SIPHAR
Società Italiana di Farmacognosia

SOCIETA' ITALIANA DI FITOCHIMICA
E DELLE SCIENZE DELLE PIANTE MEDICINALI, ALIMENTARI E DA PROFUMO

S.I. FIT
SOCIETÀ ITALIANA DI FITOTERAPIA

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SOCIETÀ ITALIANA DI FARMACOLOGIA

Società Botanica Italiana

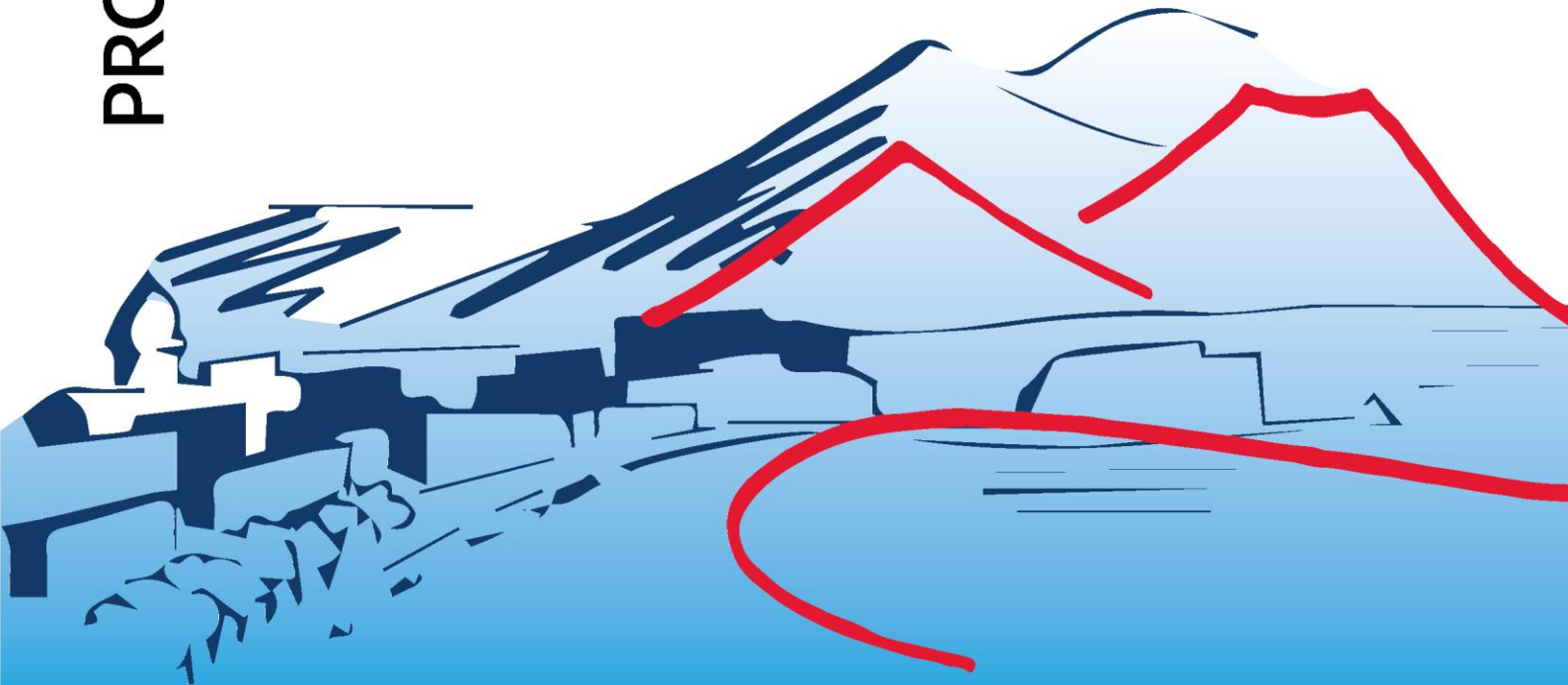
2° CONGRESSO INTERSOCIETÀ SUI PRODOTTI VEGETALI PER LA SALUTE

IL RUOLO DELLE PIANTE MEDICINALI NELLA MEDICINA MODERNA

PROGRAMMA

CENTRO CONGRESSI
FEDERICO II

Via Partenope 36, Napoli 10-12 Aprile 2025



Con il patrocinio di

Università degli Studi di Napoli Federico II

Università degli Studi di Padova

comune di Bonea

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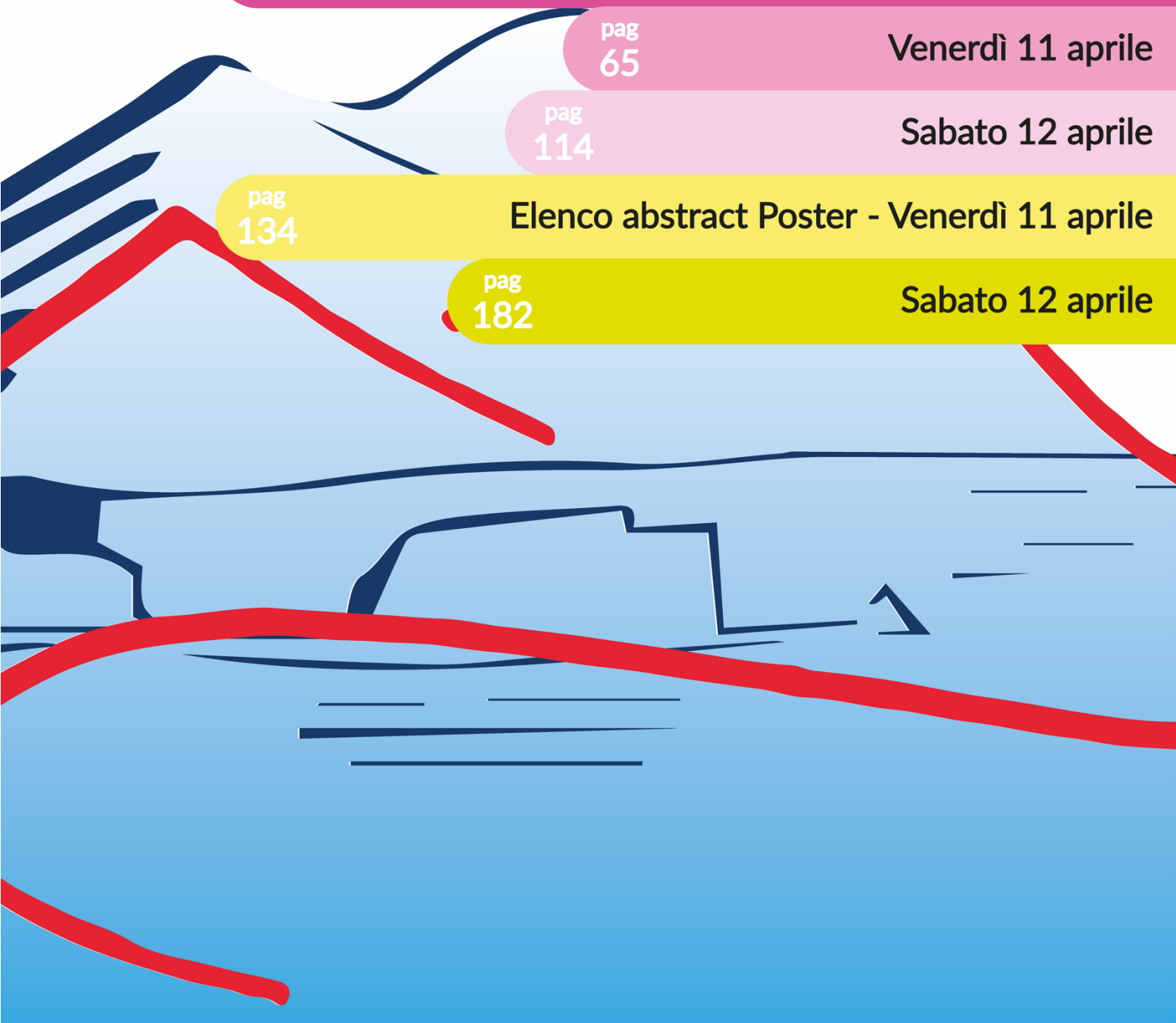


COMUNICAZIONI ORALI

Giovedì 10 Aprile

12.30-13.30
Registrazione dei Partecipanti

13.30-14.00
Saluti di benvenuto — Aula Magna

Magnifico Rettore dell'Università degli Studi di Napoli Federico II, Prof. Matteo Lorito

Presidente AIFA, Prof. Robert Giovanni Nisticò

Presidente Ordine dei Farmacisti della Provincia di Napoli, Prof. Vincenzo Santagada

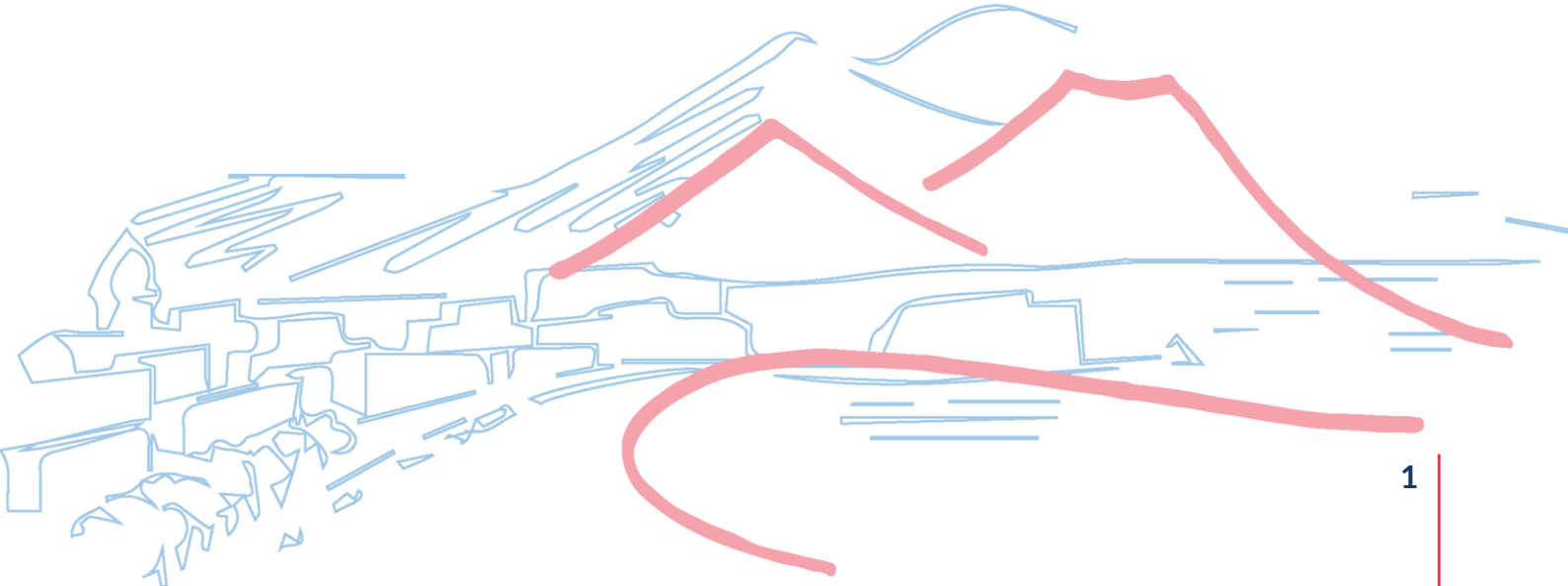
Direttore del Dipartimento di Agraria, Prof. Danilo Ercolini

Direttore del Dipartimento di Farmacia, Prof. Angelo A. Izzo

Lettura magistrale — Aula Magna

Moderatore: Sacchetti G (Ferrara)

14.00-14.30
Challenges for area-based conservation of plant diversity across Europe
Chiarucci A (Bologna)



14.30-16.15 Sessione 1 — Aula Magna

Moderatori: Bisio A (Genova) - Borrelli F (Napoli)

14.30-14.45
In vivo evidence of Cistus (_Cistus x incanus_ L.) and Chestnut (_Castanea sativa_ Mill.) extracts as gastroprotective nutraceuticals.
Piazza S, Martinelli G, Pozzoli C, Sonzogni E, Maranta N, Fumagalli M, Haddad S, Vicentini S, Nicotra G, Sangiovanni E, Dell'Agli M (Milano)

14.45-15.00
Use of medicinal plants of borage (_Borago officinalis_) and wild mallow (_Malva sylvestris_) to control gastrointestinal nematode infection in sheep.
Bosco A, Scarano P, Nappa A, Falzarano A, Lucibelli S, Quaranta G, Claps S, Sciarrillo R, Guarino C, Rinaldi L, Cringoli G (Napoli, Sannio, Basilicata)

15.00-15.15
Anti-inflammatory and immunomodulatory effects of glucoraphanin, a natural hydrogen sulfide donor, in inflammatory bowel diseases.
Indolfi C, Saviano A, Esposito E, Correale M, Marigliano N, Schettino A, Iqbal AJ, Bucci M, Mitidieri E, Maione F, Sorrentino R, d'Emmanuele di Villa Bianca R (Napoli, Birmingham)

15.15-15.30
Polyphenols-enriched extract from _Citrus medica_ L.: Is it a novel strategy to fight intestinal inflammation?
Carlucci V, Jaegerova T, Lela L, Faraone I, Hajslova J, Milella L (Potenza, Prague)

15.30-15.45
Metabolic effects of a hydroalcoholic extract from _Cymodocea nodosa_ in a murine obesogenic diet model.
Benedetti G, Flori L, Vitiello M, Carbonetti L De Leo M, Menicagli V, Lardicci C, Balestri E, Braca A, Calderone V, Nieri P, Testai L (Pisa)

15.45-16.00
Beneficial effects induced by an aqueous aged black garlic extract in rodent models of ulcerative colitis and colitis-associated visceral pain.
Recinella L, Leone S, Libero ML, Lucarini E, Ciampi C, Chiavaroli A, Acquaviva A, Nilofar N, Orlando G, Ghelardini C, Di Cesare Mannelli L, Ferrante C, Menghini L, Di Simone SC, Brunetti L (Chieti, Firenze)

16.00-16.15
The dietary compound luteolin reduces pro-inflammatory capability of M1 macrophages and colitis by targeting TRPM8
Miraglia M, Cicia D, Iannotti FA, Ferrante C, Iaccarino N, Chiavaroli A, Moriello AS , Amico R, Rinaldi MM , Randazzo A , Capasso R, Matteoli G, Izzo AA, Pagano E (Napoli, Leuven, Chieti)

14.30-16.15 Sessione 2 — Aula A

Moderatori: Mandrone M (Bologna) – Testai L (Pisa)

14.30-14.45
Antioxidant and enzyme inhibitory potential of essential oils from *Ocimum gratissimum* L., *Lippia alba* Mill., and *Lippia sidoides* Cham.
Francolino R, Amato G, Grul'ová D, Ferreira J, De Martino L, De Feo V (Fisciano, Presov, Sao Paolo)

14.45-15.00
Multifaceted study of *Vepris boiviniana*: phytochemicals, cytotoxic effects, antioxidant potential, and enzyme inhibition.
Nilofar N, Bakar K, Mohamed A, Hryć B, Sieniawska E, Ferrante C, Menghini L, Zengin G, Polz-Dacewicz M (Chieti, Konya, Comoros, Lublin)

15.00-15.15
Essential Oil of *Cannabis sativa* L.: chemical composition and antimicrobial potential against methicillin-resistant *Staphylococcus pseudintermedius* Strains.
Pieracci Y, Bozzini MF, Ascrizzi R, Fulvio F, Montanari M, Bassolino L, Paris R, Fratini F, Flamini G (Pisa, Bologna)

15.15-15.30
Chemical analysis and antimicrobial activity of the traditional food crop *Moringa oleifera* Lam.
Anzano A, De Falco B, Ammar M, Ricciardelli A, Grauso L, Sabbah M, Capparelli R, Lanzotti V (Portici, Palestine, Napoli)

15.30-15.45
Troxerutin activity against oxidative stress: a potential strategy for corneal damage.
Cucinotta L, Mannino D, Filippone A, Basilotta R, Palermo N, Paterniti I, Esposito E (Messina)

