Book of Abstracts

SALERNO

February, 15-16, 2024

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Agenda

Thursday, February 15th	
14:00	*Registration*
14:30	*Welcome addresses---G. Bianco, P. Montoro, C. Gaeta*
Session 1	**OMICS IN PLANT KINGDOM 1**
Chairperson: G. Bianco, D. Caruso	
15:30	***Plenary*** PL01 **Pierre Marie Allard**, University of Fribourg *Mass-spec, computers, and Linked Open Data for chemodiversity characterization at the global scale*
Oral communications	
16:15	OR01 **Giovanna Baron**, University of Milan *An integrated metabolic, in silico and proteomic approach to reveal the parahormetic mechanism underlying the protective health effects of grape seed proanthocyanidins*
16:30	OR02 **Manuela Giovanna Basilicata,** University of Salerno *Peptidomics profiling, identification of bioactive peptides, and assessment of the biological activities of novel nutraceutical products with a potential protective role in a doxorubicin-mediated cardiac damage model*
16:45	OR03 **Lorenza Marinaccio**, University "G. d'Annunzio"-Chieti *Valorization of grape pomace: a comparison among the phytochemical profile and antioxidant activity of grape pomace and berries of common use in food and nutraceutical industries*
17:00	Coffee Break and Poster
Session 2	**OMICS IN PLANT KINGDOM 2**
Chairperson: L. Navarini, C. Talotta	
Oral communications	
17:30	OR04 **Larissa Della Vedova**, University of Milan *Comprehensive in vitro ADME study of 5-(3,4-dihydroxyphenyl)-γ-valerolactone, the main colonic catabolite after flavonoids-rich supplements intake*
17:45	OR05 **Giovanna Aquino**, University of Salerno *Tomato (Solanum lycopersicum L.) leaves as a sustainable source of bioactive compounds: from analytical characterization, thought biological evaluation, to the formulation and characterisation of lipid nanoparticles*
18:00	OR06 **Maria Assunta Crescenzi**, University of Salerno *Metabolite Profiling, through LC-ESI/LTQOrbitrap/MS Analysis, of Antioxidant Extracts from Physalis alkekengi L*
18:15	OR07 **Eduardo Sommella**, Thermo Fischer SCIENTIFIC/University of Salerno *Pseudo-targeted and untargeted HRAM-based approaches: A case study on Hop bioactive compounds*
18:30	**End of session**
Friday, February 16th	
Session 3	**NATURAL PRODUCTS BASED DRUG DISCOVERY**
Chairperson: F. Piscitelli, P. Montoro	
9.00	***Plenary*** PL02 **Angelo Fontana**, Consiglio Nazionale delle Ricerche-Naples *Modulation of innate immune response by marine sulfolipids*
Oral communications	
9:45	OR08 **Flavio Polito,** University of Salerno *Chemodiversity and antibiofilm activity of essential oils from Tunisian eucalyptus*
10:00	OR09 **Paola Nezi**, Toscana life sciences *Hydroxyanthracene derivates citotoxicity: A differential evaluation between single molecule and whole plant extract*

10:15	OR10 **Virginia De Vito**, Alesco S.r.l. *Sucrosomial® Selenium Absorption and Biodistribution in In vivo model*
10:30	Coffee Break and Poster
Session 4	**SMALL MOLECULES ANALYSIS**
Chairperson: S. Piacente, F. Lelario	
11:00	***Keynote*** KN01 **Barbara Sgorbini**, University of Turin *Exploiting MS quantification of volatile compounds for the quality control of natural products*
Oral communications	
11:30	OR11 **Paola Di Matteo**, University "Sapienza"-Roma *Essential oils for supportive clinical aromatherapy: chemical profiling by GC-MS, ESI-MS and FT-IR analysis*
11:45	OR12 **Serena Fiorito**, University "G. d'Annunzio" Chieti *GC-MS Biocomponents Characterization of Waste Violina pumpkin and Olea europaea L. leaf extracts.*
12:00	OR13 **Vincenzo Piccolo**, University of Naples Federico II *Characterization and quantification of intact glucosinolates in Catozza rapeseeds: a promising food matrix for nutraceuticals development*
12:15	OR14 **Domingo Pastran**, Shimadzu Italia S.r.l. *Shimadzu's ready-to-use method for Metabolomics. Differentiation of Tomato Varieties*
12:30	OR15 **Giuseppe Amato**, University of Salerno *Postharvest Microwave Drying of Basil (Ocimum basilicum L.): The Influence of Treatments on the Quality of Dried Products*
13:00	Buffet lunch
Session 5	**FOOD AND NUTRACEUTICALS**
Chairperson: C. Gaeta, E. Gregori	
14:30	***Keynote*** KN02 **Gianluca Picariello**, Consiglio Nazionale delle Ricerche-Avellino *Flavone di-C-glycosides from seed germ of Leguminosae: Inhibitors of carbohydrate digesting enzymes from industrial by-products*
Oral communications	
15:00	OR16 **Paolo Della Sala**, University of Salerno *Characterization of Strawberry Extracts from Novel Cultivars*
15:15	OR17 **Antonietta Cerulli**, University of Salerno *Metabolite Profile of "Green" Extracts of Cynara cardunculus (Carciofo di Paestum PGI) leaves by LC-HRMS-Based Metabolomics*
15:30	OR18 **Alba Lasalvia**, University "Sapienza"-Roma *Edible Insects as Novel Foods: The Chemical Characterization of Acheta domesticus*
15:45	OR19 **Eleonora Truzzi**, University of Modena and Reggio Emilia *Phytochemical and functional characterization of Morus alba L. fruits from varieties grown in Italy*
16:00	OR20 **Lucia Bartella**, University of Calabria *A new method for the determination of 5-methyltetrahydrofolic acid in Citrus juices by High Resolution Mass Spectrometry*
16:15	OR21 **Mariateresa Maldini**, SCIEX, Italia *Metabolomics analysis of Moringa oleifera leaves: correlation between in vitro effect on C2C12 myotubes cell line and geographical origin.*
16:30	Conclusions

Poster Communications

PO01	***Stable isotope ratio analysis of volatile organic compounds for the botanical characterization and authentication of lavender essential oil*** *Long Chen [1,2], Purna K. Khatri, Mauro Paolini, Roberto Larcher, Luca Ziller, Dana Alina Magdas, Olivian Marincas, Alberto Roncone, Luana Bontempo* [1] Research and Innovation Centre, Fondazione Edmund Mach (FEM) [2] Centre Agriculture Food Environment, University of Trento
PO02	***Effects of different lighting conditions on growth and metabolism of basil grown under Microcosm conditions*** *Rosaria Cozzolino [1], Livia Malorni, Luigi d'Aquino, Paola Montoro* **[1]** Institute of Food Science, National Research Council
PO03	***LC-HRMS based evaluation of the effect of saline irrigation and plant-based biostimulant application on phytocannabinoid composition of fiber hemp (Cannabis sativa L.)*** *Ernesto Gargiulo[1], Carmen Formisano[1], Ida D. Mola, Nunzia Iaccarino, Nunzio Fiorentino, Giuseppina Chianese* [1]Department of Pharmacy, University of Naples Federico II
PO04	***Sweet potato (Ipomoea batatas) leaves as a valuable source of biologically active chlorogenic acid regioisomers*** *Silvia Colomban, Luciano Navarini [1]* [1]Illycaffè Spa
PO05	***Development of a new extraction method for a food supplement based on pomegranate: analysis of metabolic profile by LC-MS*** *Luciana Maria Polcaro [1,2], Milena Masullo, Sonia Piacente* [1]Department of Pharmacy, University of Salerno [2]PhD Program in Drug Discovery and Development, University of Salerno
PO06	***Pharmaceutical and cosmetic potential of Crataegus laciniata (Rosaceae) Flowers extract*** *Emanuele Rosa [1], Salvatore Mirabile, Valentina D'Angelo, Maria Paola Germanò, Shiva Pouramin Arabi, Valentina Parisi, Francesco Maria Raimondo, Nunziatina De Tommasi* [1]PLANTA/Centro autonomo di Ricerca, Documentazione e Formazione
PO07	***Bioactive sterols in innovative Brassica vegetables: an investigation by liquid chromatography and high-resolution mass spectrometry with atmospheric pressure chemical ionization*** *Valeria Cinquepalmi, Andrea Castellaneta, Ilario Losito, Beniamino Leoni, Massimiliano Renna, Pietro Santamaria, Cosima Damiana Calvano, Tommaso R.I. Cataldi* [1] Department of Chemistry. University of Bari Aldo Moro
PO08	***A chemometric investigation to study the stability of tannins by LC-QqQ: from standard analytical compounds to UVCBs***

	Sara Tamimi [1], *Claudio Marzio Quintiero, Michela Burico, Matteo Stocchero, Mattia Gianni, Luisa Mattoli* [1]Metabolomics and Analytical Sciences, Aboca S.p.A
PO09	***Extraction of polyphenols from oak bark for the tanning industry using greener ultrasonic technology*** *Vittoria Vittoria* [1 2,3], *Ilaria Quaratesi, Immacolata Bruno, Antonio Pauciulo, Andrea R. Bartiromo, Ioan Calinescu, Ioana Popa, Petre Chipurici, Rocco Gliubizzi, Carmine Gaeta, Carmen Talotta, Veronica Iuliano, Elena Badea* [1] University of Salerno, Department of Chemistry and Biology "A. Zambelli" [2] BI-QEM SPECIALTIES SPA [3] National Research&Development Institute for Textile and Leather (INCDTP)
PO10	***Cucurbita pepo var. styriaca seeds: a source of bioactive polar lipids detected by UHPLC-Q-Orbitrap-MS/MS*** *Annunziata Paolillo* [1,2,3], *Assunta Napolitano, Francesco Sottile, Milena Masullo, Sonia Piacente* [1]Department of Pharmacy, University of Salerno [2]PhD Program in Drug Discovery and Development, University of Salerno [3]Department of Architecture, University of Palermo, Piazza Marina, 61, 90133, Palermo, Italy
PO11	***Sustainable biostimulant protein hydrolysates from Chlorella vulgaris*** *Francesco del Prete* [1], *Francesca Sansone, Tiziana Esposito, Teresa Mencherini, Manuela Giovanna Basilicata, Giovanna Aquino, Annamaria di Serio, Domenico Ronga, Giacomo Pepe* [1]Department of Pharmacy, University of Salerno
PO12	***The insect Hermetia illucens: a sustainable source of molecules of high biological and economic value*** *Carmen Scieuzo* [1,2], *Rosanna Salvia, Fabiana Giglio, Roberta Rinaldi, Mariarita Rubino, Emine Derin, Federica De Stefano, Angela Pascale, Patrizia Falabella* [1]Department of Sciences, University of Basilicata, Italy [2]Spinoff XFlies s.r.l., University of Basilicata, Italy
PO13	***Antimicrobial activity of lipids extracted from Hermetia illucens larvae reared on different substrates*** *Antonio Franco* [1,2], *Carmen Scieuzo, Rosanna Salvia, Valentina Pucciarelli, Francesco Iannielli, Sofia Ouazri, Ilaria Caivano, Luca Borrelli, Fulvia Bovera, Eric Schmitt, Patrizia Falabella* [1]Department of Sciences, University of Basilicata, Italy [2]Spinoff XFlies s.r.l., University of Basilicata, Italy
PO14	***Optimization and validation of a LC-MS/MS method for the determination of Alternaria toxins in tomato products*** *Claudia Notarfonso* [1], *Emanuela Gregori, Francesca Debegnach, Martina Enza Grieco, Giuseppina Scialò e Barbara De Santis*

	[1]Laboratorio Nazionale di Riferimento per le Micotossine e le Tossine Vegetali Naturali, Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Istituto Superiore di Sanità
PO15	***Antimicrobial properties of the chitosan from different biomasses of the bioconverter insect Hermetia illucens*** *Antonio Franco [1,2], Rosanna Salvia, Anna Guarnieri, Micaela Triunfo, Dolores Ianniciello, Giovanni Lomonaco, Antonio Dolce, Miriam Viola, Carmen Scieuzo, Angela De Bonis, Patrizia Falabella* [1]Department of Sciences, University of Basilicata, Italy [2]Spinoff XFlies s.r.l., University of Basilicata, Italy
PO16	***Chitosan derived from the diptera Hermetia illucens: innovative biopolymer for application in cosmetic and pharmaceutical fields*** *Rosanna Salvia [1,2], Micaela Triunfo, Anna Guarnieri, Dolores Ianniciello, Carmen Scieuzo, Maddalena Ventura, Antonio Franco, Giovanna Donnarumma, Angela De Bonis, Patrizia Falabella* [1]Department of Sciences, University of Basilicata, Italy [2]Spinoff XFlies s.r.l., University of Basilicata, Italy
PO17	***Aqueous extracts of Vicia faba L. pod valves: a promising source of bioactive compounds and their potential use as adjuvants in the treatment of Parkinson's disease*** *Carmen Tesoro, Filomena Lelario [1], Fabiana Piscitelli, Angela Di Capua, Maria Assunta Acquavia, Giuliana Bianco, Paolo Della Sala, Paola Montoro, Mario Dell'Agli, Rosanna Ciriello* [1]Department of Sciences, University of Basilicata, Italy
PO18	***Phytochemical investigation and antibacterial activity of Thymus daenensis Celak aerial parts*** *Marzieh Rahmani Samani [1,2], Antonietta Cerulli, Florinda Fratianni, Filomena Nazzaro, Milena Masullo, Sonia Piacente* [1]Department of Pharmacy, University of Salerno [2]PhD Program in Drug Discovery and Development, University of Salerno
PO19	***Lignin extraction from natural sources*** *Hamidreza Moradi [1], Veronica Iuliano, Carmine Gaeta, Placido Neri, Carmen Talotta* [1]Department of Chemistry and Biology "A. Zambelli", University of Salerno
PO20	***Cistus × incanus L.: phytochemical profile and anti-inflammatory activity in an in vitro model of gastric inflammation*** *Marco Fumagalli [1], Giulia Martinelli, Carola Pozzoli, Giovanna Nicotra, Silvia Francesca Vicentini, Nicole Maranta, Enrico Sangiovanni, Elisa Sonzogni, Mario Dell'Agli, Stefano Piazza* [1]Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", University of Milan
PO21	***Pomodoro Riccio metabolomic profile by means of NMR and MS methodologies: a case study*** *Valeria Vergine [1], Alba Lasalvia, Maria Elisa Crestoni, Donatella Ambroselli, Cinzia Ingallina, Luisa Mannina, and Giuliana Vinci* [1]Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma

PO22	***Bioactive potential of chestnut spiny bur: early evidence for anti- inflammatory effects in intestinal cells*** *Carola Pozzoli [1], Marco Fumagalli, Stefano Piazza, Giulia Martinelli, Nicole Maranta, Marco Angarano, Vincenzo Nicolaci, Elisa Sonzogni, Enrico Sangiovanni, Mario Dell'Agli* [1]University of Milan - Department of Pharmacological and Molecular Sciences "Rodolfo Paoletti"
PO23	***Unravelling the chemical complexity of essential oils: in-depth characterization and profiling by GC×GC-MS*** *Daniela Peroni, R. Franceschini, Eligio Sebastiani [1]* [1]SRA Instruments SpA
PO24	***ESI-FT-ICR-MS-based identification of a new flavonoid in Extra Virgin Olive Oil (Ravece cv)*** *Patrizia Iannece [1], Francesco Siano, Ermanno Vasca, Gianluca Picariello* [1]Dipartimento di Chimica e Biologia "A. Zambelli", Università degli Studi di Salerno
PO25	***Study of mono and di-O-caffeoylquinic acid isomers in Acmella oleracea extracts by HPLC-MS/MS and application of Linear Equation of Deconvolution Analysis (LEDA) algorithm for their characterization*** *Maria Bellumori[1], Marco Pallecchi, Beatrice Zonfrillo, Luigi Lucio, Marta Menicatti, Marzia Innocenti, Nadia Mulinacci and Gianluca Bartolucci* [1]NEUROFARBA Department, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, via U. Schiff 6, Sesto Fiorentino, 50019 Firenze

Scientific Program

Plenary Lectures

Mass-spec, computers, and Linked Open Data for chemodiversity characterization at the global scale.

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Keywords: *mass spectrometry, linked open data, knowledge graphs, natural products, chemodiversity*

The description of natural products (specialized metabolites) usually implies a tedious experimental journey spanning from the (bio-guided) isolation of a molecule to its complete structural elucidation. On the other hand, journals in the field will most often require a molecule to be novel and or bioactive for its publication. Together, these two facts explain our severe lack of knowledge regarding the global chemodiversity. Today, metabolomics and computational mass spectrometry software offer fantastic solutions to characterize the chemical content of living systems at unprecedented precision and throughput.[1] However, the alignment of metabolomics datasets within and across laboratories still poses challenges.[2] We are currently exploring the potential of semantic web technologies to better connect metabolomics experiments among them and with external public resources.[3] Here we will present the exciting possibilities offered by adopting Linked Open Data formats in the field of metabolomics. We will also explain the central role of these approaches in the frame of the Earth Metabolome Initiative[4], an ongoing Open Science effort aiming to characterize chemodiversity at the global scale.

References

1. Beniddir, M. A., Kang, K. B., Genta-Jouve, G., Huber, F., Rogers, S., & Van Der Hooft, J. J. *Natural product reports*, **2021**, *38*(11), 1967-1993. https://doi.org/10.1039/D1NP00023C

2. Watrous, J. D., Henglin, M., Claggett, B., Lehmann, K. A., Larson, M. G., Cheng, S., & Jain, M. *Analytical chemistry*, **2017**, *89*(3), 1399-1404. https://doi.org/10.1021/acs.analchem.6b04337

3. Gaudry, A., Pagni, M., Mehl, F., Moretti, S., Quiros-Guerrero, L. M., Cappelletti, L., ... & Allard, P. M. . ChemRxiv (preprint), **2023** https://doi.org/10.26434/chemrxiv-2023-sljbt-v2

4. https://www.earthmetabolome.org/

Modulation of innate immune response by marine sulfolipids

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Innate immune cells respond to invaders (bacteria, viruses, and fungi) and intrinsic danger signals such as pathogen-derived compounds or altered self-products. This activity is crucial to protect the host from infections and harmful tissue alteration, but the response must be also tightly controlled to prevent unwarranted effects that can lead to numerous diseases. The study of these mechanisms has also opened the development of new approaches in immunotherapy. In recent years, we have reported the immunomodulatory activity of sulfavant A, a sulfoglycolipid inspired by the natural sulfoquinovosides of marine diatoms[1–4]. The molecule and its structural analogues, collectively called sulfavants, activate the response of different types of innate immune cells, such as dendritic cells and macropahges, through an unconventional mechanism involving engagement of Triggering Receptor Expressed on Myeloid cells 2 (TREM2). Here I discuss the immunological novelty of of sufavant A in triggering the immune response beyond the classic inflammatory paradigm and, with a specific emphasis on the use of MS-based approaches, I briefly report the latest results in the search of natural sulfur-containing molecules as immunomodulatory agents for the treatment of cancer, Alzheimer's disease, and other neurodegenerative diseases.

