application of UHPLC (ultra high-performance liquid chromatograph), a chromatographic fingerprint analysis developed and optimized within PlantLIBRA project, to *Passiflora incarna*ta L. This method showed high rate of reliability in correctly identifying the plant

and discriminating it from other Passiflora species.

Patrizia RESTANI in her presentation on "*Analytical identification of substances of concerns*" showed 3 cases of how some analytical techniques such as HPLC, HPTLC and mass spectrometry, can be used to detect the presence of illegal substances (e.g. the active amine "sinephrine") in PFS, especially for body weight control, for sport and with tonic-stimulant effects. In her final remarks, she stressed that the quality control cannot be limited to production, but also to



products on the market and that it is important to organize a network of laboratories sharing and coordinating their expertise in this specific field.



Mihaela BADEA (Universitatea Transilvania - Romania) explained the progress obtained by her research group in developing "Sensors" and "Biosensors" for the analytical control of contaminants and residues, such as heavy metals, mycotoxins and pesticides. More detailed information on this topic can be found in the article on page 3.

In the afternoon session of the seminar the following topics have been addressed:

Andrea PRIMAVERA, is the president of FIPPO, an Italian association of growers of medicinal plants, whose task is to coordinate the stages of production, first processing and marketing of herbal raw materials (and products thereof), providing technical assistance for the development of all the activities related to the sector of plants. In his presentation, in order to obtain quality herbal materials, he highlighted the importance to apply good agricultural practices (GMP) in all stages of the production of plant raw materials, from cultivation and harvesting to storage, and to promote the professional training of



all operators of this sector for a better understanding and resolution of the critical aspects.

♦ Bruno SCARPA of the Italian Ministry of Health illustrated the regulatory framework of PFS in



Italy/Europe highlighting the critical aspects of the health claims regulation and explained the "BELFRIT" project (BELgium, FRance and ITaly project) for the definition of a common list of plants to be used in food supplements. The Ministry of Health hopes that the traditional use and all the available scientific evidence other than human studies, are included as admissible proof in the process of evaluation of health claims on botanicals by European Commission and

EFSA (European Food Safety Authority), still temporarily suspended.

Finally, *Brunella CARRATÙ* of ISS (Istituto Superiore di Sanità, Italy), showed the progress of the working group involved in the creation of the international network of laboratories, one of the most important objectives of the European project, to select the public and private structures that requested to participate. Among them, 8 laboratories have been identified as experts in plants identification, 19 in the determination of specific compounds (beneficial or toxic compounds such as polyphenols, anthocyanins, alkaloides,



estragole, cathechins, etc., or contaminants like pesticides, fumigants, acrylamide, heavy metals, etc.), and 3 in the detection of irradiation treatment. The publication of the final list of the laboratories on the PlantLIBRA website (http://www.plantlibra.eu/web/) is scheduled for the end of the project in May 2014. These topics have been addressed by ISS research group in an article in Italian published on "EFSA Focal Point Newsletter" (Volume 1, No. 12, December 2013



Some pictures of PlantLIBRA Milan Training course

PlantLIBRA wished through this event to increase the awareness in the quality practice in all stages of production of food supplements containing botanicals and botanical preparations and share with the food operators and academics some results on the aspects related to quality being investigated by PlantLIBRA.The seminar ended with a lively debate, in which the participants expressed their interest in the continuation of the project.

The success of the initiative and the interest and appreciation for the issues discussed was confirmed by the results of a survey conducted through a questionnaire designed to collect data on the degree of satisfaction of the participants. Most of the participants who filled out the questionnaire, judged as relevant or very relevant the importance of the PlantLIBRA research on quality issues of PFS and the quality of the information and topics presented in the course. Moreover, for most of the auditors, the information supplied in the conference may stimulate important changes in their professional activity.

PlantLIBRA in Japan - January 2014

Patrizia RESTANI (University of Milan), scientific coordinator of PlantLIBRA project, had the opportunity to present the activity of PlantLIBRA project in the framework of the collaboration between the Faculty of Pharmacy of the University of Milan (Department of



Pharmacological and Biomolecular Sciences) and the University of Tokushima in Japan. The Professor was invited to participate to the different activities organized by the local university in January 2014.

January 12th, 2014



The professor attended as speaker at the symposium on the subject "*Developing drugs and pharmacy awareness and education from a regional viewpoint: Herbs, biological diversity and expression of rural community healthcare*". The issues of the conference (below) were discussed by Prof. Restani with other researchers from Germany, India, Indonesia, USA and Uzbekistan, with the aim to share and

exchange knowledge, ideas and information. During the symposium, she illustrated the EU regulatory framework of products containing botanicals describing also the activity of Policy Advisory Board in PlantLIBRA project.

Background of the symposium

Since ancient times, different people and cultures worldwide have received benefits from keeping alive traditional knowledge and techniques in cultivating and using botanical, herbal and naturally derived medicines. Unfortunately, these valuable resources are easily falling victim to exploitation of chemical technologies, used by economically strong countries, such as isolating the active ingredients and developing them into new therapeutic reagents. In order to avoid such unfair or possibly illegal situations, as well as keep alive biological diversity and resourcefulness, the Convention on Biological Diversity (CBD) has been established. In addition, international transfer of such biological resources is now restricted. International understanding and mutual agreement are now crucial in taking steps towards a beneficial relationship between "Biologically Resource-Rich" and "Technologically Resource-Rich" countries.

Aside from these international issues, many "Biologically Resource-Rich" rural areas now face other urgent concerns such as rapid aging and decrease in population. Novel and effective community-based health care with well-trained young professionals now need our urgent and immediate attention.

January 13th, 2014

The scientific coordinator of PlantLIBRA project participated to a satellite event "Student's

reports at one Thousand years forest" in Kamikatsu, a Japanese town known for its long tradition in cultivating tea. Special focus was given to the healthy properties of tea produced in the area and fermented by Lactobacillus.

January 14th, 2014

Patrizia Restani participated to a dedicated workshop with

the students (some pictures below) on the topic "Educational and research activities of the field of pharmaceutical sciences" and presented the objectives and some results of



PlantLIBRA project in the seminar "*New knowledge in the field of botanicals: experimental results from the EU project PlantLIBRA*".



NEXT EVENTS

PlantLIBRA Final Conference – Vienna, 12th-14th May 2014

Just a month to go till the end of the project and, to sum up the work done in these four years, PlantLIBRA partners will meet in Vienna (Austria) in occasion of its final conference from 12th to 14th May 2014.



The conference is open to all stakeholders (leading scientists, regulating authorities, and business representatives in the food supplement area) that are interested in integrating and sharing their knowledge in botanical science, and in benefit and risk assessment of plants food supplements (PFS), with PlantLIBRA partners.

The conference, as Patrizia Restani, scientific coordinator of PlantLIBRA, said in the presentation of the conference, "... *will present to the wider community the more integrated scientific approach to risks and benefits of PFS tailored to support policy science-based decision making, and contribute with an increased science database to the challenges faced by the PFS sector. Ultimately, it aims to increase the productivity and competitiveness of the European Knowledge-Based Bio-Economy".* The conference main topics will be:

- the advances in the science in benefit evaluation for PFS and their safety assessment;
- authentication and analytical methods for plant raw materials and final products
- Plants, demographics, and reasons of intake of the first pan European 6 country consumption survey;
- the ePlantLIBRA database for searches of quality assessed data on chemical composition, botanical information, bioactivity and toxicity of plants present in PFS;
- insights and analysis of the consumer behavior of PFS users;

- the project's results in the context of the changing regulatory environment;
- workshops for policy regulator and stakeholders
- and much more botanical science!

All the information about the final conference can be found at https://plantlibra2014.icc.or.at/.

Programme of the Final conference

Monday, May 12 – WP2 WORKSHOP

DEFINITION AND ASSESSMENT OF "BEST PRACTICE" BENEFIT/ASSESSMENT METHODOLOGY FOR USE IN FUTURE HUMAN INTERVENTION AND CLINICAL STUDIES

The workshop aims to reach consensus with policymakers, stakeholders and consumers on the "best" methodologies to evaluate and classify the benefits of PFS.

The following issues on PFS will be considered and discussed:

In vitro assays:

What is the best practice for in vitro assay design? How well can in vitro assays on plant food supplements predict an effect in humans? What can be done to improve the predictive power of in vitro assays on PFS?

Studies on volunteers:

What is the best practice methodology for human intervention studies when assessing potential benefits of plant food supplements? To what extent can studies on plant food supplements be in line with requirements of regulatory bodies such as EFSA? What is the design of an ideal intervention study on PFS?

Gary Williamson - Chair of session

9.00-9.15	The purpose of the workshop Gary Williamson – University of Leeds (United Kingdom)
9.15-9.40	Translational safety and efficacy testing of natural compounds Raymond Pieters - Utrecht University of Applied Sciences (The Netherlands)
9.40-10.05	Beneficial physiological effects of food supplements: how to measuring the unmeasurable? <i>Renger Witkamp - Division of Human Nutrition, Wageningen University</i> <i>(The Netherlands)</i>
10.05-10.30	Evaluating patients across the disease continuum: lessons learnt from PFS acting on the cardiovascular system <i>Paolo Meoni - ATuniS Development, Tunis (Tunisia)</i>
10.30-10.50	COFFEE BREAK
10.50-11.20	Experimental data from UoL Gary Williamson - University of Leeds (United Kingdom)
11.20-11.40	Experimental results from UMIL <i>Mario Dall'Agli - Università degli Studi di Milano (Italy)</i>
11.40-12.00	Experimental results from CSIR Dalu Mancama - Council for Scientific and Industrial Research Pretoria (South Africa)
12.00-12.15	Experimental results from UBA Raul Pastor - University of Buenos Aires (Argentina)
12.15-13.00	Discussion with participants and SAB contribution
13.00-14.30	LUNCH

PLANTLIBRA Final Conference

Integrating botanical science for safer products: Quality, intake patterns, benefits and risk assessment of plant food supplements

14.30-14.45	Welcome and introduction remarks <i>Roland Poms – ICC, International Association for Cereal Science and Technology</i> <i>(Austria)</i> <i>Patrizia Restani - Università degli Studi di Milano (Italy)</i>
14.45-15.15	The importance of the PlantLIBRA project from the point of view of the DG Research of the EC <i>Dieter Brigitta - EU Officer</i>

PLENARY SESSION I : Authentication and analytical methods for plant raw material and final products

Chlodwig Franz - Chair of session 15.15-15.35 Fingerprint as a tools for identification of botanical ingredients Chlodwig Franz/Johannes Novak - Univesität of Wien (Austria) 15.35-15.55 Quality control of botanicals and Plant Food Supplements Hartwig Sievers - Phytolab, Vestenbergsgreuth (Germany) 15.55-16.15 The quality systems in place for botanical health products Michel Horn - Laboratories ORTIS, Elsenborn (Belgium) **COFFEE BREAK** 16.15-16.30 16.30-16.45 Analytical approaches in identifying and confirming adverse effects to botanicals: the experience of PlantLIBRA with European Poison Centers Patrizia Restani - Università degli Studi di Milano (Italy) 16.45-17.00 New method for the detection of contaminants Mihaela Badea - Universitatea Transilvania DIN Brasov (Romania) 17.00-17.15 Quality control and safety aspects of Plant Food Supplement containing Citrus aurantium L. Chiara Di Lorenzo - Università degli Studi di Milano (Italy) 17.15-17.30 The role of an international network of laboratories in PFS guality control Brunella Carratù - Istituto Superiore di Sanità, Roma (Italy) 17.30-18.00 General discussion 18.00-19.00 SIAG Meeting A discussion on: "A possible roadmap for a specific status for botanical health products in the European Union. Proposal for an innovative approach based on the experience of pioneering member states" Participants: SMEs and PlantLIBRA partners 18.00-19.00 SAB Meeting WELCOME RECEPTION - Hotel 19.00

Tuesday, May 13			
PLENARY SESSION II : Benefit, risks, risk/benefit assessment			
Ivonne Rjetjel	Ivonne Rjetjens and Corrado L. Galli - Chairs of session		
9.00-9.25	Integrated approach to risk-benefit assessment of PFS Antonella Guzzon - Hylobates Consulting, Roma (Italy)		
9.25-09.50	On-line tools for benefit-risk assessment of PFS Jouni Tuomisto - Terveyden ja Hyvinvoinnin Laitos, Helsinki (Finland)		
09.50-10.15	The BRAFO approach in the context of risk-benefit assessment of food supplements <i>Jeljer Hoekstra - National Institute for Public Health and the Environment (The Netherlands)</i>		
10.15-10.40	The harmonization of Belgium, France and Italy lists (BELFRIT project) Mauro Serafini - University "La Sapienza", Roma (Italy)		
10.40-11.05	COFFEE BREAK		
11.05-11.25	The role of the matrix on genotoxicity of botanicals Marina Marinovich/Laura Marabini - Università degli Studi di Milano (Italy)		
11.25-11.45	The risks associated with the consumption of botanical food supplements sold on the internet <i>Marjan Willaert - NAREDI, Bruxelles (Belgium)</i>		
11.45-12.05	New concepts for the risk and safety assessment of plant food supplements (PFS) Suzanne van den Berg -Wageningen University (The Netherlands)		
12.05-12.20	General discussion		
12.20-13.00	Poster Session		
13.00-14.30	LUNCH		

PLENARY SESSION III : Understanding the use of PFS

Monique Raats and Lluis Serra Majem - Chairs of session

14.30-14.50	Usage of Plant Food Supplements across six European countries: findings from the PlantLIBRA Consumer Survey <i>Lluís Serra-Majem - Fundación para la Investigación Nutricional, Barcelona (Spain)</i>
14.50-15.10	Why European adults use Plant Food Supplements in six European countries: findings from the PlantLIBRA Consumers Survey and focus groups in three countries <i>Bernadette Egan - University of Surrey (United Kingdom)</i>
15.10-15.40	 Type of Plant Food Supplements used in six European Countries: findings from the PlantLIBRA Consumer Survey 1. Characteristics and botanical components of PFS used in six European countries: findings from the PlantLIBRA Consumer Survey - <i>Franco Maggi, Università degli Studi di Milano</i> (<i>Italy</i>) 2. Most used botanicals – which are they? With a focus on <i>Cynara scolymus</i> (artichoke) - <i>Simone de Klein, Phytolab, Vestenbergsgreuth (Germany</i>) 3. The use of PFS containing <i>Ginkgo biloba</i> in Europe – reason of use, consumer behaviours and perceptions of benefits: The PlantLIBRA study- <i>Mihaela Badea and Lorena Dima,</i> <i>Universitatea Transilvania, Brasov (Romania</i>)

15.40-16.00	Plant Food Supplements in the Media <i>Flavia Bruno - Università degli Studi di Milano (Italy)</i>
16.00-16.20	The role of images on Plant Food Supplement packaging Naomi Klepacz - University of Surrey (United Kingdom)
16.20-16.40	Communicating risk and benefits on Plant Food Supplement packaging Monique Raats - University of Surrey (United Kingdom)
16.40-16.50	Intake of plant food supplements in the netherlands based on dutch dietary supplement database and dutch national food consumption surveys <i>Elly J.M. Buurma Centre for Nutrition, Prevention and Health Services RIVM, National Institute for Public Health and the Environment Bilthoven The Netherlands</i>
16.50-17.00	General discussion
17.00-17.10	Early Career Investigators Award
17.10-17.20	COFFEE BREAK
17.15-19.00	Steering Committee Meeting
19.00-22.30	Conference Dinner (optional)

Wednesday, May 14

PLENARY SESSION IV: ePlantLIBRA		
Paul Finglas - Chair of session		
9.00-9.15	Introduction to ePlantLIBRA Paul Finglas - Institute for Food Research, Norwich (United Kingdom)	
9.15-11.15	 Live Demonstration Jenny Plumb - Institute for Food Research, Norwich (United Kingdom) 1. Composition of Bioactives in PFS - Jenny Plumb - IFR, Norwich (United Kingdom) 2. Beneficial Bioeffects of PFS - Jaqueline Lyons - Univwersity College Cork (Ireland) 3. Adverse Effects in PFS - Karin Norby - Danish Technical University (Denmark) 4. Contaminants and Residue data - Miles Thomas - Food and Environmental Research Agency (UK) 	
11.15-11.30	COFFEE BREAK AND POSTER	
11.30-12.15	 User Perspectives SIAG - Helene Kergosien - Euromed (France) PAB - Klaus Riediger - Austrian Agency For Health and Food Safety, Vienna (Austria) Poisons Centers - Hugo Kuperschmidt - Swiss Toxicological Information Center Zurich (Switzerland) 	
12.15-12.45	Updating, Access, Membership, Future plan Paul Finglas - Institute for Food Research, Norwich (United Kingdom)	

PAB MEETING	
14.30-14.45	Update on PlantLIBRA <i>Patrizia Restani - Project Coordinator</i>
14.45-15.00	Policy implications of the PlantLIBRA outcome Simon Pettman - European Advisory Services, Bruxelles (Belgium)
15.00-15.15	Existing tools for quality management of plant food supplements Patrick Coppens - European Botanical Forum, Bruxelles (Belgium)
15.15-15.30	Pitfalls in quality assurance Hartwig Sievers - Phytolab, Vestenbergsgreuth (Germany)
15.30-15.45	Practical considerations for quality assurance, experience from Plantlibra Chlodwig Franz - Univesität of Wien (Austria)
15.45-16.00	Quality of plant food supplements - Member State perspective Joris Geelen - FPS (Belgium)
16.00-16.45	Round table discussion (3 theme's)1. Is the quality assured or does it needs more efforts?2. Expectations of regulators and companies3. What are priorities in this area?
16.45-17.00	Closing remarks <i>Simon Pettman - European Advisory Services, Bruxelles (Belgium)</i> <i>Patrizia Restani - Project Coordinator</i>

Contact and more information:

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PlantLIBRA EC project number 245199

Final greetings by PlantLIBRA

The PlantLIBRA project is close to its end and, to sum up the work done in these four years, all the partners involved in the project met in Vienna (Austria) in occasion of the **International PlantLIBRA** Conference "Integrating botanical science for safer products. Quality, patterns, benefits and risk intake assessment of plant food supplements", last May 12th-14th, 2014. The final conference, hosted by ICC (International Association for Cereal Science and Technology), local partner of PlantLIBRA, in the beautiful setting of Vienna, has been organized in collaboration with SISTE (Italian society of science applied to botanicals and health products) and UMIL (University of Milan, Italy).