15.45-16.00
***Morus alba* twigs: waste no more! Unveiling antibacterial potential through untargeted metabolomic-guided phytochemical investigation.**
Gargiulo E, Chianese G, Sirignano C, Buommino E, Lembo F, Arpini S, Ribatti E, Falzoni M, Taglialatela – Scafati O (Napoli, Milano)

16.00-16.15
Efficacy of *Citrus sinensis* essential oils in the control of *Pseudomonas syringae* pv. *tomato* and *Fusarium oxysporum* f.sp. *radicis lycopersici*.
Lo Vetere M, Lanteri AP, Bisio A (Genova, Albenga)

16.15 16.45 *Coffee break*

16.45 -18.45 Sessione 3

Aula Magna

Moderatori: Conforti F (Rende) – Meli R (Napoli)

16.45-17.00
Protective effects of *Vaccinium macrocarpon* on AGE-Mediated sarcopenia: *in vitro* and *in vivo* evidence.
Paiella M, Raiteri T, Salvadori L, Manenti T, Sorci G, Prodam F, Filigheddu N, Riuzzi F (Perugia, Novara, Anghiari)

17.00-17.15
Kadsurenin F from *Piper Kadsura* exerts anti-inflammatory properties in mouse colitis.
Pace S, König S, Czapka A, Bilancia R, Troisi F, Parisi O, Cicala C, Caiazzo E, Gerstmeier J, Stuppner H, Borrelli F, Rossi A, Werz O. (Roma, Jena, Napoli, Innsbruck)

17.15-17.30
Comprehensive polar lipid profiling of "Cavolfiore della Piana del Sele" PGI (*Brassica oleracea* L. var. *botrytis*) inner leaves by LC-ESI/HRMS/MS analysis and evaluation of tyrosinase inhibitory activity.
Cerulli A, Napolitano A, Masullo M, Piacente S (Fisciano, Napoli)

17.30-17.45
Combining *Acmella oleracea* and *Boswellia serrata* extracts: a novel pharmacological approach in inflammatory vestibulodynia.
Perrone M, Fusco A, Ricciardi F, Morace AM, Bonsale R, Melake Teweldemedhin M, Di Martino E, Limongelli R, Papa A, Maione S, Guida F, Luongo L (Napoli)

17.45-18.00
Plant-based extract with vitamin D2: a novel nutraceutical strategy against hepatic lipotoxicity in MAFLD.
Varfaj I, Bartolini D, Migni A, Franco Moscardini I, Sardella R, Garetto S, Lucci J, Galli F, Marcotullio M C (Perugia, Arezzo)

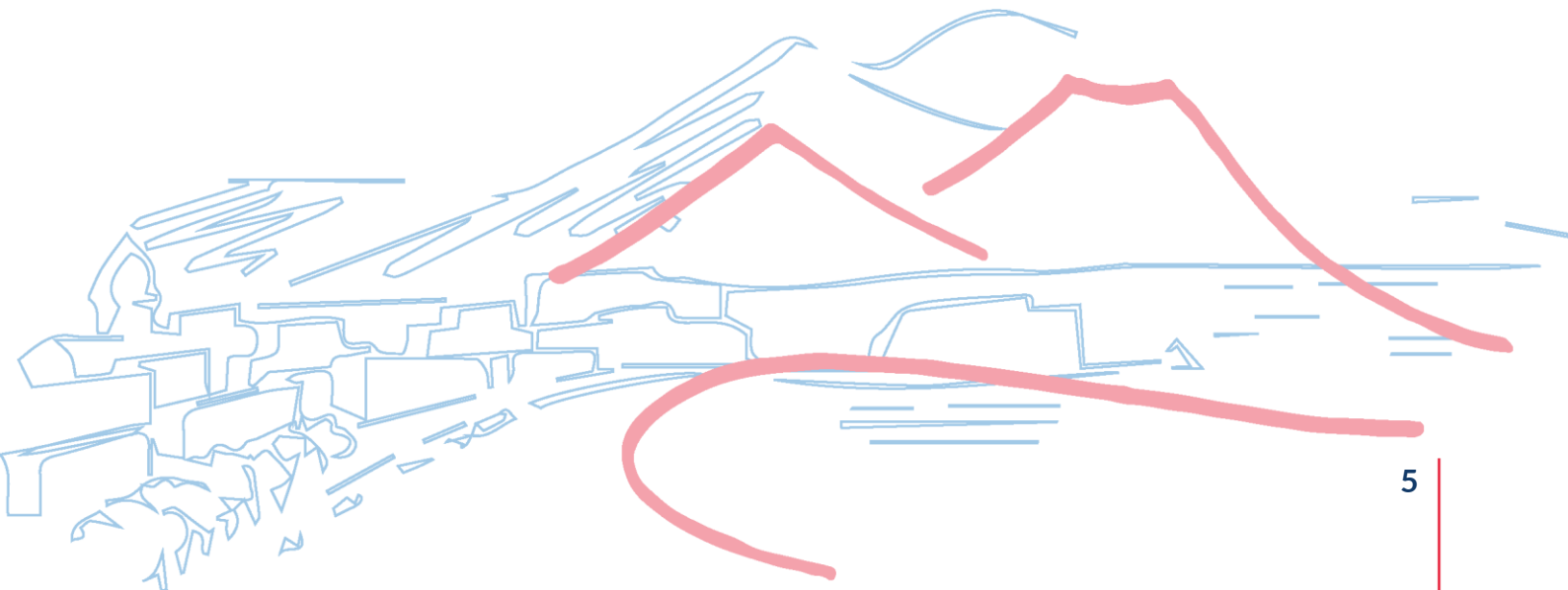
18.00-18.15
Selective Activity of Chrysin-6-C-fucopyranoside from *Cyclanthera pedata* on Peroxisome Proliferator-Activated Receptor Gamma (PPAR©).
De Vito M; Zuccolo M; Bassoli A; Borgonovo G; Giupponi L; Giorgia A; Schiano Moriello A; Iannotti FA (Pozzuoli, Edolo, Milano, Padova)

18.15-18.30
Bergamot essential oil as a potential therapeutic agent for inflammatory bowel disease: phytochemical profile, toxicity assessment, and anti-inflammatory effects.
Genovesi G, Centulio AP, Ciaramellano D, Acquaviva A, Libero ML, Di Simone SC, Tunali F, Leone S, Recinella L, Chiavaroli A, Ferrante C, Menghini L, Brunetti L, Orlando G (Chieti, Torrevecchia Teatina)

18.30-18.45

Attenuation of glucose-induced microglial neuroinflammation by a *Melissa officinalis* L. phytocomplex from *in vitro* cultured cells.

Videtta G, Pressi G, Galeotti N (Firenze, Camisano Vicentino)



16.45 -18.45 Sessione 4

Aula A

Moderatori: Bilia AR (Firenze) – Trainotti L (Padova)

16.45-17.00
Phytochemical investigation of *Withania somnifera* L. and evaluation of acetylcholinesterase inhibitory activity by spectrophotometric assay and STD-NMR analysis.
Polcaro LM, Angulo Alvarez J, Piacente S, Masullo M (Salerno, Seville)

17.00-17.15
Metabolic profiling of *Polyporus umbellatus* (Pers.) Fr.
Patriarca A, Ottaviani D J, De Rosa M, Spagnoli M, Santi L, De Vita D, Toniolo C, Frezza C, Saccoccio M, Di Rita F, Sciubba F (Roma, Monte Porzio Catone)

17.15-17.30
Revealing the phytochemistry of the Mediterranean orchid *Himantoglossum robertianum* (Loisel.) P. Delforge sampled in different ecological habitats in Sardinia Island.
Trincia S, Chiocchio I, Mandrone M, Tarozzi C, Sanna C, Cortis P, De Agostini A, Poli F (Bologna, Cagliari)

17.30-17.45
Studying the biological activities of *Roccella tinctoria* DC. lichen and its main phenolic compounds.
Corsetti L, De Vita D, Di Giacomo S, Fraioli DR, Frezza C, Di Sotto A (Roma)

17.45-18.00
Valorization of *Olea europaea* L. tree pruning waste: optimization of phytochemicals extraction by D-optimal design approach
Tumminelli E, Cavalloro V, Marrubini G, Martino E, Rossi D, Collina S (Pavia)

18.00-18.15
Phytochemical insights and biological activity of *Salvia karwinskii* Benth. exudate against phytopathogens and pests.
Devi P, Lanteri A P, Minuto A, Rosa E, Karioti A, De Tommasi N, Bisio A (Genova, Albenga, Salerno, Pisa)

18.15-18.30
Towards eco-friendly metabolomics: a NADES-guided, standard free semi-quantitative metabolomics for *Melissa officinalis* analysis.
Spaggiari C, Hiemstra I, Kazbar A, Costantino G, Righetti L (Wageningen, Parma)

18.30-18.45
Plant products as innovative tools for honeybee health in veterinary medicine.
Bava R, Lupia C, Bulotta R M, Conforti F, Statti G, Musella V, Castagna F (Catanzaro, Potenza, Rende)

19.00 Cocktail di Benvenuto

Venerdì 11 Aprile

9.00-10.30 Sessione 5 — Aula Magna

Moderatori: Roviezzo F (Napoli) – Turrini E (Bologna)

9.00-9.15
Investigation of the molecular immune mechanisms of *Baccharis dracunculifolia* DC. through the use of an integrated in silico/*in vitro* model.
Cappellucci G, Romão-Veiga M, Ribeiro-Vasques VR, Miraldi E, Baini G, Biagi M, Sforcin JM (Siena, Botucatu, Parma)

9.15-9.30
Erucin ameliorates skeletal muscle dysfunction in Duchenne Muscular Dystrophy.
Casale V, Smimmo M, D'Andrea D, Persico G, Cirino G, Filipovic M, Bucci M, Vellecco V (Napoli, Dortmund)

9.30-9.45
Protective effects induced by the association of vitamin D3, vitamin K2, resveratrol, and water extracts from *Equisentum arvense*, *Crataegus curvisepala*, *Vitex agnus-castus*, and *Glycine max*.
Acquaviva A, Di Simone SC, Libero ML, Centulio AP, Genovesi G, Ciaramellano D, Recinella L, Leone S, Brunetti L, Ferrante C, Chiavaroli A, Orlando G (Chieti)

9.45-10.00
Hydrogen sulfide enhances the therapeutic effects of resveratrol in managing asthma symptoms.
Simonelli M, Cerqua I, D'Avino D, Perna S, Corvino A, Severino B, Capasso R, Rossi A, Roviezzo F (Napoli)

10.00-10.15
Cytoprotective effects of a polyphenol-based extract from *Humulus lupulus* L. against damage induced by respiratory toxicants in airway cells.
Percaccio E, Ciarla C, Acquaviva A, Facchinetti R, Nicotra G, Di Giacomo S, Ferrante C, Di Sotto A (Roma, Chieti, Milano)

10.15-10.30
Beneficial effects induced by a blend of a new bromelain-based polyenzymatic complex plus N-Acetylcysteine in urinary tract infections.
Leone S, Recinella L, Libero ML, Ciaramellano D, Centulio AP, Genovesi G, Acquaviva A, Orlando G, Chiavaroli A, Ferrante C, Menghini L, Di Simone S, Brunetti L (Chieti)

9.00-10.30 Sessione 6 — Aula A

Moderatori: Ferrante C (Chieti) - Sorrentino R (Napoli)

9.00-9.15
***Echinacea angustifolia* DC. root extract: phytochemicals and molecular mechanisms in wound healing.**
Santopietro F, Benedetto N, Russo D, Tuseef M, Mangieri C, Carmosino M, Milella L (Potenza)

9.15-9.30
Aliophen-XP, a patented malt- and hop-based formulation, inhibits psoriasis-like inflammation in mice.
De Palma G, Spagnuolo C, Adabbo E, Russo G L, Cicala C (Napoli, Avellino, Milano)

9.30-9.45
Targeting melanoma: exploring plant extracts for tyrosinase and RAGE inhibitors
Fossati A, Cavalloro V, Papetti A, Moretto G, Collina S, Martino E (Pavia)

9.45-10.00
Polydatin reduces cardiotoxicity and enhances the anticancer effects of sunitinib by decreasing pro-oxidative stress, pro-inflammatory cytokines, and NLRP3 expression
Quagliariello V, Berretta M, Iovine M, Paccone A, Taibi R, Montopoli M, Maurea N (Napoli, Messina, Pordenone, Padova)

10.00-10.15
Anti-inflammatory effects of a commercial *Eleutherococcus senticosus* root extract: *in vitro* and *in vivo* evidence.
D'Avino D, Cerqua I, Perna S, Spinelli M, Simonelli M, Sbrogna G, Ialenti A, Sommella EM, Daglia M, Pace S, Roviezzo F, Rossi A (Napoli, Salerno, Fisciano, Roma)

10.15-10.30
Erucin and Sulforaphane, natural sulfur compounds for the management of metabolic disorders: evaluation of antioxidant effects and modulation of *de-novo* browning process on 3T3-L1 preadipocyte cells.
Flori L, Galgani G, Bray G, Pinelli R, Ippolito C, Segnani C, Pagnotta E, Ugolini L, Citi V, Pellegrini C, Bernardini N, Martelli A, Calderone V (Pisa, Bologna)

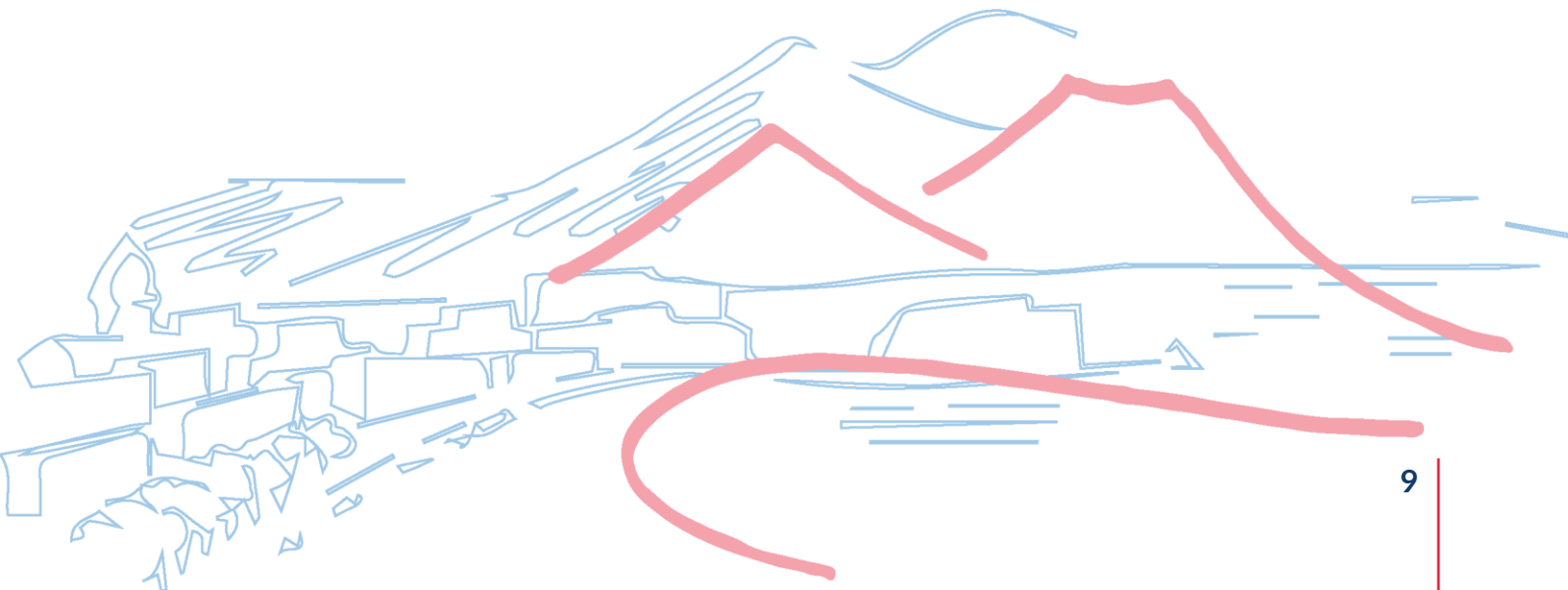
10.30-11.30 Coffee break e sessione poster

Lettura magistrale

Aula Magna

Moderatore: Calderone V (Pisa)