References

1. Manzo, E. *et al. Sci. Rep.* **2017**, 7, 6286. https://doi.org/10.1038%2Fs41598-017-05969-8
2. Gallo, C. *et al. Cell. Mol. Life Sci.* **2022**, 79, 369. https://doi.org/10.1007/s00018-022-04395-y
3. Fioretto, L. *et al. J. Colloid Interface Sci.* **2021**, 611, 129–136. https://doi.org/10.1016/j.jcis.2021.12.054
4. Gallo, C. *et al. Mar. Drugs.* **2020**, 18, 604. https://doi.org/10.3390/md18120604

Keynote Presentations

Exploiting MS quantification of volatile compounds for the quality control of natural products

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Keywords: *quantitation, volatile compounds, quality control*

The demand for quantitative data in the field of flavours and fragrances is constantly increasing. This is mainly due to the ever-increasing number of quality and safety controls required, especially in today's globalised economy. The demand for quantification arises mainly from a) international bodies and committees (e.g. EFSA, IFRA, IOFI), b) legally imposed restrictions (e.g. suspected volatile allergens, thujones etc.), c) product characterisation by/for industry, d) internal quality control and last but not least e) because of its scientific importance (e.g. studies on the biological activity of volatile compounds). The quantitative aspects of volatile compound analysis are not easy to handle, not only because quantitation has long been considered less important than the elucidation of new metabolites, but also because various aspects of quantification have been and still are underestimated [1]. Based on these considerations, mass spectrometry plays a fundamental and essential role in the quality control of natural products, allowing at the same time a complete qualitative and quantitative characterisation of volatiles in raw plant materials and their products.

Here, some case studies are presented to illustrate the role of mass spectrometry in the quantification of volatile compounds in complex mixtures, especially in the differentiation of raw plant materials of different origins, in the adulteration of essential oils and in the quantification of compounds that are legally restricted in raw plant materials and finished products [2,3].

References

1. Bicchi, C.; Liberto, E.; Matteodo, M.; Sgorbini, B; Mondello, L; d'Acampora Zellner, B; Costa, R; Rubiolo, P. *Flav. Fragr. J.* **2008**, *23*, 382-391, doi: 10.1002/ffj.1905.

2. Sgorbini, B.; Bicchi, C.; Cagliero, C.; Cordero, C.; Liberto, E.; Rubiolo, P. *J. Chromatogr. A* **2015**, *1376*, 9-17, doi: 10.1016/j.chroma.2014.12.007.

3. Capetti, F.; Marengo, A.; Cagliero, C; Liberto, E; Bicchi, C; Rubiolo, P.; Sgorbini, B. *Molecules* **2021**, *26*, 5610, doi: 10.3390/molecules26185610.

Flavone di-*C*-glycosides from seed germ of *Leguminosae*: Inhibitors of carbohydrate digesting enzymes from industrial by-products

***Gianluca Picariello*[1], *Francesco Siano*[1], *Olga Fierro*[1], *Ermanno Vasca* [2]**

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Keywords: *Leguminosae germ flours; Flavone C-glycosides; α-amylase and α-glycosidase inhibitors;*

The cotyledon (or germ) from seeds of Mediterranean carob (*Ceratonia siliqua*) and tara (*Caesalpinia spinosa*) is a by-product of the extraction of E410 (locust bean gum) and E417 (tara seed gum), which are galactomannans highly demanded by food, pharmaceutical and cosmetic industries. Carob and tara seed germ flour as well as that of seeds from other less exploited *Leguminosae*, such as various *Prosopis* species (South American "algarrobo"), are protein- (45-55%, w/w) and polyphenol-rich (8-15 g kg^{-1}) food ingredients with excellent functional and nutritional properties. Germ polyphenols were comparatively characterized by HPLC-diode array detector and HPLC coupled with high-resolution tandem mass spectrometry (MS/MS). Largely predominant phytochemicals are di-*C*-glycoside derivatives of apigenin and, at much lower extent, of other flavones (1). In particular, most abundant compounds are schaftoside (apigenin 8-*C*-arabinosyl-6-*C*-glucoside) and isoschaftoside (apigenin 6-*C*-arabinosyl-8-*C*-glucoside) in *C. siliqua* and *Prosopis* spp. and vicenin-2 (apigenin di-6,8-*C*-glucoside) in *C. spinosa*. Several complex flavone *C*-glycoconjugates, *O*-glycosyl-*C*-glycosyl mixed derivatives and *O*-acylated-di-*C*-glycosides have been putatively assigned in all the flour samples based on collision induced decay (CID) MS/MS spectra, while signature compounds distinguish *C. spinosa* germ flour from counterparts of the remaining species.

Purified apigenin di-*C*-glycosides and crude phytocomplexes are powerful antioxidants, as assessed with a recently developed coulometric method (2). Even more interestingly, they are inhibitors of pancreatic α-amylase and small intestinal α-glycosidase, with IC_{50} comparable to hypoglycemic drugs. This finding suggests the use of germ flours as ingredients for wheat-based foods (*i.e.*, pasta or bakery products) with reduced glycemic impact.

Wheat pasta fortified with 5 and 10% (w/w) of carob germ flour retained 70-80% of the polyphenol fraction upon cooking in boiling water, thus delaying the release of sugars from starch during simulated oral-gastro-duodenal digestion (3).

Carob germ flour has been used safely for human nutrition. In contrast, tara germ flour, recently introduced into protein-enriched food preparations and superfood beverages targeted at the USA and Canada large distribution, is the alleged causative agent of a of even severe diseases that have affected hundreds of individuals in North America. However, the harmful compound(s) remain unidentified. This event has raised health concerns and food safety regulatory issues with general implications, which require urgent definition (4).

References

1. Picariello, G.; Sciammaro, L.; Siano, F.; Volpe, M.G.; Puppo, M.C.; Mamone, G. *Food Res. Int.* **2017**, *99*, 730–738, doi: 10.1016/j.foodres.2017.06.05

2. Siano, F.; Sammarco, A.S.; Fierro, O.; Castaldo, D.; Caruso, T.; Picariello, G.; Vasca, E. *Antioxidants* **2023**, *12*, 1963, doi: 10.3390/antiox12111963.

3. Siano, F.; Mamone, G.; Vasca, E.; Puppo, M. C.; Picariello, G. *Food Res. Int.* **2023**, *170*, 112962, doi: 10.1016/j.foodres.2023.112962

4. Chittiboyina, A. G.; Ali, Z.; Avula, B.; Khan, S. I.; et al. *Chem. Res. Toxicol.* **2023**, *36*, 818-821, doi: 10.1021/acs.chemrestox.3c00100.

Oral Presentations

An integrated metabolic, *in silico* and proteomic approach to reveal the parahormetic mechanism underlying the protective health effects of grape seed proanthocyanidins

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Keywords: *OMICS, proanthocyanidins, parahormesis.*

Proanthocyanidins (PACs) are a class of secondary metabolites present in many plants as oligomers and polymers. Their health benefits on inflammatory-based diseases are supported by a huge number of *in vitro* and *in vivo* studies. A large portion of orally ingested PACs reach the colon where they are catabolized by colonic microflora into phenyl-γ-valerolactones, which showed anti-inflammatory activity.[1] The aim of the present study was to evaluate (i) the catabolic conversion of a standardized oligo-polymeric PACs grape seed extract (GSE), both *in vitro* and in a human intervention study, and (ii) the biological activity and molecular mechanism of the main metabolite, namely 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (VL), by an integrated metabolic, *in silico* and proteomic approach. An *in vitro* human fermentation model allowed to identify VL as the main PACs catabolite produced by colonic microbiota. VL was identified as sulfate and glucuronide phase II metabolites by LC-ESI-MS/MS in the urine (~36 μmoles/24 hours) of volunteers treated with PACs. VL was then synthesized, and its effect was evaluated by using two phenotypic cell models: HEK293 with the cell reporter for Nrf2 activation and R3/1 with cell reporter for NF-κB activation. A dose-dependent activity was observed in both experiments. The molecular mechanism of Nrf2 activation by VL was then elucidated by *in silico* studies which fully clarified the molecular engagement of VL with the thiol group of KEAP-1. The ability of VL to modulate the NF-κB and its phosphorylated form p(Ser276)-NF-κB levels was then confirmed on human colon Caco-2 cells. Quantitative proteomics studies were then carried out on Caco-2 cells to give a deeper insight into the mechanism of action of VL, in both physiological and inflammatory conditions. In physiological condition, VL improved the mitochondrial activity, by boosting the oxidative phosphorylation. When a pro-inflammatory stimulus was used (TNFα), VL reverted the expression levels of certain key genes belonging to the integrin signaling pathway, usually triggered by an inflammatory state, and regarding oxidative phosphorylation a partial reversion of the TNFα-induced downregulation of proteins inherent to the electron transport chain was observed. Moreover, the activation of the Nrf2 cascade was indirectly observed through the modulation of the downstream gene products, including COX2, upregulated in the inflammatory state and downregulated in the presence of VL. In conclusion, we demonstrated how VL can enhance body's resilience through the activation of Nrf2, which works as an hormetic mediator protecting biological systems and conserving resources through its controlled reaction, as reported for phytochemicals that act by such a mechanism that can be called "parahormesis".[2]

References

1. Ki H.S., Chung S., Song M.Y., Lim C., et al. *Molecules*, **2020,** 25, 2215. https://doi.org/10.3390/molecules25092215
2. Forman H.J., Davies K.J.A., Ursini F., *Free Radic Biol Med*, **2014**, 66. https://doi.org/10.1016/j.freeradbiomed.2013.05.045

Peptidomics profiling, identification of bioactive peptides, and assessment of the biological activities of novel nutraceutical products with a potential protective role in a doxorubicin-mediated cardiac damage model

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Keywords: *mass spectrometry; proteomics; biological activity.*

Adjuvant therapies like anthracyclines play a crucial role in treating various cancers, including breast, gastric, and esophageal cancers. However, despite their effectiveness in extending cancer-related survival, these agents can lead to drug-related cardiotoxicity. Spirulina, Reishi (Ganoderma lucidum), and Moringa are three nutraceuticals known for their anti-inflammatory effects and are currently utilized in cancer patients as complementary and alternative medicines to enhance quality of life and alleviate fatigue. We hypothesize that the nutraceutical combination of Spirulina, Reishi and Moringa (Singo) could reduce inflammation and cardiotoxicity induced by anthracyclines.
Firstly, we investigated the nutraceutical potential of Spirulina platensis, Moringa oleifera and Ganoderma lucidum extracts. To determine the protein composition, a label-free based liquid chromatography–mass spectrometry (LC–MS/MS) proteomic approach was carried out. In detail, a nanoflow ultra-high-performance liquid chromatography (UHPLC) instrument (Ultimate 3000, Thermo Fisher Scientific, Bremen, Germany) was coupled online to an Orbitrap Fusion™ Lumos™ Tribrid Mass Spectrometer (Thermo Scientific) fitted with the Nanospray Flex NG ion source (Thermo Fisher Scientific). The peptides were trapped on a PepMap trap column (Thermo Fisher) and then separated onto a C18 reversed-phase nano column (0.075 ID × 250 mm × 2.6 µm biozenTM Peptide XB-C18, Phenomenex, Bologna, Italy). The raw MS data were analyzed using Protein Discoverer™ Software version 2.5 employing the Sequest search engine. The spectra were searched against the *Arthrospira platensis* and *Arabidopsis thaliana* databases from UniprotKB. The analytical platform allowed us to determine the distribution of the molecular weight, protein sequence coverage and the profile of lengths for all identified peptides. The polysaccharide content of extracts was determined by the phenolic–sulfuric acid method while the characteristic functional groups were identified by FT-IR spectroscopy.
Female C57Bl/6 mice were subjected to different treatments: Sham, short-term doxorubicin (DOXO), Singo, or pre-treatment with Singo followed by DOXO. Various analyses, including ELISAs and immunohistochemistry, were performed to assess myocardial expressions and conditions. Human cardiomyocytes were exposed to DOXO alone or with Singo, and studies on cell viability and inflammation were conducted. In preclinical models, Singo improved cardiac function and reduced biomarkers associated with heart failure and fibrosis in DOXO-induced cardiotoxicity [1].

References

1. Quagliariello, V.; Basilicata, M. G.; Pepe, G.; De Anseris, R.; Di Mauro, A.; Scognamiglio, G.; Palma, G.; Vestuto, V.; Buccolo, S.; Luciano, A.; Barbieri, M.; Bruzzese, F.; Maurea, C.; Pumpo, R.; Ostacolo, C.; Campiglia, P.; Berretta, M.; Maurea, N. *J. Cardiovasc. Dev. Dis.*, **2022,** 9, 42. https://doi.org/10.3390/jcdd9120423

Valorization of grape pomace: a comparison among the phytochemical profile and antioxidant activity of grape pomace and berries of common use in food and nutraceutical industries

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Keywords: *food waste, HPLC-ESI-Q-TOF-MS, antioxidant activity*

Every year is estimated that 9 million tons of grape pomace are generated which counts ≈ 20% of the grape processed in terms of weight [1]. We investigated the phytochemical profile and antioxidant activity of different extracts of grape pomace comparing the results with those of berries commonly available on the market (*e.g.* cranberry, elderberry, rose hip berries, goji berries and raisin). The extracts were prepared using a mixture of EtOH:H_2O 7:3 as extraction solvent and decoction (DC), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) as extraction techniques. The total phenolic and flavonoid content was determined by performing colorimetric assays. The identification was conducted by HPLC-ESI-Q-TOF-MS working both in positive and negative ion mode and using accurate mass values, ion source fragmentation, MS/MS fragmentation patterns, comparison with analytical standards and bibliographic research. Heat Maps have been prepared for each extract. We identified a total of 67 compounds in grape pomace extracts (figure 1), 43 for cranberry extracts, 39 for rose hip berries extracts, 28 for elderberry extracts, 20 for goji berries extracts and 16 for raisin extracts. The main compounds were quantified using the UV signal, except for citric and isocitric acids which were quantified using MS. DPPH, ABTS, CUPRAC and FRAP assays were performed to determine the antioxidant activity of each extract showing the best results for grape pomace DC, UAE and MAE followed by cranberry UAE and rose hip berries DC and the worst results for goji berries and raisin DC, UAE and MAE in line with the total phenolic content of these extracts.

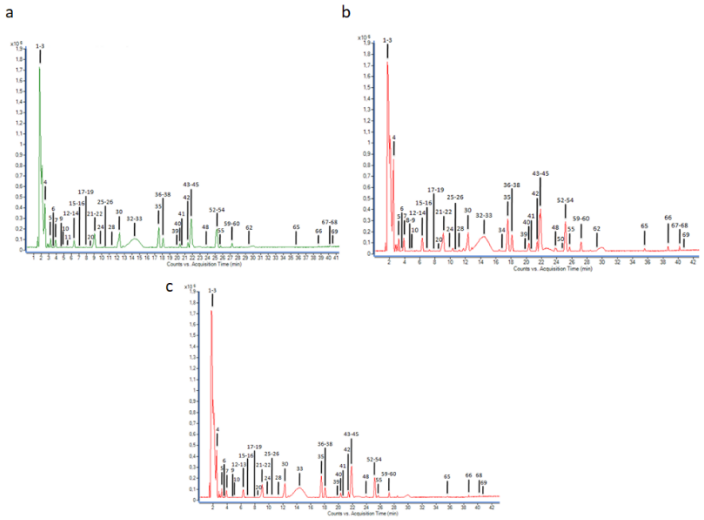

Figure 1. Base peak chromatogram of grape pomace DC (a), UAE (b) and MAE (c) obtained using HPLC-ESI-Q-TOF-MS in negative ion mode.

References

1. Schieber, A.; Stintzing, F. C.; Carle, R. *Trends in Food Science & Technology* **2001**, *12*, 401–413, https://doi.org/10.1016/S0924-2244(02)00012-2.

Comprehensive in vitro ADME study of 5-(3,4-dihydroxyphenyl)-γ-valerolactone, the main colonic catabolite after flavonoids-rich supplements intake.

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Keywords: *5-(3',4'-dihydroxyphenyl)-γ-valerolactone, in vitro ADME, DMPK.*

The interest for 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (VL) has increased recently, as it is one of the major metabolites derived from the catabolism of flavonoids by gut microbiota and is a well know compound with health-promoting effects. Nevertheless, its absorption, metabolism and transport properties and possible herb-drug interaction (HDI) risks have not been clarified yet. Hence, the purpose of this study is to investigate through the application of LC-MS analytical platforms and in vitro models PK/PD properties of VL [1].
Caco2 and Wt-MDCK monolayer models were chosen, and an UPLC-QqQ platform was used to quantify VL and evaluate its apparent permeability and efflux ratio. VL is rapidly metabolized in its sulfate metabolite valerolactone sulfate (VLS), as emerged by mass spectrometric analysis.
VLS showed in both models an apparent permeability acceptable for the oral route of administration. On the other hand, efflux ratio values found are discrepant in the two models: with Caco2 it is >2, whereas it is not with Wt-MDCK, indicating that VLS is substrate of an efflux transporter more expressed in Caco2 cell line (BCRP) [2].
Phase II and I metabolism of VL were evaluated with human liver S9 and microsome fraction (HLM). Metabolites formed were qualitative assessed by LC-HRMS system and quantified by UPLC-QqQ.
VL was found to be rapidly degraded in S9 fraction (half-life $t_{½}$ 8.72 minutes) but degraded at a slower rate in HLM (half-life $t_{½}$ 23.08 minutes). Most importantly, in S9 fraction VL over time forms two different phase II metabolites, both glucuronic adducts, on the two possible sites of the molecule; also, the sum of glucuronic conjugates represents more than 80% of total tested VL [3].
VL has been tested for its modulation of CYP3A4 and CYP1A2, demonstrating marginal inhibition of CYP3A4 (IC_{50} > 25 µM), lack of inhibition on CYP1A2, and absence of activating activity on pregnane X receptor (PXR) [4].
In conclusion, 5-(3',4'-dihydroxyphenyl)-γ-valerolactone is widely absorbed as sulfate conjugate in the intestine and reaches the systemic circulation. The sulfation pathway is reversible and the inversion in mainly mediated by arylsulfatase, which is a lysosomal enzyme with an higher mRNA expression at hepatic level [5]. Hence, in the liver VL can be further metabolized into its glucuronide adducts which represent the most abundant metabolites of the catabolite. Finally, it neither induces nor blocks major cytochromes and gene receptors that can alter their expression, nor efflux systems causing no herb-drug interaction.

References

1. Mena, P.; Bresciani, L.; Brindani, N.; Ludwig, I. A.; Pereira-Caro, G.; Angelino, D.; Llorach, R.; Calani, L.; Brighenti, F.; Clifford, M. N.; Gill, C. I. R.; Crozier, A.; Curti, C.; Del Rio, D. *Nat. Prod. Rep.* **2019**, *36* (5), 714–752. https://doi.org/10.1039/C8NP00062J.
2. Volpe, D. A.. *Future Medicinal Chemistry* **2011**, *3* (16), 2063–2077. https://doi.org/10.4155/fmc.11.149.
3. Obach, R.S.; Baxter, J.G.; Liston, T.E.; Silber, B.M.; Jones, B.C.; Macintyre, F.; Rance, D.J.; Wastall, P. J *Pharmacol Exp Ther* **1997**;283(1):46-58.a.
4. Husain, I.; Manda, V.; Alhusban, M.; Dale, O. R.; BaeJ.-Y.; Avula, B.; Gurley, B. J.; Chittiboyina, A. G.; Khan, I. A.; Khan, S. I. *Phytomedicine* **2021**, *81*, 153416. https://doi.org/10.1016/j.phymed.2020.153416.
5. Coughtrie, M. W. H.; Sharp, S.; Maxwell, K.; Innes, N. P. *Chemico-Biological Interactions* **1998**, *109* (1–3), 3–27. https://doi.org/10.1016/S0009-2797(97)00117-8.