The programme of the Conference consisted of four sessions according to the main objectives of the project:

- Plenary session I: Authentication and analytical methods for plant raw material and final products;
- Plenary session II: Benefit, risks, risk/benefit assessment;
- Plenary session III: Understanding the use of PFS;
- Plenary session IV: ePlantLIBRA.

In every session, the groups organised in eight Work Packages (WP) to carry out the scientific research of the project (WP1-WP8), showed the results of their work. Each topic has been discussed and comments and suggestions of great interest have been made to improve the PlantLIBRA research in all its aspects. Newsletter

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This report does not necessarily reflect the Commission views or its future policy on this areas.



(continued from page 1)

More than 100 participants attended the conference, including people involved in the project, representatives of the academic world and association in the food supplements sector, SMEs and regulatory authorities, interested in integrating and sharing their knowledge in botanical science, and in benefit and risk assessment of plants food supplements (PFS).

The participants showed great interest in the outcomes related to the development of analytical methods to assure the quality and the safety of PFS and to the implementation of the ePlantLIBRA database, that is considered by both the companies and the regulatory authorities an important referencing source to search all the useful information (composition data, beneficial and adverse effects, contaminants, etc.) about the plants of possible use in foods and PFS. Relevant for the success



of the project and object of discussion have been also the results related to the assessment of risks and benefits associated with plants and PFS, another important objective of PlantLIBRA.

The Conference has also been an important occasion to show the final results of the survey on the use of PFS in 6 European countries (Finland, Germany, Italy, Romania, Spain and United Kingdom) that demonstrated how the cultural differences between the countries may play a relevant role in the consumption choices of health products. Even the consumer perceptions of PFS have been considered, especially how the expectations of PFS by consumers and stakeholders may be modulated by different media sources or by information reported on the labels and packaging of products.



Dr *Dieter BRIGITTA*, invited to attend the conference as the project officer at the Directorate-General for Research and Innovation in the European Commission, in his opening speech, highlighted the importance of the results obtained by the project hoping that they can become the beginning of new research projects for future funding opportunities. In an interview published on "http://www.nutraingredients.com/", a daily online news service available as a free-access website

providing daily and weekly newsletters on various topics related to nutrition, in occasion of the PlantLIBRA final conference, he declared "I have to say based on everything I have seen, it has been very successful". He added: "It actually validated why European Commission put forward this topic four years ago... a lot of the research questions that were put forward at the start were answered but also a lot of very new, interesting research ideas have come forward and that's often a good indicator of a good project when the questions that were asked at the beginning prove to be valid questions but also very new, intriguing ways of moving forward".

The conference has been preceded, on Monday morning, May 12th, by the workshop organized by the WP2 leader Gary WILLIAMSON (University of Leeds - UK) on the "Definition and assessment of "Best practice" benefit/assessment methodology for use in future human intervention and clinical studies", in which all the research groups involved in this work package (Uol, University of Leads - UK; UMIL, University of Milan - Italy; CSIR, Council for Scientific and Industrial Research - South Africa and UBA, University of Buenos Aires – Argentina) showed their results in the development of *in vitro* assays to test the beneficial effects of plants and PFS in the gastro-intestinal tract and to identify and quantify the potential biomarkers of inflammation or cardiovascular disease, three out of the five health areas selected within the project to study the beneficial effects of PFS. The main objective of the WP2 was the achievement, application and validation of a methodology to determine the benefits of consumption of PFS using new and advanced in vitro approaches, in order to search alternative methods to complement the clinical studies, that currently are the only kinds of study accepted by EFSA (European Authority for Food Safety), in order to scientifically substantiate health claims. In the final discussion with the participants and the SAB (Science Advisory Board) members, has been underlined the importance of the data available on the tradition of use of certain plants, data that, although insufficient as such, may constitute the starting point to design new experimental *in vitro* and *in*



vivo approaches.

The conference ended with the fifth PlantLIBRA policy Advisory Board (PAB) with the presence of the representatives of the Health Ministry of Belgium (*Joris GEELEN*), Romania (*Gabriela GARBAN*) and Austria (*Klaus RIEDIGER*). It has been underlined how PlantLIBRA has created a fruitful and steady confrontation and collaboration between all the stakeholders involved in improving PFS safety and efficacy (academic world, regulatory authorities and companies), stressing the importance to reach in Europe, from the legislative point of view, the complete harmonization of the list of plants authorized in PFS, already begun with the BelFrIt project, that, although not a goal of PlantLIBRA, has been stimulated by it, and has contributed, as Stefanie Geiser (EAS - European Advisory Services – Belgium) underlined in her speech, to a common

understanding of the needs of a future policy in relation to botanicals in Europe. *Patrizia RESTANI* (UMIL), coordinator of the project, in her final remarks, visibly moved thanked all the groups involved in the project for the great work done and the satisfactory outcomes reached by each one, hoping that they may be the starting point of future and productive collaborations. She concluded that though PlantLIBRA finishes at the end of May 2014, it will only be an "*Arrivedercl*".





PlantLIBRA "Early Career Investigators Award" Vienna PlantLIBRA Conference



Poster session – Vienna PlantLIBRA Conference



Moments of conviviality and final greetings Vienna PlantLIBRA Conference

The abstracts of the works illustrated in the four sessions of the International PlantLIBRA Conference and of the posters have been included in a book, that was distributed to all participants in USB sticks together with other informative material on the project.

For a major dissemination of the topics discussed during the Vienna conference and the results of PlantLIBRA among stakeholders that did not attend the meeting, the abstracts have also been published on the Newsletter (see pages 7-29).













Some pictures of the various sessions of Vienna PlantLIBRA Conference

WP2 WORKSHOP

Definition and assessment of "best practice" benefit/assessment methodology for use in future human intervention and clinical studies

BENEFICIAL PHYSIOLOGICAL EFFCTS OF PLANT FOOD SUPPLEMENTS: HOW TO MEASURE THE UNMEASURABLE?

R.F. Witkamp, Nutrition and Pharmacology, Wageningen University

In accordance with EU legislation, plant food supplements (PFS) are intended to maintain or improve health. Their expected effects are physiological rather than pharmacological, and claims referring to the prevention or treatment of human diseases are not allowed. In this context, a 'reduction of disease risk' claim only refers to the reduction of one or more general risk factors for disease. Beneficial physiological effects should be scientifically demonstrated in representative target groups. Together, this requires an approach that is different from that with pharmaceuticals, for which effects on clinical or surrogate endpoints are often easier to establish. An important starting point is to consider the definition of health. The WHO definition of 1946 states: "health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". Although this definition acknowledges that health should not be described within the context of the presence or absence of disease or impairments, it does not provide clear directions on how to deal with the dynamics and timedependency of health. In many cases the borders between health and disease are not very sharp. To address this, "health" is increasingly considered as "the ability to adapt" to internal and external stimuli. New models are currently being developed that take into account the complexity and balance of homeostatic mechanisms. Advances in genomics and systems biology enable to measure and model biomarker profiles and to translate these into processes. Beneficial physiological effects are substantiated by demonstrating that homeostasis is normalized or stabilized, or that processes contributing to the development of disease are modulated. A "snapshot of health status" is determined by different clusters of biomarkers that reflect essential processes such as inflammation, oxidative stress or metabolism. This requires more knowledge on processes that start to deviate in an early stage. Methods based on analysis of the robustness (or resilience) of physiological homeostasis in individuals are regarded as a promising approach to measure health and beneficial physiological effects. Comprehensive multi-parametric ("omics") analysis under conditions of physiological or even psychological stress may identify key parameters that are more adequately describing healthy and compromised conditions when compared to current biomarkers, which are typically measured during steady state and representing markers of disease. A limitation of these approaches is that there will be no single challenge tests that are indicative of general health. Instead these tests will be indicative for a specific "health domain". Although the principle of these tests is not new, as seen for example with the glucose tolerance test, the combination with new bio-analytical technologies (microarray analysis and metabolomics) and calculation power makes them particularly useful to test healthimproving effects of foods and PFS. However, it should be stressed that these approaches are not technically simple or cheap. Furthermore, several issues remain to be resolved. These include the nature of the challenge, the force of the stimulus, accepted designs, statistics and validation.

Keywords: Systems biology, Homeostasis, Resilience, Challenge Test, Biomarkers, Health Effects

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EVALUATING PATIENTS ACROSS THE DISEASE CONTINUUM: LESSONS LEARNT FROM PFS ACTING ON THE CARDIOVASCULAR SYSTEM

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In European legislation, Plant Food Supplements (PFS) are defined as foodstuffs in which plant preparations are the main ingredient and for which the purpose is to supplement the normal diet by concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form. Whilst these definitions clearly point to Plant Food Supplements as "Foodstuffs" and differentiate them from medicines (whose main purpose is to diagnose, prevent or treat a disease), many clinical studies of PFS are still conducted in diseased populations and using the same outcome measures commonly used for medicinal products.

Based on a previous published survey of the scientific literature and on further review of published papers in the cardiovascular area, we will draw conclusions on the adequateness of current approaches for the clinical evaluation of PFS. Within this context, cardiovascular disease represents an extremely interesting case for the evaluation of methods assessing PFS' benefits as the transition between health and disease and the key markers of this progression has been thoroughly characterized and placed within a continuum.

The identification of panels of "continuous" biomarkers allowing the measure of the effect of PFS on the evolution from prediseased to diseased states and the inclusion of individuals in healthy or pre-disease states was found to be of particular importance in the evaluation of PFS in cardiovascular disease. Examples will be made on how these essential features of clinical studies could be extrapolated to other functional areas for PFS.

IN VITRO ASSAYS TO TEST THE BENEFICIAL EFFECT OF PLANT FOOD SUPPLEMENTS IN THE GASTRO-INTESTINAL TRACT: EXPERIMENTAL RESULTS FROM UMIL

<u>M. Dell'Agli¹</u>, C. Di Lorenzo², E. Sangiovanni¹, F. Colombo², M. Fumagalli¹, E. Colombo¹, P. Restani²

¹Laboratory of Pharmacognosy, and ²Laboratory of Food Chemistry and Toxicology, Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy

In the task Work Package 2, evidence for PFS benefit from epidemiological, clinical, and intervention have been reviewed. A number of pathological conditions, where PFS are commonly used, were identified and inflammation was one of those. Two systematic reviews aimed to summarize and critically evaluate the evidence for or against the efficacy of PFS or substances (compounds or foods) relevant to PFS for coping inflammatory conditions were published. The outcome of the evaluation process indicated for the need of other studies in particular for *Matricaria recutita* L., *Vitis vinifera* L., and *Calendula officinalis L*. While the potential benefits of green tea have been reported

in a wide range of health areas, and in particular metabolic syndrome, the consumption of green tea (Camellia sinensis Kuntze) did not affect significantly inflammation conditions; however, the effect at gastric level was not considered in deep. The in vitro assays to test the beneficial effect of botanicals in the gastro-intestinal tract have been developed and applied to the extracts from plants above described. The assays were set up in human gastric epithelial cells in order to study the beneficial effects on cytokine release (IL-8, IL-6, TNFa) and NFκB pathway (transcription and nuclear translocation) during gastric inflammation; the same assays were set up in human intestinal cell line CaCo-2, both undifferentiated (colonocytes) and differentiated to enterocytes Our results on green and black tea extracts indicate an effect on the NF-kB driven transcription induced by cytokines deeply involved in gastric inflammation; this effect was correlated to the catechin content. Vitis vinifera L. water extract inhibited NF-kB pathway and IL-8 release in gastric and intestinal cell lines; this extract should be considered promising for future studies. Results on Matricaria recutita L. water extract seem to exclude an anti-inflammatory effect in the gut, whereas the effect of Calendula officinalis L. extract is currently under investigation.

Keywords: Gastric inflammation, NF-κB, *Vitis vinifera* L., *Matricaria recutita* L., *Calendula officinalis* L., green tea, *Camellia sinensis* Kuntze,

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154.

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PHYTOCHEMICAL PROFILING AND *IN VITRO* EFFICACY SCREENING AGAINST OPPORTUNISTIC INFECTIONS OF SELECTED PLANT-BASED EXTRACTS

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Health-promoting or disease risk reducing foods, in particular plant-based food supplements, have been consumed for decades. Such products display high consumer acceptance because of a series of advantages, including their natural origin and hence perceived safety, as well as their proposed efficacy and relatively low costs. Increasingly, such substances are used to preserve optimal immune function in an effort to prevent or alleviate a range of infections, particularly amongst individuals with impaired host defences due to diseases such as leukaemia, HIV/AIDS, haematological diseases, and endocrinopathies such as diabetes. Popular examples of PFS use include Echinacea purpurea, which is believed to exert immunomodulatory effects, and products from Vaccinium oxycoccos (common cranberry) which are often used to prevent and treat urinary tract infections. However, a lack of comprehensive empirical data often exists regarding the safety and efficacy of many popular PFS products. In an effort to assess, in vitro, the biological effects of several such PFS on selected micro-organisms, extracts from Echinacea purpurea, Andrographis paniculata, Pelargonium sidoides, Matricaria recutita L., Calendula sp., and Vitis vinifera were phytochemically profiled using LC-MSMS then screened against a panel of target micro-organisms. These included adenovirus, coxsackievirus, and herpes simplex virus (I), and gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes); gram-negative bacteria (E. coli, Salmonella enteritidis, Klebsiella pneumoniae, Pseudomonas aeruginosa);

acid-fast bacilli (*Mycobacterium aurum*) and yeast (*Candida albicans*). Moderate to good efficacy was observed against herpes activity with extracts prepared from *E. purpurea* and *M. recutita*; likewise, extracts of *V. Viniferia* and *M. recutita* exhibited good activity against adenovirus. Interestingly no apparent efficacy was observed for these extracts against the bacterial and yeast strains tested. Since these studies were limited to the assessment of *in vitro* biological effects, downstream work to correlate this data with data from human-based trials would better inform our understanding of the effects of these plants on human immune function.

Keywords: plant food supplements, plant extracts, immune function, assay, opportunistic infection, viruses, bacteria

IDENTIFICATION AND QUANTIFICATION OF URINARY BIOMARKERS AFTER INGESTION OF GRAPE SEED EXTRACT IN HEALTHY ADULT VOLUNTEERS

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Several experimental and epidemiological studies suggest that a diet rich in polyphenols could reduce the risk factors for chronic diseases, such as cardiovascular disease and cancer (Hollman et al, 1999). Polyphenols are substances produced by the secondary metabolism of plants; the estimated daily consumption in the diet is approximately 1 g/day. Polyphenols are classified into 2 groups: phenolic acids and flavonoids. Phenolic acids account for about a third of the total intake and flavonoids nearly two thirds (Scalbert and Williamson, 2000). The contribution of the various subclasses of flavonoids to the total intake is uncertain and is likely to vary significantly between individuals. Proanthocyanidins, which are polymers of flavonols, are present in numerous foods containing plant derivatives, processed foods and beverages (Gu et al 2004).

Grape seed extract is a concentrated source of polyphenols, and in particular polymeric proanthocyanidins (Hayasaka et al, 2003). The available data on the absorption and metabolism of proanthocyanidins suggest a poor bioavailability (Gonthier et al, 2003). Most proanthocyanidins reach the colon, where bacteria hydrolysed them into smaller molecules, such as phenolic compounds; as a consequence, plasma levels of polyphenols are very low (Scalbert and Williamson, 2000). Various metabolites, such as hydroxyphenylpropionic acid, catechins and gallic acid have been identified as urinary biomarkers when flavonoid are consumed with the diet.

The aim of this study, performed in human healthy volunteers, was to confirm the correlation between polyphenols, consumed with grape seed extracts, and selected urinary biomarkers. In particular, the attention was payed to identifying those compounds (including metabolites), which could be considered suitable as urinary biomarkers of exposure.

Keywords: urinary biomarkers, grape seed extract, polyphenols, proanthocyanidins

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PLENARY SESSION I

Authentication and analytical methods for plant raw material and final products

"FINGERPRINT" AS A TOOL FOR IDENTIFICATION OF BOTANICAL INGREDIENTS

DNA-Based Authentication of Medicinal and Aromatic Plants

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The correct botanical identification of plant raw materials is of utmost importance to guarantee safety and efficacy of plant food supplements (PFS). The principal identification strategies are based on macro- and microscopic analysis, complemented in NEMs by phytochemical methods. Besides these methods, identification by the DNA sequences of the plants is in fast development and supplements classical methods in difficult matrices like e.g. powdered roots or even extracts.

The DNA sequence is very informative, because it is unique for every individual but in different sections conserved on certain taxonomic levels like the species level. By analyzing DNA segments conserved on the species level, it is possibly to determine the botanical identity of an unknown sample. This approach is called "DNA barcoding".

However, the classical assessment by DNA sequencing is still time-consuming and relatively expensive, a fact that can be overcome by other DNA-based methods better suitable for routine analysis. One of these methods is High-Resolution-Melting-Analysis (HRMA). The principle of this method is the separation of the two DNA-strands ('melting' of the DNA) by slowly increasing the temperature at highly constant rates ('highresolution'). The 'melting' can be measured by the decreasing intensity of a fluorescence dye intercalating the DNA resulting in a melting curve that is specific for a DNA sequence.

While using the above mentioned DNA-based approaches separate methods have to be developed for target species and frequent adulteration, the next technological revolution in DNA sequencing, termed "next-generation sequencing" enables now a completely different assessment by analyzing in one run all species that are in one sample. This is until now mainly used to study microbial communities in e.g. environmental samples ("metagenomics") but is also applicable for complex plant mixtures.

In this presentation some examples of DNA-based species identification will be shown including the applicability of this method for the quantitative determination of adulterations.