11.30-12.00
Mediterranean diet and medicinal plants as microbiota modulators
Cena H, Al-Naqeb G, Kalmpourtzidou A (Pavia)



12.00-13.15 Sessione 7

Aula Magna

Moderatori: Fimognari C (Bologna) – Romano B (Napoli)

12.00-12.15
Proceeding from animal to organoid models of triple-negative breast cancer to explore the therapeutic potential of erucin, an H_2S-realising compound from *Eruca sativa* Mill.
Bello I, Esposito C, Barile M, Bucci M, Cirino G, Grolla A, Travelli C, Panza E (Napoli, Novara, Pavia)

12.15-12.30
***Boswellia Serrata* extract as a new therapeutic strategy to slow the progression of colon cancer.**
Mannino D, Cucinotta L, Filippone A, Basilotta R, Casili G, Paterniti I, Esposito E (Messina)

12.30-12.45
Boosting curcumin analogs' cytotoxicity with light: an *in vitro* study.
Greco G, Turrini E, Belluti F, Calvaresi M, Ferrini F, Sestili P, Fimognari C (Bologna, Rimini, Urbino)

12.45-13.00
Genistein and curcumin inhibit proliferation and invasiveness in $BRAF^{V600E}$ mutant and wild-type melanoma cells: insights of the anticancer effects.
Vaccaro F, Cullotta C, Pallio G, Irrera N (Messina)

13.00-13.15
Secoiridoid-enriched extra virgin olive oil extracts enhance mitochondrial activity and antioxidant response in colorectal cancer cells: the role of oleacein and oleocanthal in PPARγ interaction.
Delre P, Leo M, Mancini C, Lori G, Ferraris I, Lucchini F, Molinario A, Leri M, Castellaneta A, Losito I, Cataldi T, Rossato M, Colantuoni V, Taddei M L, Lavecchia A, Sabatino L (Benevento, Firenze, Napoli, Verona, Bari)

12.00-13.15 Sessione 8 — Aula A

Moderatori: Martelli A (Pisa) - Montopoli M (Padova)

12.00-12.15
Protective effects of oleoylethanolamide on kidney dysfunction associated with obesity and metabolic disorders.
Comella F, Melini S, Opallo N, Navatti N, Di Napoli E, Paciello O, Meli R, Pirozzi C, Mattace Raso G (Napoli)

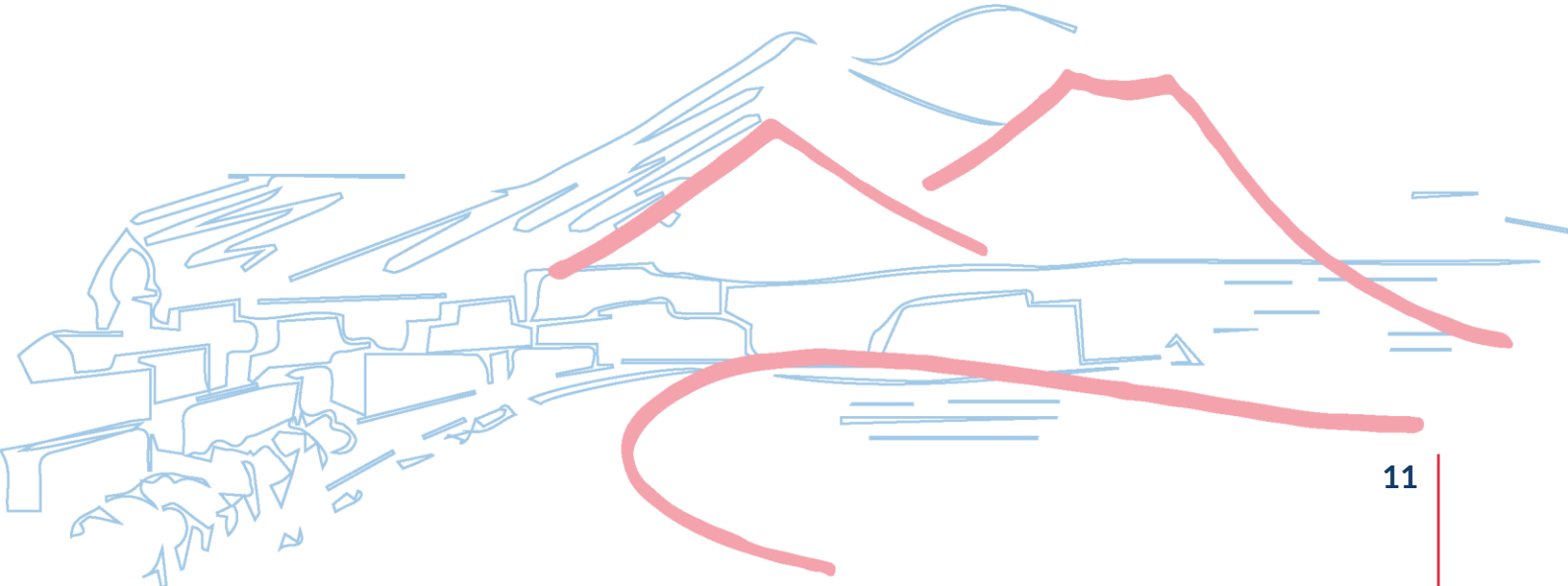
12.15-12.30
Valorization of two endemic Italian plants by the bioaccessibility of the metabolites present in their hydroalcoholic extracts using an *in vitro* digestion model.
Menzio G, Grasso S, Marengo A, Cagliero C, Sgorbini B, Sanna C, Rubiolo P (Torino, Cagliari)

12.30-12.45
Fructooligosaccharides mitigate metabolic disruptions induced by chronic fructose or galactose consumption in rats by reducing the accumulation of advanced glycation end products.
Almasri F, Collotta D, Aimaretti E, Sus N, Aragno M, Dal Bello F, Eva C, Mastrocola R, Landberg R, Frank J, Collino M (Stuttgart, Piemonte, Gothenburg)

12.45-13.00
Effect of N-acylethanolamines mixture (Olaliamid®) on fat-to-heart crosstalk compromised by obesity: *in vivo* and *in vitro* evidence.
Navatti NP, Melini S, Opallo N, Comella F, Mattace Raso G, Pirozzi C, Meli R (Napoli)

13.00-13.15
Cyanidin-3-O-glucoside beneficial effects against intestinal barrier injury induced by indomethacin.
Salamone FL, Molonia MS, Trischitta S, Saija A, Cimino F, Speciale A (Messina)

13.15-14.30 Light lunch



14.30-16.30 Sessione 9 — Aula Magna

Moderatori: Della Loggia R (Trieste) – De Tommasi N (Salerno)

14.30-14.45
Evaluation of the efficacy of *Zingiber officinale* Roscoe, and its main terpene component, zingiberene, in modulating microglial senescence.
Sasia C, Galeotti N (Firenze)

14.45-15.00
Neuroprotective effects of *Actaea racemosa* in an *in vivo* model of spinal cord injury.
Scuderi S A, Basilotta R, Filippone A, Palermo N, Paterniti I, Campolo M, Esposito E (Messina)

15.00-15.15
Photochemical behavior and degradation products of natural cannabinoids.
Bini A, Protti S, Pollastro F, Bonesi S, Merli D (Pavia, Novara, Buenos Aires)

15.15-15.30
Study on the neuroprotective activity of olive leaf extract from pruning waste against different types of stress in SH-SY5Y cells.
Vaccaro F, Rigillo G, Baini G, Cappellucci G, Miraldi E, Biagi M (Siena, Modena, Reggio Emilia, Parma)

15.30-15.45
Treatment with a mixture of standardized extracts obtained from *Centella asiatica*, *Echinacea purpurea* and *Zingiber officinale* prevents behavioural and neurochemical alterations induced by chronic social defeat stress in mice.
Costa A, Micheli L, Sordi V, Ciampi C, Lucci J, Passani MB, Provensi G (Firenze, Camerino, Sansepolcro)

15.45-16.00
Cannabidiol-loaded cationic vesicles loaded in a thermosensitive hydrogel: an efficient nanoplatform for successful intranasal administration in a rat model of hydrocephalus.
Grifoni L, Giachi G, Ciampi C, Vanti G, Fiani S, Mennini N, Bergonzi MC, Micheli L, Di Cesare Mannelli L, Ghelardini C, Bilia AR (Sesto Fiorentino, Firenze)

16.00-16.15
Prosocial effect of non-psychotropic *Cannabis sativa* oil.
Mac Sweeney E, Popescu VS, Premoli M, Ascrizzi R, Ribaudo G, Gianoncelli A, Flamini G, Pistelli L, Memo M, Bonini SA, Mastinu A (Brescia, Pisa)

16.15-16.30
PEA-OXA restores cognitive impairments associated with vitamin D deficiency-dependent alterations of the gut microbiota.
Pagano S, Iannotta M, Perrone M, Infantino R, Giorgini G, Fusco A, Marabese I, Manzo I, Belardo C, Di Martino E, Boccella S, Silvestri C, Luongo L, Di Marzo V, Guida F, Maione S (Napoli, Quebec)

14.30-16.30 Sessione 10 — Aula A

Moderatori: Piacente S (Salerno) - Pollastro F (Novara)

14.30-14.45
Optimization of the management of olive leavels from Tuscan cultivars for their up-cycling as nutraceuticals.
Ugolini T, Cecchi L, Digiglio I, Zanoni B, Mulinacci N (Firenze)

14.45-15.00
Optimization of secondary metabolites production in plant cell cultures.
Carpi A, Armellin M, Marin A, Dal Monte R, Trainotti L (Padova)

15.00-15.15
Callus culture of *Mespilus germanica* L. (Rosaceae): a biofactory for bioactive compounds.
Rosa E, Parisi V, De Tommasi N, Donadio G, Fraternale D (Fisciano, Urbino)

15.15-15.30
Circular biosolutions: compost teas as a new frontier in antiviral and antimicrobial therapy.
Verrillo M, Della Marca R, Cozzolino V, Zannella C, Galdiero M, Spaccini R, De Filippis A (Napoli)

15.30-15.45
Influence of wood distillate, a bio-stimulant, on the nutritional quality of fruits in four *Cucurbita* species.
Fedeli R, Loppi S (Siena, Palermo)

15.45-16.00
Chemical Profiling of Matcha Tea: Analysis of Principal Metabolites Variations Among Different Grades.
Toniolo C, Patriarca A, De Vita D, Frezza C, Santi L, Sciubba F (Roma)

16.00-16.15
Citrus, Pomegranate, Conifers, and More: An Integral Green Extraction Technique Enabling the Sustainable Exploitation of Valuable Plant By-Products.
Meneguzzo F, Tagliavento L, Zabini F, Testai L (Benevento, Pisa)

16.15-16.30
Diterpenes with potential anti-inflammatory properties from *Lavandula pubescens* Decne.
Pouramin Arabi S, Parisi V, Nocera R, Rosa E, Bader A and De Tommasi N (Salerno, Makkah)

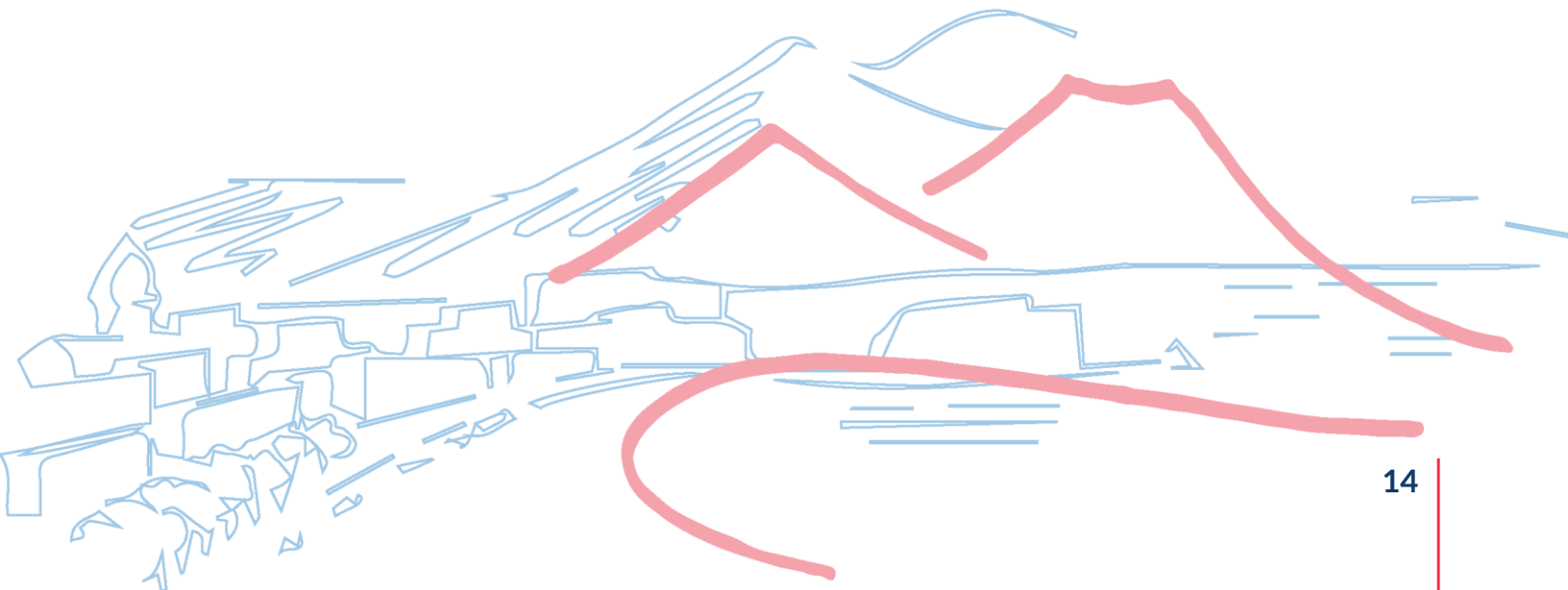
16.30-17.00 Coffee break

Lettura magistrale

Aula Magna

Moderatore: Calapai G (Messina)

17.00-17.30
Herbal medicinal products in Italy: regulatory framework and tools to increase awareness on their role in the marketplace
Assisi A. (AIFA, Roma)



17.30-19.00 Sessione 11 — Aula Magna

Moderatori: Biagi M (Parma) – Rossi A (Napoli)

17.30-17.45
Phytovigilance: Natural does not mean free of ADR.
Rizzi L, Gallo M, Bacis G, Torsello A (Milano, Bergamo)

17.45-18.00
Adverse reactions to *Ginkgo biloba* medicinal products released in European countries.
Ammendolia I, Attard E, Mannucci C, Esposito E, Calapai G, Currò M, Midiri P, Attard T, Cardia L, Calapai F (Messina, Msida, Novara)

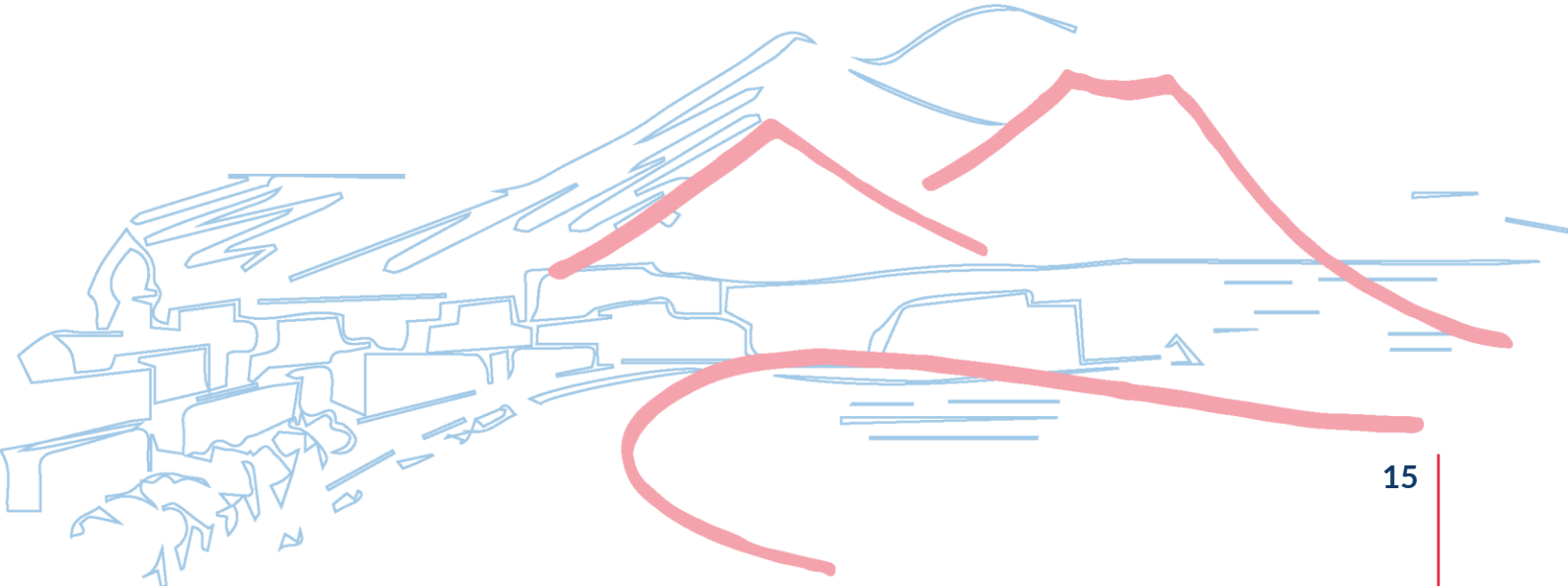
18.00-18.15
Role and impact of phytomedicines in the modulation of visceral hypersensitivity and mycobiome in DGBI
De Togni H (Schwabe Pharma Italia, Egna)