Tomato (*Solanum lycopersicum* L.) leaves as a sustainable source of bioactive compounds: from analytical characterization, through biological evaluation, to the formulation of lipid nanoparticles

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Keywords: *tomato leaves; solid lipid nanoparticles; anticancer activity.*

Food waste from food processing, nowadays, has gained a significant attention as possible sources of functional compounds with health benefits. In this context, by-products from tomatoes, including leaves, peel, and seeds are recognized as rich sources of phytochemical compounds with health-promoting properties.[1] This study aimed to valorize "Datterino PGI" and "Piccadilly PGI" tomato leaves, examining the potential anticancer properties of tomato extracts and analyzing their phytochemical composition.[2] RP-UHPLC-ESI-HR-MS/MS analysis showed an abundant of alkaloids, flavonoids, fatty acids, lipids, and terpenes.
In this regard, the potential anticancer activity of the extracts was evaluated *in vitro* by MTT assay on neuroblastoma cells. The ethanolic extracts, unlike aqueous extract, lead to a significant decrease in the percentage of cell viability on SH-SY5Y instead of OECs, without significant changes compared to the control. Since the therapeutic application is limited due to stability and bioavailability issues, tomato leaves extracts have been subjected to formulation studies.[3-4] Therefore, encapsulating them into Solid Lipid Nanoparticles (SLN) appears to be an effective strategy. Considering the potential anticancer activity, a special attention was paid to steroidal glycoalkaloid identified: α-tomatine (α-TM) and its aglycone tomatidine (TD).[5] Nanoformulations α-TM-SLN and TD-SLN were prepared by solvent-diffusion technique, their technological parameters were characterized and the effects of empty were tested using Franz diffusion cells, analyzing the acceptor phase samples using a UHPLC–ESI(+)–QqQ–MS. Results showed that the maximum α-TM (126.4 ng/mL) and TD (31.62 ng/mL) amounts, corresponding to approximately 65% and 88% of the loaded drug, were reached after 22 h, followed by a slower release until the end of the experiment. Moreover, the effect on the percentage of cellular viability in OECs and in SH-SY5Y was assessed and biological results demonstrated that the treatment with free α-TM (0.25 µg/mL) and TD (0.50 µg/mL) on SH-SY5Y cultures induced a significant decrease in the percentage of cell viability when compared with the control, instead of OECs. In particular, the effect appeared more evident when SH-SY5Y cells were exposed to treatment with α-TM-SLN and TD-SLN, confirming their potential anticancer activity. Based on these results, tomato leaves extracts could be considered as a valuable source of bioactive compounds, suitable for various applications in the food, nutraceutical, and pharmaceutical fields, and the use of nanotechnology could be regarded as a promising approach for delivering α-TM and TD and exploiting their potential anticancer properties.

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References

1. Silva-Beltrán, N.P.; Ruiz-Cruz, S.; Cira-Chávez, L.A.; Estrada-Alvarado, M.I.; Ornelas-Paz Jde, J.; López-Mata, M.A.; Del-Toro-Sánchez, C.L.; Ayala-Zavala, J.F.; Márquez-Ríos, E. *Int J Anal Chem* **2015**, 284071. https://doi.org/10.1155/2015/284071

2. Choi, S.H.; Ahn, J.B.; Kozukue, N.; Kim, H.J.; Nishitani, Y.; Zhang, L.; Mizuno, M.; Levin, C.E.; Friedman, M. *J. Agric. Food Chem.* **2012**, 60, 3891–3899. https://doi.org/10.1021/jf3003027
3. Sansone, F.; Picerno, P.; Mencherini, T.; Villecco, F.; D'Ursi, A.M.; Aquino, R.P.; Lauro, M.R. *J. Food Eng.* **2011**, 103, 188–196. https://doi.org/10.1016/j.jfoodeng.2010.10.015
4. Elizalde-Romero, C.A.; Montoya-Inzunza, L.A.; Contreras-Angulo, L.A.; Heredia, J.B.; Gutiérrez-Grijalva, E.P. *Front. Nutr.* **2021**, 8, 790582. https://doi.org/10.3389/fnut.2021.790582
5. Bailly, C. *Steroids* **2021**, 176, 108933. https://doi.org/10.1016/j.steroids.2021.108933

Metabolite Profiling, through LC-ESI/LTQOrbitrap/MS Analysis, of Antioxidant Extracts from *Physalis alkekengi* L.

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Keywords: *LC-ESI/LTQOrbitrapMS, antiradical scavenging activity, metabolic profiling.*

Physalis alkekengi L. (family Solanaceae) is a plant native to Asia and southern Europe. The formation of the fruit is fascinating: the calyx swells like a parchment paper balloon and changes color, going from the initial green to the bright red of autumn. Fruits and calyces can be eaten both cooked and raw and are the only edible part of the plant [1]. *Physalis alkekengi* L. is widely used as a dietary supplement and a raw material for tea preparation. For this reason, a comprehensive analysis of the green extracts was carried out (Figure 1). Fruits and calyces of this plant were analyzed through two different extraction methods: hydroalcoholic extraction and decoction. Metabolite profiling of *Physalis alkekengi* L. calyx and fruit was performed by LC-ESI/LTQOrbitrap/MS followed by LC-ESI/LTQOrbitrap/MS/MS to identify 58 phytocompounds using the two different extraction methods. Subsequently, the antioxidant activity of the different *Physalis alkekengi* L. extracts was evaluated by preliminary spectrophotometric assays and subsequent cell studies. The extracts of *Physalis alkekengi* L. were found to be a good source of metabolites such as flavonoids, organic acids, phenylpropanoids, physalins and carotenoids, which exhibit various biological activities, especially antioxidant activity capable of reducing the production of free radicals in Caco-2 intestinal cells. For the first time, an integrated approach, that combines a metabolomics approach with an antioxidant evaluation, was applied to the study of green extracts and decoctions of *Physalis alkekengi*, the most commonly used green extraction method in herbal preparations [2]. An interesting result was the high antioxidant activity of these extracts, especially for decoction, which allows the suggestion of a potential beneficial use of *Physalis alkekengi* L. fruit and calyx in the preparation of tea infusions.

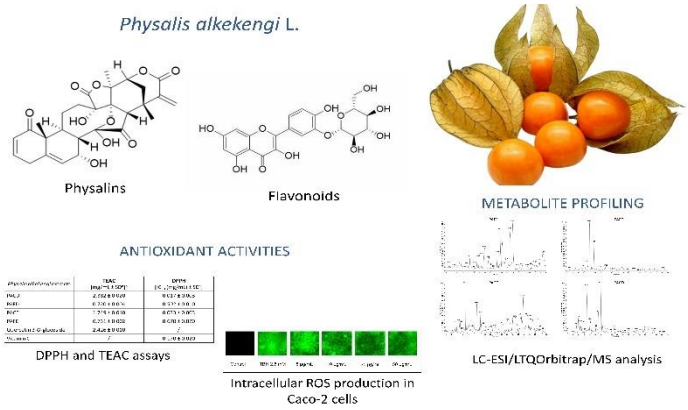

Figure 1. *Research workflow.*

References

1. Liang, L.; Li, C.; Wang, Y.; Yue, Y.; Zhang, H.; Yang, M.; Cao, X.; Zhao, M.; Du, J.; Peng, M.; et al. *Phytomedicine* **2022**, *105*, 154328. https://doi.org/10.1016/j.phymed.2022.154328

2. Crescenzi, M.A.; Serreli, G.; Deiana, M.; Tuberoso, C.I.G.; Montoro, P.; Piacente, S. *Antioxidants* **2023**, *12*, 2101. https://doi.org/10.3390/antiox12122101.

Pseudo-targeted and untargeted HRAM-based approaches: A case study on Hop bioactive compounds

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Keywords: *Humulus Lupuluds, HRAM, Metabolites*

Bitter acids, a group of prenylated phloroglucinol derivatives found in Humulus lupulus L., are renowned for their diverse health benefits. However, research on their metabolism, stability, and potential metabolic pathways remains limited. This study aimed to investigate the metabolic stability of hop α- and β-acids, identify phase I and II metabolites both in vitro and in vivo, and assess their anti-inflammatory properties. We employed a combination of pseudo-targeted and untargeted workflows utilizing high-resolution accurate mass spectrometry (HRAM). Our findings enhance understanding of the metabolic fate of bitter acids and shed light on potential metabolic pathways influenced by these compounds in inflammatory cellular models. These results underscore the versatility and utility of HRAM methods in supporting pharmacokinetic studies of natural products and untargeted metabolomics.

Chemodiversity and antibiofilm activity of essential oils from Tunisian *Eucalyptus*

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Keywords: *Essential oils, gas chromatography/mass spectrometry, antibiofilm activity*

Eucalyptus (Myrtaceae), native to Australia and Philippines, includes about 900 species nowadays distributed throughout the world (Salehi et al., 2019). During the 1950s, more than 100 species were introduced in Tunisia to fight against soil erosion and in support of the major projects of reforestation (Elaissi et al., 2010). They also have become important for the economy: as a fast-growing source of wood used for construction and as firewood, and for their unifloral honey characterized by health-promoting properties (Bobis et al., 2020). *Eucalyptus* EOs have antimicrobial (Bachir and Benali, 2012), antifungal (Gakuubi et al., 2017) antiseptic, anti-inflammatory (Ben Marzoug et al., 2011), disinfectant, mucolytic and analgesic properties (Valeriano et al., 2012). Recent studies have highlighted the activity of the essential oils (EOs) against bacterial biofilms, including EOs from species of *Eucalyptus* (Khedhri et al., 2022). These studies are of interest considering their use in medicine also to prevent the emergence of resistance to conventional drugs. The biological properties depend on the EOs phytochemical profile: *Eucalyptus* EOs are rich in monoterpenes and sesquiterpenes, especially eucalyptol (Dhakad et al., 2018). In this work, the EOs from 22 *Eucalyptus* species were obtained by hydrodistillation and their compositions were examined by GC and GC-MS. The most representative components were α-pinene, *p*-cymene, piperitenone, β-vetivenene, sphatulenol, β-eudesmol, *trans*-pinocarveol, viridiflorene and especially eucalyptol. The antibiofilm activity was evaluated with cell viability tests and inhibition of biofilm formation (Nazzaro et al., 2022). The EOs showed antibacterial activity against some important pathogenic strains such as *Acinetobacter baumannii*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and inhibited the biofilm formation, in dose dependent manner, depending both on the tested strain and the EO. The data obtained on the chemical composition of these EOs can help to shed light on the complex phytochemistry of the EOs of the *Eucalyptus* genus. EOs demonstrated an important role in fighting the bacterial biofilm both at the beginning of the biofilm formation and in more advanced stages. Their use can help in decreasing the emergence of bacterial resistance to conventional antibacterial drugs.

References

1. Bachir RG. Benali M. *Asian Pac. J. Trop. Biomed.* **2012**, 2, 739–742. https://doi.org/10.1016/s2221-1691(12)60220-2

2. Ben Marzoug HN, Romdhane M, Lebrihi A, Mathieu F, Couderc F, Abderraba M, Larbi Khouja M, Bouajila J. *Molecules* **2011**, 16, 1695–170. https://doi.org/10.3390/molecules16021695

3. Bobis O, Moise AR, Ballesteros I, Reyes ES, Durán SS, Sánchez-Sánchez J, Cruz-Quintana S, Giampieri F,Battino M, Alvarez-Suarez JM. *EFood Chem*. **2020**, 325, 126870. https://doi.org/10.1016/j.foodchem.2020.126870

4. Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. *J. Sci. Food Agric*. **2018**, 98, 833–848. https://doi.org/10.1002/jsfa.8600

5. Elaissi A, Marzouki H, Medini H, Larbi Khouja M, Farhat F, Lynene F. Harzallah-Skhiri F, Chemli R, *Chem. Biodivers*. **2010**, 7, 909–921. https://doi.org/10.1002/cbdv.201000038

6. Gakuubi MM, Maina AW, Wagacha JM. *Int. J. Microbiol*. **2017**, 2017, 8761610-8761617. https://doi.org/10.1016/j.ijfoodmicro.2017.02.014

7. Khedhri S, Polito F, Caputo L, Manna F, Khammassi M. Hamrouni L, Amri I, Nazzaro F, De Feo V, Fratianni F. *Molecules* **2022**, 27, 8227-8244. https://doi.org/10.3390/molecules27238227

8. Nazzaro F, Polito F, Amato G, Caputo L, Francolino R, Fratianni F, Candido V, De Feo V. *Antibiotics* **2022**, 11, 724. http://dx.doi.org/10.3390/antibiotics11060724

Hydroxyanthracene derivates citotoxicity: A differential evaluation between single molecule and whole plant extract

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Keywords: *hydroxyanthracene derivates, phytochemical characterization, citotoxicity*

Hydroxyanthracene derivates (HADs) are a group of natural or synthetic compounds with a wide range of biological activities (for instance, anti-inflammatory, antibacterial, and antiarthritic). In addition, because of their properties for helping the normal bowel function, HADs are widely used in constipation as pharmacological drugs and nutritional supplements. Nevertheless, during the past years, a safety usage of HAD products has been placed under consideration from European Food Safety Authority (EFSA) because some scientific studies reported that HADs are not lacking toxicity. In particular, the safety of the use of medicinal plants containing HAD in dietary supplements was re-evaluated concluding that HAD emodin, aloe-emodin, and the structurally related substance danthron have been shown to be genotoxic and carcinogenic until proven otherwise based on other studies. Thus, the first objective of this study was to shed light on the large variability in composition of botanical food supplements containing HAD by a systematic analysis of the qualitative and quantitative composition of a cohort of extracts and raw materials of plants with high levels of anthraquinones commercially available (*Cassia angustifolia, Rhamnus purshiana, Rhamnus frangula, Rheum palmatum, and Rheum raponticum)* using UPLC-MS/MS. Specifically, the targeted analysis was performed by parallel reaction monitoring (PRM). Since, the current investigations of HAD toxicity were based on *in vitro* and *in vivo* studies conducted mainly on the use of the single molecules (emodin, aloe-emodin, and rhein) rather than on the whole plant extract, the qualitative quantitative characterization was the starting point to select the most appropriate products for the treatments for an *in vitro* cell studies. Thus, the second objective of this study was the investigation, for the first time, of the toxic events of HAD used as single molecule in comparison with the whole plant extracts containing HAD occurring in an intestinal in vitro model of human colorectal adenocarcinoma cells (Caco- 2). A shotgun proteomics approach followed by a bioinformatic elaboration was applied to investigate the differential protein expression in the Caco-2 cells after a single-HAD or whole–plant extract treatment to fully understand the potential targets and signaling pathways. In conclusion, the combination of a detailed phytochemical characterization of HAD products and a largely accurate analysis of the proteomic profile of intestinal cells treated with HAD products provided the opportunity to investigate their effects in the intestinal system.

References

1. Tinti L, Cicaloni V, Nezi P, Isoldi G, Etiope P, Barlozzini B, Pecorari R and Salvini L: *Front. Plant Sci*. **2023** 14:1166075. doi: 10.3389/fpls.2023.1166075

Sucrosomial® Selenium Absorption and Biodistribution in *In vivo* model

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Keywords: Sucrosomial® Selenium, *in vivo* Study**,** Biodistribution

Selenium (Se) is an essential nutrient for human health. Se is absorbed in the gastrointestinal tract through a not well-known active transport. Selenite salt is the water-soluble compound mainly use in food supplements. Unfortunately, it combines with other food components during digestion to form insoluble complex, reducing its absorption across the intestinal wall and increasing the risk of toxicity (Ye et al., 2021). Sucrosomial® Selenium is a patented oral Se formulation based on sodium selenite salt (NaSe), associated with phospholipids and sucrester's matrix. Thanks to this innovative delivery system, Se could pass through the gastric environment reducing its interaction with other food components and decreasing its common side effects. Moreover, in the intestinal compartment Se bypass the interaction with the intestinal mucosa increasing its permeability across the epithelium. The aim of this study was to investigate Sucrosomial® Selenium absorption in blood and transport different tissues after a single oral dose of 0.25 mg Se/kg in mice. NaSe, Sucrosomial® Selenium (UltraSel®), and Sucrosomial® Selenium with sodium pyrophosphate (UltraSel NAPP®) were studied. An ICP-MS method was developed and validated for quantification of Se in serum and different tissues (thyroid, heart, brain and skeletal muscle), after microwave mineralization. Serum concentration of Se was determined at different time points (1h, 3h, 8h, 24h) by ICP-MS. Data showed a significant differences of serum Se concentration among groups. Animals treated with NaSe and Ultrasel NAPP® showed a very fast absorption with a maximal concentration (Cmax) reached at 1h, while animals treated with Ultrasel® showed a slower kinetics of absorption with a Cmax at 8h post gavage. These different formulations may impact not only on the intestinal absorption but also on Se incorporation into tissue proteins. Thyroid was the organ that more efficiently incorporated Se after 24h of a single oral dose. Se is important for thyroid hormone metabolism and subjects with low intake of this mineral may develop thyroiditis, hypothyroidism, and Graves' syndrome. In the present study, Se amount was found to be significantly higher, compared to basal levels, after a single oral administration of Ultrasel® and Ultrasel NAPP®. The latest seems to be the most effective in delivering Se to the thyroid, although the amount of the mineral was not statistically different to that observed with Ultrasel®. These formulations also demonstrated to deliver efficiently Se into the heart. Particularly, Ultrasel® determined a statistically significant increase of Se concentration in this organ. This analysis has a potential clinical impact considering that suboptimal selenium levels (<100 μg/L) are prevalent in more than 70% of patients with heart failure and are associated with lower exercise capacity, lower quality of life, and worse prognosis. Finally, Se concentration in the brain and skeletal muscle were not different among groups, although a growth trend was observed moving from NaSe to Ultrasel® and Ultrasel NAPP®. These results confirm that after a single oral dose, Sucrosomial® delivery system can influence Se intestinal absorption and biodistribution in different tissues especially in thyroid gland and heart. The absence of less impressive differences observed in the brain, and skeletal muscle can be ascribed to the very short timing point considered. A multiple dose administration may certainly provide additional information on the Se supplementation with the Sucrosomal ® Technology. Thus, for all these reasons, Sucrosomial® Selenium can be considered a valid alternative to common Se salts to improve Se supplementation effectiveness. Moreover, the developed ICP-MS method resulted to be a simple, quick, and robust method for the measurement and quantification of the trace element Se in different biological matrixes.