Keywords: DNA-based identification, DNA-barcoding, plant food supplements, DNA sequencing, high-resolution melting analysis, next-generation sequencing

QUALITY CONTROL OF BOTANICALS AND PLANT FOOD SUPPLEMENTS

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Herbs and herbal preparations have been playing an important role for both dietary and medicinal use from paleolithic huntergatherers to 21st century urban culture. People have always been aware that the variability of the properties of botanical materials depending, e.g., on the plant part used, vegetative state, weather conditions, harvesting time, postharvesting treatment and essentially the mode of preparation by, e.g., peeling, cooking, fermentation, extraction process, are no less important for the tolerability digestibility and nutritional or functional value than the choice of the plant itself. Over the past decades based on scientific findings we have started to understand what our ancestors had established on an empiric basis. We understand why, based on the absence/presence and dose of certain secondary compounds some plants/preparations are beneficial or harmful. Because of their complex composition the quality of botanical raw materials and ingredients cannot be controlled solely on the basis of analytical

procedures. However, the increasing pressure on both natural and agricultural sources of botanical raw materials as well as new scientific findings, e.g., on toxicologically relevant or functionally relevant ingredients, contaminants or potential herb drug interactions as well as growing consumer expectations represent an increasing challenge for all parties involved in the supply and marketing chain of botanical raw materials and finished products. Therefore within the PlantLIBRA project one focus has been on the revision and development of methods for the identification and quality control of botanical raw materials and ingredients. The presentation will highlight the challenges outlined above and some results from the PlantLIBRA project, Work Package 7.1.

ANALYTICAL APPROACHES IN IDENTIFYING AND CONFIRMING ADVERSE EFFECTS TO BOTANICALS: THE EXPERIENCE OF PLANTLIBRA WITH EUROPEAN POISONS CENTERS

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One of the aims of the EU PlantLIBRA Project was to investigate adverse effects to botanicals and plant food supplements (PFS) in humans via the collection of literature data and new case reports from poisons centres.

The use of PFS is increasing in the world for the possible beneficial health effects associated with their consumption, but although botanicals have natural origin, this is not synonymous of absolute safety. In fact, botanicals have side effects, which may be severe in some cases.

During the last year of activity, the PlantLIBRA project has performed the prospective study "*Biomarkers of exposure in* patients with adverse effects or poisoning after intake of plant food supplements or misidentified poisonous plants".

The rational of this project is based on the fact that there is a lack of studies in the medical literature investigating the issues of 1) PFS-related adverse effects; 2) poisoning due to misidentified plants, and 3) the methods/biomarkers, which prove the intake of a specific plant.

The aim of this study is to fill this gap by providing clinical information and biological samples of patients with adverse effects or poisoning due to intake of PFS or misidentified poisonous plants.

In the last months, some new cases were included in the study and allowed to develop new analytical methods or to modify previous published approaches in order to measure known or unknown metabolites (biomarkers) associated with the intake of PFS or botanical ingredients.

In the same period, some cases of poisoning due to botanicals were considered although not included in the prospective study because they did not fulfill the inclusion criteria. In particular, methods for the detection of *Ginkgo biloba* metabolites were developed and applied to the analysis of blood and urine from a child, who was exposed to a large number of Ginkgo seeds. Due to the unavailability of the main blood metabolite (4'-O-methylpyridoxine) in the market, a synthesis was performed to optimize the validation of methods.

This experience showed how PlantLIBRA could support Poisons Centers and clinical toxicologists not only with its analytical expertise but also by preparing synthetic metabolites when necessary.

Keywords: HPLC, botanicals, plant food supplements, adverse effects, poisoning, metabolites, biomarkers

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NEW METHODS FOR THE DETECTION OF CONTAMINANTS

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Hazardous chemical contaminants or undesirable substances in food and feed are often unavoidable Scientific literature indicates several analytical methods for detection for detection of contaminants and residues (heavy metals, pesticides, mycotoxins) from different biological matrices as: HPLC, ELISA, enzymatic methods, AAS, sensors, biosensors, Surphace Plasmon Resonance (SPR).

Bioanaytical methods request the presence of recognizing sensing layer being in an intimately contact with the sample (analyte) and with a transducer that converts the physicochemical changes occurred after the specific interaction into a measurable signal. Biorecognition use the presence of enzymes, antibodies, aptamers - single-stranded nucleic acid RNA and DNA molecules (ca.30 to 100 nucleotides) that recognize specific and with high affinity several ligands, molecular imprinted polymers (MIPs). The biomolecules are immobilized on the surphace of the working electrodes or, for some other examples, are part of the columns used for cleaning procedures of several extracts before to perform the analytical method.

Accuracy, sensitivity, specificity, stability, speed of response, the possibility to be reused (sometime after reactivation), low cost and the possibility to be used on the labs or directly on the field (telemonitoring) are some of the advantages for using (bio)sensors in different analysis. For complex samples there are recommended multiple, simultaneous and specific detections, followed by a specific mathematic calculation using artificial neural network.

Keywords: contaminants, enzymes, antibodies, aptamers, biosensors, Surphace Plasmon Resonance

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QUALITY CONTROL AND SAFETY ASPECTS OF PLANT FOOD SUPPLEMENTS CONTAINING *Citrus aurantium* L.

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The market of plant food supplements (PFSs) is expanding quickly. These products are usually labeled as natural, claim several health benefits and are perceived as safe by consumers. The high availability of PFS has raised concerns about their quality, specific composition, and safety, which can enter in parallel markets and allow also illicit activities. Furthermore, a frequent issue is the unsuitable labeling, which can omit illicit ingredients or declare lower quantity of controlled active substances. As a consequence, there is an increase of risk of adverse reactions.

Some products are more susceptible than other to adulterations and abuse, as for example, PFS aimed to reduce the body weight and improve general physical performances.

Citrus aurantium L., also known as bitter orange, is a botanical ingredient frequently used in these kinds of PFS. The most significant compounds contained in C. aurantium are active amines, such as octopamine, synephrine, tyramine, N-methyltyramine and hordenine. The C. aurantium extract is generally allowed in food supplements, but in several countries limits have been established. With the decree dated July 09 2012, the Italian Ministry of Health established a limit of synephrine in C.aurantium-based products, in order to maintain the daily intake \leq 30 mg, whereas the sum of other amines, including octopamine, should be lower than 1/8 (12.5%) synephrine (Ministry of Health, 2012). The scientific literature reports some cases of adverse effects, after the intake of PFS containing C. aurantium extract; they involved mainly the cardiovascular system due to the sympathomimetic properties of the amines. After the FDA ban of ephedrine containing supplements, C. aurantium extracts appeared as an alternative to Ephedra in herbal supplements aimed to weight control and energetic enhancement. On these bases, quality control of PFS is of crucial importance also taking into consideration that illicit use of C. aurantium and/or its synthetic amines is not infrequent (Angell and Kassirer, 1998; Haller and Benowitz, 2000).

The principal aim of our work was to set up and validate analytical approaches for the quality control of PFS containing *Citrus aurantium*, in order to offer new tools for both industries involved in PFS production and laboratories responsible for the post-marketing quality control. High Performance Thin Layer Chromatography (HPTLC) was used for a first qualitative screening of active amines in complex matrices such as PFS. A High Performance Liquid Chromatography (HPLC) approach, coupled with a fluorimetric detector, was then developed for quantitative analysis and validated according to FDA guidelines. This analytical protocol allowed to eliminate the interferences due to other ingredients present in the samples analysed.

Keywords: active amines, *Citrus aurantium*, HPTLC, HPLC, plant food supplements

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THE ROLE OF AN INTERNATIONAL NETWORK OF LABORATORIES IN PFS QUALITY CONTROL

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Over the past decade important safety issues associated with the use of plant food supplements (PFS) have resulted in regulatory action world-wide in an effort to protect public health.

The safety problems emerging with PFS products reflect a growing market where many of the safety concerns arise in quality control phase.

Indeed herbal ingredients present a number of specific problems when quality control aspects are considered. This situation is further complicated by the traditional practice of using combinations of herbal ingredients.

Concerns about poor quality and safety of herbal ingredients highlighted the need for stricter regulations and controls of botanical products also due to the increasing volume of online marketing.

For this reason, many specialized laboratories are required in performing difficult and diverse analysis of this type of products due to the many complex matrices.

PlantLIBRA has created an international network of laboratories with the expertise in the analysis of plant/extract/food supplements with the aim of providing an integrated resource made available for decision-makers, both in the public and private sectors.

The main goals of the network are:

 provide reliable high quality analytical data for the identification of plant material, analysis of bioactive constituents that have positive or negative health effects, detection of environmental contaminants or residues from agriculture activities, analysis of biological markers useful to evaluate environmental exposure, physiological activity, adverse effects and/or misidentification and adulteration;

- support the work of international organizations involved in the food safety together with food manufacturers in achieving high quality products;
- provide analytical tools to European Poison Centres for rapid identification of adverse effects due to plant ingredients

The network includes laboratories that provided comprehensive information and that, have demonstrated adequate competencies in the different analytical fields. In the structuring of the network, laboratories are classified on the basis of the specific competencies: plant/herb identification, determination of specific compounds, and detection of irradiation treatment.

PLENARY SESSION II

Benefit, risks, risk/benefit assessment

INTEGRATED APPROACH TO RISK-BENEFIT ASSESSMENT OF PFS

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Plant food supplements (PFSs) have been used for decades to improve health and well-being. They are present on the market in various forms (e.g. capsules, pills, tablets) in which bioactive compounds occur in high concentration to enhance the expected health benefit. On the other hand, due to this concentrated form, toxic compounds may be present at levels that might raise safety concern. Clearly, an evaluation of both safety and beneficial effects of plant extracts, used in food supplements, is needed to guarantee their consumption is safe and protect consumers' health.

Approaches exist that have addressed these two aspects separately, thus only risks or only benefits of botanical preparations used in PFSs, while tools to evaluate both risks and benefits are not yet available. To fill this gap, within the EC-funded PlantLIBRA project an integrated approach to the risk and benefit assessment (RBA) of PFSs was developed. Starting from previous methodologies available for assessing risks and benefits of food products by food safety authorities, such as EFSA (2010), and EC-funded PRO safety authorities, was developed, taking into account the specific features of these products.

The RBA methodology for PFS is based on a scientificallydriven, stepwise approach that takes into account all the scientific data available (tradition of use, in vitro studies, in vivo studies on experimental animals and humans, history of safe use) on the botanical preparation of interest and on its bioactive compounds. The scientific evidence available for the risks and benefits is evaluated for the quality and strength of study design and experimental results. The approach includes also the systematic analysis of the chemical composition of the botanical preparation of interest. This is a pivotal step considering that a botanical preparation may vary in composition among different food supplements and that, as consequence, the intake of bioactive compounds providing the beneficial, or adverse, health effects might be different.

The RBA methodology was applied to several case studies: the essential oil extracted from the dried bark of *Cinnamomum verum* J. S. Presl, the extract of the dried fruits of *Foeniculum vulgare* Miller and the extract of the dried leaves of *Ginkgo biloba* L. The results of the systematic analysis on composition, on the identification and characterisation of adverse and beneficial effects of the botanical preparations are presented, together with the evaluation of the scientific evidence available and the related source of uncertainty.

Keywords: Risk and benefit assessment, plant food supplements, cinnamon essential oil

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A RISK-BENEFIT METHODOLOGY (BRAFO), A CALCULATION TOOL (QALIBRA) AND A CASE STUDY (PLANT STEROLS)

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The BRAFO tiered approach describes a tiered benefit-risk methodology. The method consists of 4 steps, preceded by a preassessment and problem formulation stage. The tiered approach stops as soon as the risk-benefit question is sufficiently answered. Tier 1 identifies and characterizes the risks and benefits. Tier 2 makes a qualitative assessment. Eventually in tier 3 benefits and risks are expressed in a common health metric and compared. If uncertainties are large benefits and risk are probabilistically assessed and integrated, in tier 4. The QALIBRA software is a calculation tool that supports the quantitative assessments of BRAFO tiers 3 and 4. The tool and methodology are illustrated by an assessment of plant sterols enriched margarine. Plant sterols lower cholesterol level and thus may reduce cardiovascular risk but as a potential adverse effect may also indirectly decrease vitamin A levels.

Keywords: tiered risk-benefit methodology, RB calculation tool, plant sterols

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THE HARMONIZATION OF BELGIUM, FRANCE AND ITALY LISTS (BELFRIT PROJECT)

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European legislation for food supplements with botanicals is not harmonized and not adapted to meet the particular challenges of these heterogeneous ingredients. Faced with this situation, the Belgian, French and Italian authorities, on 2011, each assisted by a renowned scientific expert, have decided to develop a common approach for the evaluation of botanicals in the 'BELFRIT' project. Plants in original lists were: Belgium 645, France 548, Italy 1182. Therefore 2045 botanicals names had to be verified. A first step in this initiative was the compilation of a list of plants whose use in food supplements could be possible, provided that the necessary measures to ensure consumer safety are respected. It provides a precise identification of the plants, indicates some key points in the production to be controlled, while also taking traditional knowledge into account. This harmonized list can be a pragmatic tool for risk managers and operators and an important piece of the puzzle for harmonization of this field. Nevertheless, it is not a legally binding instrument and cannot be opposed to legal provisions, including those of the Member States involved in the project. On 2014, March 27th , the Italian Government issued a decree concerning the adoption of BELFRIT List as List of Botanicals allowed in Food Supplements.

Keywords: Botanicals, Harmonized Lists, France, Belgium, Italy

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THE ROLE OF THE MATRIX ON GENOTOXICITY OF BOTANICALS

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Alkenylbenzene compounds are naturally constituents of a number of plants such as basil, nutmeg, tarragon, star anise, and fennel. Human exposure to these chemicals may then occur through the consumption of foods, food flavored with these aromatic plants or their fractions (e.g. essential oils), some herbal medicines and food supplements.

The steady increase in the market sales of herbal medicines and above all botanical food supplements has raised concerns about the possible effects on human health of cumulative doses of these chemicals and because some of the alkenylbenzenes have been shown to be genotoxic *in vitro* and carcinogenic in rodent studies and therefore, restrictions in their use were established

Rodent toxicological studies were conducted using single compounds at very high doses in comparison to actual amount present in the plant, whereas human exposure is generally characterized by relatively low concentrations together with other constituents of a complex food matrix, which can influence the final biological activity.

The modulating effect of the matrix has been already reported in studies evaluating the formation of adducts between hydroxy derivatives of alkenylbenzenes and DNA.

We evaluated the cytotoxicity and genotoxicity by of a series of alkenylbenzenes, and their hydroxy metabolites, thought to be the proximate mutagens, by means of Comet and micronucleus assays in HepG2 (human hepatoma) cells, and some extracts of commercial food supplements containing the parent compounds.

In addition, the compounds resulted positive in the mutagenicity tests were re-assessed in the presence of supplement extract.

The results confirmed that the parent compounds are not genotoxic *per se*, differently from their OH derivatives, that resulted active in vitro at doses between 50 -600 microM; the food supplement extracts tested were not cytotoxic and genotoxic and, when evaluated in combination, inhibited the genotoxicity of hydroxy metabolites.

Considering that the alkenylbenzenes are not completely converted into the proximate mutagens, that these latter are genotoxic *in vitro* at high concentrations and that food matrix seems to modulate in a protective way this toxic activity, we can conclude that the risk deriving from exposure through the diet to these compounds is acceptable. Keywords: alkenylbenzenes, genotoxicity, risk assessment

THE RISKS ASSOCIATED WITH THE CONSUMPTION OF BOTANICAL FOOD SUPPLEMENTS SOLD ON THE INTERNET

Marjan Willaert - President NAREDI (www.naredi.be), Brussels (Belgium)

The trade of food supplements on the internet as such is not illegal. Moreover, there are several Belgian companies who opt for this distribution channel and do so in full respect of the law. It looks that their market share will increase in future.

Unfortunately today we can only conclude that many food supplements reach through the internet the Belgian market which are not only **illegal** (not conform the Belgian and European legislation), but many of which are also **deceptive** (not containing the listed ingredients or dosages) or simply **dangerous** (containing non authorized ingredients or toxic dosages). We note that the sale of these products is performed from abroad (outside Europe), not transparent, not controlled and without respect of the applicable law.

NAREDI wants to dissociate herself from this illegal trade and encourages the Belgian and European authorities to act strongly upon it.

These practices put a negative shadow on the food supplement sector in Belgium and in Europe and represent an unfair competition to the European food supplements operators.

NAREDI is willing to cooperate with the authorities wherever possible to stop this plague, particularly in raising awareness to consumers.

NAREDI, the Belgian national federation, is the official representative and spokesperson for the food supplement sector in Belgium. Most of her members are small and medium sized enterprises.

NEW CONCEPTS FOR THE RISK AND SAFETY ASSESSMENT OF PLANT FOOD SUPPLEMENTS (PFS)

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Although botanicals and botanical preparations including plant food supplements (PFS) are well accepted by consumers, the use of such products might raise a potential health concern. In fact, some botanicals and/or products made thereof may contain compounds that are known to be genotoxic and carcinogenic. To foster the safe use of PFS containing compounds that are both genotoxic and carcinogenic, adequate procedures for risk and safety assessment are essential. However, risk and safety assessment of such compounds is accompanied with difficulties (EFSA, 2005; van den Berg et al., 2011a). An international consensus on the best approach for risk assessment of such compounds is not (yet) available (EFSA, 2005). For these reasons, new concepts for risk and safety assessment of botanicals and/or botanical preparations such as PFS were tested within WP3 of the PlantLIBRA framework. These concepts included the margin of exposure (MOE) concept, the threshold of toxicological concern (TTC) concept, a mode of action based concept and a matrix effect concept. Compounds classified as alkenylbenzenes were selected as our model compounds. Testing the different concepts, it was concluded that in addition to the TTC concept, especially the MOE concept is a useful approach to perform risk and safety assessment of PFS. The MOE approach can be used for priority setting by risk managers (van den Berg et al., 2011b; van den Berg et al., 2014). In addition, in vitro experiments and mode of action based physiologically based kinetic (PBK) modeling can be applied to facilitate read across making it possible to perform risk and safety assessment by the MOE approach for botanical compounds for which the toxicological database is limited (van den Berg et al., 2012). Moreover, using this PBK modeling based approach matrix-derived combination effects can be taken into account when evaluating the risk and safety of PFS (van den Berg et al., 2013). In conclusion, the new concepts tested within WP3 of the PlantLIBRA framework may contribute to reducing the number of animal experiments required for the risk and safety assessment of botanicals and botanical preparations including PFS.