18.15-18.30
***Ginkgo biloba*: from tradition to modern approaches in favour of efficacy and safety**
Tongiani S (Indena SpA, Milano)

18.30-18.45
Pharmaco-toxicological profile of "Aglianico del Vulture" red wine polyphenolic extract (RWP) as dietary supplement for preventing obesity-associated cardiometabolic complications
Desantis V, Andriano A, Caradonna IC, Marozzi MLS, Cicco S, Infantino V, Nacci, Potenza MA, Montagnani M (Bari, Potenza)

18.45-19.00
Benzyl isothiocyanate suppresses development of thyroid carcinoma by regulating both autophagy and apoptosis pathway.
Basilotta R, Scuderi SA, Casili G, Mannino D, Filippone A, Lanza M, Paterniti I, Esposito E (Messina)

20.30 Cena



Sabato 12 Aprile

9.00-10.30 Sessione 12 — Aula Magna

Moderatori: Milella L (Potenza) - Dell'Agli M (Milano)

9.00-9.15
Neuroprotective effect of nutritional supplements in animal models of glaucoma.
Adornetto A, Bagetta G, Nucci C, Russo R (Rende, Roma)

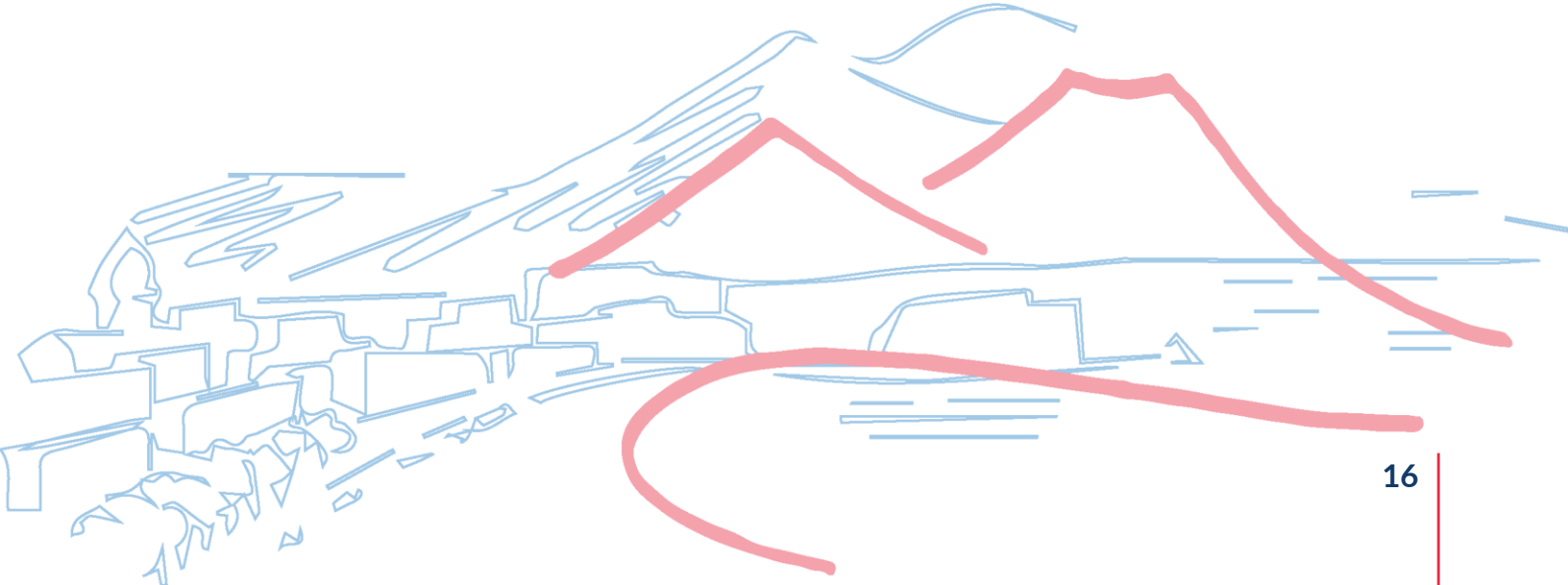
9.15-9.30
***Eremurus* species as new sources of metabolites with neuroprotective activity.**
Cavalloro V, Marchesi N, Ahmed KM, Ambrosio FA, Costa G, Alcaro S, Collina S, Martino E (Pavia, Kurdistan Region, Catanzaro)

9.30-9.45
Olive leaf extract reduces mast cell-mediated allergic inflammation.
Somma F, Romano B, Maresca DC, Ianaro A, Ercolano G (Napoli)

9.45-10.00
Exploring the anti-inflammatory and antioxidant properties of *Vitis vinifera* L. leaves extract: a sustainable approach to wine byproducts utilization.
Morandini S, Pucci M, Tirelli E, Popescu V S, Mastinu A, Peron G, Ribaudo G, Gianoncelli A, Puglisi R, Bongioni G, Cucchi L, Corsini S, Novello M, Cenadelli S, Uberti D, Abate G (Brescia, Rivolta d'Adda, Montichiari)

10.00-10.15
***Ulva pertusa* modulated colonic oxidative stress markers and clinical parameters: a potential adjuvant therapy to manage side effects during 5-FUrRegimen.**
Ardizzone A, Scuderi SA, Basilotta R, Capra AP, Campolo M, Paterniti I, Esposito E (Messina)

10.15-10.30
Roots and aerial parts: a comparative study on the biological activity and composition of *Cistus monspeliensis* L.
Popescu VS, Mac Sweeney E, Chiocchio I, Mandrone M, Sanna C, Bilo F, Morandini S, Borgese L, Trincia S, Poli F, Abate G, Mastinu A (Brescia, Bologna, Cagliari, Brescia)



9.00-10.30 Sessione 13 — Aula A

Moderatori: Governa P (Siena) - Cicala C (Napoli)

9.00-9.15
Protective effect of *Salvia officinalis* L. hydrodistillation wastewater against *E. coli*-induced damage in Caco-2 cells.
Molonia MS, Napoli E, Salamone FL, D'arrigo M, Cristani MT, Trischitta S, Saija A, Speciale A, Cimino F (Messina, Catania)

9.15-9.30
Effects of N-acylethanolamine association on metabolic dysfunction and cellular stress responses driven by obesity.
Melini S, Pirozzi C, Navatti NP, Del Piano F, Comella F, Opallo N, Mattace Raso S, Pietrocola F, Meli R (Napoli, Stockholm)

9.30-9.45
Erucin, a natural hydrogen sulfide (H_2S) donor, ameliorates vascular dysfunctions associated with metabolic syndrome.
Smimmo M, Casale V, Persico G, Mitidieri E, d'Emmanuele di Villa Bianca R, Bello I, Panza E, Brancaleone V, Indolfi C, Cirino G, Bucci M, Vellecco V (Napoli, Basilicata)

9.45-10.00
***In vitro* test to evaluate the anthelmintic efficacy of hazelnut and pomegranate by-products on *Trichostrongylus colubriformis* and *Haemonchus contortus* in sheep.**
Amato R, Bosco A, Martone G, Nappa A, Musella V, Castagna F, Cutrignelli MI, Policastro G, Fabbricino M, Rinaldi L (Napoli, Catanzaro)

10.00-10.15
Effect of *Fabiana imbricata* Ruiz et Pav. essential oil in prostate cancer cells.
Avola R, Graziano ACE, Cardile V, Madrid A, Russo A (Enna, Catania, Valparaìso, Catania)

10.15-10.30
Neuroprotective effects of a novel plant-based formulation with magnesium and vitamin B6.
Libero M.L, Di Simone S.C, Acquaviva A, Nilofar N, Tunali F, Centulio A, Ciaramellano D, Genovesi G, Leone S, Recinella L, Menghini L, Orlando G, Ferrante C, Chiavaroli A (Chieti, Torrevecchia Teatina)

10.30-11.30 Coffee break e Sessione poster

11.30-13.00 Sessione 14 — Aula Magna

Moderatori: Statti G (Rende) – Smeriglio A (Messina)

11.30-11.45
Novel gram-scale production of dietary isothiocyanate moringin, a slow H_2S donor.
De Nicola GR, Rollin P (Pescia, Orleans)

11.45-12.00
Circular Economy and valorization of waste products from agri-food production: Calabrian liquorice leaves as a functional component in baked products.
Zicarelli L, Fucile M, Alexa E, Conforti F, Statti G (Cosenza, Timisoara)

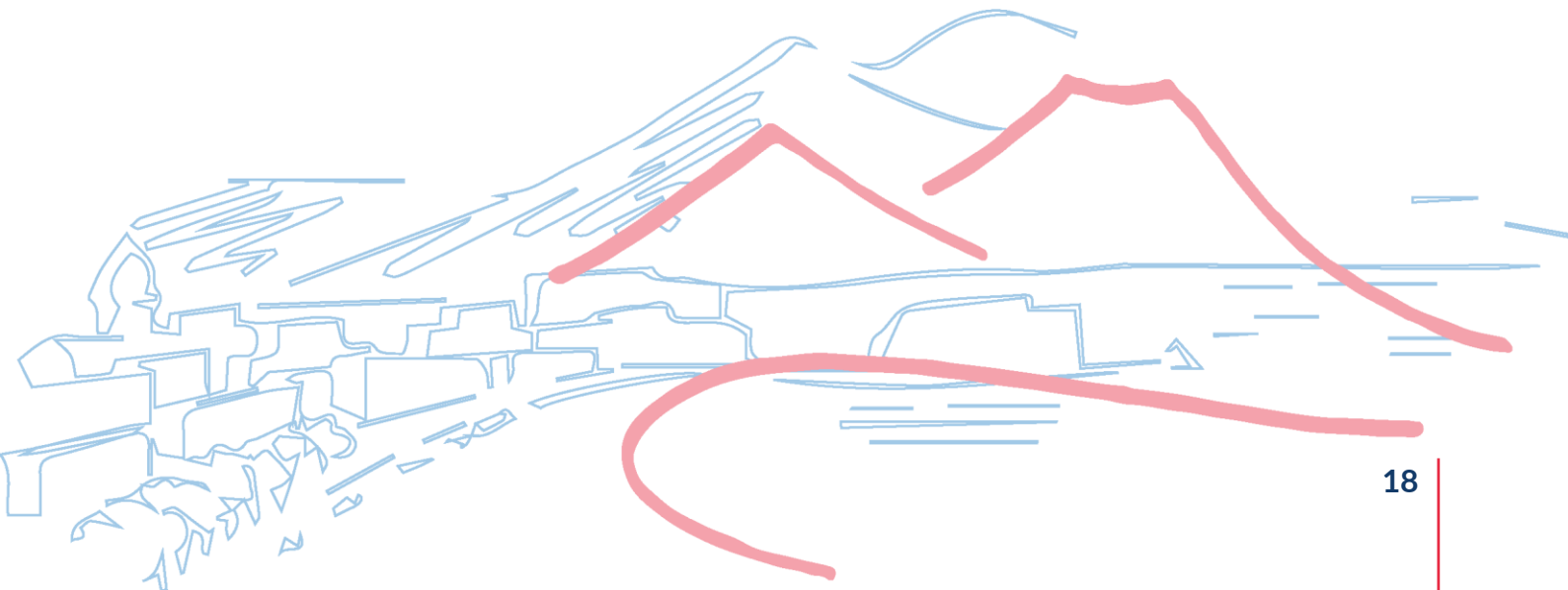
12.00-12.15
White mulberry *in vitro* cultures for healthy product development.
Dalla Costa V, Piovan A, Brun P, Filippini R (Padova)

12.15-12.30
Ellagic and Punicic acid reduce oxidative stress and neuroinflammation in a kainic acid rat model of epilepsy.
Pallio G, Cullotta C, Irrera N, Bitto A (Messina)

12.30-12.45
From trauma to tranquility: how myrcene helps the brain bounce back.
Bonsale R, Teweldemedhin M, Guida F, Luongo L, Maione S (Napoli)

12.45- 13.00
***Aegonychon calabrum* (Ten.) Holub: a source of bioactive compounds with antioxidant, anti-inflammatory, and antidiabetic potential**
Fucile M, Di Bella F, Puntillo D, Statti G, Conforti F (Rende)

13.15
Premiazione e Chiusura del Convegno



POSTER

Venerdì 11 Aprile

10.45-11.45

Sessione 1

Moderatore: Sangiovanni E (Milano)

P1. **Beneficial effects induced by a blend of biologically active compounds in an *ex vivo* model of prostatitis.**
Ciaramellano D, Centulio AP, Genovesi G, Libero ML, Acquaviva A, Leone S, Recinella L, Orlando G, Chiavaroli A, Ferrante C, Menghini L, Di Simone S, Brunetti L (Chieti, Torrevecchia Teatina)

P2. **Cannabidiol and beta-caryophyllene alone or in combination in an *in vitro* model of inflammation: a possible synergic effect.**
Mazzantini C, Pellegrini-Giampietro DE, Landucci E (Firenze)

P3. ***In Vitro* Anti-Inflammatory effects of ethyl acetate and n-butanol extracts from Aglianico Grape Pomace (*Vitis vinifera* L.).**
Perna S, D'Avino D, Kretzschmar E, Simonelli M, Cerqua I, Forino M, Roviezzo F, Capasso R, Rossi A (Napoli, Jena, Avellino)

P4. **Discovery of a marine-derived Natural Matrix from the Indonesian ascidian *Polycarpa aurata* with anti-inflammatory properties as a source of hydrogen sulfide.**
Correale M, Indolfi C, Esposito E, Bello I, Casertano M, Panza E, Menna ML, Imperatore C, Sorrentino R, Mitidieri E, d'Emmanuele di Villa Bianca R (Napoli)

P5. **Exploring the anti-inflammatory and immunomodulatory properties of a *Mangifera indica* L. extract on murine and human macrophages.**
Marigliano N, Schettino A, Begum J, Mansour AA, Rimmer P, Iqbal TH, Scognamiglio G, Iqbal Jilani A, Bucci MR, Saviano A, Maione F (Napoli, Birmingham, Abha)

P6. **Exploring the anti-inflammatory and protective properties of *Achillea erba-rotta* subsp. *moschata* (Wulfen) I.Richardson in brain endothelial cells**
Mercuriali B, Bottoni M, Milani F, Muluhie M, Giuliani C, Rzemieniec J, Castiglioni L, Fico G, Sironi L (Milano)

Sessione 2

Moderatore: Pagano E (Napoli)

P7. **Brassicaceae-derived Erucin, an H_2S-releasing isothiocyanate, exerts anticancer effects on human triple negative breast cancer cells.**
Barile M, Bello I, Esposito C, D'Ariano M, Smimmo M, Bucci M, Cirino G, Panza E (Napoli)

P8. ***In vitro* study of the enhancement of anthracycline cytotoxic activity through photoactivation.**
Neggiani G, Greco G, Turrini E, Calvaresi M, Fimognari C, Maffei F (Rimini, Bologna)