Reference:

1. Ye R Huang JWang Z, Chen Y,Dong Y. *Int. J. Mol. Sci.* **2021**, 22, 11708 https://doi.org/10.3390/ijms222111708

Essential oils for supportive clinical aromatherapy: chemical profiling by GC-MS, ESI-MS/MS and FT-IR analysis

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Keywords: *Mass Spectrometry Fingerprinting; Aroma Profile; Rose-Jasmine-Lotus Essential Oils.*

Essential oils are highly concentrated plant extracts consisting of complex mixture of compounds belonging to different chemical classes including alcohols, aldehydes, esters, ethers, ketones, phenols, amines, amides and terpenes. These compounds come from the secondary metabolism of various plants and have, in most cases, antibacterial, antifungal and antioxidant properties. The composition of essential oils varies, both in qualitative and quantitative terms, with the species, and it is influenced by climatic and geographical factors as well as by production process. Essential oils are currently used in various industries such as food and beverages, cosmetics, pharmaceutical, and in aromatherapy due to their health benefits. In particular, the essential oils market demand in medical application is driven by their characteristic of no major effect compared to the traditional medicine. Clinical aromatherapy is a fast-growing complementary therapy worldwide. It is used for the rising stress-related health problems or medical side effects of cancer care, such as anxiety, depression, nausea, vomiting and insomnia [1]. In a clinical setting, essential oil quality and safety are mandatory and they are strictly related to the composition. In this perspective, the authenticity assessment is very important, too, because essential oils may be adulterated due to their high economic evaluability and beneficial properties.
The present study aims to characterize the chemical profile of rose, jasmine and lotus essential oils, from different manufacturers, by Gas Chromatography coupled with mass spectrometry (GC-MS) [2], Electro Spray Ionization Tandem Mass Spectrometry (ESI-MS/MS) [3-4] and Fourier Transform Infrared Spectroscopy (FT-IR) analysis, for clinical aromatherapy in oncological application, poorly reported in literature, in order to investigate on their quality characteristics, differences and on the presence of bioactive compounds.
GC-MS analysis of essential oils provided the separation and identification of compounds in the aromatic fraction and evidenced differences among the oils, the presence of adulterants in some cases, and compounds with potential biological effect. ESI-MS/MS analysis provided the fingerprint of the essential oils methanolic extract, and the fragmentation pattern of the main evidenced peaks, evidencing diagnostic ions useful for fast evaluation of samples from different plant species and manufacturers. FT-IR analysis provided important information on the authenticity and discrimination of essential oils, too. The herein proposed multi-analytical approach can be considered a powerful tool for an overall insight into the essential oils, from the chemical composition to potential biological properties and clinical application.

References

1. Farrar A.J., Farrar F.C., *Nurs. Clin. North Am*. **2020**, 55(4):489-504, doi: 10.1016/j.cnur.2020.06.015.
2. Di Matteo P., Bortolami M., Di Virgilio L., Petrucci R., *J. Food Sci. Technol*. **2022,** Nov, 59(11):4553-4562. doi: 10.1007/s13197-022-05536-8.
3. Di Matteo P., Bortolami, M., Curulli A., Feroci M., Gullifa G., Materazzi S., Risoluti R., Petrucci R., *Front. Biosci*. **2023**, (Landmark Ed) 28(1), 3.doi.org/10.31083/j.fbl2801003.
4. Petrucci R., Di Matteo P., De Francesco G., Mattiello L., Perretti G., Russo P., (2020), *J. of Agr. And Food Chem.* **2020**, 68 (30) 7984-7994. https://doi.org/10.1021/acs.jafc.0c02802

GC-MS Biocomponents Characterization of Waste *Violina pumpkin* and *Olea europaea* L. leaf extracts.

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Keywords: *leaf extract, GC-MS, biocomponents.*

Traditionally, leaf extracts are characterized by the presence of many potential bioactive compounds with antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties and, for this reason, used to mitigate or treat a wide range of disorders. On the other hand leaves stand out among the most common waste generated by agri-food industries and, quite recently, much attention have been devoted to the valorization of this processing by-products [1].
Pumpkins and olive oil represent two of the most consumed and investigated foods from a nutraceutical and therapeutic point of view but, to better define and characterized the potentiality of the waste materials, their leaf have been collected and subjected to different extraction procedures.
Gas chromatography-mass spectrophotometry (GC-MS) which is recognised to possess a very wide field of applications, has been used for the analysis of such obtained extracts. For *Curcubita moschata* pumpkin leaf extract this technique represents an usefull tool for the identification of some interesting biologically active compounds among them, *p*-coumaric acid contained in Fraction 7 and coming from the whole acetone subextract, showed a great potentiality in the treatment of intervertebral disc degeneration (IDG) *in vitro* [2].
On the other hand, GC-MS analysis of *Olea europea* L. leaf aquouse extract, resulting from a long green and innovative process, help us to identify the elenolic acid, which is the monoaldehydic dihydropyran part of the most common and aboundant oleuropein. Investigations on elenolic acid are also of great importance for its promising therapeutic potential, displaying *in vitro* and *in vivo* effects as an anti-diabetic, hepatoprotective, and anti-influenza virus agent [3].

References

1. Selim, S; Albqmi, M.; Al-Sanea, M.M.; Alnusaire, T.G.; Almuhayawi, M.S.; AbdElgawad, H.; Al Jaouni, S.K.; Elkelish, A.; Hussein, S.; Warrad, M.; El-Saadony, M.T. *Front. Nut.* Sec. *Food Chem.* **2022**, 9, DOI: 10.3389/fnut.2022.1008349

2. Lambertini, E.; Penolazzi, L.; Notarangelo, M.P.; Fiorito, S.; Epifano, F.; Pandolfi, A.; Piva, R. *Int. J. of Mol. Med.* **2023**, *51(39*), DOI: 10.3892/ijmm.2023.5242.

3. Salamanca, A.; Almodóvar, P.; Jarama, I.; González-Hedström, D. *Antiv. Chem. and Chemoth.* **20221**, 29, DOI: 10.1177/20402066211063391

Characterization and quantification of intact glucosinolates in *Catozza* rapeseeds: a promising food matrix for nutraceuticals development

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Keywords: *Glucosinolates, Rapeseeds, Myrosinase*

Glucosinolates (GSLs) are anionic β-thioglucoside N-hydroxysulfate derivatives of the Brassicaceae family. GSLs coexist in plant tissues with the enzyme myrosinase, which is stored separately in the near myrosin cells.[1] Upon cell-damage, GSLs encounter the enzyme myrosinase, that hydrolyzes them activating a substrate-enzyme defense mechanism against herbivores and pathogens for which GSLs breakdown products (e.g., isothiocyanates) are toxic.[2] GSLs analysis has historically represented a major chromatographical challenge due to their physico-chemical properties. Therefore, in order to perform an accurate quantitative GSLs analysis, the aim of this project has been the development and validation of a method for the extraction and the analysis of intact GSLs. The quantitative analysis was performed using a targeted HPLC-HESI-MS/MS method in negative acquisition mode, developed by using a mixture of 6 GSLs analytical standards. The chromatographic method was validated by the assessment of its linearity, limits of detection (LODs), limits of quantification (LOQs), precision, and accuracy. The validation was performed according to the ICH validation guideline (ICH.Q2[R1], 1995). The precision (% CV) values ranged from 0.2 to 4.8% and from 0.2 to 8.0% for intraday and interday precision, respectively. The accuracy (% bias) values ranged from -8.8% to 1.9% and from -8.8 to 3.9% for intraday and interday accuracy, respectively. According to the low values obtained for both % CV and % bias, the developed method may be considered a reproducible and reliable protocol for the identification of 17 intact GSLs and quantification of 6 intact GSLs by HPLC-HESI-MS/MS analysis. After the development and validation of the HPLC-HESI-MS/MS method, the first issue was the preparation of the food matrix for the intact GSLs quantification. Seeds were selected for their highest GSLs content in plants. Among all species, *Catozza* rapeseeds (Brassica rapa L. var. rapa DC.) were selected to re-evaluate the commercial potential of native horticultural ecotypes of Campania region (Southern Italy). In order to provide a reliable quantification of intact GSLs, several parameters were assessed, including myrosinase inactivation conditions (e.g., freeze-drying and heating treatments), rapeseeds grinding methods, and extraction procedures. The optimization provided the best myrosinase inactivation conditions by a 30 minute heating treatment at 80 °C, leading to the highest GSLs content. Two extractions methods were performed on the rapeseed meal to obtain hydroalcoholic *Catozza* rapeseed extracts (HCRE) and food-grade aqueous *Catozza* rapeseed extracts (ACRE). Therefore, the aqueous extraction method for ACRE preparation has been validated by matrix effect and recovery assays to assess the efficacy of the extraction method. To assess the nutraceutical potential of these optimized extracts, due to the ability of GSLs to release hydrogen sulfide (H_2S), preliminary assays were performed to investigate the release of H_2S in a cell-free assay and the vasorelaxing effects in an *ex vivo* mouse aorta assay. The two extracts showed a concentration-dependent and endothelium-independent relaxing effect in mice aorta by releasing H_2S. This study represents the starting point for further in-depth investigation of *Catozza* rapeseeds use for GSLs-based formulations for nutraceuticals purpose.

References

1. Clarke, D. B. *Analytical Methods* **2010**, *2* (4), 310–325. https://doi.org/10.1039/b9ay00280d.

2. Agerbirk, N.; Olsen, C. *Phytochemistry*. **2012**, pp 16–45. https://doi.org/10.1016/j.phytochem.2012.02.005

Shimadzu’s ready-to-use method for Metabolomics. Differentiation of Tomato Varieties

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Keywords: *Metabolomics, chemometrics, tomato*

Metabolomics analysis can be a challenge in analytical laboratories, especially for newcomers to the field. Shimadzu Corporation offers an extensive array of instruments and application tools to simplify and enhance efficiency analysis, so users can begin a metabolomic study without time-consuming evaluation of method parameters. Shimadzu’s LC-MSTOF and LC/GC-MSMS systems together with the Multi-Omics Analysis Package supports the entire metabolomic analysis process: from sample preparation to result and data interpretation, including all chromatographic and MS instrumental settings. Different varieties of tomato were studied using Shimadzu's Package and a GC-MSMS system. Results clearly categorized four types of tomatoes and highlighted their differences in amino acids and sugars content, demonstrating this method package as a useful tool for the metabolomic analysis of plants. Moreover, untargeted analysis of metabolites can be performed using Shimadzu’s QTOF systems and, unlike other technologies such as ion trap instruments, it can be developed for quantitative analysis to establish and quantify biomarkers for each sample. All chemometrics tools: PCA, HCA, Volcano Plots and other multivariate statistical plots are automatically generated using Shimadzu's platform, as well as metabolic mapping, network analysis and projection on the metabolic map. In this manner, a significant metabolomic study of tomatoes was conducted, revealing distinct metabolomic differentiations using a ready-to-use method from sample preparation to chemometric results.

Postharvest Microwave Drying of Basil (*Ocimum basilicum* L.): The Influence of Treatments on the Quality of Dried Products

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Keywords: *Ocimum basilicum L.; process sustainability; drying methods;*

Edible herbs are used in the human diet both for their pleasant flavors and their countless benefits to human health. These properties are due to their components which mainly have antioxidant and anti-inflammatory therapeutic functions [1]. Since aromatic herbs are highly perishable materials due to their high water content, to ensure that the products are safe and stable over time they must be treated with appropriate techniques. The application of microwave-assisted drying, a promising technique in terms of process sustainability, for the stabilization of the aromatic herb *Ocimum basilicum* L. was studied [2,3]. The activities were carried out by applying different operating conditions in order to evaluate the impact of the time/temperature combination on the final quality of dried basil. The volatile fraction of the dried sample was obtained by steam distillation. Subsequently, through the use of gas chromatography coupled to mass spectrometry (GC-MS), the chemical profiles and the differences between the different treatments were evaluated. Conventional convective processes were also applied to make comparisons between dried basil products both at the production stage and from a quality preservation perspective. The results showed that microwave heating is suitable as a drying method, as expected, due to the known interaction between the plant tissue (rich in water) and the electromagnetic field; and that drying methods have a different influence on the chemical composition of essential oils extracted from dried products, in terms of number (varying from 41 to 18 components in different dried samples) and percentage (up to 67% in linalool and 21% in -*trans*-bergamotene in several dried samples) of its constituents.

The advantage of the qualitative-quantitative variability, linked to the drying method, allows us to obtain essential oils with peculiar characteristics that can be used in the food sector (flavor enhancers), in the cosmetic sector (creams and soaps) and in the healthcare sector (phytotherapeutic formulations).

References

1. Borges, C.V.; Minatel, I.O.; Gomez-Gomez, H.A.; Lima, G.P.P. In *Medicinal Plants and Environmental Challenges*, **2017**; pp. 259–277. https://doi.org/10.1007/978-3-319-68717-9_15
2. Calin-Sanchez, A.; Lech, K.; Szumny, A.; Figiel, A.; Carbonell-Barrachina, A. *Food Res. Int.* **2012**, *48*, 217–225. https://doi.org/10.1016/j.foodres.2012.03.015
3. Thamkaew, G.; Sjöholm, I.; Gómez Galindo, F. *Food Sci. Nutr.* **2021**, *61*, 1763–1786. https://doi.org/10.1080/10408398.2020.1765309

Characterization of Strawberry Extracts from Novel Cultivars

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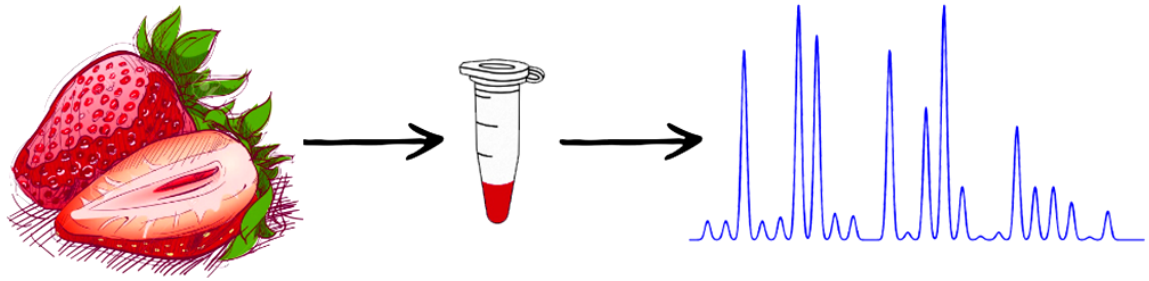
Keywords: *Metabolomics, High-resolution mass spectrometry, Strawberry extracts.*

Strawberries contain bioactive compounds that contribute to their unique characteristics and promote several health benefits [1]. Gaining a thorough understanding of the metabolic profile of a novel strawberry cultivar becomes crucial to obtain specific information regarding its phytochemical content, quality, and traceability [2]. Moreover, this study investigates strawberry extracts from novel cultivars, examining the concentrations of callistephin in different harvests [3,4]. Employing High-Performance Liquid Chromatography (HPLC), we quantified the levels of callistephin, revealing changes throughout the harvesting process. Additionally, High-Resolution Mass Spectrometry (HRMS - positive and negative mode) and Liquid Chromatography-Mass Spectrometry (LC-MS) studies were conducted to identify the compound classes and confirm molecular structures [5]. This study gives valuable insights into the levels of anthocyanidins across various cultivars. Through a comprehensive understanding of anthocyanidin composition in different cultivars, there is potential to optimize cultivation methods, thereby reducing resource inputs and environmental impact.



References

1. The research is part of the broader **RESO** project (**RE**silienza e **SO**stenibilità), which focuses on enhancing our understanding its applications in nutraceutical and pharmaceutical domains.

2. Wang, S. Y.; Lin, H. S. *J. Agric. Food Chem.* **2000**, *48*, 140-146, doi: 10.1021/jf9908345

3. Enaru, B.; Drețcanu, G.; Pop, T. D.; Stănilă, A.; Diaconeasa, Z. *Antioxidants* **2021**, *10*, 1967. doi: 10.3390/antiox10121967

4. Hollman, P. C. *J. Sci. Food Agric*. **2001**, *81*, 842- 852. doi: 10.1002/jsfa.900

5. Onzo A, Acquavia MA, Pascale R, Iannece P, Gaeta C, Lelario F, Ciriello R, Tesoro C, Bianco G, Di Capua A. *Anal Bioanal Chem.* **2022,** *414*, 7805-7812. doi: 10.1007/s00216-022-04314-x

Metabolite Profile of "Green" Extracts of *Cynara cardunculus* (Carciofo di Paestum PGI) leaves by LC-HRMS-Based Metabolomics

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Keywords: *leaves of "Carciofo di Paestum" PGI; green extracts; LC-HRMS-based metabolomics.*

Globe artichoke (*Cynara cardunculus* L. var. *scolymus* L.), belonging to the family Asteraceae, is a perennial plant cultivated in the Mediterranean area. The globe artichoke has been widely investigated for its chemical profile and valued for its nutraceutical and medicinal properties [1]. "Carciofo di Paestum", an Italian traditional cultivar, is a labeled PGI (Protected Geographical Indication) product of the Campania region, representing an important economic resource [2].

With the aim to give an interesting opportunity to use artichoke by-products for the development of nutraceutical and/or cosmetic formulations, the investigation of leaves of "Carciofo di Paestum" has been carried out.

In the first step, different extractions have been performed, using EtOH, EtOH: H_2O (75:25, 50:50) as solvents and conventional (maceration at room temp) and unconventional (Naviglio and ultrasound-assisted extraction UAE) extraction procedures. In the second step, in order to identify the primary and specialized metabolites occurring in the extracts of "Carciofo di Paestum*"* leaves, an analytical approach based on high-performance liquid chromatography coupled to multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS), in negative ion mode, has been carried out. According to their accurate mass, characteristic fragmentation pattern, retention time, and literature data, more than 35 metabolites have been identified: in particular, primary metabolites belonging to polar fatty acids class and specialized metabolites belonging to flavonoids, phenylpropanoids, sesquiterpenoids as well as triterpenoids.

Successively, an approach based on LC-HRMS metabolomics with Multivariate Data Analysis (MVDA) was used to identify the metabolite variation among the "green" extracts obtained by different extraction procedures. Literature data shows how caffeoylquinic acid and dicaffeoylquinic acid derivatives are able to modulate lipid and glucose metabolisms in metabolic-related disorders [3]. Considering the interesting activity reported for these compounds, this work highlights that the extracts obtained using 50:50 EtOH:H_2O and 75:25 EtOH:H_2O by Naviglio extraction resulted the most abundant in caffeoyl-, dicaffeoyl quinic acid derivatives.

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References

1. Zayed, A.; Serag, A.; Farag, M.A. *J. Funct. Foods* **2020**, 69, 103937, doi: 10.1016/j.jff.2020.103937.

2. Cerulli, A.; Masullo, M.; Pizza, C.; Piacente, S. *Molecules* **2022**, 27, 3328, doi: 10.3390/molecules27103328.

3. Naveed, M.; Hejazi, V.; Abbas, M.; Kamboh, A.A.; Khan, G.J.; Shumzaid, M.; Ahmad, F.; Babazadeh, D.; Xia, F.F.; Modarresi-Ghazani, F.; et al. *Biomed. Pharmacother*. **2018**, 97, 67–74, doi: 10.1016/j.biopha.2017.10.064.