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PLENARY SESSION III Understanding the use of PFS

USAGE OF PLANT FOOD SUPPLEMENTS ACROSS SIX EUROPEAN COUNTRIES: FINDINGS FROM THE PLANTLIBRA CONSUMER SURVEY

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Background: The popularity of botanical products is on the rise in Europe, with consumers using them to complement their diets or to maintain health, and products are taken in many different forms (e.g. teas, juices, herbal medicinal products, plant food supplements (PFS)). However there is a scarcity of data on the usage of such products at European level (.

Objective: To provide an overview of the characteristics and usage patterns of PFS consumers in six European countries.

Design: Data on PFS usage were collected in a cross-sectional, retrospective survey of PFS consumers using a bespoke frequency of PFS usage questionnaire.

Subjects/setting: A total sample of 2359 adult PFS consumers from Finland, Germany, Italy, Romania, Spain and the United Kingdom.

Data analyses: Descriptive analyses were conducted, with all data stratified by gender, age, and country. Absolute frequencies, percentages and 95% confidence intervals are reported.

Results: Overall, an estimated 18.8% of screened survey respondents used at least one PFS. Characteristics of PFS consumers included being older, well-educated, never having smoked and self-reporting health status as "good or very good". Across countries, 491 different botanicals were identified in the PFS products used, with *Ginkgo biloba* (Ginkgo), *Oenothera biennis* (Evening primrose) and *Cynara scolymus* (Artichoke) being most frequently reported; the most popular dose forms were capsules and pills/tablets. Most consumers used one product and half of all users took single-botanical products. Some results varied across countries.

Conclusions: The PlantLIBRA consumer survey is unique in reporting on usage patterns of PFS consumers in six European countries. The survey highlights the complexity of measuring the intake of such products, particularly at pan-European level. Incorporating measures of the intake of botanicals in national dietary surveys would provide much-needed data for comprehensive risk and benefit assessments at the European level.

Keywords: PlantLIBRA, plant food supplements, herbal dietary supplements, European survey, botanicals, consumer; usage

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WHY EUROPEAN ADULTS USE PLANT FOOD SUPPLEMENTS IN SIX EUROPEAN COUNTRIES: FINDINGS FROM THE PLANTLIBRA CONSUMERS SURVEY AND FOCUS GROUPS IN THREE COUNTRIES

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Recent years have witnessed an increase in the use of plant food supplements in Europe but consumer-reported intake data is limited and often occurs in the more general context of surveys of dietary supplement intake or use of complementary or alternative medicine (Skeie, Braateen, Hjartaker & Lentjes; 2009; Vargas-Murga, Garcia-Alvarez, Roman-Vinas, Ngo, Ribas-barbara et al; 2011).

A retrospective, cross-sectional survey of consumers of plant food supplements was conducted in 24 cities in six European countries (Finland, Germany, Italy, Romania, Spain, UK). Eligible consumers were identified using a brief screening questionnaire and subsequently provided detailed information on their product usage in the preceding 12 months, including frequency of use, reasons for use and sources of product information.

Consumer perceptions of the role and efficacy of plant food supplements were explored in a separate study in which a number of focus groups were conducted in three countries (Italy, Romania and UK), with both consumers and non-consumers. The effect of information presented on packaging on the assessment of benefits and risks was also explored in the focus groups.

A total of 2359 consumers participated in the survey (48.4% male; 51.6% female), reporting on the consumption of 1288 products, containing 491 different botanical ingredients, across the six countries. Overall the principal health reasons for which plant food supplements were used were defence/immunity (33%), energy (15%), digestion (14%), sleeping (10%) and flu/cold (10%). Consumers over 65 are more likely to use products for cholesterol, digestion, joints and bones, heart and circulation, respiratory issues and prostate than their younger peers. Consumers under 65 are more likely to list issues like weight, hair and skin, flu/cold and energy as their reasons for use. The most trusted sources of information are nutritionists, complementary therapists and scientific journals; the least trusted are friends and family, magazine and the internet.

Two focus groups were conducted in each of three countries; one with users (n=5-6) and one with non-users (n=5-6) of plant food supplements. Overall plant food supplements were perceived as natural products, and perceived as unlikely to cause any harm. Users viewed plant food supplements as compensating for deficiencies associated with demanding modern lifestyles; non-users believed that eating an adequate diet and having a healthy lifestyle negated the need for such products. Trust was an issue for both groups with users referring to brand trust and non-users perceiving a lack of scientific evidence on efficacy. The source of information cited most frequently was 'word of mouth'. Product packaging that evoked naturalness and simplicity and the plant name in the title was preferred by users. Information on pack perceived to be useful included benefits, instructions for use, ingredients and warnings.

Keywords: plant food supplements, usage, naturalness, lifestyle, packaging

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CHARACTERS AND BOTANICAL COMPONENTS OF PFS USED IN SIX EUROPEAN COUNTRIES: FINDINGS FROM THE PLANTLIBRA CONSUMER SURVEY.

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The PlantLIBRA PFS consumption survey has been conducted in six European countries and involved a final sample of 2359 consumers, according to the criteria defined by the study group. From the completed interviews the PFS consumed per country and all the botanical included in these products have been extracted, with these data a product/botanical database has been created and analysed using the SPSS 18.0 statistical software.

An overall of 1288 different products were identified, and significant differences were observed between countries (higher number of different products in Italy and Spain, lower in UK). The number of different botanical ingredients was 491, and also in this case UK differs from the other five countries with approximately one quarter of different botanical consumed. Approximately 57% of the PFS are products with only one botanical ingredient, range between countries being: UK=84.5%; Finland=20.5%. Less multi-botanical products were used by consumers over 60 than by those aged 18-59.

The most consumed dose forms were capsules (38.3%) and pills/tablets/lozenges (36.8).

Considering a maximum of 5 products used by each consumer, Ginkgo biloba and Oenothera biennis are the more used botanical in the 6 countries as a whole, and mostly as a single ingredient in the PFS (66% and 80.9% respectively). When data are stratified by gender, men seems to favour Ginkgo and Panax ginseng, while evening primrose (Oenotera biennis) and artichoke (Cynara scolimus) are the two most consumed botanicals by women. Looking at country level, in Germany and Romania ginkgo is the most common botanical. In Finland, PFS containing soy are the most consumed products (in 86.3% of the cases, multi-botanical products). In Italy Aloe vera is the first botanical, both as a single or multi-ingredient PFS, moreover it has a widespread use in Romania, UK and Germany, while in Spain and Finland it does not appear among the first 25 plants. In Spain artichoke is the most consumed botanical, in Germany it is the second one. Data from UK deviate from the data of other EU partners, since evening primrose represents more than one third of the counts, suggesting a more focalised consumption of PFS by the UK's responders. When comparing the country list of most consumed botanicals, however, the different legal frameworks about botanical must be kept in mind, since in countries like Germany many plants are present in the market as herbal medicinal products (HMP), and these products have been consistently excluded from the survey results.

MOST USED BOTANICALS – WHICH ARE THEY? WITH A SPECIAL FOCUS ON ARTICHOKE

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PlantLIBRA Work package 1: Intake estimation of Plant Food Supplements (PFS)

One of the objectives of PlantLIBRA and especially of this work package was to estimate the plant food supplement (PFS) intake in Europe. Therefore, a cross-sectional retrospective PFS consumer survey was conducted in six European countries. In this survey PFS consumers were asked about their consumption behaviour regarding PFS and about lifestyle factors to describe a consumer profile.

The PFS consumers described their consumed products. These data were recorded in an own database (consumer database). Then every single reported product was checked if it is a PFS or not and the botanical ingredients were recorded. For this analysis further databases had to be built up. A combined botanical database was created. Each botanical was coded with a unique identification code and the scientific, English and local name. In another database each product was coded for its botanical ingredients (product-botanical database). By linking all three databases analyses and comparisons between countries are possible for the botanical ingredients.

This way, a total number of 491 botanicals were identified as ingredients of plant food supplements consumed by the participants of the PlantLIBRA PFS consumer survey. The botanicals were ingredients of single or multi-botanical products. Some botanicals have been reported more often than others (n = 1 to 194).

Artichoke (Cynara scolymus) is one of the most consumed botanicals after Ginkgo (Ginkgo biloba) and Primrose (Oenothera biennis). It is among the eleven botanicals that were reported more than a 100 times with regard to the results of all countries (rank 3).

Products containing artichoke are in particular popular in Spain and Germany (rank 1 and 2) and also in Romania and Italy (rank 7 and 10). In contrast, they were not stated by British consumers. They are taken more often by females than by males but there could be observed no difference in the consumption behaviour of younger and older consumers.

The most frequent reason for the intake of products containing artichoke reported by consumers was support of the digestive function. This is also true regarding the data of the respective single countries except for Spain, where body weight management was reported as the main reason. Other important reasons for intake are cholesterol management and liver and urinary tract support.

Conclusion: Data about plant food supplements were obtained by means of a cross-sectional retrospective PFS consumer survey in six European countries. The botanicals used as ingredients in food supplements could be identified from the primary data by respective analysis techniques. This way, a wide variety of botanicals (491) used in PFS could be identified. However, the analysed frequency of the botanicals in PFS differs in a wide range (n = 1-194).

Artichoke (Cynara scolymus) is one of the most consumed botanicals (rank 3). Products containing artichoke are popular in Spain, Germany, Romania and Italy. They are taken more often by females than by males. The most frequent reason for the intake reported by consumers was support of the digestive function. Other important reasons for intake are body weight management, cholesterol management and liver and urinary tract support.

Keywords: Botanicals, artichocke, Cynara scolymus, plant food supplements (PFS), PFS consumption survey, PlantLIBRA

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THE USE OF PFS CONTAINING GINKGO BILOBA IN EUROPE- REASON OF USE, CONSUMER BEHAVIOURS AND PERCEPTIONS OF BENEFITS: THE PLANTLIBRA STUDY

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Many consumer studies focus on the use of dietary supplements in general and not specifically on those that are plant-derived. Plant food supplements are often used for medicinal purposes and not specifically as dietary supplements.

The work will analyse the data obtained by the 6-coutries European survey (Italy, Spain, Romania, UK, Finland, Germany), performed in the frame of PlantLIBRA project, concerning the consumers using PFS containing Ginkgo biloba. Data on consumer's characteristics, patterns of consumption (frequency of use, dosage, co-administration with drugs), reasons of use, and consumers' perception on benefits were compared with scientific data obtained from epidemiological, preclinical and clinical studies. The data were presented for general European group, as well as for each country, identifying if some differences by age groups, gender or other criteria appeared.

Keywords: Ginkgo biloba, consumers, plant food supplements

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PLANT FOOD SUPPLEMENTS (PFS) IN THE PRINT MEDIA: A SURVEY IN ITALY, ROMANIA AND UK

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Print media are an influential source of information for PFS. We conducted a detailed analysis of the messages on PFS in print media.

The participating countries designed collectively the framework for the analysis to avoid discrepancies. Each country selected at least two newspapers and two magazines. All articles were evaluated by two independent researchers: contents, headlines, leads and related articles were recorded.

Broadly articles fell into three categories:

(1) reporting recent research findings about benefits and/or risks of a specific PFS; the contents and tone of the article were dictated by the findings or the journalist's interpretation of them.

(2) presenting either the benefits of a specific plant or a particular benefit category, for example energy boosters, most often positive in tone.

(3) providing an overview of some aspects of the market (much rarer).

<u>Italy.</u> We noted a difference between newspapers and magazines in how stories are told, which could be due to the difference in professional authorship: articles in newspapers were derived mainly from industrial sources (70%) or scientists (30%) mediated by journalists; in magazines more frequently scientific experts and sources were used. In both cases the communication is clear and positive Articles in magazines are often accompanied by images. In both types of print media, the tone of the articles was positive. Benefits are always outlined with positive wordings (enhancement, favoring, etc); risks or interactions are rarely presented.

Romania. Articles are addressed to general people, women and athletes. The themes concerning energy, well-being, body detox or natural weight loss indicated the benefits of herbals and PFS and only 40% possible risk/adverse effects. Sometimes doctors and herbalists wrote the articles, using a positive tone. Sources of information are rarely mentioned. Data presented are similar at advertising and data packaging of the products. A few articles indicated scientific data.

<u>UK.</u> Articles in main pages in newspapers were summaries by journalists of recently published research. By contrast, articles in the "health" section, written by doctors and specialists, were more similar in tone and content to magazine articles, which either described a specific herb or depict it as a possible remedy for a particular condition. Shorter articles focused on either the health benefits of PFSs or risks associated (not working or unwanted effects). Longer articles generally described a plant's

potential benefits using approved wording and included a warning of risks.

Print media appear to be a reliable source of information on PFS, in the three countries, with the limitation of the lack of reference to risks and interactions with drugs in Italy and Romania. The choice of information to include, messages to emphasize and whether to provide context to make that information meaningful varied according to individual publications and journalists.

Keywords: plant food supplement, print media, health, analysis, medical journalism, benefits, risks

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HEALTH IMAGES ON PRODUCT PACKAGING INFLUENCE CONSUMER'S UNDERSTANDING OF THE PRODUCT'S HEALTH-RELATED FUNCTIONS.

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Food product packaging frequently carries health-related information - either in the form of a written health claim or a pictorial, graphic or symbolic representation – so it is therefore potentially an important source of information for consumers. However, consumers generally spend minimal time attending to product packaging at the point of sale (Grunert, Wills & Fernádez-Celemin, 2010). Even when adequately attended to, product packaging may confuse consumers into drawing inappropriate health inferences (Urala, Arvola & Lähteenmäki, 2003) and thus might sometimes act as a source of misinformation instead of a source of information. It is therefore important to understand how all aspects of the packaging

environment interact to influence consumer's beliefs about a product's function.

Front-of-pack labelling has been studied quite extensively in relation to consumers' thinking and reasoning about food products. However, little research has focused on the role of pictorial, graphic or symbolic representations on front-of-pack labelling in shaping consumer thinking and reasoning. Furthermore, such research is limited in understanding consumer's thinking and reasoning about dietary supplements.

This research aimed to investigate whether the presentation of pictorial, graphic or symbolic representations on the labels of fictitious dietary supplements would lead people to falsely recognise health claims as being associated with those products. Furthermore, it aimed to investigate whether these recognition errors could be reduced through the use of an explicit warning.

In two laboratory experiments participants viewed fictitious dietary supplement packaging on some of which pictorial representations of the products' supposed health-related function were added. These product labels were viewed alongside a variety of health claims, and subsequently participants undertook a recognition memory test comprising previously seen and novel health claims. In a subsequent replication of this experiment, half of the participants were given an explicit warning that the pictorial representations were meaningless.

Findings suggest that when a pictorial representation was present on the product label, participants were significantly more likely to erroneously recognise novel health claims that were implied by the images. Guessing did not fully account for these recognition errors; rather, participants were frequently confident that they had seen these claims and, in some cases, claimed to actually remember seeing them. Furthermore, although the addition of an explicit warning appeared to reduce these recognition errors, the reduction was not statistically significant.

We conclude that pictorial representations on product packaging can implicitly prime the formation of expectations regarding a product's function, and that viewers' cognitions might be guided by these expectations even when explicitly instructed to ignore this information. We propose that memory-based measures such as ours could offer a useful way of assessing consumers' understanding of product labels without depending on explicit (and often unreliable) self-reports.

Keywords: visual images, health claims, memory, consumer behaviour

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PLENARY SESSION III Understanding the use of PFS

USAGE OF PLANT FOOD SUPPLEMENTS ACROSS SIX EUROPEAN COUNTRIES: FINDINGS FROM THE PLANTLIBRA CONSUMER SURVEY

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Background: The popularity of botanical products is on the rise in Europe, with consumers using them to complement their diets or to maintain health, and products are taken in many different forms (e.g. teas, juices, herbal medicinal products, plant food supplements (PFS)). However there is a scarcity of data on the usage of such products at European level.

Objective: To provide an overview of the characteristics and usage patterns of PFS consumers in six European countries.

Design: Data on PFS usage were collected in a cross-sectional, retrospective survey of PFS consumers using a bespoke frequency of PFS usage questionnaire.

Subjects/setting: A total sample of 2359 adult PFS consumers from Finland, Germany, Italy, Romania, Spain and the United Kingdom.

Data analyses: Descriptive analyses were conducted, with all data stratified by gender, age, and country. Absolute frequencies, percentages and 95% confidence intervals are reported.

Results: Overall, an estimated 18.8% of screened survey respondents used at least one PFS. Characteristics of PFS consumers included being older, well-educated, never having smoked and self-reporting health status as "good or very good". Across countries, 491 different botanicals were identified in the PFS products used, with *Ginkgo biloba* (Ginkgo), *Oenothera biennis* (Evening primrose) and *Cynara scolymus* (Artichoke) being most frequently reported; the most popular dose forms were capsules and pills/tablets. Most consumers used one product and half of all users took single-botanical products. Some results varied across countries.

Conclusions: The PlantLIBRA consumer survey is unique in reporting on usage patterns of PFS consumers in six European countries. The survey highlights the complexity of measuring the intake of such products, particularly at pan-European level. Incorporating measures of the intake of botanicals in national dietary surveys would provide much-needed data for comprehensive risk and benefit assessments at the European level.

Keywords: PlantLIBRA, plant food supplements, herbal dietary supplements, European survey, botanicals, consumer; usage

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WHY EUROPEAN ADULTS USE PLANT FOOD SUPPLEMENTS IN SIX EUROPEAN COUNTRIES: FINDINGS FROM THE PLANTLIBRA CONSUMERS SURVEY AND FOCUS GROUPS IN THREE COUNTRIES

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Recent years have witnessed an increase in the use of plant food supplements in Europe but consumer-reported intake data is limited and often occurs in the more general context of surveys of dietary supplement intake or use of complementary or alternative medicine (Skeie, Braateen, Hjartaker & Lentjes; 2009; Vargas-Murga, Garcia-Alvarez, Roman-Vinas, Ngo, Ribas-barbara et al; 2011).