P9. **Identification and validation of antisenescence activity of *Salvia haenkei* extract using a high throughput screening.**
Dieni C, Giacomini I, Cocetta V, Tinazzi M, Pinzerato M, Ragazzi E, Montopoli M (Padova)

P10. **Mediterranean extract of *Sidnyum elegans*: a potential novel therapeutic agent against the triple-negative breast cancer.**
D'Ariano M, Esposito C, Barile M, Bello I, Casertano M, Imperatore C, Menna M, Sorrentino R, Mitidieri E, D'Emmanuele di Villa Bianca R, Panza E (Napoli)

P11. ***C. cardunculus* L. subsp. *cardunculus*: antiangiogenic activity and formulation strategies.**
Cacciola A, De Gaetano F, D'Angelo V, Stancanelli R, Fais A, Tuberoso CIG, Ventura CA, Germanò MP (Messina, Monserrato)

P12. ***Castanea sativa* Mill. bark extract: a chemopreventing agent inducing cytodifferentiation of a leukemic promyelocytic cell line.**
Rombolà F, Lenzi M (Bologna)

P13. **Biological investigation of ferulenol and prenilated coumarin from toxic giant fennel (*Ferula communis* L.).**
Camola A, Sanna C, Rosa A, Pollastro F (Novara, Cagliari)

Sessione 3

Moderatore: Marcotullio CM (Perugia)

P14. ***Helichrysum microphyllum* subsp. *tyrrhenicum*: a possible correlation between volatile compounds and phloroglucinols.**
Pollastro F, Allegrone G, Sanna C (Cagliari)

P15. **Aronia melanocarpa fruits: preliminary studies on chemical composition and biological activity.**
Era B, Fais A, Smeriglio A, Floris S, Trombetta D, Porcedda S, Piras A (Monserrato, Messina)

P16. **From olive mill processing waste to plant-based complexes for nutraceutical applications.**
Najlaoui A, Ingegneri M, Imbesi M, Trombetta D, Smeriglio A (Messina)

P17. **Chemical markers in Italian propolis: chrysin, galangin and CAPE as indicators of geographic origin.**
Allodi M, Cappellucci G, Baini G, Pistone E S, Miraldi E, Costantino G, Spaggiari C, Biagi M (Parma, Siena)

P18. **Non-volatile terpenoids and lipophilic flavonoids from *Achillea erba-rotta* Subsp. *moschata* (Wulfen) I. Richardson.**
Salamone S, Rosa A, Pollastro F (Novara, Cagliari)

P19. **Preliminary phytochemical investigations on *Prunus domestica* L. fruit.**
Giordano A, Baini G, Cappellucci G, Niccolucci V, Vaccaro F, Vidotto F, Miraldi E (Siena)

Sessione 4

Moderatore: Martino E (Padova)

P20. **Recovery of bioactive compounds from the biomass of aromatic plants after distillation Using NADES: a sustainable alternative extraction method.**
Truzzi E, Bertelli D, Catellani B, Darvishi Jazi D, Benvenuti S (Modena)

P21. **Health benefits of traditional infusions based on alpine *Artemisia* species: Chemical profile and biological potential.**
Argentieri MP, Cappellari O, Cristiano E, Diella MV, Iriti M, Vitalini S (Bari, Milano)

P22. **Health-Promoting effects, phytochemical constituents and molecular genetic profile of the Purple Carrot 'Purple Sun' (*Daucus carota* L.).**
Maresca V, Capasso L, Rigano D, Stornaiuolo M, Sirignano C, Piacente S, Cerulli A, Marallo N, Basile A, Nebbioso A, Giordano D, Facchiano A, De Masi L and Bontempo P (Roma, Napoli, Fisciano, Avellino, Portici)

P23. **Valorization of shea butter from local production in Benin: a phytochemical and pharmacological study.**
Di Sotto A, Percaccio E, Garzoli S, Minacori M, Corsetti L, Eufemi M, Stabile D, Giuliano M, Romano A (Roma, Campania)

P24. **Phytochemical profile and *in vitro* bioactivities of the Italian tomato landrace Riccio di Parma.**
Di Sotto A, Vergine V, Baldani C, Corsetti L, Vitalone A, Mannina L, Ingallina C, Ambroselli D, Crestoni ME (Roma)

P25. **Nutritional and bioactive potential of borlotto bean pod: a sustainable source for nutraceutical applications**
Imbesi M, Ingegneri M, Rando R, Mandrone M, Chiocchio I, Poli F, Smeriglio A, Trombetta D (Messina)

Sessione 5

Moderatore: Argentieri MP (Bari)

P26. **Valorization of *Citrus bergamia* by-products: a sustainable approach for the prevention and treatment of metabolic diseases.**
Romeo M, Fucile M, Conforti F, Statti G (Rende))

P27. **Effects of different cereals on diet-induced metabolic abnormalities: a focus on wheat and rye.**
Almasri F, Aimaretti E, Porchietto E, Schéle E, Dickson S, Landberg R, Frank J, Collino M (Hohenheim, Torino, Sweden)

P28. **Investigating the bioactive potential of rice-bran-derived gamma Oryzanol in gut health.**
Tirelli E, Morandini S, Pucci M, Mac Sweeney E, Popescu V, Ribaudo G, Gianoncelli A, Mastinu A, Eleutieri A M, Uberti D, Cecarini V, Abate G (Brescia, Camerino)

P29. **Zn-Spirulina Platensis as strategy to counteract advanced glycation end products accumulation and metabolic imbalance in a murine model of hypercaloric diet.**
Ferreira Alves G, Aimaretti E, Porchietto E, Mantegazza G, Gargari G, Collotta D, Einaudi G, Marzani E, Algeri A, Dal Bello F, Aragno M, Guglielmetti S, Mastrocola R, Collino M, Cifani C (Camerino, Torino, Milano, Mantova)

P30. **Validation of the traditional use of aromatic plants from Valtellina (SO) by *in vitro* model of *H. pylori*-related gastritis.**
Maranta N, Martinelli G, Piazza S, Fumagalli M, Pozzoli C, Sonzogni E, El Haddad S M, Parolo G, Sangiovanni E, Dell'Agli M (Milano, Sondrio)

P31. **Exploring the dual role of *Mangifera indica* L. in alleviating inflammation and abdominal pain in inflammatory bowel disease.**
Schettino A, Lucarini E, Marigliano N, Smimmo M, Romano F, Iaccarino N, Lombardi S, Izzo L, Scognamiglio G, Begum J, Mansour AA, Randazzo A, Greco KV, Iqbal AJ, Bucci M, Ghelardini C, Mannelli LDC, Saviano A, Maione F (Napoli, Firenze, Birminghan, Abha, UK, Sao Paolo)

P32.**The impact of nutritional supplementation on dysbiosis: an *in vitro* evaluation of probiotics, vitamins, and plant extracts formulation in modulating intestinal barrier function.**
Tinazzi M, Sacilotto A, Cocetta V, Giacomini I, Pisciottano M, Raso F, Bulferi G, De Togni H, Lanza R, Consolo P, Berretta M, Montopoli M (Padova, Milano, Egna, Messina)

Sessione 6

Moderatore: Orlando G (Chieti)

P33. **Astaxanthin and environmental stress: a preliminary study in keratinocytes and *Caenorhabditis elegans*.**
Pagliarani B, Pruccoli L, Balducci M, Tarozzi A (Rimini)

P34. **Resins and essential oils formulated in lipid nanovesicles for dermatological applications.**
Vanti G, Panagiotidou E, Dina E, Grifoni L, Giachi G, Gardikis K, Aligiannis N, Bilia AR (Firenze, Athens)

P35. **Officinal plants as new frontiers of cosmetic ingredients.**
Vitalone A, D'Andrea L, Di Sotto A, Caruso A, Parente R (Roma)

P36. ***In vitro* and in silico study of *Sedum telephium* (L.) leaf juice: skin absorption and wound healing ability.**
Vidotto F, Cappellucci G, Baini G, Biagi M, Giordano A, Vaccaro F, Miraldi E (Siena, Parma)

P37. **Non-volatile secondary metabolites from *Araucaria* Juss. species: exploring their potential for medicinal applications.**
Frezza C, De Vita D, Sciubba F, Toniolo C, Scintu C, Santi L, Attorre F (Roma)

P38. **Hydrogen sulphide in erucin for the development of new treatments of psoriasis.**
Rizzi L, Martelli A, Pagnotta E, Ugolini L, Bresciani E, Meanti R, Sartori E, Calderone V, Torsello A (Milano, Pisa, Bologna)

P39. **Isolation and characterization of exosome-like nanoparticles and their evaluation as agents/carriers in functional cosmetics**
Montagna S, Costantino G (Parma)

Sessione 7

Moderatore: Vitalone A (Roma)

P40. **GABAA receptors are involved in the anti-epileptic effects of a polyphenol-rich extract of white grape juice.**
Farina M, Maugeri A, Russo C, Citraro R, Leo A, De Sarro G, Navarra M (Messina, Catanzaro)

P41. **Evaluation of plant extracts on paclitaxel-treated C8-D1A mouse astrocytes, a potential new strategy against neuropathy.**
Spezzini J, Flori L, Di Cesare Mannelli L, Ghelardini C, Pagnotta E, Righetti L, Lanni C, Preda S, Brunetti L, Trabace L, Calderone V, Martelli A (Pisa, Firenze, Bologna, Pavia, Chieti, Foggia)

P42. **PEA and DHA, two natural compounds in the autism spectrum disorder management.**
Filogamo F, Liguori FM, Cristiano C, Russo R (Napoli)

P43. **A combination of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and curcumin synergistically reduces neuroinflammation in microglia by targeting the NLRP3 inflammasome and the NOX2/Nrf2 signaling pathway.**
Marcolin E, Chemello C, Ragazzi E, Zusso M (Padova)

P44. **Evaluation of the combined effect of sulforaphane and isoliquiritigenin in a cellular model of neuroinflammation.**
Mantini M, Barbalace MC, Rinaldi I, Hrelia S, Malaguti M, Angeloni C (Bologna)

P45. **Beneficial effects of a customized botanical dietary supplement produced by an innovative vertical farming system in an *in vitro* model of neuroinflammation.**
Camillò L, Capitani L, Marzani E, Bastianello A, Algeri A, Spampinato SF, Collino M (Torino, Guidizzolo)

Sabato 12 Aprile

10.30-11.30

Sessione 8

Moderatore: Trombetta D (Messina)

P46. **Brain boosters from cannabis: CBGA and CBDA show promise in beating memory loss.**
Limongelli R, Vitale RM, Morace AM, Ricciardi F, Fusco A, Boccella S, Guida F, Iannotti FA, Luongo L, Amodeo P, Maione S (Napoli)

P47. **Effect of cannabidiol in combination with alpha lipoic acid and resveratrol the psychitric changes associated with social isolation.**
Teweldemedhin MM, Belardo C, Infantino R, Boccella S, Morace AM, Perrone M, Guida F, Maione S, Luongo L (Napoli)

P48. **Elucidating the molecular mechanisms of oleocanthal in neuroinflammation.**
Rinaldi I, Barbalace MC, Freschi M, Mantini M, Malaguti M, Ortore G, Hrelia S, Giusti L, Digiacomo M, Angeloni C (Bologna, Meldola, Pisa, Camerino)

P49. **Silybin overcomes doxorubicin resistance in colorectal cancer cells by targeting GLUT1**
Cocetta V, Giacomini I, Tinazzi M, Gabbia D, Carrara M, Montopoli M (Padova)

P50. **Activity of a phytocomplex from in vitro cell culture of *Zanthoxylum piperitum* (L.) DC. on human fibroblasts.**
Baini G, Rigillo G, Pressi G, Cappellucci G, Biagi M (Siena, Modena, Camisano Vicentino, Parma)

P51. **Pinosylvin identified in *Pinus nigra* subsp. *laricio* inhibits LPS-induced inflammation in RAW 264.7 cells by suppressing pro-inflammatory cytokines and mediators and by downregulating the JAK/STAT Signaling Pathway**
Perri MR, Statti G, Conforti F (Rende)

Sessione 9

Moderatore: Mastinu A (Brescia)

P52. **Antioxidant and wound repairing effect of protolichesterinic acid from *Cetraria islandica* in an *in vitro* model of intestinal inflammation.**
Rispoli RM, Villicaña González E, Brizzi A, Schwaiger S, Marzocco S (Fisciano, Innsbruck)

P53. **Antibacterial, antioxidant and anti-inflammatory properties of essential oils in combination.**
Galletta B, Ginestra G, Micale N, Nostro A, Naccari C, Cristani M (Messina, Catanzaro)

P54. **Determination of phenolic content, antioxidant activity, and brine shrimp toxicity of the aerial part extracts from *Sinapis alba* and *Sinapis arvensis* (Brassicaceae) growing wild in Sicily (Italy).**
Davì F, Taviano MF, Raimondo FM, Ragusa S, Spadaro V, Miceli N (Messina, Palermo)

P55. **Exploring new lamiaceae extracts to counteract oxidative stress and cellular senescence in *in vitro* models.**
Pinzerato M, Giacomini I, Cocetta V, Tinazzi M, Dieni C, Ragazzi E, Montopoli M (Padova)

P56. **Correlation between sustainable agronomic practices and antioxidant potential: focus on leaves' extracts of three medicinal mediterranean wild species.**
Trabalzini A, Fornaciari M, Sardella R, Varfaj I, Sorci G, Orlandi F (Perugia)

P57. **Role of purple corn anthocyanins against Doxorubicin-induced cardiotoxicity: an insight into NF-κB pathway.**
Toccaceli M, Marinelli A, Tonelli C, Petroni K (Milano)

Sessione 10

Moderatore: Lenzi M (Bologna)

P58. **Exploring the chemical diversity of *Cannabis sativa* essential oils: a study on four selected genotypes.**
Bozzini MF, Pieracci Y, Ascrizzi R, Paris R, Bassolino L, Paganelli S, Panzani S, Dìaz Guerrero P, Venturi F, Turchi B, Fratini F, Flamini G (Pisa, Bologna, Modena, Reggio Emilia)

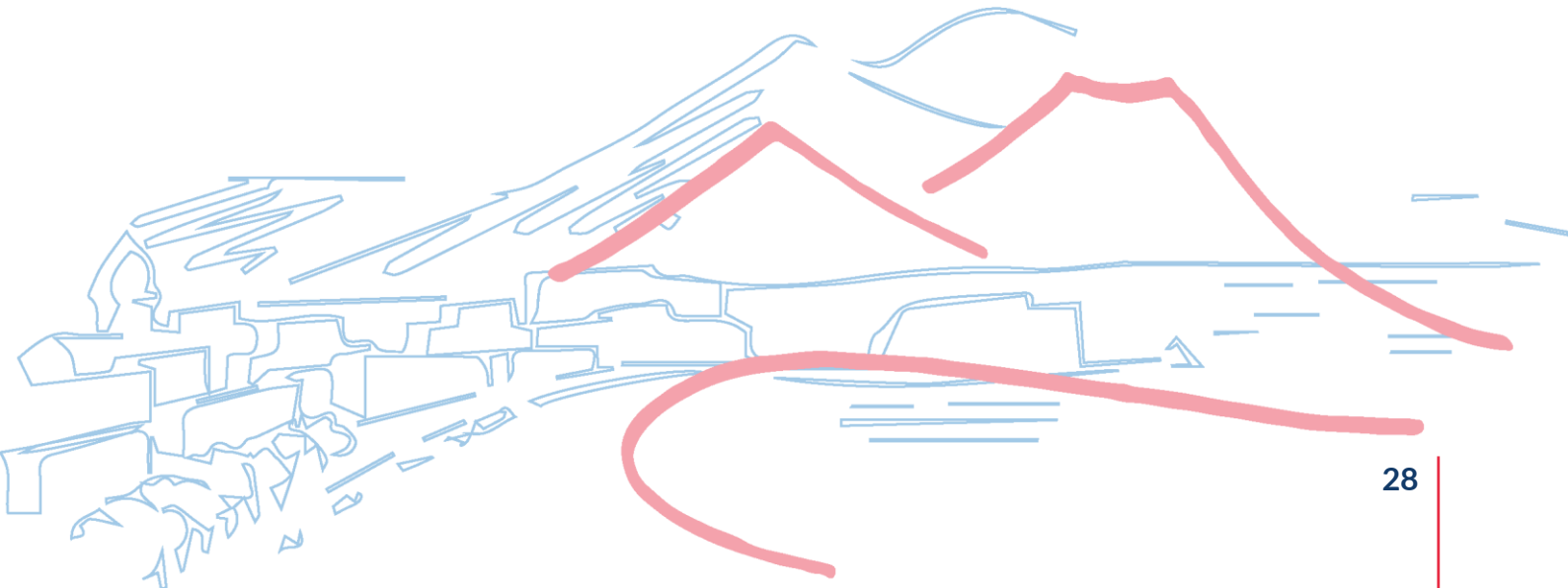
P59. **Phytochemical analysis and biological activities of *Odontites vulgaris* Moench.**
Baldani C, Percaccio E, Frezza C, De Vita D, Sciubba F, Foddai S, Di Sotto A (Roma)