Edible Insects as Novel Foods: The Chemical Characterization of *Acheta domesticus*

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Keywords: *Novel Foods, Metabolomics, FT-ICR MS.*

The expected large population increase in the very next future and the necessity of reducing the ecological problems related to the intensive food production result in a growing request for alternative protein sources. In this context, edible insects, with their environmental and nutritional advantages, represent a new and innovative food source able to satisfy both sustainability and nutritional demands [1]. Entomophagy deserves to be promoted for three main reasons: i) health, since insects are nutritious alternative to traditional food sources, rich in proteins, fatty acids and minerals; ii) environmental, since they require lower water and feed consumption and their farms generate few greenhouse gases and ammonia; iii) economic factors, since insect rearing needs smaller spaces for farming and it can be a low-tech and low-capital investment option. In the present study, for the first time, a spray-dried *A. domesticus* (house cricket) powder produced in Italy has been investigated through a multimethodological approach. An untargeted characterization was achieved thanks to the powerful performances of: 1) Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) that allows a fast single measurement, the identification of several classes of metabolites present in the matrix [2]; Nuclear Magnetic Resonance (NMR) spectroscopy, for accurate quantification and molecular assignement. Furthermore, targeted Gas-Chromatography Mass Spectrometry (GC-MS) was employed to obtain complementary information. After extraction with Bligh-Dyer procedure and appropriate dilution, the samples were directly submitted to electrospray ionization (ESI) source. High resolution mass spectrometry analyses have been carried out by using a Bruker BioApex FT-ICR both in positive and negative ionization mode. Complementary information for determination of fatty acids was gathered by using ESI (-) coupled to a linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific). Then, the putative attributions have been confirmed by CID experiments and by cross-reference with online libraries. Analyses of the hydroalcoholic and organic extracts revealed the presence of more than 500 molecular formula. The qualitative analysis was accomplished by graphic instruments such as van Krevelen diagrams (vKd) and histograms of relative frequencies. VKd allows to get an immediate overview of several molecular family density showing that both the extracts came out rich in lipids, terpenoids and polyketides, followed by amino acids with less hits. The averaged relative frequency distribution histogram revealed that both extracts were largely populated by CHO (e.g. lipids) and CHNO (e.g. amino acids) with more entries in organic extract, followed by CHNOS and CHOP species with more entries in hydroalcoholic extract. The improved knowledge of the chemical profile of this Novel Food opens up new horizons both for the use of the cricket products and for the use of specific components that could be isolated for the production of new formulations. At the same time, the application of this multimethodological approach has allowed to highlight how edible insects represent a very innovative and low environmental impact food source.

References

1. Baiano, A. *Trends Food Sci. Technol*. **2020**, 100, 35–50, doi.org/10.1016/j.tifs.2020.03.040

2. Lasalvia, A.; Cairone, F.; Cesa, S.; Maccelli, A.; Crestoni, M.E.; Menghini, L.; Carradori, S.; Marinacci, Gallorini, M.; Elsallabi, O.; Pesce, M.; Patruno, A. *Antioxidants.***2022** Oct 22;11(11):2088. doi: 10.3390/antiox11112088.

Phytochemical and functional characterization of *Morus alba* L. fruits from varieties grown in Italy

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Keywords: *Metabolomics; Multivariate statistical analyses; Functional foods.*

Mulberry belongs to the genus *Morus* (Moraceae family) which includes eight species. Among these, the most important species used for its well-known pharmacological effects is *M. alba* which has been exploited for centuries in Eastern Asia folk medicines. *Morus* leaves and fruits are important sources of polyphenols and 1-deoxynojirimycin (DNJ). Polyphenols from *M. alba* were demonstrated to exert antioxidant activities and reduce the production of advanced glycation end products (AGEs), which play a central role in the initiation and progression of diabetes, and to increase the activity of DNJ synergistically. The iminosugar DNJ is an alkaloid capable of strongly inhibiting the α-glucosidase, an intestinal digestive enzyme responsible for the release of glucose from disaccharides. Also, the anthocyanins in *M. alba* fruit extracts were proven to inhibit the α-glucosidase in vitro.
In the present work, the type and level of phytochemicals and functional properties of the fruits from thirteen *M. alba* cultivars grown in Italy were characterized, since there are few available data about their nutraceutical properties. *M. alba* fruits were extracted in acid methanol and their phytochemical profile was investigated via ultrahigh-performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (HRMS). The putative identification of fifty polyphenolic compounds was achieved using the Compound Discoverer software. The total organic acid, phenolic, flavonoid, and anthocyanin contents and the antiradical, iron (II) chelating, and iron (III) reducing activities were determined. Also, the α-glucosidase inhibitory activity was evaluated *in vitro*. The 1-DNJ and the most concentrated anthocyanins were detected by multiple reaction monitoring and quantified by an external standard calibration method in liquid chromatography coupled with a triple quadrupole mass spectrometer. The fruits of M. alba cultivars grown in Italy displayed variable polyphenolic contents and different biological activities. The antioxidant and the α-glucosidase inhibitory activities did not have relevant positive or negative correlations with the quantitative results in Pearson's test probably due to the different expression of metabolites in the fruits. To better understand the correlation between the metabolites and the biological activities, the areas of the most concentrated polyphenols in *M. alba* fruit extracts were exported by using Compound Discoverer software and multivariate statistical regression models were generated. The semi-quantitative results obtained through the Compound Discoverer were used as x-block for correlating the antioxidant results (y-block). Several multivariate statistical models were built on the dataset and the most satisfactory (higher values of R^2) were examined to understand which variables (polyphenolic compounds) were correlated to the antioxidant activity. Regression models built on UHPLC-HRMS results revealed a strong correlation between the expression of quercetin derivatives, cyanidin 3-O-glucoside, caffeoyl methyl quinates, and 5,5'-dehydrodivanillic acid among the others, and fruit anti-radical, iron (III) reducing activities and α-glucosidase inhibitory capacity.
The achieved results on the compositional and functional characterization of mature M. alba fruits might improve their consumption and their economic value in Italy.

A new method for the determination of 5-methyltetrahydrofolic acid in Citrus juices by High Resolution Mass Spectrometry

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Keywords: *5-methyltetrahydrofolic acid, citrus juices, high resolution mass spectrometry*

A new and accurate method relying on high resolution mass spectrometry has been developed for the quantitative determination of 5-methyltetrahydrofolic acid (5-MTHFA) in various citrus juices.
5-Methyltetrahydrofolic acid is part of the folate family and stands the most representative form within green vegetables, legumes, and citrus fruits. Folates exhibit a chemical structure consisting in a pteridine ring with a *p*-aminobenzoate portion linked to glutamyl chains. Variations in folates are observed based on the reduction and substitution degree of the pterin cycle and the length of glutamate chain [1]. They are essential micronutrients, classified as water-soluble vitamins, and offer numerous biological activity and beneficial effects on human health. The significance of these effects is also underscored by the recent European regulation EU 432/2012, which endorses some health claims on foods and beverages containing specific amounts of folates (30 µg and 15 µg per 100 g, respectively). Herein, we present an innovative analytical approach designed for the measurement of 5-MTHFA in different citrus juices, placing a particular emphasis on the distinctive citrus varieties of Calabria region: *Bergamot*, *Citron*, *Clementina*, and *Lemon*, in order to comply with the above-mentioned EU regulation and enhance the overall quality of these citrus products.
The method has been attained by using high-performance liquid chromatography coupled with high resolution mass spectrometry (HPLC-HRMS) based on Orbitrap technology.
The quantitative assay has been established by means internal standard calibration method, using folic acid - naturally missing in foods - as standard. The MS analyses have been conducted in positive polarity and in fullscan mode. Prior to applying the developed approach to the real samples, key analytical parameters have been assessed: accuracy, precision, matrix effect and robustness. Accuracy values ranged from 95 and 105%, while both repeatability and reproducibility were less than 15%, confirming the consistency and the precision of the proposed analytical procedure. The measured quantity of 5-methyltetrahydrofolic acid found in real samples ranged from 100 µg/L to 200 µg/L, highlighting that bergamot, citron and clementine fruits are excellent source of folates. Furthermore, to confirm the robustness of the entire analytical procedure, the examined samples have been also submitted to a classical HPLC-MRM method, yielding comparable results.

References

1. N. Delchier, A. L. Herbig, M. Rychlik, C. M. G. C. Renard, *Comprehensive Reviews in Food Science and Food Safety*, **2016**, *15*, 506.
2. European Union Regulation, N. 432/**2012**.
3. P. M. Thomas, V. P. Flanagan, *Journal of Agricultural and Food Chemistry*, **2003**, *51*, 1293.

Metabolomics analysis of *Moringa oleifera* leaves: correlation between in vitro effect on C2C12 myotubes cell line and geographical origin.

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Keywords: *plant metabolomics, zenoTOF, antioxidant activity*

Moringa oleifera Lam. is an important, multipurpose plant, widely used traditionally for its nutritional and medicinal properties due to the presence of active biomolecules in its parts. In particular, the leaf extract is used for its ability to in regulate metabolism and its antioxidant activity.

It has been reported that *Moringa oleifera* leaf extract (MOLE) has beneficial properties that may mitigate pathological conditions including diabetes. MOLE treatment has been shown to increase oxidative energy metabolism and possibly favors mitochondrial biogenesis through the SIRT1/PPARα-pathway [1].

This work aims to test the antioxidant capacity of *Moringa oleifera* leaves from different geographical areas on C2C12 myotubes and correlate the phenotypic activity to the metabolomics profile.

An untargeted metabolomics analysis of MOLE samples from each different geographical area has been performed to identify primary and secondary metabolites that may be responsible for the biological activity. Accurate mass spectrometers, such as time-of-flight (TOF) instruments, are often used, given their capability for thorough identification and confident characterization using MS/MS data. Here, a sensitive quantitative and qualitative method has been optimized and developed by using a ZenoTOF 7600 system.

The generation of highly sensitive MS/MS spectra together with the high-quality fragmentation spectra enables confident identification of low abundant compounds by using MS/MS library matching. Their presence and/or variation can result in finding marker compounds of the geographical origin and/or be linked to the biological activity.

References

1. Duranti G., Maldini M., Crognale D. et al.. *Phy. Plu.* **2021**, *1*, *100014–100023*. https://doi.org/10.1016/j.phyplu.2020.100014.

Poster Presentations

Stable isotope ratio analysis of volatile organic compounds for the botanical characterization and authentication of lavender essential oil

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Keywords: *authenticity, stable isotope ratios, lavender essential oil*

In this study, we developed a combination of Gas Chromatography-tandem Mass Spectrometry (GC-MS/MS) and Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS) techniques to detect and prevent misrepresentation in lavender essential oil. The method involved an optimized chromatographic run to effectively separate pure reference standards within 17 minutes and the investigation of the isotope ratios of carbon ($\delta^{13}C$) and hydrogen ($\delta^{2}H$) in a wide range of volatile organic compounds (VOCs) found in lavender oil. An extensive application of the validated method was performed on a large number of essential oils to analyze the most distinctive VOCs and their corresponding stable carbon and hydrogen isotope ratios ($\delta^{13}C$ and $\delta^{2}H$) in lavender, lavandin, and commercial lavender essential oil samples. Using sPLS-DA models which incorporate VOCs, $\delta^{13}C$ and $\delta^{2}H$ values, or their combination for data analysis, we achieved 100% accuracy in classifying lavender and lavandin. These preliminary results highlight the potential of VOCs and compound-specific $\delta^{13}C$ and $\delta^{2}H$ as promising tools for the authentication and botanical classification of lavender essential oil and the detection of potential adulteration with synthetic compounds.

References

1. Perini, M., Pianezze, S., Strojnik, L., Camin, F., *J. Chromatogr. A.*, **2019**, 1595, 168–173. https://doi.org/10.1016/j.chroma.2019.02.032.
Guo, X., Wang, P. *Molecules*, 2020, 25, 23. https://doi.org/10.3390/molecules25235541

Effects of different lighting conditions on growth and metabolism of basil grown under Microcosm conditions

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Keywords: *indoor farming, plant metabolomics, precision agriculture*

Indoor farming of basil (*Ocimum basilicum* L.) under artificial lighting to support year-round produce demand is meeting an increasing interest worldwide. Diverse lighting conditions differently affect downstream metabolic pathways that influence basil growth, development and metabolism. Several experiments were carried out growing basil from the seedling stage to fully developed plants in Microcosm (EP 3 236 741 B1), an innovative device aimed at growing plants indoor as in natural conditions [1]. In a first experiment, plants were grown under white (W) or blue-red (BR) light with a photosynthetic photon flux density (PPFD) of 255 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The results indicated that a greater plant biomass was produced under the W light, whereas higher concentrations of phenolic compounds were detected under the BR light [2]. In a second experiment, the effects of two different PPFD values, 250 and 380 $\mu mol \cdot m^{-2} \cdot s^{-1}$, on the plant secondary metabolism, including volatile organic compounds (VOCs), were investigated. Principal component analysis (PCA) of volatiles and non-volatiles metabolites showed that the samples can be discriminated according to the different PPFD levels. Specifically, samples from plants grown under 380 $\mu mol \cdot m^{-2} \cdot s^{-1}$ displayed higher levels of all oxygenated monoterpenes, including 1,8-cineole, linalool and eugenol, that have been reported to be among the most representative components in basil cultivars and among the most relevant compounds responsible for the persistent odour in the basil overall aroma [3-4]. In a third experiment, the effects of two different photoperiods (16/24 and 24/24 h light) under the same PPFD of 380 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the secondary metabolism were investigated. The PCA results indicated that the two lighting periods induced different VOCs profiles, while they slightly affected the phenolic composition of the samples. In particular, the metabolic profile of plants grown under 24/24 h light was correlated with most of the identified volatiles, with the exception of *cis*-3-hexenal and 1-octen-3-one that appeared to be overexpressed in plants grown under 16/24 h light.

References

1. d'Aquino, L.; Lanza, B.; Gambale, E.; Sighicelli, M.; Menegoni, P.; Modarelli, G.C.; Rimauro, J.; Chianese, E.; Nenna, G.; Fasolino, T.; D'Urso, G.; Piacente, S.; Montoro, P. *Sci. Hortic.* **2022**, 299,111035. doi.org/10.1016/j.scienta.2022.111035
2. d'Aquino L.; Cozzolino, R.; Nardone, G.; Borelli, G.; Gambale, E.; Sighicelli, M.; Menegoni, P.; Modarelli, G.C.; Rimauro, J.; Chianese, E.; Nenna, G.; Fasolino, T.; D'Urso, G.; Montoro, P. *Plants* **2023**, *12*, 1450, doi.org/10.3390/plants12071450
3. Lee, S.J.; Umano, K.; Shibamoto, T.; Lee, K.G. *Food Chem.* **2005**, *91*, 131-137. doi.org/10.1016/j.foodchem.2004.05.056
4. D'Alessandro, A.; Ballestrieri, D.; Strani, L.; Cocchi, M.; Durante, C. *Molecules* **2021**, *26*, 3842. doi.org/10.3390/ molecules26133

LC-HRMS based evaluation of the effect of saline irrigation and plant-based biostimulant application on phytocannabinoid composition of fiber hemp (*Cannabis sativa* L.).

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Keywords: *Phytocannabinoids, secondary salinization, bioeffectors.*

Phytocannabinoids are a class of over 200 meroterpenoids and represent the hallmark of the secondary metabolism of *Cannabis sativa*. These metabolites show a surprising diversity of biological targets and qualify as privileged structures for biomedical research [1]. Their chemical variability and relative content are the result of genetically encoded pathways optimized by evolution and can be affected by agrotechnique (irrigation, fertilization, cultivar) and modulated by pedo-climatic factors acting as elicitors. For example, salt stress can modify plant morphology, anatomy and physiology [2, 3] while inducing a variety of differentially expressed genes (DEGs) in hemp [4], associated with the biosynthesis of phytocannabinoids. According to this evidence, hemp cultivation addressed to the production of biomolecules for pharmaceutical applications can involve poorly fertile saline-degraded soils that are not suitable for food production.

In our study, aimed at the evaluation of the effect of secondary salinity and biostimulant application on the growth and the metabolism of cannabis plants, mesocosms (Ø=50 cm) sowed with hemp (*Cannabis sativa* L.) var. Felina were arranged in the facilities of the University of Naples, Dept. of Agricultural Sciences. A medium fertility sandy soil was used and 4 water salinity levels (NaCl solutions with an Electrical Conductivity (EC) of 0, 2.0, 4.0 and 6.0 dS m^{-1}) of irrigation water were tested in combination with 2 biostimulant treatments over a randomized complete block design. Hemp biometric parameters were monitored at full flowering and showed that plant growth was significantly affected by both tested treatments. Saline irrigation significantly reduced biomass production only with EC4 and EC6 treatments. Methanolic extracts of both inflorescences and leaves were obtained by the development of a green and rapid extraction procedure assisted by ultrasonication. The phytocannabinoid composition, of all the obtained samples, was investigated by LC-HRESIMS^2 analysis allowing the detection of 13 characteristic secondary metabolites with an accuracy error below 10 ppm. As expected, in all the analysed samples, the main component was cannabidiol, together with other neutral and acidic phytocannabinoids (Δ^9-THC, CBN, CBC, and others), cannflavins A and B, and canniprene. Multivariate unsupervised PCA analysis revealed that the phytocannabinoid composition is significantly affected by the origin of the samples is the main factor influencing the phytocannabinoid composition, followed by salinity levels of the irrigation water and by biostimulant application.

References

1. Hanuš, L. O.; Meyer, S. M.; Muñoz, E.; Taglialatela-Scafati, O.; and Appendino, G. *Nat. Prod. Rep*. **2016,** *33*, 1357–1392. doi:10.1039/c6np00074f
2. Akram, N.A., Shafiq, F., Ashraf, M., Iqbal, M., and Ahmad, P. *J. Plant Growth Regu*. **2021,** *40*, 891-905. doi.org/10.1007/s00344-020-10158-5
3. Guerriero, G., Behr, M., Hausman, J. F., and Legay, S. *Genes*, **2017,** *8*(10), 242. https://doi.org/10.3390/genes8100242
4. Gao, C., Cheng, C., Zhao, L., Yu, Y., Tang, Q., Xin, P., ... & Zang, G. *Int. J. Genom*. **2018,** Article ID 3057272. https://doi.org/10.1155/2018/3057272

Sweet potato (*Ipomoea batatas*) leaves as a valuable source of biologically active chlorogenic acid regioisomers

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Keywords: *chlorogenic acids, sweet potatoes leaves, LC-MS/MS*

Plant extracts are a source of valuable phytochemicals. These secondary metabolites play a remarkable role in the human diet and have been shown to have several health benefits, therefore there is growing interest in using biologically active compounds found in plants as functional foods and ingredients for nutraceutical products [1,2]. Chlorogenic acids (CGAs) are among the most abundant polyphenols, and they have recently attracted the attention of several research groups also thanks to their biological activity including antioxidant, anti-inflammatory and antiviral properties [3]. Much of the literature on acyl-quinic acids focuses on different tissues of coffee plants including seed endosperm and leaves, which contain substantial amounts of these secondary metabolites [4]. Thanks to our analytical expertise on coffee chlorogenic acids and with special attention to agrifood by-products, we have taken into consideration plant matrices different from coffee as an alternative source of CGAs [5] and sweet potato leaves are the focus of this work. The sweet potato (*Ipomoea batatas*) belongs to the Convolvulaceae family. Long cultivated for its edible root tubers, sweet potato is an important carbohydrate source in the tropics, especially in Central America and New Guinea. Young shoots, leaves and tubers are all edible matrices even if in Italy only the latter is commercially exploited. Following the experimental protocol already used for coffee leaves [6], several mono- and di-caffeoylquinic acid regioisomers have been identified and quantified in sweet potato leaves by UHPLC-UV and UHPLC-MS/MS. In addition to providing quantitative data, the present study highlights some inconsistencies regarding the nomenclature of chlorogenic acids adopted in describing the CGAs profile of sweet potato leaves reported in the literature. The identification of *p*-coumarylquinic acid and feruloylquinic acid regioisomers in sweet potato leaves has been performed for the first time. The identification of 3,4,5-tricaffeoylquinic acid has also tentatively been attempted. It is important to note that the content varies significantly with leaf age: young leaves are characterized by a higher content of secondary metabolites, as previously observed with coffee leaves and this aspect must be taken into consideration for the exploitation of this plant matrix in both food and nutraceutical applications. Moreover, in view of its peculiar CGAs profile, sweet potato leaves may represent a valuable surrogate standard of non-commercially available chlorogenic acids for analytical applications.