A retrospective, cross-sectional survey of consumers of plant food supplements was conducted in 24 cities in six European countries (Finland, Germany, Italy, Romania, Spain, UK). Eligible consumers were identified using a brief screening questionnaire and subsequently provided detailed information on their product usage in the preceding 12 months, including frequency of use, reasons for use and sources of product information.

Consumer perceptions of the role and efficacy of plant food supplements were explored in a separate study in which a number of focus groups were conducted in three countries (Italy, Romania and UK), with both consumers and non-consumers. The effect of information presented on packaging on the assessment of benefits and risks was also explored in the focus groups.

A total of 2359 consumers participated in the survey (48.4% male; 51.6% female), reporting on the consumption of 1288 products, containing 491 different botanical ingredients, across the six countries. Overall the principal health reasons for which plant food supplements were used were defence/immunity (33%), energy (15%), digestion (14%), sleeping (10%) and flu/cold (10%). Consumers over 65 are more likely to use products for cholesterol, digestion, joints and bones, heart and circulation, respiratory issues and prostate than their younger peers. Consumers under 65 are more likely to list issues like weight, hair and skin, flu/cold and energy as their reasons for use. The most trusted sources of information are nutritionists, complementary therapists and scientific journals; the least trusted are friends and family, magazine and the internet.

Two focus groups were conducted in each of three countries; one with users (n=5-6) and one with non-users (n=5-6) of plant food supplements. Overall plant food supplements were perceived as natural products, and perceived as unlikely to cause any harm. Users viewed plant food supplements as compensating for deficiencies associated with demanding modern lifestyles; non-users believed that eating an adequate diet and having a healthy lifestyle negated the need for such products. Trust was an issue for both groups with users referring to brand trust and non-users perceiving a lack of scientific evidence on efficacy. The source of information cited most frequently was 'word of mouth'. Product packaging that evoked naturalness and simplicity and the plant name in the title was preferred by users. Information on pack perceived to be useful included benefits, instructions for use, ingredients and warnings.

Keywords: plant food supplements, usage, naturalness, lifestyle, packaging

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CHARACTERS AND BOTANICAL COMPONENTS OF PFS USED IN SIX EUROPEAN COUNTRIES: FINDINGS FROM THE PLANTLIBRA CONSUMER SURVEY

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The PlantLIBRA PFS consumption survey has been conducted in six European countries and involved a final sample of 2359 consumers, according to the criteria defined by the study group. From the completed interviews the PFS consumed per country and all the botanical included in these products have been extracted, with these data a product/botanical database has been created and analysed using the SPSS 18.0 statistical software.

An overall of 1288 different products were identified, and significant differences were observed between countries (higher number of different products in Italy and Spain, lower in UK). The number of different botanical ingredients was 491, and also in this case UK differs from the other five countries with approximately one quarter of different botanical consumed. Approximately 57% of the PFS are products with only one botanical ingredient, range between countries being: UK=84.5%; Finland=20.5%. Less multi-botanical products were used by consumers over 60 than by those aged 18-59.

The most consumed dose forms were capsules (38.3%) and pills/tablets/lozenges (36.8).

Considering a maximum of 5 products used by each consumer, Ginkgo biloba and Oenothera biennis are the more used botanical in the 6 countries as a whole, and mostly as a single ingredient in the PFS (66% and 80.9% respectively). When data are stratified by gender, men seems to favour Ginkgo and Panax ginseng, while evening primrose (Oenotera biennis) and artichoke (Cynara scolimus) are the two most consumed botanicals by women. Looking at country level, in Germany and Romania ginkgo is the most common botanical. In Finland, PFS containing soy are the most consumed products (in 86.3% of the cases, multi-botanical products). In Italy Aloe vera is the first botanical, both as a single or multi-ingredient PFS, moreover it has a widespread use in Romania, UK and Germany, while in Spain and Finland it does not appear among the first 25 plants. In Spain artichoke is the most consumed botanical, in Germany it is the second one. Data from UK deviate from the data of other EU partners, since evening primrose represents more than one third of the counts, suggesting a more focalised consumption of PFS by the UK's responders. When comparing the country list of most consumed botanicals, however, the different legal frameworks about botanical must be kept in mind, since in countries like Germany many plants are present in the market as herbal medicinal products (HMP), and these products have been consistently excluded from the survey results.

MOST USED BOTANICALS – WHICH ARE THEY? WITH A SPECIAL FOCUS ON ARTICHOKE

<u>S. de Klein</u>, E. M. Meissner PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany PlantLIBRA Work package 1: Intake estimation of Plant Food Supplements (PFS)

One of the objectives of PlantLIBRA and especially of this work package was to estimate the plant food supplement (PFS) intake in Europe. Therefore, a cross-sectional retrospective PFS consumer survey was conducted in six European countries. In this survey PFS consumers were asked about their consumption behaviour regarding PFS and about lifestyle factors to describe a consumer profile.

The PFS consumers described their consumed products. These data were recorded in an own database (consumer database). Then every single reported product was checked if it is a PFS or not and the botanical ingredients were recorded. For this analysis further databases had to be built up. A combined botanical database was created. Each botanical was coded with a unique identification code and the scientific, English and local name. In another database each product was coded for its botanical ingredients (product-botanical database). By linking all three databases analyses and comparisons between countries are possible for the botanical ingredients.

This way, a total number of 491 botanicals were identified as ingredients of plant food supplements consumed by the participants of the PlantLIBRA PFS consumer survey. The botanicals were ingredients of single or multi-botanical products. Some botanicals have been reported more often than others (n = 1 to 194).

Artichoke (Cynara scolymus) is one of the most consumed botanicals after Ginkgo (Ginkgo biloba) and Primrose (Oenothera biennis). It is among the eleven botanicals that were reported more than a 100 times with regard to the results of all countries (rank 3).

Products containing artichoke are in particular popular in Spain and Germany (rank 1 and 2) and also in Romania and Italy (rank 7 and 10). In contrast, they were not stated by British consumers. They are taken more often by females than by males but there could be observed no difference in the consumption behaviour of younger and older consumers.

The most frequent reason for the intake of products containing artichoke reported by consumers was support of the digestive function. This is also true regarding the data of the respective single countries except for Spain, where body weight management was reported as the main reason. Other important reasons for intake are cholesterol management and liver and urinary tract support.

Conclusion: Data about plant food supplements were obtained by means of a cross-sectional retrospective PFS consumer survey in six European countries. The botanicals used as ingredients in food supplements could be identified from the primary data by respective analysis techniques. This way, a wide variety of botanicals (491) used in PFS could be identified. However, the analysed frequency of the botanicals in PFS differs in a wide range (n = 1-194).

Artichoke (Cynara scolymus) is one of the most consumed botanicals (rank 3). Products containing artichoke are popular in Spain, Germany, Romania and Italy. They are taken more often by females than by males. The most frequent reason for the intake reported by consumers was support of the digestive function. Other important reasons for intake are body weight management, cholesterol management and liver and urinary tract support.

Keywords: Botanicals, artichocke, Cynara scolymus, plant food supplements (PFS), PFS consumption survey, PlantLIBRA

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THE USE OF PFS CONTAINING GINKGO BILOBA IN EUROPE- REASON OF USE, CONSUMER BEHAVIOURS AND PERCEPTIONS OF BENEFITS: THE PLANTLIBRA STUDY

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Many consumer studies focus on the use of dietary supplements in general and not specifically on those that are plant-derived. Plant food supplements are often used for medicinal purposes and not specifically as dietary supplements.

The work will analyse the data obtained by the 6-coutries European survey (Italy, Spain, Romania, UK, Finland, Germany), performed in the frame of PlantLIBRA project, concerning the consumers using PFS containing Ginkgo biloba. Data on consumer's characteristics, patterns of consumption (frequency of use, dosage, co-administration with drugs), reasons of use, and consumers' perception on benefits were compared with scientific data obtained from epidemiological, preclinical and clinical studies. The data were presented for general European group, as well as for each country, identifying if some differences by age groups, gender or other criteria appeared.

Keywords: Ginkgo biloba, consumers, plant food supplements

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PLANT FOOD SUPPLEMENTS (PFS) IN THE PRINT MEDIA: A SURVEY IN ITALY, ROMANIA AND UK

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Print media are an influential source of information for PFS. We conducted a detailed analysis of the messages on PFS in print media.

The participating countries designed collectively the framework for the analysis to avoid discrepancies. Each country selected at least two newspapers and two magazines. All articles were evaluated by two independent researchers: contents, headlines, leads and related articles were recorded.

Broadly articles fell into three categories:

 reporting recent research findings about benefits and/or risks of a specific PFS; the contents and tone of the article were dictated by the findings or the journalist's interpretation of them.
 presenting either the benefits of a specific plant or a particular benefit category, for example energy boosters, most often positive in tone.

(3) providing an overview of some aspects of the market (much rarer).

<u>Italy.</u> We noted a difference between newspapers and magazines in how stories are told, which could be due to the difference in professional authorship: articles in newspapers were derived mainly from industrial sources (70%) or scientists (30%) mediated by journalists; in magazines more frequently scientific experts and sources were used. In both cases the communication is clear and positive Articles in magazines are often accompanied by images. In both types of print media, the tone of the articles was positive. Benefits are always outlined with positive wordings (enhancement, favoring, etc); risks or interactions are rarely presented.

Romania. Articles are addressed to general people, women and athletes. The themes concerning energy, well-being, body detox or natural weight loss indicated the benefits of herbals and PFS and only 40% possible risk/adverse effects. Sometimes doctors and herbalists wrote the articles, using a positive tone. Sources of information are rarely mentioned. Data presented are similar at advertising and data packaging of the products. A few articles indicated scientific data.

<u>UK.</u> Articles in main pages in newspapers were summaries by journalists of recently published research. By contrast, articles in the "health" section, written by doctors and specialists, were more similar in tone and content to magazine articles, which either described a specific herb or depict it as a possible remedy for a particular condition. Shorter articles focused on either the health benefits of PFSs or risks associated (not working or unwanted effects). Longer articles generally described a plant's potential benefits using approved wording and included a warning of risks.

Print media appear to be a reliable source of information on PFS, in the three countries, with the limitation of the lack of reference to risks and interactions with drugs in Italy and Romania. The choice of information to include, messages to emphasize and whether to provide context to make that information meaningful varied according to individual publications and journalists.

Keywords: plant food supplement, print media, health, analysis, medical journalism, benefits, risks

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HEALTH IMAGES ON PRODUCT PACKAGING INFLUENCE CONSUMER'S UNDERSTANDING OF THE PRODUCT'S **HEALTH-RELATED FUNCTIONS**

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Food product packaging frequently carries health-related information - either in the form of a written health claim or a pictorial, graphic or symbolic representation - so it is therefore potentially an important source of information for consumers. However, consumers generally spend minimal time attending to product packaging at the point of sale (Grunert, Wills & Fernádez-Celemin, 2010). Even when adequately attended to, product packaging may confuse consumers into drawing inappropriate health inferences (Urala, Arvola & Lähteenmäki, 2003) and thus might sometimes act as a source of misinformation instead of a source of information. It is therefore important to understand how all aspects of the packaging environment interact to influence consumer's beliefs about a product's function.

Front-of-pack labelling has been studied quite extensively in relation to consumers' thinking and reasoning about food products. However, little research has focused on the role of pictorial, graphic or symbolic representations on front-of-pack labelling in shaping consumer thinking and reasoning. Furthermore, such research is limited in understanding consumer's thinking and reasoning about dietary supplements.

This research aimed to investigate whether the presentation of pictorial, graphic or symbolic representations on the labels of fictitious dietary supplements would lead people to falsely recognise health claims as being associated with those products. Furthermore, it aimed to investigate whether these recognition errors could be reduced through the use of an explicit warning.

In two laboratory experiments participants viewed fictitious dietary supplement packaging on some of which pictorial representations of the products' supposed health-related function were added. These product labels were viewed alongside a variety of health claims, and subsequently participants undertook a recognition memory test comprising previously seen and novel health claims. In a subsequent replication of this experiment, half of the participants were given an explicit warning that the pictorial representations were meaningless.

Findings suggest that when a pictorial representation was present on the product label, participants were significantly more likely to erroneously recognise novel health claims that were implied by the images. Guessing did not fully account for these recognition errors; rather, participants were frequently confident that they had seen these claims and, in some cases, claimed to actually remember seeing them. Furthermore, although the addition of an explicit warning appeared to reduce these recognition errors, the reduction was not statistically significant. We conclude that pictorial representations on product packaging can implicitly prime the formation of expectations regarding a product's function, and that viewers' cognitions might be guided by these expectations even when explicitly instructed to ignore this information. We propose that memory-based measures such as ours could offer a useful way of assessing consumers' understanding of product labels without depending on explicit (and often unreliable) self-reports.

Keywords: visual images, health claims, memory, consumer behaviour

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PLENARY SESSION IV ePlantLIBRA

USERS AND APPLICATIONS OF ePLANTLIBRA AN ONLINE PLANT FOOD SUPPLEMENTS DATABASE

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Summary

ePlantLIBRA is a comprehensive web-based database containing information on the content of bioactive compounds in plant food supplements (PFS), as well as their beneficial and adverse biological effects (http://eplantlibra.eurofir.eu). The information in ePlantLIBRA has been extracted from peerreviewed publications, evaluated for quality and inputted into the database by trained evaluators. The database is of practical use to researchers, epidemiologists, clinical toxicologists, poisons information specialists, food regulatory authorities and product developers in the PFS industry. Since the database contains many of the technical, exposure and toxicological effects data required by the European Food Safety Authority to assess the safety of botanicals for use in PFS, the database is particularly useful to national bodies representing PFS manufacturers, and to regulators.

H. Kergosien will be presenting the use of ePlantLIBRA database as a tool for PFS Manufacturers to meet National and European Regulation requirements, covering how the database could help French Botanical Food Supplements Manufacturers to meet requirements included in their Decree which will be implemented soon. This regulatory text has an Annex I with a list of 550 plants with special warnings about possible toxic substances or special labelling requirements and an Annex III about safety. The ePlantLIBRA database can also be useful to substantiate Botanical Health claims.

K Riediger will be presenting the user perspectives on behalf of the PlantLIBRA Policy Advisory Board, the board consists of representatives of regulatory bodies in the European member states and non-European counterparts.

H Kupferschmidt will present the use of ePlantLIBRA for Poisons centres and users with specific interest in the potential adverse effects of PFS.

Keywords: plant food supplements; bioactive; botanicals; database; adverse effects; regulations

THE ePLANTLIBRA DATABASE: COMPOSITION AND **BIOLOGICAL EFFECTS DATA ON PLANT FOOD SUPPLEMENTS**

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Background

A primary aim of the PlantLIBRA project (www.plantlibra.eu) was to improve the plant food supplement (PFS) scientific knowledge base to better enable regulators and stakeholders to assess the risks and benefits of PFS. An objective of the project was to develop a comprehensive database containing up-todate, validated scientific information on PFS bioactive compounds with reported health benefits or adverse effects, and on the contaminants and residues that may be associated with them. This has been achieved through development of the unique, web-based ePlantLIBRA meta-database.

Methods

PlantLIBRA has made use of the methodologies and databases established during two EU FP6 funded projects (EuroFIR and MoniQA) in developing the ePlantLIBRA meta-database. ePlantLIBRA is identical in structure and function to the EuroFIR eBASIS database (http://ebasis.eurofir.eu), which contains composition and biological activity data for bioactive compounds in plant foods. Plants in ePlantLIBRA have been connected to corresponding commodities in the MoniQA database (www.moniqa.eu), with links to pesticide Maximum Residue Limits and other contaminants. Data on global occurrences of safety issues from the HorizonScan database (www.horizonscan.com) have also been linked to ePlantLIBRA plants to demonstrate the range of residues and contaminants likely to be encountered in such commodities.

Results

ePlantLIBRA offers a user-friendly and efficient interface for searching, extracting and exporting data on PFS, with links back to original published references. To date, data from over 400 publications have been evaluated for quality and entered into ePlantLIBRA, covering 49 PFS or their botanical ingredients. All plants within the database have corresponding contaminant and pesticide residue data.

Conclusions

The ePlantLIBRA meta-database is a powerful tool for expert users. Combining information on bioactive compounds, analytical methods, case-reports of adverse events, literature on beneficial effects and potential contaminants in a single platform, it is a valuable resource for food regulatory bodies, risk authorities and researchers, as well as product developers within the food industry.

Keywords: ePlantLIBRA, plant food supplements, database, bioactive compounds, contaminants.

POSTER SESSION

VOLTAMMETRIC DETECTION OF L-ASCORBIC ACID FROM PHARMACEUTICAL SAMPLES AND PLANT FOOD SUPPLEMENTS

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Ascorbic acid (vitamin C) is important in some biochemical pathways, and during the last years as active compounds used in several pesticides (L-ascorbic acid is used as a fungicide on glasshouse tomato, potato, and field and glasshouse flower bulbs) is a challenge for groups of researchers to develop new, rapid and sensitive method for its detection.

Voltammetric methods for the detection of ascorbic acid were developed and optimised. There were used differential pulse voltammetry (DPV) and cyclic voltammetry (CV) using screenprinted carbon-electrodes and modified carbon-printed electrodes with cobalt phthalocyanine, as mediator.

Detection of ascorbic acid in different media: phosphate buffer (pH 7) and acid media (hydrochloric acid 2% and 3.65%) were studied.

Results showed that the method developed for ascorbic acid can be used to test different samples as biological, pharmaceutical samples, as well as teas and plant food supplements containing *Hippophae rhamnoides* (seabuckthorn).

Keywords: L-ascorbic acid, differential pulse voltammetry, cyclic voltammetry, *Hippophae rhamnoides*

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PLANT ORIGINATED FOOD¹ AS A TOOL OF PERSONALIZED MEDICINE FOR PREVENTION OF INFLAMMATION ASSOCIATED DISEASES

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Today the investigation of plants based food products, which is a source of prebiotic biologically active substances and probiotics is very relevant.