P60. **Characterization and quality control of herbal drugs.**
Marra F, Pesce J, Ghiotti D, Lambertini S, Solimeo I, Cersosimo A (Rende, Cassina Rizzardi)

P61. **Investigating the anti-aging benefits of *Salvia haenkei*: an *in vivo* approach.**
Giacomini I, Sarill M, Cocetta V, Tinazzi M, Alimonti A, Giori AM, Montopoli M (Padova, Svizzera)

P62. **Innovative breeding system to develop new crop strategies to obtain pharmaceutical metabolites.**
Radice RP, De Fabrizio V, Padula F, Iannelli V, D'Arienzo G, Martelli G (Salerno, Basilicata)

P63. **Exploring the medicinal value of nature reserves: the case of Bosco Siro Negri**
Cavalloro V, Fossati A, Bracco F, Collina S, Martino E (Ferrara, Palermo, Pavia)



Sessione 11

Moderatore: Antognoni F (Bologna)

P64. **Ecosystem services and phytotherapeutic valorization of resilient species.**
Santandrea A, Lupia C, Fucile M, Zicarelli L, Canora G, Toma C, Conforti F & Statti G (Rende, Castelluccio Superiore, Sersale, Arad, Fisciano)

P65. **Hemp byproducts: optimizing supercritical extraction and unveiling antimicrobial potential.**
Foletti ME, Kupe A, Sacchetti G, Tacchini M (Ferrara)

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Elenco abstract comunicazioni orali

Giovedì 10 aprile

Lettura Magistrale

Challenges for area-based conservation of plant diversity across Europe

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Protection of biodiversity is one of the challehges of the next decades, with most of the natural ecosystems showing degradation and biodiversity loss. In Europe, there is an almost total lack of untouched nature and this is affecting the way we are addressing biodiversity conservation. In this lecture, I will discuss a) the conservation capacity of the the Natura 2000 (N2K) network of protected areas, the world largest coordinated network of protected areas estabilished under the Habitats and Birds Directives by the European Union (EU), and b) the coverage of strictly protected areas, as those in which the natural ecological processes can proceed without human interferece, across the EU countries. The first gioals is addressed by using over 1.2 million vegetation plots from the European Vegetation Archive obtaining over 14.2 million of species occurrences. The first goal is addressed by analysing the political, biogeographical and elevational distribution of 9,382 protected areas procted under the IUCN categories Ia, Ib and II in comparison to the target of strictly protecting 10% of the area of EU member states..
I show how the N2K network hosts around 90% of the native vascular flora of the EU countries, showing that a large proportion of European plant diversity is found within the network. Yet, significant variation exists across countries and biogeographical regions, indicating that the N2K sites are not equally representative of plant biodiversity across the EU. In addition, the N2K network contains, on average, more species per area than unprotected land. On the other hand, the coverage of strictly protected areas is extremely far from the 10% target and this can threat the long term persistence of undistrubed natural processes. A new perspective for the conservation of the most fundamental aspects of plant diversity in Europe should be soon developed.

Comunicazioni orali - Sessione 1

In vivo evidence of Cistus (*Cistus x incanus* L.) and Chestnut (*Castanea sativa* Mill.) extracts as gastroprotective nutraceuticals

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Cistus (*Cistus x incanus* L.) and Chestnut (*Castanea sativa* Mill.) are Mediterranean plants traditionally used for gastrointestinal inflammatory disturbs. Plant extracts from these plants are characterized by the predominance of polyphenols, with specific reference to condensed and hydrolysable tannins, respectively. Our group previously demonstrated that hydroalcoholic extracts from Cistus areal parts and Chestnut leaves exerted antimicrobial and anti-inflammatory activity in a co-culture model of human gastric epithelial cells (GES-1) and H. pylori. The combination of the two plant extracts guaranteed the coexistence of anti-inflammatory and anti-adhesive properties against H. pylori infection, at concentrations lower than 100 µg/mL (Martinelli et al., 2024).

With the present work, we aimed at translating the evidence obtained in vitro to in vivo model of gastric ulcer. Rats were orally treated for 10 days with 12.5 or 25 mg/Kg/day of each extract, and their combination, before the induction of gastric ulcer by ethanol administration. The Ulcer Index measured by ulcer count suggested that Cistus extract played a more relevant role in gastroprotection than Chestnut. Moreover, Cistus alone, but also its combination with Chestnut (12.5 mg/Kg), inhibited the production of CXCL-1 (the homologous of CXCL-8 in rat) and increased the activity of catalase in the gastric mucosa.

This work sustains the potential role of the combination of Cistus and Chestnut for gastroprotection, thus paving a solid base for clinical investigation against gastric disorders, with focus on H. pylori-related gastritis.

Martinelli et al. (2024): https://doi.org/10.3390/foods13010040

Use of medicinal plants of borage (*Borago officinalis*) and wild mallow (*Malva sylvestris*) to control gastrointestinal nematode infection in sheep.

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Gastrointestinal nematodes (GINs) are ubiquitous in grazing small ruminants and cause significant costs due to production losses. Moreover, anthelmintic resistance (AR) is now widespread throughout Europe and poses a major threat to the sustainability of modern small ruminant livestock farming (Charlier et al., 2022. Adv Parasitol). For this reason, the exclusive use of commercial anthelmintics for the treatment of GIN infections in ruminants is less sustainable due to AR, as well as the problem of drug residues in animal products and the environment. Therefore, an integrated and complementary therapeutic approach is needed, including the search for alternatives to synthetic anthelmintic drugs (Maurizio et al., 2023. Parasitol). The aim of this study was to evaluate the possibility of using the aqueous macerates of borage (*Borago officinalis*) and wild mallow (*Malva silvestris*) to control GIN infections in sheep. The borage and wild mallow plants present in seminatural pastures of southern Italy were sampled. After drying, the two plants were subjected to aqueous extraction processes using the conventional maceration technique in order to extract different bioactive compounds.

The *in vivo* trial was conducted in a farm of southern Italy (Campania region), where the prevalence of GINs was high. Sheep with natural-mixed infection and different worm burdens were used. For the trial, sheep were divided into four homogeneous groups by age, body weight and grazing season (n = 12 animals/group): G_Bor: 0.5 liter of borage macerate (2.5 g of extract) in a single administration; G_Mal: 0.5 liter of wild mallow macerate (2.5 g of extract) in a single administration; G_ALB: 3.8 mg/kg of albendazole (positive control); G_CNT: 0.5 liter of water (negative control). Individual faecal samples were collected rectally before treatment (D0) and 7, 14 and 21 days after treatment (D7, D14 and D21) and analysed using the Mini-FLOTAC technique (Cringoli et al., 2017. Nat Protoc). The coprocultures were performed for each group in order to identify the GIN genera.

The faecal egg count reduction (FECR) was calculated for each group using the formula FECR = 100 × (1 − [T2/C2]). The results showed the infection of different GIN genera in the farm examined: *Trichostrongylus* (26%), *Teladorsagia* (37%), *Haemonchus* (28%) and *Chabertia* (9%). The FECRT showed a mean reduction of GIN eggs in the G_Bor and G_Mal groups of 50.2% and 64.4% at Day 7, 47.7% and 48.5% at Day 14, 24.5% and 9.9% at Day 21, respectively. The FECR in the G_ALB group was 89.8 %.

Due to their complex chemical compositions, numerous bioactive ingredients, and natural origin, herbal formulations represent a potentially valuable alternative for the control of GINs in sheep. In this context, the results of the present study showed that both the aqueous macerates of borage and wild mallow are promising candidates.

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Anti-inflammatory and immunomodulatory effects of glucoraphanin, a natural hydrogen sulfide donor, in inflammatory bowel diseases.

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Over the last two decades, hydrogen sulfide (H_2S) has emerged as an endogenous regulator of a broad range of physiological functions. It is a signalling molecule endogenously produced by L-cysteine through the action of three enzymes: cystathionine-γ-lyase (CSE), cystathionine-ß-synthase (CBS) and 3-mercaptopyruvate sulfotransferase (3-MST) [1]. There are certain diseases where H_2S levels are abnormally low due to reduced biosynthesis and/or increased degradation. In these cases, a novel experimental therapeutic approach involving H_2S replacement (through H_2S donors) has been developed and is being translated into clinical practice [1]. In this view, there is a growing interest in identifying natural donors of H_2S [2]. Glucoraphanin, a glucosinolate found in cruciferous vegetables, has been identified as a slow-releasing H_2S donor that exerts beneficial effects in model of neuropathic pain and sarcopenia [3-4]. Here we investigated the effect of glucoraphanin in inflammatory bowel disease (IBD) using different approaches in *in vitro* and *in vivo* models. Blood samples were obtained from untreated IBD patients, including those with ulcerative colitis (UC), irritable bowel syndrome (IBS), Crohn's disease (CD), and healthy controls (HC). The ability of glucoraphanin to modulate macrophage polarization (M1/M2) was evaluated in both patient and HC samples. Additionally, blood samples treated with glucoraphanin (30 µM) were stimulated with lipopolysaccharide (LPS 0.1 µg/ml) for 3 hours to assess IL-17 and TNF-α levels. The in vivo anti-inflammatory action of glucoraphanin was investigated in a murine zymosan-induced peritonitis model, where glucoraphanin (50 mg/kg; i.p.) was administered 30 minutes before the injection of zymosan (500 mg/kg; i.p.). At 24 h from treatment mice were euthanized and the peritoneal exudate was collected for flow cytometry and molecular studies. In addition, the effect of glucoraphanin (30 µM) was evaluated in murine macrophagic cells (J774) stimulated with LPS (10 µg/ml), focusing on inflammatory pathways (iNOS, COX-2, NF-κB, IL-6, and PGE2), as well as H2S and NO levels. Glucoraphanin treatment significantly decreased TNF-α levels in UC and IBS patients upon LPS stimulation, without effects observed in CD patients. IL-17 levels remained unchanged across all groups. In HC, glucoraphanin induced a comparable reduction in TNF-α levels. Furthermore, glucoraphanin decreased TNF-α levels in M1 macrophages derived from both IBD patients and HC, while enhancing IL-10 levels in M2 macrophages from HC. In the murine model, glucoraphanin effectively inhibited inflammatory monocyte recruitment and transient macrophage populations, accompanied by increased H_2S and decreased NO levels in peritoneal exudates. In J774 cells, glucoraphanin suppressed LPS-induced iNOS, COX-2, and NF-κB expression, while reducing IL-6 and NO levels but not PGE2. Importantly, glucoraphanin restored H_2S levels diminished by LPS stimulation. Taken together, the results of this study reveal a novel role for glucoraphanin, a natural H2S donor, in positively influencing the inflammatory and immunological disturbances involved in the onset and progression of IBD. These findings offer promising therapeutic implications for managing inflammatory dysregulation in these diseases.

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[4] Micheli L et al., Int J Mol Sci. 2022.

Polyphenols-enriched extract from *Citrus medica* L.: Is it a novel strategy to fight intestinal inflammation?

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Inflammatory bowel diseases (IBDs) are pathological conditions characterized by chronic inflammation of the gastrointestinal tract. Although the etiology is multifactorial, alteration of the intestinal microbiota and mucosal barrier function is a common feature. This condition determines an increase in the absorption of bacterial antigens, inducing a greater inflammatory response[1]. Currently, there are treatments to reduce inflammation, however these have adverse effects. In recent years, there has been a notable increase in research activity focused on the complexity and diversity of natural products, largely due to their potential as pharmaceutical agents. Among these, *Citrus medica* L. is characterized by the rich presence of flavonoids, terpenoids, and coumarins which exhibit anti-inflammatory properties[2]. However, despite being a source of active metabolites, as far as is known, the properties of the whole fruit in preventing or counteracting the characteristic aspects of IBD have never been defined. Therefore, the aim of the study was to identify novel solutions by defining the phytochemical profile and protective properties of an optimized extract of *Citrus medica* L. on the intestinal epithelium. By applying the Response Surface Modeling (RSM), 27 extracts using different extraction parameters were carried out to obtain an optimized extract (OE) with high total phenolic content and antioxidant activity. Optimal conditions obtained by RSM for the extraction of compounds with high antioxidant activity were extraction time of 1h, 85.30% EtOH and 47.22°C.

A targeted LC-HRMS/MS approach on OE allowed the identification of 29 compounds including phenolic acids, flavonoids, coumarins, limonoids and fatty acids. Nomilin was estimated to be the most abundant compound in the OE with 286.63 ± 6.13 mg/100g DW, followed by limonin (91.32 ± 3.81 mg/100g DW), citropten (73.91 ± 2.90 mg/100g DW), scoparone (32.69 ± 4.76), *p*-coumaric acid (69.52 ± 3.19 mg/100g DW), diosmin (50.83 ± 6.64 mg/100g DW) and eriocitrin (32.36 ± 8.97 mg/100g DW). A widely used model to study intestinal inflammation *in vitro* is represented by the human Caco-2 cell line stimulated by LPS. *Citrus medica* optimized extract suppressed LPS-induced inflammatory response by downregulating the expression of phosphorylated p38, NF*k*B and TLR-4 and reducing the levels of nitric oxide and pro-inflammatory cytokines (TNF-*α*, IL-1*β*, and IL-6, IFN-γ). These effects were observed for the first time in this study. *Citrus medica* holds significant potential in mitigating inflammation and restoring intestinal homeostasis. Further research and clinical studies are necessary to fully elucidate its mechanisms and therapeutic efficacy.

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Metabolic effects of a hydroalcoholic extract from *Cymodocea nodosa* in a murine obesogenic diet model.

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Metabolic syndrome is a multifactorial pathology that predisposes to the onset of other diseases, including obesity, cardiovascular disease and type 2 diabetes mellitus. This syndrome is associated with an increase in visceral adipose tissue, characterized by adipocytes with a pro-oxidant and pro-inflammatory phenotype, which is closely correlated to the regulation of metabolic homeostasis[1]. Besides lifestyle change as the most viable strategy to modify the course of the disease, and the wide availability of synthetic compounds already approved for the treatment of obesity and related conditions, the phytotherapeutic-nutraceutical approach finds a rationale for use as an early intervention, but also as a source of potential new therapeutic agents.

In this scenario *Cymodocea nodosa* (Ucria) Asch. (Cymodoceaceae), a dioecious marine angiosperm, represents a possible phytotherapeutic-nutraceutical alternative for the treatment of metabolic disorders. Several studies have been carried out to evaluate the metabolic effects of the polysaccharide fraction of this aquatic phanerogamous, known for its beneficial properties[2-3]; in contrast, the number of studies on the hydroalcoholic (polar) fraction is still limited. Therefore, in this study the properties of a hydroalcoholic (EtOH-H_2O 8:2 v/v) *Cymodocea nodosa* aerial parts extract (CYM extract) collected in the Ligurian Sea (Italy) were investigated. Chromatographic analysis revealed that this extract was rich in polyphenolic compounds, such as flavonoids and phenolic acids, and was titrated in chicoric acid (AC) 12.3±2.4 mg AC/g dry extract, which represents half of the total phenolic content. CYM extract was tested in a murine model of cardiometabolic disorder induced by a high fat diet for 9 weeks (CYM extract dosage of 500 mg/kg/day).