References

1. Crozier, A.; Clifford, M. and Ashihara, H. *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet* **2006**, doi:10.1002/9780470988558.
2. Liang, N.; Kitts, D.D. *Nutrients* **2016**, *8*, 16, doi:10.3390/nu8010016.
3. Sinisi, V.; Stevaert, A.; Berti, F.; Forzato, C.; Benedetti, F.; Navarini, L.; Camps, A.; Persoons, L.; Vermeire, K. *Planta Med*. **2017**, 83, 615, doi:10.1055/s-0042-119449.
4. Farah, A. and Donangelo, C. *Brazilian Journal of Plant Physiology* **2006**, *18*, doi:10.1590/S1677-04202006000100003.
5. Gutiérrez Ortiz L. A., Berti F., Navarini L., Crisafulli P., Colomban S., Forzato C. *Journal of Chromatographic Science* **2018**, *56*, 753–760, doi:10.1093/chromsci/bmy041.
6. Monteiro, Â.; Colomban, S.; Azinheira, H.G.; Guerra-Guimarães, L.; Do Céu Silva, M.; Navarini, L.; Resmini, M. *Antioxidants* **2020**, *9*, 6, doi:10.3390/antiox9010006.

Development of a new extraction method for a food supplement based on pomegranate: analysis of metabolic profile by LC-MS

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Keywords: *Green extraction; LC-MS analysis; Punica granatum*

Punica granatum L., commonly known as pomegranate, is an ancient fruit widely consumed all over the world as fresh fruit or juice [1]. The pomegranate market has steadily grown, due to the increasing demand of health-conscious consumers for products with health-promoting effects [2]. The beneficial effects of pomegranate are mainly due to the occurrence of phenolic compounds like phenolic acids, tannins, flavonols, anthocyanins and hydrolyzable tannins [1]. In recent years, a growing interest in developing ecological and environmentally friendly methods for natural product extraction has led to the investigation of extraction procedures aimed at obtaining a higher extraction yield using a lower amount of solvents and energy, also reducing the extraction time [3]. Herein, a new extraction procedure was developed for the peels of *Punica granatum*, cultivar "Dente di Cavallo" which were extracted through a non-conventional extraction technique like solid-liquid dynamic extraction (SLDE-Naviglio) using pomegranate juice and ethanol (30:70) as extraction solvent. The extract was analyzed by a combined approach based on LC-ESI/QExactive/MS/MS analysis. Furthermore, its chemical profile was compared with those of pomegranate juice and of the extract obtained from peels by SLDE-Naviglio technique using as solvent a mixture of ethanol and water (70:30). LC-MS analysis allowed the identification of different classes of specialized metabolites including hydrolyzable tannins, flavonoids, ellagic acid and phenol glucoside derivatives. Moreover, the extracts were evaluated for their total phenolic, tannin, and flavonoid content by spectrophotometric assays. The results indicated the highest phenolic content (484.27 mg GAE/g ± 1.291) and flavonoid content (432.33 mg rutin/g ± 1.595) in the extract obtained by SLDE-Naviglio using pomegranate juice and ethanol (30:70) as extraction solvent.

References

1. Yisimayili, Y., Chao, Z., *Food Chemistry*, **2022**, 395, 133600. doi:10.1016/j.foodchem.2022.133600
2. Topalović, A., Knežević, M., Ivanović, L., Gačnik, S., Mikulic-Petkovsek, M., *Journal of Food Composition and Analysis*, **2021**, 100, 103933. doi:10.1016/j.jfca.2021.103933
3. Gallo, M., *Separations*, **2022**, 9, 287. doi:10.3390/separations 9100287

Pharmaceutical and cosmetic potential of *Crataegus laciniata* (Rosaceae) Flowers extract

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Keywords: *Crataegus Sicilian flower, LC-MS-HR, zebrafish embryo*

Crataegus laciniata Ucria (Rosaceae) is a plant widely spread, particularly in South Italy, in North Europe and temperate regions. Recently, *C. lacinata* fruits were investigated for their beneficial effects on the cardiovascular system, including hypotensive activity and hypocholesterolemic and hypolipidemic effects [1]. In this study, the chemical profile and biological activity of *C. lacinata* flower extract (CLF) were investigated. Through LC- MS-HR (LTQ-Orbitrap XL) operating in negative- and positive-ion modes, the characterization of specialized metabolites was obtained. The chemical profile showed that the extract was rich in flavonoids, both *C* and *O* glycosides; being the most abundant CLF metabolites, such as hyperoside and vitexin moreover the presence also of luteolin and quercetin derivatives with varied glycosylation patterns was highlighter. Other identified polyphenols were anthocyanidins, such as delphinidin and cyanidin, or their polymers. These compounds are well known for their antioxidant activity [2] and the extract was tested by DPPH, ABTS and FRAP assay and it showed strong scavenger activity. CLF extract showed in vitro health properties through the evaluation of inhibition of the key enzymes involved in diabetes (α-amylase and α-glucosidase). The no-mutagenesis of extract was demonstrated with *Danio rerio* (zebrafish), an ideal in vivo model due to its high correlation with humans in response to pharmaceutical and cosmetic testing. This in vivo assay showed the cosmetical potential of the extract (25–100 µg/mL) as depigmentation compared to phenylthiourea (PTU) and safety and no-toxic effect was proved because the absence of malformations was observed. The obtained results outline that CLF extract is a rich source of bioactive compounds, which can be used for the treatment of metabolic disorders, as well as skin hyperpigmentation. In addition, its favourable safety profile observed during in vivo experiments might promote future applications in the pharmaceutical and cosmeceutical fields.

References

1. Nazhand, A., Lucarini, M., Durazzo, A., Zaccardelli, M., Cristarella, S., Souto, S. B., Silva A. M., Severino P., Souto E. B. & Santini, A., *Forests*, **2021**, 11, 564. doi.org/10.3390/f11050564
2. Alirezalu, A., Salehi, P., Ahmadi, N., Sonboli, A., Aceto, S., Hatami Maleki, H., & Ayyari, M., I*nternational journal of food properties*, **2018**, 21, 452-470. doi.org/10.1080/10942912.2018.1446146

Bioactive sterols in innovative Brassica vegetables: an investigation by liquid chromatography and high-resolution mass spectrometry with atmospheric pressure chemical ionization

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Keywords: *plant sterols, APCI, HPLC-FTMS*

Plant sterols (PSs) are secondary metabolites originating from isoprene, occurring in plants in various forms, including free sterols (FSs), esters of fatty acids, steryl glycosides (SGs), and acyl steryl glycosides [1]. PSs are minor constituents of plant cell membranes, where they preserve the integrity, fluidity and permeability of the lipid bilayer by increasing stress resistance [2]. Nutraceutical properties have been extensively claimed for PSs, due to their ability to lower blood cholesterol levels and exert anti-obesity, anti-diabetic and anti-inflammatory activities [3]. In the last decade, with the increasing attention to healthy eating and lifestyle, the interest in fresh, ready to eat innovative vegetables, such as microgreens and baby leaves, including compounds with a nutraceutical potential, like PSs, has been on the rise. Very recently, a targeted metabolomic approach useful to characterize PSs, including FSs and SGs, based on reversed-phase liquid chromatography coupled to high-resolution Fourier-transform mass spectrometry with atmospheric pressure chemical ionization (RPLC-APCI-FTMS) has been undertaken in our laboratories. The attention has been focused on microgreens and baby leaves of *Brassicaceae* species such as broccoli raab and kale, already known as sources of bioactive compounds [4], including PSs, recently identified in these products using GC-MS [5].
In the present communication, the ability of RPLC to enhance the separation of PSs and of APCI to efficiently ionize these compounds will be demonstrated. The developed RPLC-APCI-FTMS method enabled the detection and quantitation of both major plant sterols, such as campesterol, β-sitosterol and stigmasterol, and of less abundant species, like brassicasterol, isofucosterol, cholesterol and lanosterol, in the extracts of broccoli raab and kale microgreens and baby leaves. Notably, the extracted ion current (EIC) chromatograms obtained from RPLC-APCI(+)-FTMS data, referred to major ions resulting from APCI of sterols (*i.e.*, protonated/dehydrated forms), revealed the occurrence of several isomeric species, especially for stigmasterol, brassicasterol and lanosterol, deserving further investigation. Moreover, campesteryl, β-sitosteryl and stigmasteryl glucosides, generating the corresponding protonated aglycones as the major ions during the APCI process, were identified, thus confirming the potential of RPLC-APCI-FTMS as a powerful analytical approach for a comprehensive characterization of sterols in complex matrices.

References

1. Valitova J. N. *et al.*, *Biochem.*, **2016,** *81*, 819–834, doi: 10.1134/S0006297916080046.
2. Du Y. *et al.*, *Int. J. Mol. Sci.*, **2022**, *23*, 1-22, doi: 10.3390/ijms23042332.
3. Poudel P. *et al.*, *Natural Secondary Metabolites*, **2023**, *2*, 285-319, doi:10.1007/978-3-031-18587-8_8.
4. Ramirez D., *et al.*, *Int. J. Mol. Sci.*, **2020**, *21*, 1–21, doi: 10.3390/ijms21061998.
5. Castellaneta A. *et al.*, *J. Steroid Biochem. Mol. Biol.*, **2023**, *232*, 106361, doi: 10.1016/j.jsbmb.2023.106361.

A chemometric investigation to study the stability of tannins by LC-QqQ: from standard analytical compounds to UVCBs

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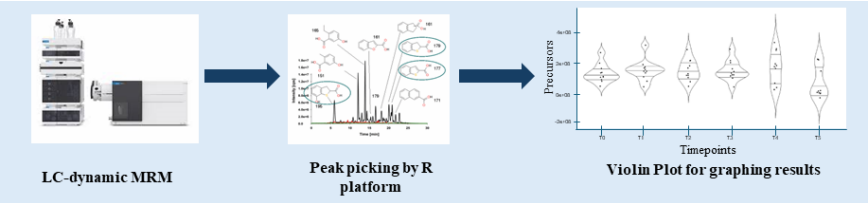
Keywords: *chemometrics, stability, tannins*

Complex natural products (CNPs) based on complex natural matrices are used all over the world to realize food supplements, traditional medicines and medical devices [1]. These complexes, not fully definable, are classified as substances of unknown or variable composition, complex reaction products, or biological materials (UVCBs sub-type 3) [2, 3]. Their high complexity in terms of composition makes the task of guaranteeing quality, efficacy and safety requirements challenging. In general, analytical characterization of UVCBs is technically demanding because of the limited commercial availability of standards and the large number of constituents, which makes their composition partly unknown and variable.[3] Studies and experience are needed for the analytical characterization of UVCBs with methods that include analysis of representative constituents, as well as read-across methods for groups of analogous compounds, then HRMS broadband analytical and cheminformatics approaches.[3] Here we show a chemometric approach for automating a stability study of tannins, polymeric polydisperse class of substances with a large variety of different chemical structures. The tendency to react to each other when used in analytical mix led us to investigate their degradation during the time through an LC-QqQ method, starting from single analytical solutions of the available standards, using the R platform for the extraction of the typical transitions at different time points. After the peak picking of two transitions, optimized in the LC-dynamic MRM method for each available standard compound, their intensity detected at different time points were then normalized by the Probabilistic Quotient Normalization "PQN" algorithm.



Other approaches are reported to study tannins in CNPs[4]. However, this work represents the first chemometric method for studying the stability of tannins which can be applied to UVCBs according to a read-across method.[4] Validation steps must also be performed but, in the future, it could be applied to other classes of compounds with the aim of describing the stability of UVCBs, or CNPs.

References

1. Yuan, H.; Ma, Q.; Ye, L.; Piao, G. *Molecules* **2016**, *21*, 559, doi:10.3390/molecules21050559.
2. Lai, A.; Clark, A. M.; Escher, B. I.; Fernandez, M.; McEwen, L. R.; Tian, Z.; Wang, Z.; Schymanski, E. L. *Environ. Sci. Technol.* **2022**, *56*, 7448–7466, doi:10.1021/acs.est.2c00321.
3. Salvito, D.; Fernandez, M.; Jenner, K. et al. *Environ. Toxicol. Chem.* **2020**, *39*, 2097–2108, doi:10.1002/etc.4846.
4. Burico, M.; Fodaroni, G.; Flamini, E.; Ascani, N.; Proietti, G.; Tamimi, S.; Quintiero, C. M.; Massa, L.; Gianni, M.; Mattoli, L. *J. Mass Spectrom.* **2022**, 57, e4878, doi:10.1002/jms.4878.

Extraction of Polyphenols From Oak Bark for the Tanning Industry Using Greener Ultrasonic Technology

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Keywords: *Biomass valorization, lignocellulosic biomass, Tanning industry,*

Hide is a perishable material derived as a by-product of the food industry, transformed into a durable textile through tanning processes [1]. The leather production industry poses significant environmental challenges, employing hazardous chemicals and excessive water and energy consumption. Conventional tanning relies on heavy metals like chromium (III) or fossil-based agents, emphasizing the urgency for developing non-toxic, biodegradable, and bio-based alternatives.

Biomass stands out as an ideal resource due to its abundant, cost-effective, structurally diverse, biocompatible, and biodegradable nature. Recent advancements showcase the potential of alginate, a biodegradable polysaccharide from brown algae, as a viable candidate for tanning material synthesis [2]. Building on this, our work presents a sustainable approach to creating a novel tanning agent through microwave-assisted esterification of oak bark polyphenols with modified sodium alginate.

The process involves oxidizing sodium alginate with H_2SO_4, then controlled hydrolysis in a microwave reactor. Oak bark polyphenolic extract is obtained using ultrasound-assisted flow extraction with a dilute NaOH solution. The esterification occurs at low pressure and mild temperature in the same microwave reactor. The efficacy of alginate-based tanning materials in interacting with collagen within hide is assessed through micro-differential Scanning Calorimetry, FTIR-ATR, HR-MS and NMR MOUSE techniques.

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References

1. Covington A. D., *Chem. Soc. Rev*. **1997**, *26*, 111−126, doi.org/10.1039/CS9972600111.
2. Lee K. Y.; Mooney D. J., *Prog. Polym. Sci.*, **2012**, *37*, 106, doi.org/10.1016/j.progpolymsci.2011.06.003.

Cucurbita pepo var. *styriaca* seeds: a source of bioactive polar lipids detected by UHPLC-Q-Orbitrap-MS/MS

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Keywords: *Seeds; Polar lipids; UHPLC-Q-Orbitrap-MS/MS analysis*

Pumpkin plants, belonging to the Cucurbitaceae family, have been cultivated since the earliest times of humanity and are known throughout the world. In Austria, a special Styrian pumpkin variety (*Cucurbita pepo* var. *styriaca*) has been cultivated for decades; it was imported in Europe in the post-Columbian era and it is still farmed today as a food in almost all warm and temperate areas of the world [1]. The seeds of *Cucurbita pepo* are rich in fatty acids, phytosterols, vitamins, carotenoids and tocopherols with antioxidant properties capable of soothing skin inflammation [2]. In recent studies, it has been shown that *Cucurbita pepo* var. *styriaca* oil can be used as an exogenous dermocosmetic supplement due to its high antioxidant content in acne therapy and it is very effective in protecting the skin from UV rays, especially the most sensitive skin like that of children [3]. In addition, the oil of *C. pepo* var. *styriaca* has been shown to exhibit good antibacterial activity against *P. aeruginosa and S. aureus* [4]. In order to obtain a deep understanding of the chemical composition of *C. pepo* var. *styriaca* seeds, with particular attention to the composition of polar lipids (major components of seeds), the seeds ethanolic extract was investigated by an analytical approach based on liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). The analysis of the HRMS/MS spectra acquired by using an UHPLC-Q-Orbitrap instrument operating both in negative and in positive ion mode allowed the identification of a high number of metabolites belonging to the classes of oxylipins, phospholipids, sphingolipids, and glycolipids, here described for the first time in the seeds of this variety of *Cucurbita pepo*. On the basis of the biological activities reported in literature for these lipid classes, these data support the use of pumpkin seeds in human nutrition as a food supplement rich in bioactive lipids having potential beneficial effects on human health.

Referenze

1. Fruhwirth, G. O., Hermetter, A. *European Journal of Lipid Science and Technology*, **2007**,*109 (11), 1128- 1140.* doi:10.1002/ejlt.200700105
2. Oliveira, M. L. M., Nunes-Pinheiro, D. C. S., Bezerra, B. M. O., Leite, L. O., Tomè, A. R., Girão, V. C. C. *Acta Scientiae Veterinariae*, **2013**, *vol.41, pub.1168.* redalyc: articulo.oa?id=289031817074
3. Nakavoua, A. H. W., Enoua, G. C., Manhan-Iniangas, S., Chalard, P., Figuérédon, G. *Green and Sustainable Chemistry*, **2021**, *11(02), 49-58.* doi:10.4236/gsc.2021.112005
4. Enoua, G. C., Nakavoua, A. H. W., N'goka, V., Pouambeka, T. W., Mahoungou, P. M. D., Obaya, N. N., Vouidibio, A. B. M., Makomo, H., Ongoka, R. P. *Green and Sustainable Chemistry*, **2022**, *12(04), 104-117.* doi:10.4236/gsc.2022.124009

Sustainable Biostimulant Protein Hydrolysates from *Chlorella vulgaris*

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Keywords: *Microalgae, Protein hydrolysates, Label-free based LC–MS/MS Proteomic Approach*

Across the past decades microalgae rich of bio-active compounds were explored as food and biomasses for health and wellbeing products. More recently, the use in agriculture to improve crop productivity has been studied (1). The present study focused on Protein Hydrolysates (PHs) of *Chlorella vulgaris* as sustainable bio-stimulant product. This resilient photosynthetic unicellular organism adapts to harsh environments (wastewater), absorbing and transforming pollutants while preserving its nutritional and nutraceutical content (pigments, polysaccharides, proteins).
C. vulgaris has been cultivated in an integrated photobioreactors system (PBRs), in modified BG-11 medium and in wastewater-enriched media, showcasing a good daily productivity, satisfactory average specific growth rate and, therefore, phytoremediation potential. The cells were separated from the culture medium through continuous centrifugation and stabilized by spray drying. Using sustainable extraction procedure (mechanical cell wall breakdown and hot water extraction), the polysaccharide fraction (486.45 ± 8.4 mg carbohydrates/g CHL-P) was obtained.
From the residual biomass, PHs were produced through enzymatic hydrolysis, involving the overnight incubation at 37°C with trypsin and pepsin at different ratio [2]. PHs were characterized by a label-free based LC-MS/MS proteomic approach. A nanoflow ultra-high-performance liquid chromatograph (UHPLC, Ultimate 3000) was coupled online to an Orbitrap Fusion™ Lumos™ Tribrid Mass Spectrometer fitted with the Nanospray Flex NG ion source (all from Thermo Fisher Scientific). The peptides were trapped on a PepMap trap column and then separated onto a C18 reversed-phase nano column. The raw MS data were analysed using Protein Discoverer™ Software version 2.5 employing the Sequest search engine. The spectra were searched against the *Chlorella vulgaris* (Green alga) database from UniprotKB.
The influence of different PHs on cress seed germination was assessed by a phytotoxicity test (36 h at 25 °C). The number of germinated seeds and the average length of roots were recorded calculating a Germination Index (GI%) and analysed by Analysis of Variance (ANOVA), using GenStat 17th ed. software. Differences between the means were analysed using the Duncan test at $p < 0.05$. Results demonstrated that no PHs displayed phytotoxicity (values > 50%) while increasing the seedling root growth. Treatment 1 (CHL trypsin 1:10) showed the best bio-stimulant effects at 1:100 dilution, while treatment 2 (CHL pepsin 1:10) at 1:1000. More experiments are needed to exploit the use of this renewable natural and environmentally friendly product as enhancer of crop productivity.