The components of "healthy food" are characterized mainly by selective ability to influence microbiocenosis in human intestine, causing its correction in the direction of decrease in number of pathogenic and opportunistic microorganisms, and thus directly regulating body's metabolism [1]. Molecular-cellular action mechanisms of biologically active

substances of six strongest extracts of edible plants (dill, pomegranate, persimmon, kale, nettle and Sideritis scardica) to the immune response in experimental mice and human intestinal microbiota have been investigated. We had demonstrated that edible plants extracts both stimulate beneficial strains of bacteria and inhibit the growth of opportunistic microorganisms in a strain specific way [2]. Defined plants extracts induce a shift in expression of pro-/antiinflammatory CD1a molecules on a surface of human DCs derived from peripheral blood monocytes, moDCs [3]. Differentiation of mononuclear cells into CD1a- negative dendritic cells under the influence of kale, pomegranate and persimmon plant extracts in vitro is an important proof of their anti-inflammatory properties. Sideritis scardica extract does not significantly affect dendritic cell activity, but tends to act similarly to pomegranate and persimmon extracts. In this case, the best results were found during exposure control with relevant extracts for 6 h. In addition we were demonstrated that commensal gut bacteria are able modulate human moDCs on dose-dependent and strain-specific manner [4].

Based on the received data we have designed innovative foods that will be used for application of personalized nutrition, preventive and medical diets, depending on the individual indicators of consumers and patients. The potential of these products is a part of public health prevention strategy. **Keywords**: plant originated food, biologically active substances (BAS), preventive personalized medicine.

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SIMULTANEOUS DETERMINATION OF AFLATOXIN B2 AND OCHRATOXIN A BY HPTLC-PHOTODENSITOMETRY AND HPTLC-IMAGE PROCESSING

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Mycotoxins are secondary metabolites of fungi which occur in a variety of plant products. They have received considerable attention due to their significance in human health. Among the mycotoxins that are known to cause human diseases, aflatoxins and ochratoxins have been most studied. Thin layer chromatography was employed for simultaneous determination of aflatoxin B2 and ochratoxin A. As stationary phases, HPTLC RP-18 WF245 and HPTLC Silica gel 60 were used. The composition of the mobile phases Methanol-Water and Toluene - Ethyl acetate - Formic acid - Chloroform respectively were optimized in order to obtain a good separation of the analytes and sharp spots. Plate evaluation/documentation was performed in two different ways: classical method - photodensitometry (excitation at 333 nm and emission at 460nm) and applying a new technique - image processing. The developed methods were validated in terms of linearity, sensitivity (LOD and LOQ), precision and accuracy.

Keywords: aflatoxin B2, ochratoxin A, HPTLC, photodensitometry, image processing

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NEW METHODS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY FOR THE SCREENING OF FOOD SUPPLEMENTS AND THEIR BOTANICAL INGREDIENTS

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Botanicals and botanical derivatives are widely consumed in Western diets from various sources; among them, plant food supplements (PFS) are the most usual commercialized forms. The wide diffusion of these products requires new analytical methods to control their quality, guarantee their safety, and contribute to the scientific studies aimed to evaluate their beneficial effects.

PlantLIBRA project (acronym of PLANT food supplements: Levels of Intake, Benefit and Risk Assessment) aims to foster the safe use of plant food supplements. One of the objectives of this project is the characterization of botanical as raw materials, extracts, or ingredients of PFS present in the market. High Performance Thin Layer Chromatography (HPTLC) is a fast and suitable method for the screening of different groups of molecules, allowing the characterization of several plant preparations [Reich and Schibli, 2006]. Another objective of this research was the application of HPTLC technique to the measure of biological properties: among these positive results have been obtained for the semi-quantitative estimation of the antioxidant activity of active molecules contained in *Camellia sinensis* L. and *Vitis vinifera* L.

For this purpose, HPTLC methods for determining antioxidant activity were developed and validated in parallel on plates, using the radical DPPH (1,1-diphenyl-2- picrylhydrazyl) as derivatization agent [Reich et al.,2008]. The two screening methods were performed in the same chromatographic conditions.

HPTLC The semi-quantitative analysis allowed the characterization of catechins and caffeine in different samples of Camellia sinensis, and anthocyanins, flavonols and phenolic acids in samples of Vitis vinifera. Two extracts (from Camellia sinensis and Vitis vinifera), used as controls, were kindly PhytoLab (Vestenbergsgreuth, Germany). supplied by Comparing the results obtained from different HPTLC methods a good correlation between the antioxidant activity and catechins profile was observed. The concentration of catechins measured in green tea resulted higher than in black tea, as reported in literature [Sharma and Rao, 2009] and confirmed by quantitative HPLC analysis.

HPTLC demonstrated to be a fast and simple method for the characterization of different botanical preparations and a valid tool for routine quality control. Furthermore, the HPTLC technique allowed the association of the fingerprint with a fast evaluation of relative antioxidant activity.

Keywords: HPTLC, fingerprint, antioxidant activity, plant food supplements

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DEVELOPMENT, VALIDATION AND APPLICATION OF A HPLC-UV METHOD FOR THE CHARACTERIZATION OF PLANT FOOD SUPPLEMENTS CONTAINING Camellia sinensis L.

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One of the objectives of the PlantLIBRA project is to promote the validation of analytical methods suitable for the characterization of herbal extracts used as ingredients in Plant Food Supplements (PFS). Camellia sinensis L., including green and black tea, is a plant widely used in food supplements. Even though with different levels according to the fermentation applied to the tea leaves, the most abundant and active components are catechins, and in particular epigallocatechin gallate. Catechins have been investigated in several studies for their antioxidant and anti-inflammatory properties [Dell'Agli et al., 2013] and positive effects on body weight, cardiovascular diseases and Central Nervous System [Chacko et al., 2010]. However, there are conflicting scientific evidences in relation to their activity on human health. One of the reasons of unsatisfactory correlation between results from different human studies is the insufficient characterization of plant extracts used.

In this work, a HPLC-UV method was developed to quantify the main active molecules present in *Camellia sinensis* raw material, extracts and PFS. The compounds analyzed were catechins (epicatechin, epigallochatechin, epicatechin gallate and epigallocatechin gallate) and caffeine. The method was validated for all analytical parameters according to the FDA guidelines (selectivity, precision, recovery, accuracy, stability, sensibility, linearity) [FDA, 2013]. Subsequently, the method was applied to the analysis of green tea raw materials and some commercial food supplements. The sample of raw green tea leaves, used as a control, was kindly supplied by Phytolab (Vestenbergsgreuth, Germany). Then, the results were compared.

The method developed was in agreement with the FDA guidelines, suitable, effective and with some advantages when compared with previous methods; the new method was more performing in relation to the chromatographic separation and resolution, time of analysis and composition of mobile phases. Food supplements containing *Camellia sinensis* presented a wide range of catechin and caffeine content, showing a relative correlation with the amount of green tea extract reported in the label.

Keywords: *Camellia sinensis* L., green tea, HPLC-UV, method validation, plant food supplements, catechins, caffeine.

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ANTI-INFLAMMATORY ACTIVITY OF AN AQUEOUS EXTRACT OF *VITIS VINIFERA* L. (VAR. TEINTURIERS) IN GASTRIC EPITHELIAL CELLS

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Helicobacter pylori (H. pylori) is the bacterium main involved in the pathogenesis of gastric diseases such as peptic ulcer and gastritis; in gastric inflammation, macrophages produce proinflammatory cytokines, including TNFa and IL-1B, which determine the activation of NF-KB in gastric epithelial cells (Mai, U.E.). NF-KB is an important transcription factor that controls the expression of numerous genes involved in inflammatory processes, including the gene of IL-8, at the level of gastric epithelium (Yasumoto, K.). Today, antacids and proton pump inhibitors are mainly used to fight gastritis, often in combination with antibiotics in the case of H. pylori infection; however, it has also been shown that the intake of this drugs causes severe adverse effects (Yang, Y.X., Laheij R.G.). For this reason, it is necessary to search for new strategies that are useful in the treatment of this pathology. The water extract of Vitis vinifera L. leaves is a component of various food supplements, but currently there are no studies in the literature on antiinflammatory activity in the gastrointestinal tract. The aim of this study was to evaluate the effect of the extract of Vitis vinifera L. on gastric inflammation. The water extract was obtained from dried leaves of Vitis vinifera L., var. teinturiers and characterized by pH differential method and HPLC-DAD analysis; the extract was assayed on human gastric epithelial cells (AGS) stimulated with TNF α and IL-1 β (both at 10 ng/mL). NF- κ B driven transcription was evaluated by transient transfection with a plasmid containing the luciferase gene. NF-KB nuclear

translocation and IL-8 secretion were performed by an ELISA assay, after 1 and 6 hours treatment respectively. Quantitative analysis showed that kaempferol-3-O-glucoside was the most abundant flavonoid, and cyanidin-3-O-glucoside the main anthocyanoside. The extract inhibited NF-KB driven transcription in concentration-dependent way, after stimulation with both TNF α and IL-1 β , with statistically significant inhibition starting from 5 µg/mL and 25 µg/mL, respectively. One hour pretreatment with the same concentrations of the extract, followed by 6 hours stimulus lead to an inhibitory effect on NF-KB driven transcription. The extract inhibited NF-kB translocation in concentration-dependent manner; the translocation was reported at the level of unstimulated control at the highest concentration assayed (50 μg/mL), after stimulation with TNFα and IL-1β. IL-8 secretion was inhibited in dose-dependent way after stimulation with TNFa while, after stimulation with IL-1β, the extract inhibited IL-8 secretion, in statistically significant way, only at the concentration of 200 µg/mL. In conclusion, this study indicates that the extract of Vitis vinifera L. is able to exert a possible beneficial effect on gastric inflammation; if this effect will be confirmed by in vivo studies, the extract of Vitis vinifera L. could be used in the treatment of gastric inflammations.

Keywords: Gastric inflammation, NF-KB, Vitis vinifera L., Leaves.

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POLYPHENOL PROFILE OF GERMAN CHAMOMILE (Matricaria recutita) AND ITS INHIBITION OF COX-2 ACTIVITY IN VITRO

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Plants have been used to ease common ailments for centuries in developing countries, and this practice is growing in Western Society (Bodeker and Kronenberg, 2002). Today, plants are used as botanical preparations or plant food supplements which have been suggested to impact positively on human health (Crozier et al., 2009, Williamson et al., 2011). German chamomile (Matricaria recutita, GC) has been reported to show anti-inflammatory activity, wound healing and sedative properties among other efficacies (Paulsen, 2002). However, its composition has not been properly established and reported, and many investigators just account for apigenin. A few analytical methods have been used to analyse GC composition (Novakova et al., 2010, Lin and Harnly, 2012), but information regarding the compounds produced during the initial stages of metabolism are inconclusive. Here we report the analysis of GC by LC-ESI/MS 2 based on previously reported transitions of precursor ions; before and after enzymatic hydrolysis. Commercially available standards were used to quantify 8 predominant polyphenols in the unconjugated form(s) before and after the hydrolysis. Furthermore, GC showed inhibition of cyclooxygenase (COX-2) activity in vitro, indicative of potential anti-inflammatory activity.

Keywords: german chamomile, cox-2 inhibition, antiinflammatory, lc-esi/ms, polyphenol

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PLANT FOOD SUPPLEMENT CONSUMPTION IN FINLAND – DIFFERENCES IN USAGE PATTERNS COMPARED TO FIVE OTHER EUROPEAN COUNTRIES

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Introduction: Plant food supplements (PFS) have traditionally been widely used in many European countries. They have high consumer acceptance because of their potential health benefits. However, the data on PFS consumption on the European level has been limited. The PlantLIBRA Consumer Survey, conducted in Finland, Germany, Italy, Romania, Spain and the United Kingdom, is unique in reporting on usage patterns of PFS consumers. This presentation highlights the differences in the PFS consumption between the Finnish subsample and the other five countries.

Materials and methods: A cross-sectional 12-month retrospective survey was conducted in Finland as part of the PlantLIBRA Consumer Survey. The sample size in Finland was 401 regular PFS consumers. Participants were all adults aged between 18 to over 60 years with 48 % males and 52 % females. Consumers were recruited from four cities located in different regions of the country. **Results:** There were a lot of similarities in the use of PFS

Results: There were a lot of similarities in the use of PFS among the six European countries. In addition, there were also some country specific features. The total number of the PFS consumed by the Finnish consumers was 213 containing 196 different botanicals. In Finland, the consumption of two or more different PFS simultaneously was more common than in other countries (40.2 % of the respondents). The Finnish consumers also tended to use more often multi-ingredient PFS than consumers in other countries (38.2 % of the respondents). The periodic usage of PFS was typical to Finnish consumers.

Finnish consumers reported health problems more often than consumers in other countries. Only 28.4 % of the Finnish respondents reported no health problems, while the figures ranged between 42.8 and 53.4 % in other countries. Almost half of the Finnish respondents used regular medication. On the other hand Finnish consumers informed less frequently their general physician or pharmacist about their use of PFS (10.7 % and 6 % respectively).

Conclusions: The risks and benefits associated with the consumption of PFS are not fully known. There is evidence that consumers might not always inform their physician about the use of PFS. This is noteworthy because the use of certain PFS while taking medication may in some cases produce adverse effects. Therefore it is very important to increase our knowledge about the consumption patterns of PFS.

 $\label{eq:keywords:consumers, plant food supplements, usage patterns$

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ELABORATION OF FOOD PRODUCT WITH ANTIOXIDANT ACTIVITY BASED ON THE NATURAL POLYPHENOLIC COMPLEX OF RED WINE

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Hyperglycemia is the main diagnostic index, irrespective of diabetes type. It is the leading cause of the excessive accumulation of nonenzymatic glycosylation products in cells, increase in free radical formation and, on the whole, it stimulates processes which can be characterized as chronic oxidative stress. Induced oxidative stress stimulates the interaction of superoxide anion with nitric oxide (NO), a product of NOsynthase, and thus leads to formation of peroxynitrite, the oxidant number one in biological systems. Peroxynitrite causes cytotoxicity and leads to oxidative-nitrate stress. Peroxynitrite modifies proteins by nitrosylation of tyrosine residues, which in turn leads to loss of their biological functions. Nitrative stress causes progressive dysfunction and damage to cells, organs on organism on the whole. Therefore, the level of nitrosylated proteins modified by tyrosine nitrosylation is one of the markers that allows to evaluate the pathological changes in the biological systems.

Increased accumulation of NT (3-nitrotyrosine) and PARylated [poly(ADP-ribosyl)ated] proteins in the tissues of diabetics are associated with diabetes complications (diabetes neuropathy, nephropathy and retinopathy). Red wine (its polyphenols are considered to be the main active components) can act as ROS (reactive oxygen species) scavengers, iron chelators and enzyme modulators.

The overall objective of this work is elaboration of new food product from red wine and to define the molecular mechanisms of action of natural antioxidants found in red wine in protection of peripheral nervous system, retina, glomeruli and tubuli of the renal cortex and peripheral blood cells from oxidative-nitrate stress under type 1 diabetes. This study is novel in investigating the effect of red wine in preventing the accumulation of NT and PARylated proteins in the sciatic nerve, DRG (dorsal root ganglia), spinal cord, kidney and retina of diabetic animals. We have shown that during the experiment the body weight of control and diabetic groups of rats with consumption of red wine was significantly increased, by 52% and 19% accordingly. The significant increase in the content of NT in the sciatic nerve, DRG, spinal cord, kidney and retina, and PARylated proteins in the sciatic nerve, renal glomeruli and retinae of diabetic rats was partly or completely prevented by treatment with red wine.

The investigated corrective substances can potentially become active ingredients of new generation drugs for treatment of diabetes mellitus complications. Red wine and its polyphenol preparations might be a promising option in the prevention and treatment of diabetic complications.

Keywords: natural polyphenolic complexes, oxidative-nitrate stress, diabetes mellitus

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IN VITRO GENOTOXICITY EVALUATION OF PLANT FOOD SUPPLEMENT (PFS) CONTAINING ALKENYLBENZENE COMPOUNDS

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The consumption of botanicals containing naturally occurring substances as phytochemicals or in food supplements and related products to enhance health is greatly increased. The PFS derived from basil, parsley, fennel, nutmeg and sassafras contain alkenylbenzene compounds (estragole, methyleugenol, safrole, etc.), known for their genotoxic and carcinogenic properties, due to the proximate carcinogen 1' hydroxymetabolite (Chen et al. 2011).

In order to discriminate the activity of these substances when tested alone or in the supplement itself, we tested the genotoxicity potential of individual chemicals and their toxic metabolites (50-600 μ M) by using both alkaline comet assay (strand breaks, alkali labile sites, and cross linking evaluation) and micronucleus test (clastogenic and aneugenic damage) after 24 hrs treatment in HepG2 (human hepatoma) cells. The results were compared with methanolic extracts of PFS (obtained from the laboratory of Division of Toxicology Wageningen University Tuinlaan, The Netherlands) containing mixtures of the above substances at known concentrations. The methanolic extracts were dried in a vacuum sealed oven at 50°C and after diluted in medium (DMSO 0.1%).The extracts studied are reported in the table.

	Conc. Extract µM	mg/g supplement	Conc. Samples tested µM	Extract Range µg/ml
BASIL OIL (Pranarom)	E 45,007 ME 179	184 0.84	E 3280 ME 12.8	20-700
BASIL (Herbal gardens)	E 286 ME 200 SA 57	1.21 0.007 0.21	E 11 ME 0.531	825- 1650
TOURNISTAROM (Pranarom)	E 8,257 ME 34	28.65 0.143	E 520 ME 2.18	153-615
FENNEL (Fushi)	E 1,796	6.65	E 8	870
NUTMEG (Herbal gardens)	SA 490 ME 174 ELE	1.97 0.77 0.41	SA 8	870
SASSOFRASS (Herbal gardens)	SA 30.74	0.13	SA 1.44	600

E estragole, ME Methyleugenol, S safrole, ELE Elemicin

At the tested concentrations the methanolic extracts of PFS and the parent compounds did not show neither cytotoxic nor genotoxic activity unlike 1'-hydroxymetabolites (estragole, safrole, myristicin,). Moreover, they PFS extracts inhibited, in a dose-dependent way, the DNA damage induced by the actual corresponding active 1'-hydroxymetabolites (estragole, safrole, myristicin). The results confirm the fundamental role of metabolism in the genotoxicity of alkenylbenzenes, and highlighted that, in the case of complex food matrix, the expected toxicity is not the result of the sum of the activities of single substances, and that the presence in PFS of protective agents as nevadensine, and sulfotransferase inhibitors like PCP (pentachlorophenol) (Van den Berg et al. 2013, Alhusainy et al. 2010) should be taken into account in the risk characterization.