The results obtained showed that CYM extract contained the increase in body weight induced by an obesogenic diet. In fact, differences were revealed in adipose tissues: a containment of white adipose tissue mass, an increase in brown adipose tissue and a significant reduction of lipidic droplets sizes at perivascular level were observed. The supplementation with CYM extract showed a positive modulation of the glycemic profile, reducing blood glucose levels and glycated hemoglobin. Concerning the metabolic profile, CYM extract increased the blood levels of irisin, but no changes on circulating levels of insulin were detected. Finally, histological and ponderal analysis revealed a preventive effect on the cardiac hypertrophic process associated with the dietary regimen. Via biochemical assays, the irisin/AMPK pathway appeared to be stimulated by supplementation with CYM extract and could likely be responsible, at least in part, for these beneficial effects.

Future experiments will allow a better characterization of the nutraceutical potential of this marine species and will explore the mechanisms underlying the metabolic stimulation. In this context, marine pharmacology assumes a particularly fascinating role, offering potential innovative resources for the development of effective therapies against the complications of metabolic disorders.

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Beneficial effects induced by an aqueous aged black garlic extract in rodent models of ulcerative colitis and colitis-associated visceral pain.

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Inflammatory bowel disease (IBD) is a morbid condition characterized by relapsing-remitting inflammation of the gastrointestinal tract, accompanied by persistent gut dysmotility and abdominal pain. Aged black garlic (ABG) has been suggested to exert multiple biological properties, including antinflammatory and antioxidant effects. The present study aimed to investigate potential protective effects exerted by an aqueous ABG extract (ABGE) on colon damage caused by inflammation in both *ex vivo* and *in vivo* experimental models.

We investigated the antinflammatory effects induced by ABGE (1-500 □g/ml) on a validated *ex vivo* experimental model of ulcerative colitis constituted by rat colon specimens exposed to *E. coli* lipopolysaccharide (LPS). We determined gene expression of various biomarkers involved in inflammation, including interleukin (IL)-1□, IL-6, nuclear factor-kB (NF-kB), and tumor necrosis factor (TNF)-□. Moreover, we studied the acute effects of ABGE (0.03-1 g/kg p.o.) on visceral pain associated with colitis induced by 2,4-di-nitrobenzene sulfonic acid (DNBS) injection in rats.

Our results showed that ABGE (50-500 □g/ml) suppressed LPS-induced gene expression of IL-1β, IL-6, NF-kB, and TNF-α. In addition, the acute administration of ABGE (0.1-1 g/kg) dose-dependently relieved post-inflammatory visceral pain, with the higher dose (1 g/kg) able to significantly reduce both the behavioural nociceptive response and the entity of abdominal contraction (assessed by electromyography) in response to colorectal distension after the acute administration in DNBS-treated rats.

In conclusion, the present findings showed that ABGE could represent a potential strategy to counteract colitis-associated inflammatory processes and visceral pain. The antinflammatory and antihyperalgesic effects induced by the extract could be related to its content in polyphenolic compounds, with particular regard to gallic acid and catechin.

The dietary compound luteolin reduces pro-inflammatory capability of M1 macrophages and colitis by targeting TRPM8

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Inflammatory bowel disease (IBD) refers to chronic disorders of the gastrointestinal tract, mainly characterized by dysregulated immune responses to the gut microbiome[1]. Plant-derived natural products have long been utilized for their healing properties, including antioxidant, anti-inflammatory, and immunomodulatory effects, as well as their role in modulating gut microbiota[2]. Here, we demonstrate that luteolin, a natural flavone compound present in vegetables and fruits, alleviates intestinal inflammation by blocking the temperature-sensitive cation channel TRPM8 (*transient receptor potential melastatin type-8*) in both *in vitro* and *in vivo* models.

The potential affinity of a subset of dietary compounds for TRPM8 receptor was evaluated by using a molecular docking analysis. *In silico* analysis was validated by measuring changes of the intracellular [Ca^{2+}] in HEK-293 cells stably overexpressing recombinant TRPM8, showing that luteolin was the most promising TRPM8 blocker with an IC_{50} of 3,091±0,326 μM.

The anti-inflammatory effect of luteolin was assessed in wild type (WT) and *Trpm8*$^{-/-}$ bone marrow derived macrophages (BMDMs), polarized toward pro-inflammatory phenotype M1 (with IFN-γ plus LPS). Pro-inflammatory [i.e., interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α] and anti-inflammatory (i.e., IL-10) cytokine levels were quantified in macrophages treated or not with luteolin. Further, the luteolin effect on the metabolic status of M1 macrophages was evaluated by Seahorse and NMR-based metabolomic analysis. Finally, the luteolin (3-30 mg/kg) was tested in a dextran sodium sulphate (DSS) model of colitis. The immune cells from colonic lamina propria isolated by DSS and DSS+luteolin - treated mice were characterized via flow cytometry.

We have shown that luteolin pre-treatment (10 μM) reduced IL-1β, IL-6, and TNF-α production in M1 WT macrophages and increased IL-10 at early time points. To note, luteolin did not affect cytokine production of *Trpm8*$^{-/-}$ BMDM, confirming its TRPM8-dependent mechanism. Also, luteolin induced a metabolic reprogramming of M1 macrophages as shown by the reduction of glycolysis (measured as extracellular acidification rate) and a decrease in production of metabolites (*e.g.*, succinate and glutamate) known as regulators of the pro-inflammatory response in macrophages.

Finally, the luteolin oral administration reduced colitis severity as shown by the reduction of weight loss, disease activity and colonic damage in DSS-treated mice. FACS analysis showed that luteolin induced a significant reduction in the number of $Ly6G^{+}$ neutrophils and $Ly6C^{hi}$ monocytes, and a significant increase of mature $Ly6C^{-}MHCII^{+}$ macrophages during DSS colitis.

In conclusion, our findings suggest that targeting TRPM8 with dietary interventions may help to prevent chronic inflammatory diseases symptoms, mitigating colitis severity and immune response.

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Comunicazioni orali - Sessione 2

Antioxidant and enzyme inhibitory potential of essential oils from *Ocimum gratissimum* L., *Lippia alba* Mill., and *Lippia sidoides* Cham.

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Medicinal plants are an important source of bioactive compounds with therapeutic potential. *Ocimum gratissimum* L. (as African basil), *Lippia alba* Mill. (plumier's verbena), and *Lippia sidoides* Cham. (Mexican oregano) are widely distributed in tropical and subtropical regions, especially in South America. These plants which are known for their antimicrobial, anti-inflammatory, and antioxidant properties, have been studied for their essential oils, which contribute to these beneficial effects.

In this research, the chemical composition of the essential oils was analyzed by GC-MS. Linalool was the main compound in *O. gratissimum* EO; *L. sidoides* EO contained a high concentration of thymol; finally, eugenol was the dominant component in *L. alba* EO.

The antioxidant activity of the EOs was assessed by three different assays, using Trolox as standard. In the DPPH assay, *O. gratissimum* EO showed comparable activity (EC_{50} = 6.74 µg/mL) to Trolox (EC_{50} = 3.65 µg/mL), while *L. sidoides* EO had a much weaker response (213.57 µg/mL). Instead, *L. alba* EO showed no activity. In the FRAP assay, *O. gratissimum* EO exhibited highest Fe^{2+} reducing power (18.50 mmol Fe^{2+} equivalents/g EO) compared to Trolox (11.28 mmol Fe^{2+} equivalents/g), while *L. sidoides* EO had less activity (8.80 mmol Fe^{2+} equivalents/g EO). In the ABTS test, *L. sidoides* EO showed the highest Trolox equivalent value (744.33 mg TE/g EO), while *O. gratissimum* EO displayed a lower value (694.90 mg TE/g EO). These results highlight the strong antioxidant potential of *O. gratissimum* and *L. sidoides* EOs, suggesting their possible application in combating oxidative stress-related disorders. The essential oils were also evaluated for their inhibitory effects on α-amylase and α-glucosidase, enzymes involved in carbohydrate metabolism. *L. sidoides* EO exhibited the strongest inhibition against α-amylase (EC_{50} = 445.30 µg/mL). In contrast, both *O. gratissimum* and *L. alba* EOs showed no activity. However, the *L. sidoides* EO demonstrated a lower inhibitory potential compared to the reference inhibitor Acarbose (EC_{50} = 1.75 µg/mL). Against α-glucosidase, *O. gratissimum* EO (EC_{50} = 1.36 mg/mL) and *L. sidoides* EO (EC_{50} = 1.19 mg/mL) showed moderate inhibition, while *L. alba* EO was less effective (EC_{50} = 1.92 mg/mL), in comparison to Acarbose (EC_{50} = 0.89 mg/mL). The stronger inhibition of α-glucosidase over α-amylase suggests that these EOs could help modulate postprandial glucose absorption, reducing blood sugar spikes. This selective inhibition may also reduce gastrointestinal side effects, which are commonly associated with α-amylase inhibitors.

Further studies are currently underway to assess the ability of these EOs to inhibit acetylcholinesterase and butyrylcholinesterase, key enzymes involved in the development of neurodegenerative diseases such as Alzheimer's. Inhibitors of these enzymes are actively being studied as potential therapeutic agents for cognitive disorders, as they enhance cholinergic neurotransmission by preventing the breakdown of acetylcholine.

These findings suggest that *O. gratissimum* and *L. sidoides* EOs are promising candidates for further investigation into the management of oxidative stress-related diseases, including metabolic disorders.

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Multifaceted study of *Vepris boiviniana*: phytochemicals, cytotoxic effects, antioxidant potential, and enzyme inhibition.

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Research on bioactive plant molecules has become a global priority as interest in natural, plant-based products rises among researchers and consumers, especially with the emergence of new diseases. The discovery of unexpected side effects from synthetic drugs has further increased the focus on studying plants with pharmaceutical potential worldwide (1).

In this study, we conducted a comprehensive analysis of *Vepris boiviniana* leaf and stem bark various extracts using LC-MS chemical profiling and evaluated their antioxidant activity, cytotoxicity, and enzyme inhibition activity. Based on LCMS results, a total of 60 bioactive compounds across all extracts were identified. The 80% ethanolic stem bark extract demonstrated the highest antioxidant activity in the ABTS assay, measuring 551.82 mg TE/g. The stem bark infusion consistently showed remarkable antioxidant activity in four antioxidant assays, with values ranging from 137.39 mg TE/g to 218.46 mg TE/g. In enzyme inhibition assays, aqueous extracts from both bark and leaves exhibited significant inhibition of acetylcholinesterase, measuring 2.41 mg GALAE/g and 2.25 mg GALAE/g, respectively. The 80% ethanolic leaf extract had the lowest cytotoxicity in VERO cells (CC_{50}: 613.27 µg/mL) and demonstrated selective cytotoxicity against cancer cells, particularly H1HeLa cells, suggesting potential therapeutic specificity (2). Conversely, the 80% ethanolic bark extract showed higher toxicity in VERO cells but lower anticancer selectivity. The n-hexane extracts, especially from the leaves, displayed the highest toxicity towards non-cancerous cells with selectivity for H1HeLa and RKO cells. In conclusion, our research highlights the diverse bioactive properties of *Vepris boiviniana* extracts, showcasing significant antioxidant, enzyme inhibitory, and selective cytotoxicity potential against cancer cells.

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Essential Oil of *Cannabis sativa* L.: chemical composition and antimicrobial potential against methicillin-resistant *Staphylococcus pseudintermedius* Strains.

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Cannabis sativa L. is an annual herb belonging to the Cannabaceae family, whose cultivation, especially of the chemotypes with a low content of Δ^9-tetrahydrocannabinol (Δ^9-THC), was reintroduced at the beginning of this century. However, only in recent years has hemp gained significant attention, mainly for fiber and seed production. Indeed, *C. sativa* represents a versatile and multipurpose crop, capable of growing in a wide geographical range of areas; it is resistant to pests and diseases, and requires a low level of irrigation and fertilization. These features, together with its high biomass yield, have led to a growing interest in hemp cultivation [1]. Despite its low environmental impact, hemp supply chains still generate large amounts of waste, chiefly represented by inflorescences and leaves, whose reutilization is desirable to pursue the circularization of the economy. Hemp inflorescences are particularly valuable by-products, as they synthesize and store several secondary metabolites. In recent times, many studies have focused on hemp essential oil (EO), thanks to its numerous applications in various fields [2]. Interestingly, recent studies have highlighted the antimicrobial potential of *C. sativa* EO on both Gram-positive and Gram-negative bacteria, as well as yeasts [3]. However, limited information is available on its effectiveness oagainst animal pathogens, such as *Staphylococcus pseudintermedius*, in particular on methicillin-resistant strains. *S. pseudintermedius* is a coagulase-positive Gram-positive bacterium responsible for otitis externa and pyoderma, mainly affecting dogs, though it can also cause zoonotic infections. These conditions have usually been treated with systemic antibiotics, but an increasing number of methicillin-resistant staphylococci (MRS) strains has limited the application of conventional antimicrobial drugs, posing a major challenge in veterinary medicine [4].

In recent years, EOs have attracted attention as alternative therapeutic approaches to limit the use of conventional antibiotics; moreover, there is a growing scientific interest on hemp EO. The present study aimed to evaluate the *in vitro* inhibitory and bactericidal activity of the EO obtained from waste products of two hemp genotypes against 21 strains of *S. pseudintermedius* isolated from canine pyoderma and otitis externa. The complete chemical composition of the EOs was assessed and the major compounds were quantified and tested on the isolated bacterial strains.

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Chemical analysis and antimicrobial activity of the traditional food crop *Moringa oleifera* Lam.

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Moringa oleifera Lam., a traditional food crop, is renowned for its resilience in harsh environments, high nutritional value,[1] and medicinal properties, including cardioprotective, anti-inflammatory, antioxidant, and antimicrobial effects.[2] This study aimed to investigate the phytochemical composition of *M. oleifera* leaves and seeds throughout an untargeted NMR and GC/MS-based metabolomics approach.

A total of 29 metabolites were identified in polar extracts, including amino acids, organic acids, and carbohydrates. Amino acids were predominantly found in leaves, while carbohydrates, such as glucomoringin and glucosinolates, were more abundant in seeds. Apolar extracts were rich in fatty acids, with oleic acid being the most prevalent in seeds, alongside stearic and palmitic acids. GC-MS analysis also revealed the presence of terpenoids, such as methyl abietate derivatives.

The antimicrobial activity of the extracts was evaluated against two Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and two Gram-negative (*Pseudomonas aeruginosa*, *Salmonella enterica*) pathogens. Among the tested extracts, apolar seed extracts exhibited significant dose-dependent activity against *S. aureus* and *S. epidermidis*, reducing bacterial viability by up to 50% at a concentration of 4 mg/mL.

This study highlights the advantage of combining metabolite profiling and biological assays to identify bioactive compounds in plant extracts. The findings emphasize the pharmacological potential of *M. oleifera*, particularly its seed apolar extracts, as a source of antimicrobial agents. Future research could explore the therapeutic applications of these findings in addressing antimicrobial resistance and developing natural alternatives to synthetic antibiotics.

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Troxerutin activity against oxidative stress: a potential strategy for corneal damage.

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Oxidative stress and inflammation are key underlying factors in corneal damage, contributing to ocular surface disorders such as dry eye disease (DED), keratitis, and corneal erosion. These factors contribute to an imbalance in tear composition and structural alterations in the cornea, leading to symptoms such as discomfort, irritation, pain, and visual abnormalities, which collectively impair patients' quality of life. In this context, approaches centered on natural compounds with antioxidant and anti-inflammatory properties have shown potential in supporting ocular health and mitigating the effects of corneal damage.

Here, we investigated the role of troxerutin (TX), a natural flavonoid, in containing oxidative damage and restoring the architecture of corneal tissue.

An H_2O_2-induced oxidative stress model was carried out by using human corneal epithelial cells (HCE) SkinEthicTM 3D tissues. Cells were pre-treated with TX and after 24 hours stimulated with H_2O_2 for 10 minutes.

Our findings showed that TX protected corneal tissue from oxidative damage and supported re-epithelialization through the upregulation of tight junction proteins ZO-1 and Occludin. Furthermore, TX contributed to mitigate inflammatory signals by modulating the activity of adhesion molecules such as ICAM-1 and P-selectin. These results emphasize the potential of TX as a natural ally in preserving corneal health and addressing the managing of corneal damage.

Morus alba twigs: waste no more! Unveiling antibacterial potential through untargeted metabolomic-guided phytochemical investigation.