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References

1. Esposito T., Mencherini T., Sansone F., Del Prete F., Cannoniere A. L., Pane C., Manganiello G., Aquino R. P. Valorization of a green extract IV Convegno AISSA #Under40, 12nd-13rd July 2023 Fisciano, Italy
2. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini, L.; Canaguier, R.; et al. *Sci. Hortic.* **2015**, *96*, 28–38. doi: 10.1016/j.scienta.2015.08.037

The insect *Hermetia illucens*: a sustainable source of molecules of high biological and economic value

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Keywords: *antimicrobial peptides, transcriptomic approach, proteomic approach*

To date, the worldwide growing demand for protein sources for farm animals breeding can no longer be satisfied by the intensive fishing for production of high-protein fishmeal, and by the intensive use of agricultural land for protein crops (e.g. soy). Insect proteins can play an important role in the progressive substitution of soy and fishmeal proteins, commonly used to feed animals, and prospective in the progressive integration as novel food for humans. The greatest advantage of insect breeding is the lower environmental impact: less greenhouse gases emissions than any other conventional animal farming, lower water footprint per gram of produced protein and, moreover, some insect species are able to consume different types of organic waste. This is the case of the dipteran Hermetia illucens, a scavenger insect able to bioconvert organic waste and agrifood by-products, whose larvae, flours and transformed animal proteins can be used in aquaculture feed (Reg. EU 893/2017). The bioconversion process and the valorization of agrifood by-products, also allow to obtain secondary products of high biological and economic value, including lipids, chitin and antimicrobial peptides (AMPs). The larvae of *H. illucens* have a lipid content about 40% of the larval biomass, but it is variable in relation to the food substrate. For their composition the lipids deriving from *H. illucens* are used in animal feed and properly functionalized for the production of biodiesel, as vegetable additives of plastics and for the formulation of products suitable for personal care (soaps, detergents, shampoos). Chitin, and its derivatives, due to their properties, like biodegradability, bio-compatibility, non-toxicity, adsorption, find many applications in the industrial, and biomedical field. In addition, due to their attractive biological activities and bioadhesivity, they are widely used as absorption promoters and hydrating agents, as well as for film production and wound healing. Their applications include uses in a variety of areas, such as food industry, wastewater treatment, agriculture, tissue engineering, cosmetics, pharmaceutical and medical applications, paper production, and textiles. AMPs, small bioactive proteins, naturally produced by all living organisms as components of their innate immune system. These biomolecules provide numerous advantages over conventional antibiotics such as: low levels of resistance, broad-spectrum activity with minimal host toxicity, synergistic effects on antimicrobial activity of antibiotics and rapid killing of the microorganism. AMPs are produced by all organisms, but insects, and particularly *H. illucens*, are among the richest and most innovative sources. In pur research, *H. illucens* larvae are reared on different by-products from the agri-food chain in order to valorize these by-products, producing molecules of high economic value, with a view to circular economy.

Antimicrobial activity of lipids extracted from *Hermetia illucens* larvae reared on different substrates

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Keywords: *fats, fatty acid, beauty-care products*

In light of the escalating issue of antimicrobial resistance, there is a growing interest in exploring antimicrobial products derived from natural sources, particularly those obtained from innovative and eco-friendly materials. In this context, insect lipids stand out due to their unique fatty acid composition, which categorizes them as natural antimicrobial compounds. To investigate the antibacterial efficacy of lipids extracted from the larvae of *Hermetia illucens*, we examined this component across various feeding substrates and characterized its properties. Furthermore, we conducted an analysis of the fatty acid composition of the feeding substrates to determine the potential impact on the antimicrobial activity of the lipid component. The most abundant fatty acids detected in *H. illucens* are lauric, myristic, palmitic, and oleic acids, regardless of feed substrate. Lauric acid is a key ingredient in soaps because of its antibacterial qualities and capacity to produce a foamy soap. *H. illucens* lipids are appropriate for shower gels and soaps because their fatty acid composition is comparable to that of coconut oil and palm kernel. Several fatty acids are commonly used in the beauty industry, including lauric acid, myristic acid, oleic acid, palmitic acid (which is used as an emollient and emulsifier), and linoleic acid, have noteworthy qualities and functional benefits that support the skin barrier. The assessment of antimicrobial activity was performed against both Gram-positive *Micrococcus flavus* and Gram-negative *Escherichia coli* bacteria. Upon analyzing the fatty acid profiles of larval lipids exhibiting activity against the two bacterial strains, notable differences were observed for C4:0, C10:0, C16:1, C18:3 N3 (ALA), and C20:1. The most powerful antimicrobial activity was observed against *M. flavus*, with lipids extracted from larvae reared on strawberry, tangerine, and fresh manure substrates displaying growth inhibition zones. Interestingly, only the larvae reared on manure exhibited an inhibitory effect against *E. coli*. As the search for alternative antimicrobial agents gains interest, understanding these intricate relationships between insect lipids, feeding substrates, and antimicrobial activity becomes crucial for the development of effective and sustainable solutions in combating antimicrobial resistance. *H. illucens* can be a source of bioactive compounds that can be used as natural cosmetic ingredients.

Optimization and validation of a LC-MS/MS method for the determination of *Alternaria* toxins in tomato products

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***Keywords**: tomato, Alternaria toxins, QuEChERS*

Alternaria species are fungi present in the soil as normal components of the microflora and are both saprophytic and plant pathogenic. They can infect oilseeds such as sunflower and canola, as well as tomatoes, apples, citrus fruits, olives, and many other fruits and vegetables. *Alternaria* species are able of producing more than 70 secondary metabolites, some of which are chemically characterized and reported as mycotoxins, *Alternaria* toxins (ATs). The most common *Alternaria* toxins in food products are alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and tentoxin (TEN). Thus, this work aims to optimize and validate a LC-MS/MS method for the determination of *Alternaria* toxins (AOH; AME; TeA and TEN) in tomato products.

The operating parameters of the mass spectrometer were optimized through the direct infusion of single solutions of the different ATs studied. During tuning, the temperature and voltage values applied to the source, as well as the flow and temperature of the desolvation gas, were optimized. Intensities of the precursor ions were monitored, evaluating both positive and negative ionization. AOH, AME, TeA and TEN gave a greater signal intensity in negative ionization and the most abundant precursor ion was found to be, for all 4 analytes, [M-H]-.

Furthermore, during the infusion, for each compound studied, two characteristic fragments were identified to be used for quantification (quantifier, Q) and identification (qualifier, q) and the respective collision energies. The choice of product ions was made by preferring transitions characterized by the best intensity and repeatability of the signal, also in terms of ion ratio (IR).

The tests for the optimization of the sample preparation were performed on a non-contaminated fresh tomato sample. Different extraction mixtures and different QuEChERS composition were tested.

The optimized method was fully validated has been validated. In particular, the identification criteria of the molecules were defined and the linearity of the response was verified. Furthermore, the matrix effect was evaluated and the accuracy and precision were estimated. LOD and LOQ values and the method concentration range were identified. Finally, the uncertainty was estimated with a metrological approach.

The values of the characteristic parameters of the method obtained were judged very positively for both accuracy and precision when compared with what is required in the EURL-MP guideline. LOQ values obtained were judged adequate, and in accordance with what is indicated in Recommendation 2022/553, which for tomato based products requires a LOQ not exceeding 4 µg/kg for AOH and AME and not exceeding 20 µg/kg for TeA. TEN is not included in the Recommendation, therefore there are no reference values, however, the LOQ value obtained is considered adequate as it is lower than that validated for AOH and AME.

The validated method was applied to the analysis of 46 samples of tomato-based products, pulp and peeled tomatoes (N=17), puree (N=10), double concentrate (N=16) and triple concentrate (N=3). The indicative levels reported in Recommendation (EU) 2022/553 are exceeded for two samples in the "Puree" category for AOH, while the measured AME concentration level did not exceed the indicative levels in any of the analyzed samples. As for TeA, 4 samples showed have contamination above the indicative levels, 2 in the "Puree" category and 2 in the "Double concentrate" category. However, considering the mean values, always lower than the indicative level, the picture is not alarming.

Antimicrobial properties of the chitosan from different biomasses of the bioconverter insect *Hermetia illucens*

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Keywords: *fats, fatty acid, beauty-care products*

Chitin and its deacetylated derivative, chitosan, have various applications in biomedical and pharmaceutical fields. The market needs to have these two biopolymers readily available has led to the search for alternative sources to crustaceans, the commercial source on the industrial scale. Insects, and among them the bioconverter diptera *Hermetia illucens*, is one of the most popular, thanks to the possibility of recovering waste materials (pupal exuviae and dead adults) from its breeding to extract chitin and convert it into chitosan. Chitina and chitosan have some important properties such as biocompatibility, biodegradability, non-toxicity, antioxidant, humectant and antimicrobial activity. It is exactly the latter that makes chitosan particularly versatile for pharmaceutical and medical applications.
Some pathogens have acquired new mechanisms of drug resistance, leading to antimicrobial resistance, that makes the human body progressively weaker to fight and deal with common infections. Because of this, antibiotics are becoming more and more ineffective and drug resistance is spreading widely, leading to increasingly difficult-to-treat infections. New antibacterial molecules are needed to tackle this problem. Among them, natural ones can be a safe alternative solution.
After protonation in acid conditions, chitosan can inhibit the proliferation of many bacteria, fungi and yeasts. The mechanism of action involves an electrostatic interaction between the NH_3^+ groups of chitosan and the negatively charged portions of the membranes of bacteria, both Gram-negative and Gram-positive. The antimicrobial activity of chitosan depends on certain of its chemical-physical characteristics, mainly molecular weight and degree of deacetylation, and on some specific experimental conditions, such as temperature and pH.
The evaluation of the antimicrobial activity of chitosan was carried out through two types of experiment: agar diffusion test and microdilution assay. Bleached and unbleached chitosan from larvae, pupal exuviae and dead adults of *H. illucens* induced the formation of inhibition zones. This data is an indication of the biopolymer's ability to inhibit microbial growth. This important property of all chitosan samples was also confirmed by microdilution assay. By this experiment both against Gram-negative and Gram-positive bacteria, it has been possible to identify the minimum inhibitory concentration (MIC) values, ranging between 0.3 mg/ml and 0.15 mg/ml.

Chitosan derived from the diptera *Hermetia illucens*: innovative biopolymer for application in cosmetic and pharmaceutical fields

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Keywords: *antimicrobial activity, antioxidant activity, anti-inflammatory activity*

Chitin and chitosan are natural polymers of great technological and economic interest, having numerous applications in different fields. Chitin is a structural component of the exoskeleton of arthropods and the cell wall of fungi and yeasts; due to its insolubility, chitin is converted into its deacetylated derivative, chitosan. Currently, chitin is industrially extracted from fishing waste, mainly crustacean shells. The debate on the sustainability of this resource and the steady increase in market demand for chitin and chitosan have prompted a search for alternative sources. Insects are gaining great interest, particularly bioconverter insects such as *Hermetia illucens*. Currently, *H. illucens* is reared for protein feed production but its farming also generates large amounts of chitin-rich waste biomass, such as exuviae left over from moulting processes and dead adults, that could be exploited as a source for the extraction of this polymer. This work was aimed at developing a suitable procedure for chitin purification and chitosan production from different biomasses generated from the farming of the dipteran *H. illucens*. From larvae, pupal exuviae, and adults, chitin was extracted with yield, chemical characteristics and purity similar to that commercially available from crustaceans. Pupal exuviae were the richest biomass, with the 25.5% of chitin and also the most easily collected from insect farm, thus representing the chosen biomass for the chitosan production. From chitin, chitosan was produced by heterogeneous and homogeneous deacetylation; the two methods showed significant differences in deacetylation efficiency, yield, deacetylation degree and crystallinity degree in support of the heterogeneous method. Spectrometric, diffractometric and morphological characterization of different chitosans confirmed their similarity to the commercial polymer, from which they vary in lower viscosity and molecular weight. The different chitosan samples produced from *H. illucens* exhibited different characteristics dependent particularly on the deacetylation method and chitin decolorization, which lead to a change in deacetylation degree and molecular weight, respectively. The biological properties of chitosan useful for biomedical and cosmetic applications were also evaluated. IC_{50} values (mg/mL) showed good radical scavenging activity of chitosans from *H. illucens*; all chitosans, particularly heterogeneous ones, were able to reduce the expression of IL-6, IL-8, IL-1α and TNF-α, proving to be good anti-inflammatory agents already at 6h of treatment. Furthermore, all chitosan samples positively modulated the expression of the antimicrobial peptide HBD-2, demonstrating an indirect antimicrobial activity that can be associated with the direct one already proved. Starting from this study, we will try to relate the specific physical-chemical, morphological and biological features of chitosans from *H. illucens* to specific applications of interest.

Aqueous extracts of *Vicia faba* L. pod valves: a promising source of bioactive compounds and their potential use as adjuvants in the treatment of Parkinson's disease

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Keywords: *L-dopa, LC-ESI/LTQ-Orbitrap/MS^2, neuroprotection effects*

Broad bean (*Vicia faba* L.) is a vegetable of the Leguminosae family consumed worldwide for human and animal nutrition due to its low cost, ease of cultivation and edible seeds, rich in nutritional compounds: carbohydrates, proteins, fibres and secondary metabolites such as polyphenols. It is also recognized as a consistent natural source of L-Dopa, a dopamine precursor and first-line treatment for Parkinson's symptoms [1]. Due to the neurotoxic effect of synthetic L-dopa and its side effects, natural sources of this bioactive compound can be used as adjuvants to reduce the unpleasant effects, in particular the "on-off" motor fluctuations typical of severe forms of Parkinson's disease. Beans from *Mucuna pruriens*, known as the richest natural source of L-dopa, have been described as a useful therapeutic agent in various diseases of the human nervous and reproductive systems in the medical system known as Ayurveda [2]. However, there are only few reports on the effects of *Vicia faba* preparations in humans [3]. Recent studies have confirmed that also broad bean by-products can be an attractive natural source of bioactive compounds [4]. Therefore, this work focuses on the characterization of aqueous extracts of pod valves: 2D van Krevelen diagrams were used as a tool to obtain molecular formula maps useful for a rapid and comprehensive analysis of the more representative metabolite classes; LC-ESI/LTQ-Orbitrap/MS^2 was used to identify L-dopa and major polyphenolic compounds. This study confirmed the higher content of L-dopa in pods compared to beans, with values even comparable to those of *Mucuna pruriens*. It also highlighted the nutraceutical advantage due to a very low content of vicine and convicine, the two metabolites responsible for the haemolytic crisis in favism patients [5]. Consequently, to determine their potential use as an adjuvant in the treatment of Parkinson's disease, a neuroprotective assay was carried out against the human neuroblastoma cell line SH-SY5Y. Based on the data obtained, the extracts studied proved to be good candidates as adjuvants, since they did not show cytotoxic activity even at relatively high concentrations.

References

1. Tesoro, C.; Ciriello, R.; Lelario, F.; Di Capua, A.; Pascale, R.; Bianco, G.; Dell'Agli, M.; Piazza, S.; Guerrieri, A.; Scrano, L.; Bufo S.A.**;** Acquavia, M.A. *Molecules* **2022**, *27*(21), 7468, doi: 10.3390/molecules27217468

2. Rai, S.N.; Chaturvedi, V.K.; Singh, P.; Singh, B.K.; Singh, M.P. *Biotech* **2020**, *10*, 1-11. doi: 10.1007/s13205-020-02532-7

3. Apaydin H. l., Ertan S., Özekmekçi S. *Movement Disorders*. 2000;15(1):164–166. doi: 10.1002/1531-8257(200001)15:1<164::AID-MDS1028>3.0.CO;2-E

4. Krenz, L.M.M.; Grebenteuch, S.; Zocher, K.; Rohn, S.; Pleissner, D. *Biomass Conversion and Biorefinery* **2023**, 1-18, doi: 10.1007/s13399-023-03779-9

5. Huang, Y., & Bu, Q. Adverse Effects of Phytochemicals. In *Nutritional Toxicology* **2022**, pp. 355-384. Singapore: Springer Nature Singapore.

Phytochemical investigation and antibacterial activity of *Thymus daenensis* Celak aerial parts

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Keywords: *Thymus daenensis Celak; LC-ESI/LTQOrbitrap/MS/MS, metabolite profiling; antibacterial activity*

Thymus daenensis Celak is an Iranian endemic species known as denaian thyme. Infusion and decoction of its aerial parts are used as a carminative, digestive, antispasmodic, and anti-inflammatory remedy and for the treatment of cold in Iranian traditional medicine [1,2]. Investigation of essential oil of *T. daenensis* aerial parts has been carried out, but little is known about its non-volatile constituents. In order to achieve deeper insight into the polar compounds of *T. daenensis* aerial parts, a phytochemical investigation of this species was performed.
High-performance liquid chromatography coupled to multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS/MS) analysis, in negative ion mode, of methanol (MeOH) extract of *T. daenensis* aerial parts led to the identification of thirty-two compounds belonging to monoterpene, flavonoid, indole, megastigmane, phenolic, phenylpropanoid and fatty acid classes, which were isolated and unambiguously characterized by Nuclear Magnetic Resonance (NMR) spectroscopy. These were identified as two previously unreported thymol derivatives along with twenty-nine known specialized metabolites.
Based on the traditional uses of *T. daenensis*, MeOH extract has been investigated for the phenolic content, assayed by the Folin-Ciocalteu method, and for the radical scavenging activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Trolox Equivalent Antioxidant Capacity (TEAC) assays. Moreover, the evaluation of the biofilm inhibitory activity of extract and isolated compounds against mature biofilms of *Acinetobacter baumannii*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, and their influence on the metabolism of sessile bacterial cells was evaluated, evidencing the ability of some compounds to exert antibacterial activity.