Keywords: Plant food supplements (PFS), Alkenylbenzenes, genotoxicity,matrix-derived combination effect

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FUNCTIONAL FOOD POWDERS FROM PLANT MATERIAL

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Environmental degradation around the world resulted in a significant increase in the popularity of preventive products, particularly to strengthen human body immune system and improve health. Powders from plant material take a special place among these and find increasingly wider application in enrichment of food products and use as an independent supplement.

The authors have developed a technology for functional food powder from grains, legumes, vegetables and fruits in special composite mixtures and their further use for enrichment of food bioactive components. The drying of vegetative raw material by convective drying method to humidity 6-8 % with percussing crumbling, allows to get powders, which is easy to include into food stuff. These powders are divided into 4 groups. In antioxidant powders in concentrated form a maximum number of carotenoids, vitamin C, vitamin E and betanin get preserved, which act as antioxidants. Antioxidant powders are those derived from: carrots, pumpkins, persimmons, jujube, mix of oats and carrot and mix of beets and rhubarb. Prebiotics are defined as non-digestible food ingredients that positively affect the host organism through selective stimulation of growth and activity of one or more types of bacteria in the large intestine. Prebiotic powders have high content of fiber, e.g. beet press cake is comprises 95% of dietary fiber. Prebiotic powders include powders from apple, citrus and grape pomace, beet and apple-and-squash press cake. Phytoestrogens are the substances found in the native plant material. They contain several classes of chemical compounds. Among them, the most prominent are the following three classes: isoflavones, coumestans and lignins. Isoflavones such as genistin, daidzein, glycine are found in almost all vegetables, but soy and pea are the main source of their supply. Therefore powders that contain these vegetables belong to phytoestrogens. Although in some cases isoflavones act as antioxidants like quercetin.

Phytoestrogenic powders include apple, pear, celery leaf, celery root, mixes of soy and sugar beet, soy and carrot, soy and pumpkin, soy and onion, bean and carrot, pea and carrot powders. Powders, in particular those derived from spinach, potatoes, bananas, nettle carrot and celery mix contain large amounts of folate.

Thus, addition of functional plant powders to foods is the most promising for creation of preventive products. They can be raw materials for confectionery, baked and dairy products, and fast foods, production of pills and granules and herbal tea.

Keywords: antioxidants, folates, prebiotics, phitoestrogenes, functional powders.

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CORRELATION BETWEEN CATECHIN CONTENT AND NF-KB INHIBITION BY GREEN AND BLACK TEA INFUSIONS

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Tea obtained from the dried leaves of *Camellia sinensis* L. (family Theaceae) is one of the most widely consumed beverages in the world (Chan EWC). Among Western

populations, dyspeptic disorders including gastritis and peptic ulcer are widespread and the consumption of tea has been suggested for both prevention and treatment of the associated symptoms. Gastritis and ulcer are often due to infection with *Helicobacter pylori* (Bodger K). Inflammation caused by bacterial infection, chemical injury or stress, is associated with a massive production of cytokines by immune cells (i.e. tumour necrosis factor alpha: TNF α) and gastric epithelium (i.e. IL-8); this response depends on activation of NF- κ B (Nuclear FactorkappaB), a critical regulator of genes involved in inflammation, cell proliferation, and apoptosis.

This study investigates whether infusions and water extract from green and black tea inhibit the NF- κ B driven transcription in human epithelial gastric cells (AGS). Extracts were prepared from different brands of green and black tea, with or without caffeine, available on the Italian market. For comparison, an industrially prepared freeze-dried aqueous extract of green tea was also tested. For each sample catechin and caffeine content, was evaluated by HPLC; in addition, the total phenol content, antioxidant activity and stability of catechins 3 months after the expiry date were measured.

The NF- κ B driven transcription and the free radical scavenger activity were inhibited by water extracts, and this effect was related to catechin levels. The potency of epigallocatechin 3-gallate (EGCg) in inhibiting NF- κ B driven transcription was so great that tea extracts low in EGCg were still highly active. In one decaffeinated sample of green tea, the phenol and catechin content was very low, probably as a consequence of caffeine removal. The decrease in catechin levels after 3 months did not reduce the inhibition of NF- κ B driven transcription by tea extracts. These are the first results reporting the inhibitory effect of NF- κ B by commercial green and black infusions at the gastric level, evaluating their short-term stability as well.

Keywords: Tea, Catechins, Gastric inflammation, NF-KB

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QUALITY CONTROL OF MATRICARIA RECUTITA L. PRODUCTS BY PCR METHODS

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Many species of the Asteraceae are known to cause allergic reactions like contact dermatitis or eczema. German Chamomile (*Matricaria recutita* L., tribe Anthemideae) is suspected to cause these skin diseases because of the content of the sesquiterpene lactone anthecotulide, which is considered to be a potent contact allergen. In fact it is doubtful if anthecotulide is indeed a main constituent of German Chamomile, or, as it was reported by [1], misidentifications or contaminations with *Anthemis* species are responsible for allergic reactions.

Therefore we developed two PCR methods to identify samples of *Matricaria recutita* and to discriminate them from other species of this genus and other genera of the tribe Anthemideae (*Anthemis, Tanacetum (Chrysanthemum), Tripleurospermum* and *Leucanthemum*). A multiplex-PCR was designed in the way that a set of primers amplify different parts of the trnL gene in one PCR-reaction. Two general primers (binding in all plants) amplify an approx. 500 to 700 bp long fragment as internal positive control (the exact length is species specific). One *M. recutita* and *M. discoidea* specific primer results with one of the general primers in an approx. 200 bp long fragment. A second *Anthemis* specific primer results with one of the general primers in an approx. 350 bp long fragment. The banding patterns can be easily visualised on 2% agarose gels.

For the second method primers suitable for high-resolution melting-curve analysis (HRM) were designed. With the application of two primer pairs *M. recutita* can be identified and distinguished from all other *Matricaria* species tested and from other genera.

Both methods are able to detect admixtures and contaminations to a certain degree, what was tested with experimental mixed samples. We also tested several trade samples including eight herbal teas, two extracts and four samples of pills/dry extracts (two of them combination products).

Seven of eight herbal teas showed no contamination with *Anthemis*. One sample, a combination drug of fennel and chamomile, showed a contamination with *Anthemis*. The amplification of DNA extracted from extracts was problematic and showed no satisfying results in a standard assessment.

Keywords: Chamomile, Matricaria recutita, DNA barcoding, authentication, PCR

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DNA-BASED IDENTIFICATION OF VALERIANA OFFICINALIS L. S.L.

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Some valerian species are important drugs in different parts of the world. The widely distributed Eurasian *Valeriana officinalis* has a lower content of valepotriates compared to the North American *Valeriana edulis* Nutt. and the Southeast Asian *V. wallichii* DC. Therefore a reliable identification of drugs used as pharmaceutical raw material is very important. Although several studies about the pharmaceutical activity or the relationship of valerian species were performed, there exists not yet a reliable identification system.

For this study two identification systems based on the chloroplast atpB-rbcL intergenic spacer were developed, a multiplexed amplification-refractory mutation system (MARMS) and a high-resolution melting curve analysis (HRM). With both methods it was possible to distinguish *V. officinalis* from all other tested species of *Valeriana, Valerianella* and *Centranthus ruber*. Compared to the MARMS, visualised on agarose gels, the HRM showed a higher sensitivity and a better feasibility of the analysis, especially of degraded plant material. The amplification of relatively long PCR products necessary for sequencing or MARMS failed at several reference samples taken from old herbarium material, but the amplification of short products used for HRM allowed a positive result and an unambiguous identification in almost all samples.

Keywords: valerian, Valeriana officinalis, DNA barcoding, authentication, PCR, MARMS, HRM

FLAVONOID PROFILES OBTAINED BY UPLC-UV USED FOR THE PHYTOCHEMICAL IDENTIFICATION OF PASSIFLORA INCARNATA IN BOTANICALS

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Passiflora incarnata, commonly known as passion flower, is a perennial vine native in the southern United States, Mexico and parts of South America. Traditionally, the fresh or dried aerial parts (Passiflorae herba) have been used as herbal medicine to treat nervous anxiety and sleeping disorders. Recent studies highlight a positive effect of its methanolic extract in reducing symptoms of opiate withdrawal.

Plant samples of different *Passiflora* species and of specified origin were obtained from Europe and North America, moreover, additional samples and botanicals containing *Passiflora incarnata* of unknown provenience were provided from different countries in Europe for a total amount of 100 samples. All the samples were extracted with acetonitrile (30%, v/v) as solvent in an ultrasonic bath and the analyses were performed with UPLC H-class (Waters).

Comparing the chromatographic flavonoid profiles of the samples, it is possible to distinguish samples of different *Passiflora* species as well as samples coming from North America from the European samples.

Keywords: Passiflora incarnata, phytochemical identification, botanicals, flavonoids profile, UPLC

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IDENTIFICATION AND QUANTITATIVE ANALYSIS BY UPLC-UV OF HARPAGOSIDE; CAFFEIC ACID DERIVATIVES AND **COUMAROYL DERIVATIVES IN HARPAGOPHYTUM** PROCUMBRENS RADIX AND BOTANICAL

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Harpagophytum procumbrens, commonly known as Devil's claw, is a herbaceous plant species, native to the Kalahari Desert region of Southern Africa, with high medicinal value. The secondary roots contain mailny harpagoside and other compounds that have been found to be effective in the treatment of degenerative rheumatoid arthritis, tendonitis, kidney inflammation and heart diseases.

Plant samples of Harpagophytum procumbrens radix and botanicals were obtained from Europe and South Africa. All the samples were extracted with acetonitrile (30%, v/v) as solvent in an ultrasonic bath and the analyses were performed with UPLC H-class (Waters).

The comparison of the chromatographic profiles of the radix samples and botanical samples shows a good accordance.

Keywords: Harpagophytum procumbrens, harpagoside, UPLC

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INTAKE OF SELECTED BIOACTIVE COMPOUNDS FROM FENNEL-CONTAINING PLANT FOOD SUPPLEMENTS **AMONG FINNISH CONSUMERS**

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Plant food supplements (PFS) have a long tradition of use, and they are widely consumed in many European countries. However, little is known about the risks and benefits associated with the consumption of PFS. Fennel (Foeniculum vulgare) is a perennial aromatic herb, which has been used in traditional medicine to treat many kinds of symptoms. According to the results of the PlantLIBRA PFS Consumer Survey conducted in Finland, Germany, Italy, Romania, Spain and the United Kingdom, fennel ranked sixth in the pooled list of most consumed botanicals. Among other compounds, fennel contains trans-anethole and estragole. Trans-anethole has tumorigenic properties in laboratory animals, and estragole is carcinogenic and mutagenic at high doses.

The aim of the present study was to estimate the intake of estragole, trans-anethole and other selected bioactive compounds from fennel-containing PFS among the Finnish consumers.

Information on the consumption of fennel-containing PFS was based on the Finnish subsample of the PlantLIBRA Consumer Survey, a cross-sectional 12-month retrospective survey. The sample size in Finland was 401 PFS consumers. They had been recruited from four cities located in different parts of the country. The study participants were regular PFS consumers. Information on the fennel concentration in PFS was collected from the manufacturers/distributors of the products. Data on the concentration of bioactive compounds in fennel was extracted from the ePlantLIBRA database.

Results: The estimated average intake of estragole was 0.20 mg/d (range 0.01-7.95 mg/d), of trans-anethole 1.15 (range 0.05-63.1) mg/d, of rosmarinic acid 0.09 (0.00-5.61) mg/d, of pcoumaric acid 0.0068 (0.00-0.38) mg/d, of kaempferol 0.0034 (0.00-0.19) mg/d, of luteolin 0.0525 (0.00-2.88) µg/d, of quercetin 0.0246 (0.00-1.35) mg/d, of matairesinol 0.0066 (0.00-0.36) µg/d and of mixture of lignans 0.0412 (0.00-2.26) µg/d.

Significance: In conclusion, the intakes of kaempferol, quercetin, luteolin, matairesinol and lignans from fennelcontaining PFS were low in comparison to what is known of their dietary supply in different populations. The intake of estragole, a potentially harmful compound, from fennel-containing PFS was usually moderate when compared with intake from other dietary sources. However, the individual range of intake was quite large, and a heavy consumption of fennel-containing PFS may lead to a comparably high intake of estragole. Similarly, the intake of trans-anethole from PFS with fennel did not exceed the acceptable daily intake value defined for use asa food additive, but PFS sources should be taken into account when assessing the total exposure. To the best of our knowledge, this study presents the first intake estimates of trans-anethole, p-coumaric acid and rosmarinic acid in human populations.

Keywords: intake, bioactive compounds, fennel, plant food supplements

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DEVELOPMENT OF A SURFACE PLASMON RESONANCE **METHOD FOR OTA IN PLANT EXTRACTS**

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Ochratoxins are a group of mycotoxins produced as secondary metabolites by fungi which contaminate a large variety of food and feed commodities. The European Food Safety Authority (EFSA) has established maximum allowable levels presented in food and feed products and in raw materials (EC No 123/2005) (10 g/kg in dried vine fruits and instant coffee, 5 g/kg in raw cereal grains and roasted coffee, 3 g/kg in processed cereal foods and 2 g/kg in grape juice and all types of wine [1]. There is an increasing need to establish simple and sensitive methods for detecting these toxins.

Here we report the development of a rapid and sensitive surface plasmon resonance (SPR) assay of ochratoxin A (OTA). Surface plasmon resonance is an optical method that is very sensitive to small variations in the refractive index in the close vicinity of a thin metallic layer and allows detecting binding events in real-time [2].

SPR was previously used for the detection of many toxins and allergens relevant for food safety [3]. Our detection method is based on the competition between free OTA and OTA-HRP (OTA labeled with horseradish peroxidase) in solution for the sites of the anti-OTA antibody, previously immobilised on the surface of the SPR chip. In order to allow covalent

immobilisation of the OTA antibody by carbodiimide chemistry and to prevent non-specific adsorption, the SPR chip was coated with a self-assembled monolayer of a thiol containing six ethylene glycol groups and a carboxylic end group. In the present configuration, OTA-HRP conjugate was flown on the surface of the SPR sensor for 15 minutes, in the absence or presence of different concentrations of free OTA. The higher concentration of free OTA in solution, determine a lower signal as less of the OTA-HRP conjugate is bound to the anti-OTA antibody. A solution containing 11 ng/mL OTA lead to a decrease of the sensor response (46%) as compared to the signal in the absence of OTA. Method optimisation is described. Analysis of plant extracts in various solvent mixtures has allowed identifying acetonitrile: PBS as the best extraction solvent compatible with our detection format by SPR. Signal amplification procedures need to be considered further for improving the sensitivity of the method.

Keywords: ochratoxin A, surface plasmon resonance, plant extracts

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LOTUS LEAF ALKALOIDS CAN STRONGLY INHIBIT CYP2D6 ISOENZYME ACTIVITY

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Background: The herb lotus (Nelumbo nucifera) leaves is a commonly used traditional Chinese medicine for summer heat syndrome, to assuage thirst, and to cure both diarrhea and fever in China. It is well demonstrated that the herb has various pharmacological effects, such as anti-obesity, antihyperlipidemia, anti-oxidant, anti-microbial, and antihypoglycemic activities. The herb is currently more popular in China as a "tea drink" or a main ingredient of some herbal formulations for the purposes of losing body weight and reducing blood lipids. Therefore, lotus leaves or its products are more likely to be concurrently administered with some conventional medicines. However, its potential inhibitory effect on human cytochrome P450 (CYP) activities has not been systemically investigated to date. The present study was performed to assess the inhibitory effects of lotus leaf extracts, fractions and individual compounds, on the five CYP isoenzymes (2C9, 2C19, 2D6, 2E1, and 3A4), for predicting its potential herb-drug interactions. Methods: Five probe substrates were incubated with human liver microsomes (30 mg protein /ml) in an incubation buffer system, in the presence or absence of tested samples, including its water extract (WE), ethanol extract (EE), flavonoid fraction (FF), alkaloid fraction (AF), as well as major individual alkaloids, nuciferine (NF), Nnornuciferine (N-NF), and 2-hydroxy-1-methoxyaporphine (HMA). After incubation, the relative substrate metabolite concentrations were analyzed by using a LC-MS/MS method. The inhibition on the isoenzymes was calculated by comparing the concentrations of produced metabolites between the present and absence of tested samples. Results: EE produced a very strong inhibition effect on CYP2D6 with IC50 value of 12.05 µg/ml and relative weak effect on other isoenzymes (IC₅₀ 61.43 -98.01 µg/ml). WE was not found obvious inhibition effect on these enzymes, just with IC_{50} value 107.20 $\mu g/ml$ for CYP2D6 and higher than 500 µg/ml for others. EE was further separated into two fractions, FF and AF, for the identification of CYP2D6 inhibitors. FF was found to significantly inhibit CYP2D6 activity (IC_{50,} 0.96 $\mu\text{g/ml}),$ whereas AF just exerted a weak effect. Three pure alkaloids, NF, N-NF, and HMA, isolated from the AF, was found significantly inhibit CYP2D6 with IC₅₀ values of 3.78, 3.76,

and 3.15 μ M, respectively. Their Lineweaver-Burk plots and Dixon plots showed that they competitively inhibited CYP2D6 activity with *K* values of 1.88, 2.34, and 1.56 μ M, respectively. **Conclusion**: Lotus leaf ethanol extract has a potent inhibitory activity on CYP2D6 isoenzyme, and its alkaloid components contribute to the effect. The possible herb-drug interactions from lotus leaves or its preparations and conventional medicines should be taken into account. [Supported by the Ministry of Science and Technology of China (1108 and 2012ZX09301002-001) and EU FP7 Project PlantLIBRA (245199)].

Keywords: Lotus leaves, cytochrome P450, herb-drug interactions

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POTENTIAL MODULATION ON INTESTINAL P-GLYCOPROTEIN BY SEMEN ZIZIPHI SPINOSAE: IN VITRO AND IN VIVO STUDIES

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Background: Concomitant administration of Chinese medicine (TCM) that is P-glycoprotein (P-gp) substrates with drugs may produce significant herb-drug interactions. The seeds of Ziziphus jujube, Semen Ziziphi Spinosae (known as Suan Zao Ren in Chinese) are one of the most popular traditional Chinese herbs and health food for its sedative and hypnotic effects. The major components of Semen Ziziphi spinosae are flavonoids, saponins and alkaloids. However, the potential effects of Semen Ziziphi Spinosae on P-gp activity have yet been reported. Therefore, the purpose of this study was to investigate the inhibitory effect of Semen Ziziphi Spinosae extracts on Pglycoprotein activity. Methods: Cytotoxicity of Semen Ziziphi Spinosae extracts (total saponins STS, alcohol extract SAE, ethyl acetate extract SEAE) was assessed by MTT method. Caco-2 cell monolayer model was applied for evaluating the inhibition of P-gp in vitro by using rhodamine 123 as fluorescent probe. Pharmacokinetic of rhodamine-123 following oral administration alone and co-administration with Semen Ziziphi Spinosae extracts were studied in rats. Results: Semen Ziziphi Spinosae extracts at concentration of up to 50 µg/ml appeared not to be cytotoxic to Caco-2 cells. Extracts (STS, SAE and SEAE) were found to exhibit a dose-dependent inhibitory effect on P-gp activity. STS at 50 µg/ml increased Papp (ap-bl) from 5.77±0.63 to 8.53±0.87× 10-6cm/s (p<0.05, compared with control group) with the increasing extent of Papp being comparable to that by verapamil (50µM). The pharmacokinetic results showed that when co-administered with verapamil (15 mg/kg) promoted a significant increase in the maximum plasma concentration (Cmax) and the area under concentration-time curve (AUC (0-t)) of the rhodamine-123 by 2.1- and 2.5-fold. respectively. In addition, STS, SAE and SEAE at 12g/kg of crude herbs increased not only the AUC0-t of rhodamine 123 by 223% (P<0.01), 141% and 167% (P<0.05), but also the Cmax by 177% (P<0.05), 124% and 163% (P<0.05), respectively. Mechanism studies indicated that Semen Ziziphi Spinosae extracts modulated the activities of P-gp by inhibiting P-gp ATPase. Conclusions: Semen Ziziphi Spinosae may act as a potential P-gp modulator and co-administration may potentially affect the safety and effectiveness of drugs. However, more studies are needed on the underlying mechanism and the active inaredients.

Keywords: Semen Ziziphi Spinosae; Rhodamine 123; P-glycoprotein.

LATEST EVENTS

PlantLIBRA Buenos Aires Training course: quality and regulatory aspects of plant raw materials for Food Supplements.

On April 24, 2014, it was held in Buenos Aires (Argentina) the PlantLIBRA training course on: *"Plant for Food Supplements: quality and regulatory aspects"*. Organized by SISTE (Italian society of science applied to botanicals and health products) as part of the dissemination events for the

International Partner Countries involved in the project (Argentina, Brazil, China and South Africa); the course was hosted by UBA, Hospital de Clinicas "José de San Martín", University of Buenos Aires, IV Chair of Internal Medicine Hospital Clinic.

The conference provided an excellent opportunity for a debate among the large number of participants (more than 110) that attended the seminar included researchers, local authorities and



stakeholders on important topics, such as the Industry requirements to ensure the best quality of herbs and Plant Food Supplements (PFS).

The main objective of the PlantLIBRA training course in Buenos Aires was the promotion of the required approaches to obtain the highest quality in all stages of production of food supplements



containing botanicals and botanical preparations. It has also been an important occasion to communicate to SMEs and food operators the results of the project.

Prof. *Roberto IERMOLI*, head of the IV Chair of Internal Medicine, opened the training course describing the main activities of the University of Buenos Aires. He presented a very interesting overview on the cultural and scientific history of Argentina.

Patrizia RESTANI, scientific coordinator of the PlantLIBRA project continued with an overview of the project, describing the principal

objectives identified at the beginning of the project and the main results obtained. The presentation included the following aspects:

- Studies performed with the aim to improve the knowledge in the fields of healthy properties of PFS and the associated development of experimental approaches to scientifically substantiate "health claims". In particular, it was stressed the necessity of identifying new strategies to integrate tradition of use, studies *in vitro* and human intervention studies.
- 2. Studies on adverse effects, starting from the collection of data from the scientific literature and continuing with a survey on data at disposal of Poisons Centers (before and after the beginning of the project).
- 3. The integration of risks and benefits in a new model of assessment.

In her second presentation, *Patrizia RESTANI* summarized the main results obtained in the consumption survey on PFS conducted in six EU countries (Finland, Germany, Italy, Romania,

Spain and the United Kingdom), where some main objectives were identified: the kind of products mainly consumed in Europe and their botanical ingredients; the usage patterns of PFS; and the socio-demographic profile of PFS consumers. Finally, she also gave a short description of ePlantLIBRA, the meta-database of the project, which contains information on several botanicals in the area of PFS composition, healthy effects, adverse effects, main active compounds, etc.

In his presentation at the seminar, *Raul PASTOR*, contact person for PlantLIBRA at the University of Buenos Aires, explained the role of oxidative stress as risk factor in some human diseases, such as cardiovascular disorders, and the possible use of PFS in reducing these risk factors for chronic diseases. The group of Hospital de Clinicas has been involved in a research program on wine and health aiming at the assessment of the risk-benefit of wine consumption and of food





supplements deriving from the grape on human health, in particular in the prevention of cardiovascular diseases and metabolic syndrome.

Elisabeth NASCIMENTO, contact person of the University of San Paulo (Brazil), other partner of PlantLIBRA, described in more details the possible sources of risk (contamination by e.g. foreign matter, pesticides residues, heavy metals, etc., and adulteration) of plant raw materials used in the production of

PFS and the practical approaches (e.g. WHO guidelines) for minimizing them. As regards the quality of plants and botanical preparations, *Patrick COPPENS*, representative of EBF (European Botanical Forum), involved in the policy implications of the project scientific results and member of the PlantLIBRA policy Advisory Board (PAB), illustrated all the aspects related to the quality of PFS as detailed in the document "Quality Guide for Botanical Preparations" recently published by this association. He also presented the regulatory framework of PFS in Europe with the well-known contradictions and difficulties in classifying botanical preparations as PFS or herbal traditional





medicines.

The differences/similarities of the Argentine legislation with EU rules in regulating PFS were illustrated by María SOLEDAD ECHARRI of the Depto. de Evaluación Técnica of INAL/ANMAT (Administración Nacional de Medicamentos, Alimentos y Tecnoloia Médica). The Argentine Ministry of Health is working at amending of the current legislation (CAA: Código alimentario argentino) for food supplements, also containing botanicals.

In the last part of the training course, a round table discussion with industrial participants was organized. Microsules Argentina SA and SIDUS SA, two well-known

pharmaceutical companies, also producing dietary supplements, presented the PFS market in Argentina and underlined the efforts spent to maintain the highest quality in this area, which is in great development in South America.

NEWS FROM THE PROJECT

PlantLIBRA Young Investigator's Award: SOT's 53rd Annual Meeting, March 23–27, 2014, Phoenix, AZ

of Suzanne van den Berg, Division of Toxicology, Wageningen University (The Netherlands)

Thanks to the PlantLIBRA Young Investigator's Award, consisting of a grant to attend an international scientific conference, I attended the 53rd annual meeting of the Society of Toxicology (SOT). The meeting was held from March 23-27 in Phoenix, Arizona, USA. The program consisted of several interesting keynote lectures, platform sessions, education-career development sessions, symposium sessions,



workshops and poster presentations. This year, the meeting included five different themes: 1) Advancing Clinical and Translational Toxicology and Application of Biomarkers, 2) Enhancing



Strategies for Risk Assessment, 3) New Science and Perspectives Surrounding Environmental and Occupational Exposures, 4) Safety Assessment: Mechanisms and Novel Methods, and 5) Stem Cell Models for Integrated Biology. Several sessions were of special interest for the PlantLIBRA project of which some are briefly mentioned below. The complete program can be found online at http://www.toxicology.org/AI/Pub/Prog/2014Program.pdf

Among the great amount of posters presented during the SOT annual meeting, several works were of particular interest for the

PlantLIBRA project because they were on topics related to botanicals. For example, on Tuesday Morning, March 25, a poster session on natural products (using *in vitro* and *in vivo* studies) was organized. Moreover, a poster session on the toxicity of chemical mixtures took place on that same

day. In addition, poster sessions on risk assessment were organized on Monday, Wednesday and Thursday.

On Wednesday afternoon, I presented my own poster entitled "*New concepts for risk and safety assessment of plant food supplements (PFS)*" describing the work performed in WP3 of the PlantLIBRA project. Many people were interested in the poster including people from industry, academia, EFSA and the



US FDA. The abstract of this poster, and all other posters presented during the SOT's annual meeting, are online available at http://www.toxicology.org/AI/PUB/Tox/2014Tox.pdf.

On Wednesday morning, March 26, a workshop on supplements was scheduled which was entitled "*Improving the safety of dietary supplements and natural health products by assessing effects in*

humans". During this workshop presentations were given that focused on the clinician's perspectives (Dr. L. Nelson), natural product post-market surveillance (Dr. R. Kingston), the clinical utility of dietary supplement case reports and adverse event reports (Dr. B. Gurley), an example was given on the systematic analyses of kava's hepatotoxicity risk (Dr. C. Xing), and one of the presentations focused on the collection, interpretation, and utilization of adverse event data within a global dietary supplement company (Dr. V. Frankos).

Overall, the program of the SOT annual meeting was very interesting and added to strengthening my knowledge and networks in the field of toxicology, risk assessment and the science of botanicals. PlantLIBRA is gratefully acknowledged for awarding me the PlantLIBRA Young Investigator's Award thereby facilitating my attendance to this international scientific conference.

Thanks to Suzanne van den Berg for this fine contribution.

PUBLICATIONS

The PlantLIBRA project is close to its end and many efforts have been made by all the partners to disseminate the results of their work to the stakeholders (scientific world, regulatory sector and food chain operators) through the publication of articles on peer-reviewed journals, the participation at national and international congresses with posters or oral presentations. The list of publications of PlantLIBRA partners can be examined on the PlantLIBRA website (http://www.plantlibra.eu/web/) in the public area "Publications".

Many other articles on the results of PlantLIBRA in its multitasking aspects are currently in progress and, when published, they can be downloaded from the website in the public area "Publications".

Contact and more information: SISTE: info@sisteweb.it See: www.plantLIBRA.eu





UNIVERSITÀ DEGLI STUDI DI MILANO

THE DEPARTMENT OF PHARMACOLOGICAL AND BIOMOLECULAR SCIENCES

presents



Tuesday, June 3rd 2014

Via Balzaretti 9, Milan

Registration is free, just email at **nextstep5.register@gmail.com** Deadline for registration is **May 15th 2014**



UNIVERSITÀ DEGLI STUDI DI MILANO DIPARTIMENTO DI SCIENZE

FARMACOLOGICHE E BIOMOLECOLARI

Registration is free, just email at nextstep5.register@gmail.com Deadline for registration is May 15th 2014



Tuesday, June 3rd 2014 - SCIENTIFIC PROGRAMME

8:00 - 8:45	Registration
8:45 – 9:00	Opening

9:00 - 10.00

Prof. Giorgio Racagni, DiSFeB Director Oral presentations, 1st session – Aule A e B

Neuroscience Field Chairs: Alessia Luoni, Jennifer Stanic	Cardiovascular Field Chairs: Lorenzo Arnaboldi, Laura Castiglioni, Patrizia Amadio
9.00 – 9.15 Simona Melfi GABA-B receptor mediated effects in non-myelinating Schwann cells 9.15 – 9.30 Francesca Guzzetti Encoding synaptic GluN2A-containing NMDA receptor activation: the role of ring finger protein 10 9.30 – 9.45 Juliet Richetto Long term effects of late prenatal immune activation: transcriptome analysis and relevance for psychiatric disorders 9.45 – 10.00 Riccardo Cristofani Inhibition of dynein ATPase activity reduces aggregation of misfolded proteins involved in motoneuron diseases 10.00 – 10.15 Davide Marangon Gene regulation of GPR17, a checkpoint receptor in oligodendroglial differentiation 10.15 – 10.30 Mara Seguini Antidepressant treatments induce different and time-dependent modulation of rat hinpocampal miBNome	9.00 – 9.15 Cecilia Vitali Role of LCAT in brain lipoproteins metabolism 9.15 – 9.30 Malvina Hoxha A new gateway for rheumatoid arthritis: COXIBs with a safety cardiovascular profile 9.30 – 9.45 Elena Sommariva Does a misregulated lipid metabolism endorse Arrhythmogenic Right Ventricular Dysplasia (ARVD) adipogenic differentiation? Characterization of the lipid profile of cultured cardiac stromal cells from ARVD vs NON-ARVD patients 9.45 – 10.00 Laura Rossetti Prothrombotic phenotype of CAD patients with diabetes: platelet Tissue Factor and thrombin generation 10.00 – 10.15 Giulia Ganzetti ApoA-I depletion in chow-fed ApoE-KO mice severely enhances aortic and coronary atherosclerosis development 10.15 – 10.30 Susanna Fiorelli Human monocyte-derived macrophages are beterogenous: proteomic profile of
	different morphotypes

10:30 - 11.00	Coffee break
11.00 - 12.00	Lecture – Prof. Graziella Messina
	Department of Biosciences, University of Milan
12.00 - 13.00	Round table – «DiSFeB incontra…»
13.00 - 14.00	Lunch
14.00 – 17.00	Oral presentations, 2 nd and 3 rd sessions – Aule A e B

Neuroscience Field	Nutraceutical Field
Chairs: Lucia Caffino, Manuela Mellone	Chairs: Chiara Di Lorenzo, Elisa Colombo
 14.00 - 14.15 Giuseppe Giannotti Life-long adaptive changes following repeated exposure to cocaine during adolescence: focus on cortical neuroplastic mechanisms 14.15 - 14.30 Luca Castelnovo Nerve regeneration and anti-neuropathic effects exerted by GABA-B ligands 14.30 - 14.45 Silvia Pelucchi CAP2, a regulator of actin filament dynamic, is a novel ADAM10 interactor 14.45 - 15.00 Andrea Rossetti The activation of the immune/inflammatory system is associated with the stress-induced anhedonia in rats: effect of pharmacological intervention 15.00 - 15.15 Giusy Coppolino Fate mapping of the oligodendroglial precursor cells expressing the GPR17 receptor in Experimental Autoimmune Encephalomyelitis, a mouse model of Multiple Sclerosis 15.15 - 15.30 Paolo Tornese Corticosterone application in vitro to synaptosomes mimics the ex vivo effect of stress on the glutamate presynaptic machinery 	 14.00 - 14.15 Anna Parolari <i>y</i>-Conglutin, the lupin seed glucose-lowering glycoprotein, interferes on insulin pathway in Hep G2 cells 14.15 - 14.30 Monica Marzagalli Type I GnRH receptor mediates the antitumor effect of GnRH III-bioconjugates on prostate cancer cells 14.30 - 14.45 Rossella Calò Evaluation of Protective effect of thymol against UVA- and UVB-induced damage using an In Vitro (human keratinocytes) and an ex-vivo human skin tissue model 14.45 - 15.00 Marco Fumagalli In vitro anti-inflammatory activity of tannin-enriched extracts from Fragaria spp 15.00 - 15.15 Gianfranco Frigerio Ginkgolides and 4'-O-methylpyridoxine as biological markers of Ginkgo biloba poisoning 15.15 - 15.30 Yuri Manzoni Molecular approaches to evaluate the suitability of oat varieties for gluten-free diet
Immunology Field	Metabolism Field
Chairs: Alessandro Villa, Silvia Franchi	Chairs: Sara Della Torre, Gaia Cermenati, Paola Corsetto
 15.30 – 15.45 Federica Dellera ApoA-I deficiency in ApoE-KO mice severely alters lymph nodes and modulates T-lymphocyte subsets 15.45 – 16.00 Giovanna Luisa Pepe Genome-wide analysis of estrogen action in macrophages 16.00 – 16.15 Giorgia Calderazzi Study of M2 polarization in mouse brain 16.15 – 16.30 Paola Roderi Repeated intratracheal instillation of PM10 induces lipid reshaping in lung parenchyma and in extra-pulmonary tissues 16.30 – 16.45 Angela Papale Molecular mechanisms involved in chemical allergen-induced IL-18 production in a human keratinocyte cell line 16.45 – 17.00 Elena Bizzotto SIRT1 activity disjoins anti-inflammatory and chemopreventive activity of NSAIDs 17.00 – 17.15 Massimiliano Bissa DNA and fowlpox-based recombinants as a prophylactic/therapeutic vaccine against Human Papillomavirus (HPV)-related cancers 	 15.30 – 15.45 Alessandra Ferrari Epigenetic regulation of energy metabolism in a model of diet induced obesity: focus on class I HDACs 15.45 – 16.00 Erika Fiorino Histone deacetylases (HDACs) and cholesterol catabolism: effect of HDAC7 deletion on lipid and lipoprotein profile 16.00 – 16.15 Roberta Fontana Hepatic estrogen receptor activity and metabolic disorders 16.15 – 16.30 Simone Romano Effects of diabetes on brain structures 16.30 – 16.45 Matteo Audano Zc3h10 orchestrates C2C12 skeletal muscle cells differentiation 16.45 – 17.00 Chiara Macchi Could iron overload affect the reproductive axis? Evidence from in vitro and in vivo models 17.00 – 17.15 Stefania Moretti Signaling pathways responsible for the regulation of the glutamate transporter 1 (GLT1/EEAT2) in pancreatic beta cells

17:30 - 18.00 Closing remarks and prizes for best oral presentations Happy Hour

18.00