References

1. Rahimmalek, M.; Afshari, M.; Sarfaraz, D.; Miroliaei, M. *Industrial Crops and Products*. **2020**, *154*, 112640, doi.org/10.1016/j.indcrop.2020.112640.
2. Emami Bistgani, Z.; Hashemi, M.; Akbari, P.; Mumivand, H. *Crop Science*. **2023**, *63*, 921–935, doi.org/10.1002/csc2.20901.

Lignin extraction from natural sources

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Keywords: *Biomass valorization, GVL-extracted lignin, Circular Economy*

Renewable plant-based sources are promising raw materials for energy, chemicals and may add a revenue source for manufacturers based on circular economy. Among lignocellulosic biomass, agricultural residues provide an appealing feedstock because of its readily obtainable, cost-effectiveness, and worldwide availability. Lignin, the second-most prevalent component in lignocellulosic biomass, forms a biopolymer network interface between hydrophobic lignin and amphiphilic cellulose microfibers. Apart from being depolymerized into low-value biofuels when exposed to severe and corrosive conditions lignin can be polymerized into high-value vitrimer adhesives, polyesters, and polyurethane-based foam and other materials. As a result, there is an increasing need for high-quality lignin products, resulting in the need for an improvement in the fractionation of materials that are identical to native lignin. The organic solvent (Organosolv) fractionation methods, especially those using γ-valerolactone (GVL), were shown to be the most effective approach out of the methodologies which investigated. Due to its high lignin solubility and lower partial pressure during reactions, GVL is a useful renewable solvent. As a result, the main objective of this study is to use GVL-extracted method to revalorize lignin. In this study, the application of mass spectrometry emerges as a powerful tool for the comprehensive characterization of lignin extracted through the GVL-extraction method, offering a nuanced insight into its molecular structure and paving the way for enhanced understanding and utilization in sustainable material development.

References

1. Cheng, F.; Liu, S.; Karlen, S. D.; Kim, H.; Lu, F.; Ralph, J.; Vázquez Ramos, L. M.; Huber, G. W.; Dumesic, J. A., *Green Chem*. 2023, *25*, 336–347. https://doi.org/10.1039/D2GC03446H

2. Timokhin, V. I.; Regner, M.; Motagamwala, A. H.; Sener, C.; Karlen, S. D.; Dumesic, J. A.; Ralph, J., *ACS Sustain. Chem. Eng*. **2020**, *8*, 17427–17438. https://doi.org/10.1021/acssuschemeng.0c05651

3. Tian, Q.; Xu, P.; Huang, D.; Wang, H.; Wang, Z.; Qin, H.; He, Y.; Li, R.; Yin, L.; Chen, S.; Zhao, Y., *J. Environ. Chem. Eng*. **2023**, *11*, 109719. https://doi.org/10.1016/j.jece.2022.109093

Cistus × *incanus* L.: phytochemical profile and anti-inflammatory activity in an *in vitro* model of gastric inflammation

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Keywords: *Cistus × incanus L., LC-MS/MS, gastric inflammation*

Plants of the genus *Cistus* have been traditionally used in the Mediterranean area for inflammatory and infectious disorders, including gastrointestinal diseases [1]. Among them, *Cistus* × *incanus* L. is one of the most frequently cited species in the literature for a variety of biological activities which include inflammatory-based diseases. *Cistus* spp. aerial parts are rich in polyphenols such as condensed and hydrolysable tannins, procyanidins, and flavonoids [2].
The aim of the study was to characterize the chemical composition of an hydroalcoholic extract from *Cistus* × *incanus* L., obtained from aerial parts of the plant, and to investigate the biological activity of this extract in an *in vitro* model of gastric inflammation induced by *Helicobacter pylori (H. pylori)*. Colorimetric assays were performed to identify the class of compounds occurring in the extract, while the quantification of the individual molecules was performed through LC-MS/MS analysis. The activity of the extract against *H. pylori*-induced IL8 release and p65 nuclear translocation was measured with an ELISA assay and immunofluorescence, respectively. LC-MS/MS analysis allowed to identify and quantify different compounds, including catechins, procyanidins, ellagitannins (i.e. punicalagin) and flavonols (i.e. quercetin). *Cistus* extract showed the ability to reduce IL8 release (IC_{50}: 23.99 µg/mL) and p65 nuclear translocation induced by *H. pylori* in GES-1 cells, moreover the extract was able to inhibit the adhesion of the bacterium to the surface of the cells and to act directly on the microorganism reducing the bacterial growth. These biological activities were, in part, maintained after *in vitro* gastric digestion of the extract. These findings suggest the ability of *Cistus* extract to counteract gastric inflammation and *H. pylori* infection.

References

1. Tomou, E.-M.; Lytra, K.; Rallis, S.; Tzakos, A.G.; Skaltsa, H. An updated review of genus Cistus L., *Phytochemistry Reviews* **2022**, *21*, 2049-2087, doi:10.1007/s11101-022-09827-y.
2. Wittpahl, G.; Kolling-Speer, I.; Basche, S.; Herrmann, E.; Hannig, M.; Speer, K.; Hannig, C., *Planta Med* **2015**, *81*, 1727-1735, doi:10.1055/s-0035-1557822.

Pomodoro Riccio metabolomic profile by means of NMR and MS methodologies: a case study

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Keywords: *tomato; metabolomics; food safety.*

Tomatoes and tomato-based food are worldwide diffused foodstuffs, whose nutritional importance is related to their contents of many antioxidant compounds, like carotenoids, polyphenols, and vitamins. [1-3] However, tomatoes are a food category extremely exposed to safety risks that can be related to the presence of chemical residuals, like pesticides [4], and microbial contaminants, including bacteria and fungi. [1,5-6] In this context, the present study, carried out in the frame of the ONFOODS consortium (Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security – Working ON Foods) [7] stemming from the National Recovery and Resilience Plan (NRRP), is aimed at the metabolomic profile and possible identification of new and (re)-emerging hazards of an old tomato fruit ecotype, i.e., "Pomodoro Riccio". This is a cultivar particularly suited to grow up on clay soils, with a low demand for water, that could ultimately mean a lower intake of contaminants. In fact, it has been proven that water is the primary source of heavy metals and pesticide residues, [4] thus, this old cultivar has the potential to enhance the quality of tomatoes that emerges as a matter of priority for customer's safety. Pomodoro Riccio samples (fresh tomatoes, tomato paste and waste) harvested in two different years were provided by "La Sbecciatrice" company (Caserta, Campania). The hydroalcoholic and organic Bligh-Dyer extracts have then been subjected to the multimethodological analytical protocol comprising untargeted (NMR, FT-ICR MS) and targeted (HPLC-DAD, HPLC MS/MS) methodologies. In particular, the high sensitivity and mass accuracy typically achieved with FT-ICR MS implies that elemental formulas of many metabolites and harmful compounds present in trace amounts, like pesticides, agrochemical derivatives and metals, can be determined. Overall, a broad chemical and metabolomic profile have been gathered to monitor traceability and quality of tomatoes.

References

1. Concina, I.; Falasconi, M.; Gobbi, E.; Bianchi, F.; Musci, M.; Mattarozzi, M.; Pardo, M.; Mangia, A.; Careri, M.; Sberveglieri, G. *Food Control*, **2009**, *20*(10),873-880, doi: 10.1016/j.foodcont.2008.11.006.
2. Diplock, A. T.; Charuleux, J. L.; Crozier-Willi, G.; Kok, F. J.; Rice-Evans, C.; Roberfroid, M.; Stahl, W.; Vina-Ribes, J. *British journal of nutrition*, **1998,** *80*(S1), S77-S112, doi: 10.1079/BJN19980106.
3. Ingallina, C.; Maccelli, A.; Spano, M.; Di Matteo, G.; Di Sotto, A.; Giusti, A.M.; ... & Mannina, L. *Antioxidants*, **2020,** *9*(10), 1027, doi: 10.3390/antiox9101027.
4. Gambacorta, G.; Faccia, M.; Lamacchia, C.; Di Luccia, A.; La Notte, E. *Food control*, **2005,** *16*(7), 629-632, doi: 10.1016/j.foodcont.2004.07.002.
5. Bishop, R. H; Duncan, C. L.; Evancho, G. M.; Young, H. *Journal of Food Science*, **1982,** *47*(2), 437-439, doi: 10.1111/j.1365-2621.1982.tb10098.x.
6. Jiang, W.; Sandahl, J.; Dubois, J.; Flavin, M.; Reddy, S.; Neigh, A.; ... & Gore, A. *Bulletin of Environmental Contamination and Toxicology*, **2023,** *110*(2), 45, doi 10.1007/s00128-023-03692-x.
7. Funder: Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods".

Bioactive potential of chestnut spiny bur: early evidence for anti-inflammatory effects in intestinal cells

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Keywords: *chestnut by-product, intestinal health, ellagitannins*

Crohn's disease involves inflammation and excessive reactive oxygen species production, activating NF-κB and promoting proinflammatory mediators [1]. Several phytochemicals, targeting inflammation pathways, reduce transcription of mediators like IL-1β, TNF-α, and IFN-γ [2]. The HIF family, an emerging therapeutic target for intestinal inflammatory disease, mediates the hypoxic response [3]. Chestnut spiny bur, rich in vitamin E, amino acids, ellagic acid, and tannins, showed anti-inflammatory properties [4-5]. This study characterized the spiny bur extract, focusing on its chemical profile, antioxidant activity, and anti-inflammatory effects in intestinal cells.
Spiny bur was collected, ground, extracted, and subjected to LC-MS/MS for polyphenolic profiling using an HPLC/LTQ ion trap system in ESI negative ionization. Ellagitannins were quantified using HPLC/TripleQuad TM/MS/MS in ESI negative ionization. Human intestinal cells (CaCo-2), stimulated with IL-1β and IFNγ, were used as an in vitro model to evaluate biological activities. Antioxidant activity measured through colorimetric assays.
Polyphenolic profile of bur extract allowed to identify several compounds belonging to the class of hydrolysable tannins, mostly ellagitannins. Ellagitannins were therefore quantified, with an amount of vescalagin and castalagin of 7.4 ± 0.7 and 32.4 ± 0.5 μg/g (mean ± SEM), respectively.
Spiny bur extract inhibited cytokine secretion (1.4 ± 0.74, 65.5 ± 2.0 and 15.3 ± 2.8 % vs IL-1β-IFNγ for CXCL-10, MCP-1, IL-8, respectively) induced by IL-1β-IFNγ in colonocytes at 50 μg/mL. No activity was found on NF-κB or HIF-driven transcription. Antioxidant capacity was measured by DPPH and ORAC assays, with values of 1.39 ± 0.14 and 17.1 ± 0.1 mmol Trolox equivalent/g (mean
± SEM), respectively.
This study explores chestnut spiny bur as a potential anti-inflammatory co-adjuvant in intestinal diseases, with future analysis aimed at elucidating mechanisms and involved compounds.

References

1. Schottelius, A., Baldwin Jr., A., Int J Colorect Dis., **1999**, 14, 18–28. doi:10.1007/s003840050178.
2. Laurindo, Lucas Fornari, et al. Metabolites 13.1. (2023), doi:10.3390/metabo13010096.
3. Bourgonje, Arno R., et al., Redox Biology, **2023**, 60, 102603. doi:10.1016/j.redox.2023.102603.
4. Chiocchio, Ilaria, et al. *Metabolites*, **2020**, 10, 408. doi:10.3390/metabo10100408
5. Marrazzo, Pasquale, et al. *Antioxidants*, **2023**, 12, 808. doi:10.3390/antiox12040808.

Unravelling the chemical complexity of essential oils: in-depth characterization and profiling by GC×GC-MS

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Keywords: *GC×GC-MS, essential oils analysis, sample classification*

Essential oils are highly complex matrices characterize by the presence of a large number of compounds and different chemical classes. Conventional gas chromatography coupled to mass spectrometry (GC-MS) is often employed for the analysis of such products, nevertheless detailed characterization remains challenging at best, in particular for determination of compounds present at low level that might have a significant impact on quality or sensory properties.

Comprehensive two-dimensional gas chromatography (GC×GC) is an advanced separation technique that offers significantly enhanced separation power and peak capacity by exploiting two independent separation mechanisms in a single analysis. Therefore, it is a very powerful methodology for unravelling hight complexity that exceeds the resolution possible with a mono-dimensional approach.

In this study, we investigated the chemical composition of lavender and rosemary oils from plants harvested in Italy produced by steam extraction in small batches. Commercial essential oils were also analysed as reference material to evaluate the impact of isolation process.

Analyses were performed by GC×GC-MS to tackle high sample complexity and achieve insightful profiling. The enhanced two-dimensional separation, combined with the third dimension added by MS data, allowed to profile the composition for all leaf essential oils with elevated degree of detail. Individual compounds and chemical groups were distributed across a 2D space, reducing co-elutions with beneficial effect on identification thanks to the cleaner spectra.

In addition, data processing was performed with commercial software and included statistical tools such as Principal Component Analysis (PCA) for sample classification. The multivariate analysis tools offered proved useful to extend the applicability of advanced data processing in an effective yet accessible manner.

ESI-FT-ICR-MS-based identification of a new flavonoid in Extra Virgin Olive Oil (*Ravece* cv)

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Keywords*: Extra Virgin Olive Oil; Biophenols; Flavonoids; ESI-FT-ICR-MS*

Phenolic compounds, also referred to as "biophenols" are among the main responsible for the health-promoting properties of extra virgin olive oil (EVOO), also affecting sensorial traits and chemical stability. Qualitative and quantitative profiles of EVOO biophenols vary both qualitatively and quantitatively depending on a complex series of biotic, abiotic, technological and storage factors. The most abundant polyphenols in EVOO are secoiridoid derivatives of tyrosol and hydroxytyrosol, such as oleuropein, olecanthal, oleacin, ligstroside [1].
According to the EFSA indications, EVOO can be commercialized with a biophenols-specific health claim, *i.e.*, "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress", if it contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil [2].
The EFSA-recommended method to determine EVOO biophenols involves extraction of "polar" compounds in hydroalcoholic solutions (*e.g.*, 80% methanol, v/v) and analysis by HPLC-UV (280 nm) using syringic acid as the internal standard [3]. In spite of the extensive research on the topic, the chemical structure of some minor components of the EVOO polar fraction still remains unknown [4].
HPLC-UV chromatograms at 340 nm of the polar extracts from some EVOO Ravece *cultivar* samples contained a previously undescribed component eluting as a sharp peak at retention time of 39.4 min, with variable intensity. This component exhibits a strong UV absorbance max at 338 nm, suggesting a flavonoid structure. Consistently, the flow direct inject Electrospray Ionization-Fourier Transform-Ion Cyclotron Resonance-Mass Spectrometry (ESI-FT-ICR-MS) analysis of the unfractionated hydroalcoholic extracts of the same samples showed the presence on a previously unassigned signal at $[M+H]^+$ = 493.1338, with predicted molecular formula $C_{23}H_{24}O_{12}$. ESI-FT-ICR-MS of the HPLC-isolated peak at retention time 39.4 min demonstrated that this peak corresponded to the unknown compound, while the MS/MS fragment $[M+H]^+$ = 331.0811 revealed the loss of *O*-hexose moiety (-162 uma). Based on the MS information, this compound was assigned to eupalitin-3-*O*-galactoside, and it was definitely characterized by HPLC and MS/MS analysis of the authentic standard.
Eupalitin-3-*O*-galactoside was found in several but not in all EVOO samples of the Ravece *cultivar*, which is a Campanian olive biotype used for producing top-quality EVOO also awarded the DOP. Thus, it remains to establish if eupalitin-3-*O*-galactoside could be considered a molecular marker associated with the *cultivar* or with the extraction process, or, alternatively, with the combination of these two factors.

References

1. Siano, F., Picariello, G., Sammarco, A.S., Celano, G., Caruso, T., Vasca, E. *Molecules* **2023**, 28, 3108, doi: 10.3390/molecules28073108.
2. European Commission Regulation EC No. 432/2012. *Official Journal of European Union* L136,1-40, **2012**.
3 IOC/T.20/Doc No. 29, International Olive Council, Madrid, **2009**.
4 Capriotti, A.L., Cavaliere, C., Crescenzi, C., Foglia, P., Nescatelli, R., Samperi, R., Laganà, A., *Food Chem*. **2014**, 158, 392-400. doi:10.1016/j.foodchem.2014.02.13

Study of mono and di-*O*-caffeoylquinic acid isomers in *Acmella oleracea* extracts by HPLC-MS/MS and application of Linear Equation of Deconvolution Analysis (LEDA) algorithm for their characterization

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One of the most distinguished members of the genus *Acmella* is *Acmella oleracea* (L.) R.K. Jansen, an annual herb native to Brazil that is occurring throughout tropical and subtropical regions around the world. Different extracts of *A. oleracea* have been reported to hold numerous important biological activities, including a local anesthetic property, which is the main reason why this plant has been used since ancient times to relieve toothache. In addition, anti-inflammatory, analgesic, cytotoxic, antioxidant, antimicrobial, anthelmintic, antiwrinkling, aphrodisiac, and insecticidal/acaricidal properties are reported [1]. Chlorogenic acids, the esters of caffeic and quinic acids, are the main phenolic acids detected in *Acmella oleracea* extracts and have gained increasing interest in recent years due to their important biological activities [2,3]. Given their structural similarity and instability, the correct analysis and identification of these compounds in plants is challenging. This study aimed to propose a simple and rapid determination of the *A. oleracea* caffeoylquinic isomers, applying an HPLC-MS/MS method supported by a mathematical algorithm (Linear Equation of Deconvolution Analysis, LEDA) [4].
The three mono- and the three di-caffeoylquinic acids in roots of Acmella plants were studied by an ion trap MS analyzer. A separation by a conventional chromatographic method was firstly performed and an MS/MS characterization by energetic dimension of collision-induced dissociation mechanism was carried out. The analyses were then replicated using a short HPLC column and a fast elution gradient (ten minutes). Each acquired MS/MS data were processed by LEDA algorithm which allowed to assign a relative abundance in the reference ion signal to each isomer present. Quantitative results showed no significant differences between the two chromatographic systems proposed, proving that the use of LEDA algorithm allowed the distinction of the six isomers in a quarter of the time.

References

1. Uthpala, T.G.G.; Navaratne, S.B. *Food Rev. Int.* **2020**, *37*, 399–414.
2. Nascimento, L.E.S.; Arriola, N.D.A.; da Silva, L.A.L.; Faqueti, L.G.; Sandjo, L.P.; de Araújo, C.E.S.; Biavatti, M.W.; Barcelos-Oliveira, J.L.; Dias de Mello Castanho Amboni, R. *Food Chem.* **2020**, *332*, 127393.
3. Bellumori, M.; Zonfrillo, B.; Maggini, V.; Bogani, P.; Gallo, E.; Firenzuoli, F.; Mulinacci, N.; Innocenti, M. *J. Pharm. Biomed. Anal.* **2022**, *220*, 11499.
4. Pallecchi, M.; Braconi, L.; Menicatti, M.; Giachetti, S.; Dei, S.; Teodori, E.; Bartolucci, G. *Int. J. Mol. Sci.* **2022**, *23*, 13139.

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