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Despite the cultivation of *Morus alba* (Moraceae) is still mainly intended for feeding silkworms, fruits and leaves have found applications in the food and pharmaceutical industries, but stem and twigs are still viewed as agricultural wastes with a negative environmental impact, representing an untapped reservoir of bioactive compounds.[1,2]

Faced with rising antimicrobial resistance (AMR),[3] we investigated the antibacterial potential of *M. alba* twigs through an untargeted metabolomic-guided phytochemical investigation to convert waste into a valuable resource.

Our phytochemical investigation, guided by a combined LC-HRMS/MS and Molecular Networking approach, allowed the isolation and the characterization of 17 secondary metabolites including stilbenoids, flavonoids and flavanones.

Isolated metabolites were tested for their antimicrobial activity against *Staphylococcus* spp. The most active compound resulted to be kuwanon C, exhibiting a MIC of 8 µg/ml against *S. aureus* ATCC 43300 (methicillin resistant, MRSA) and *S. epidermidis* ATCC 3598 (a biofilm producers' strain). The same concentration resulted bactericidal. We also observed an additive interaction between 4 µg/ml kuwanon C in combination with low oxacillin dosage against the MRSA.

Thanks to the high chemical structure similarity of isolated metabolites, structure-activity relationships of these versatile scaffolds have been postulated.

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Efficacy of *Citrus sinensis* essential oils in the control of *Pseudomonas syringae* pv. *tomato* and *Fusarium oxysporum* f.sp. *radicis lycopersici*.

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Essential oils represent a promising option for pathogen control due to their natural antimicrobial properties.

This study evaluated the antibacterial and antifungal activity, against phytopathogenic species, of two varieties of essential oils extracted from *Citrus sinensis* peel: *C. sinensis* "Navel Orange" and *C. sinensis* "Tarocco".

As regards antibacterial activity, after determining the minimum inhibitory concentration, equal to 5% (w/w), against *Pseudomonas syringae* pv. *tomato*, the causative agent of bacterial spot on tomatoes, curative efficacy tests were conducted. The inoculated plastic surfaces were treated with essential oils and subsequently analyzed to verify the presence of the pathogen by plating on selective media and specific polymerase chain reaction.

The antifungal activity of the same essential oils was studied against *Fusarium oxysporum* f.sp. *radicis lycopersici*, the agent responsible for root rot in tomatoes. Tomato seeds were exposed during germination to different concentrations of suspended essential oils. After a few days, blackening of the roots, if present, was observed as an indicator of disease.

The results highlighted the significant effectiveness of essential oils in both assays, suggesting their potential use as sustainable tools for the management of fungal infections in agriculture and for the disinfection of surfaces promoting the reuse of agricultural artifacts with a view to circular economy.

Comunicazioni orali - Sessione 3

Protective effects of *Vaccinium macrocarpon* on AGE-Mediated sarcopenia: *in vitro* and *in vivo* evidence.

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The Western diet (WD) has become a widespread dietary habit, primarily due to its palatability and affordability. However, it is characterized by an excess intake of ultra-processed foods rich in saturated fats, refined grains, salt, and fructose, making it a significant risk factor for age-related diseases, including sarcopenia. One of the key detrimental factors in WD is its high content of advanced glycation end products (AGEs), which are formed both endogenously (eAGEs) and ingested through the diet (dAGEs). The accumulation of AGEs in the body, particularly in skeletal muscle, contributes to oxidative stress, inflammation, and tissue damage, primarily through their interaction with the receptor RAGE.

In recent years, natural compounds have emerged as promising candidates for the treatment of aging-related diseases due to their bioactive properties. Among them, Vaccinium Macrocarpon (VM) has been identified through preliminary ad hoc screening as a potent inhibitor of AGE accumulation and activity, suggesting its potential therapeutic role in counteracting AGE-induced muscle wasting.

The aim of the present study is to evaluate the effect of AGEs on sarcopenia and the putative role of VM in counteracting these detrimental effects. To this end, murine C2C12 myotubes were treated with eAGEs and dAGEs, revealing a dose-dependent induction of muscle atrophy, seen as the reduction of myotube diameters. Moreover, both AGEs increased the expression of atrogenes (*Fbxo31 and Trim63)*, master inducer of skeletal muscle atrophy, induced mitochondrial dysfunction, and increased ROS production. Notably, VM treatment effectively mitigated these AGE-induced detrimental effects, preserving myotube size, reducing atrogenes expression, decreasing ROS accumulation, and maintaining mitochondrial function.

Consistently, *in vivo* experiments showed that mice consuming a WD with 21% butter fat, a known source of dAGEs, exhibited significant skeletal muscle atrophy, seen as reduced muscle mass, increased *Fbxo31* expression, and lower MyHC-II protein levels. The presence of AGEs in both blood and muscle tissues, along with RAGE upregulation, indicated localized activation of the AGE-RAGE axis as a potential driver of muscle wasting. Notably, VM supplementation in WD-fed mice effectively inhibited AGE accumulation and prevented muscle wasting.

These findings strongly suggest that WD-derived AGEs play a crucial role in skeletal muscle atrophy, exacerbating sarcopenia through oxidative stress and mitochondrial impairment. The accumulation of AGEs in muscle tissue highlights the importance of dietary choices in age-related muscle deterioration. Encouragingly, VM extract appears to counteract these adverse effects, offering a promising dietary intervention for mitigating AGE-induced sarcopenia. Further studies are needed to explore the clinical potential of VM and its broader applications in combating AGE-related pathologies.

Kadsurenin F from *Piper Kadsura* exerts anti-inflammatory properties in mouse colitis.

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Inflammatory bowel disease (IBD), including Chron´s disease and ulcerative colitis, is a chronic condition of the gastro-intestinal tract that has emerged as a global disorder over the last two decades (1). Although several therapeutic advances have been made in the treatment of IBD, there is still an urgent need for the control of intestinal inflammation (1). Here, we show results on the anti-inflammatory activity of Kadsurenin F (Kads F) in two experimental models of IBD (DNBS and DSS-induced colitis in mice). Kads F is a neolignan present in *Kadsura piper* (Japanese pepper) and it is widely used in the Chinese herbal medicine for the treatment of asthma and arthritic conditions. When Kads F was administered intraperitoneally to mice (at the dose of 10 mg/kg) it was evident a substantial improvement of the colitis-associated parameters (i.e., weight of the animals, food intake, diarrhea). Also, analysis *ex vivo* of the colons revealed that Kads F reduced leukocyte infiltration and intestinal permeability comparable to the positive control dexamethasone. Moreover, the treatment with Kads F potently inhibited the release of pro-inflammatory TNFα, IL-1β and MCP-1. On the cellular level, in activated human monocytes, Kads F reduced with IC_{50} values in nanomolar range the formation of pro-inflammatory cytokines as well as impaired cell migration. Together, we show that Kads F displays potent anti-inflammatory activity in experimental models of colitis calling for consideration for further evaluation as potential treatment of IBD.

1. https://doi.org/10.1016/S2468-1253(24)00355-8

Comprehensive polar lipid profiling of "Cavolfiore della Piana del Sele" PGI (*Brassica oleracea* L. var. *botrytis*) inner leaves by LC-ESI/HRMS/MS analysis and evaluation of tyrosinase inhibitory activity.

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Cauliflower (*Brassica oleracea* var. botrytis), belonging to Brassicaceae family, is a widely cultivated and consumed vegetable known for its edible, compact head consisting of undeveloped flower buds [1]. In Italy, cauliflower is a highly cultivated and valued vegetable; in some regions, there are exclusive varieties that are known for their special characteristics, traditionally characterized by the local climate and soil, and farming practices. Recently, "Cavolfiore della Piana del Sele" received Protected Geographical Indication (PGI) label by the EU [2]. This variety is known for its rounded shape (minimum 13 cm), compact, and crunchy texture. It is available fresh in various forms: leafy, crowned, semi-crowned, defoliated, and naked. The primary growing area includes towns in the Campania region, Southern Italy, such as: Albanella, Battipaglia, and Eboli [2]. The inner and outer leaves of cauliflower are a significant byproduct during the processing of cauliflower, typically making up about 40-50% of the total plant biomass [3].

With the aim of exploring these by-products as a potential source of bioactive compounds, a microwave-assisted extraction (MAE) method was chosen to prepare eco-sustainable EtOH and EtOH:H_2O (50:50) extracts from the inner leaves of "Cavolfiore della Piana del Sele" PGI cauliflower. An ultra-high-performance liquid chromatography system, coupled with a hybrid quadrupole-Orbitrap mass spectrometer (LC-(-)ESI/QExactive/MS/MS) was used to highlight polar lipids, that could contribute to the bioactivity profile of cauliflower leaves. In detail, by analysing molecular formulae, fragmentation patterns with class-diagnostic product ions, chromatographic behaviour, in comparison with literature data, a wide range of polar lipids—including oxylipins, glycolipids, phospholipids, and sphingolipids—were identified.

Finally, since polar lipids, including phospholipids and glycolipids, play a crucial role in neuroprotection by maintaining neuronal membrane integrity, protecting against oxidative stress, modulating inflammation, and supporting neurogenesis [4]. Tyrosinase is an enzyme involved in melanin synthesis, mainly in the skin, but also in the brain, where it aids dopamine production and neuromelanin synthesis in the substantia nigra (SN). Overexpression of tyrosinase leads to neuromelanin build-up, damaging dopaminergic neurons and reducing dopamine levels, causing tremors, rigidity, and cognitive decline [5]. Therefore, supported by these premises, herein, for the first time, tyrosinase inhibitory activity of the extracts was tested, highlighting an interesting activity of EtOH and EtOH:H_2O (50:50) extracts which exhibited IC_{50} values of 55.00 and 61.62 µg/ml, respectively, compared to kojic acid (IC_{50} = 11.25 µg/ml) used as reference compound.

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Combining *Acmella oleracea* and *Boswellia serrata* extracts: a novel pharmacological approach in inflammatory vestibulodynia.

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Vulvodynia is a chronic pain condition that affects the vulvar area, often resulting in significant discomfort and a reduced quality of life. Current treatments for vulvodynia are limited, and there is a need for more effective therapeutic options. *Acmella oleracea*, known for its spilanthol content, and *Boswellia serrata*, rich in boswellic acids, have been explored for their potential analgesic properties in pain management. In this study, vulvodynia-like symptoms were induced in female mice using Complete Freund's adjuvant (CFA). After the induction of symptoms, the mice were treated with a combination of *Acmella oleracea* and *Boswellia serrata* extracts (AO + BS). Behavioral pain assessments were conducted to monitor the effects of the treatment. Additionally, biochemical and functional evaluations were performed to measure spinal microgliosis and neuronal overexcitation. The combination of *Acmella oleracea* and *Boswellia serrata* (AO + BS) resulted in a significant reduction of vulvar hypersensitivity in mice. Besides alleviating pain, AO + BS therapy also reduced spinal microgliosis and neuronal overexcitation in mice with vulvodynia. The findings suggest that the AO + BS combination has the potential to alleviate vulvodynia associated pain through mechanisms involving the reduction of spinal microgliosis and neuronal overexcitation. These results point to the therapeutic promise of these plant extracts for chronic pain conditions like vulvodynia. The combination of *Acmella oleracea* and *Boswellia serrata* shows potential as a treatment for vulvodynia. However, further studies are needed to explore the underlying mechanisms and to optimize the dosage for clinical use.

Plant-based extract with vitamin D2: a novel nutraceutical strategy against hepatic lipotoxicity in MAFLD.

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Hepatic lipotoxicity has been identified as a pivotal factor in the genesis and progression of metabolic-associated fatty liver disease (MAFLD). The present study aimed to identify natural ingredients that are rich in vitamins or that possess vitamin-like properties, with a view to developing innovative nutraceutical formulations that have the potential to protect the liver.

Three nutraceutical formulations were developed, based on mushroom powder (extract [1], [2]) and yeast extract [3], containing from 0.03 to 0.1%of vitamin D2 (VD2). These were then compared with a commercial synthetic Vitamin D3 (VD3, DIBASE) formulation to assess their cytoprotective effect. Due to the poor solubility of the formulations, an optimised *in vitro* digestion protocol simulating the oral, gastric, and intestinal phases was performed on all three samples under investigation as well as on the commercial formulation. Subsequent to this, high-performance liquid chromatography (HPLC) analysis was employed to assess vitamin D (VD) stability, recovery, and quantification. The results obtained from this analysis revealed that the recovery of VD2 after the simulated digestion process (*i.e.*, the intestinal phase) was found to be 50–52%, while the recovery of VD3 from the commercial formulation was found to be 70%.

Secondly, we tested the extacts of intestinal phese on hepatic in vitro model of steatosis induced with free fatty acids (FFA), specifically palmitic acid (PA) and oleic acid (OA). We evaluated the cytoprotective effects of the VD-containing extracts in pre-treatment manner and then we analyzed the effect on lipotoxicity in terms lipid droplet formation, reactive oxygen species (ROS) production, and hepatotoxicity.

Concerning the pretreatment with the extracts, it was observed that there was no direct effect on ROS levels, except for yeast extract [3], which significantly reduced iROS at 5nM VD2. The intestinal digestion of the commercial sample (20nM VD3) effectively lowered extracellular H_2O_2 levels. In the *in vitro* fatty liver model, mushroom extract [1] (20 nM VD2) and yeast extract [3] (1-5 nM VD2) reduced iROS induced by OA treatment. Furthermore, extracts from samples [1] (20 nM VD_2), [3] (5 nM VD_2), and DIBASE were found to mitigate iROS levels caused by PA or combined OA+PA exposure. With regard to H_2O_2 efflux reduction, only extract [1] (20 nM VD2) exhibited significant effects against OA or PA treatment, albeit to a lesser extent than the DIBASE extract, which demonstrated robust activity across all FFA treatments. The observed cytoprotective effects were consistent with improvements in cell viability and lipid droplet accumulation.

This study highlights the potential of natural VD_2-rich formulations to mitigate hepatocyte lipotoxicity. The findings support the development of nutraceuticals aimed at counteracting oxidative stress and lipid accumulation in MAFLD, paving the way for further preclinical investigations.

Selective Activity of Chrysin-6-C-fucopyranoside from *Cyclanthera pedata* on Peroxisome Proliferator-Activated Receptor Gamma (PPAR©).

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Caigua [*Cyclanthera pedata* (L.) Schrad.], a plant originating from the Andean region, is now cultivated in various countries worldwide, including Italy, particularly within the Alpine valleys of Lombardy by small-scale farmers and hobby horticulturists. In the alpine Camonica Valley, a local variety of caigua has been documented since 1960. In Central and South America, the plant is widely used for its therapeutic properties, notably as an antidiabetic remedy. It also possesses antioxidant and anti-inflammatory effects and is used to manage hypertension and other circulatory conditions. Additionally, caigua fruit is known to effectively reduce serum cholesterol levels. However, despite its widespread use, the phytochemical profile of the plant, especially its secondary metabolites, remains largely unexplored.

In this study, we focused on isolating flavone glycosides from caigua leaves grown in Italy's Camonica Valley using flash chromatography. We then assessed their potential activity on Peroxisome Proliferator-Activated Receptors (PPARs) and Transient Receptor Potential (TRP) channels through luciferase and intracellular calcium assays. These two receptor classes play crucial roles in regulating essential body functions, including immunity, inflammation, cell excitability, differentiation, and the metabolism of sugars and lipids, among others. Our analysis indicated that among the flavone glycosides studied, chrysin-6-C-fucopyranoside exhibited the most potent and selective agonist activity for the Peroxisome Proliferator-Activated Receptor gamma (PPARγ). Notably, it showed no affinity for the other two PPAR subtypes (PPARα and PPARβ/δ) or TRP ion channels, such as TRPV1, TRPA1, and TRPM8.

These results are promising, suggesting that compounds derived from caigua may offer a safer and more targeted alternative to current PPARγ agonists like thiazolidinediones (TZDs). These drugs, prescribed to diabetic patients to enhance insulin sensitivity in muscle and adipose tissue, are often limited by significant side effects, including weight gain, fluid retention, and cardiovascular risks, due to off-target interactions or prolonged receptor activation.

Bergamot essential oil as a potential therapeutic agent for inflammatory bowel disease: phytochemical profile, toxicity assessment, and anti-inflammatory effects.

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Inflammatory bowel disease (IBD), which includes conditions such as Crohn's disease and ulcerative colitis, is a significant public health concern. Despite advancements in conventional treatments, there remains a continuous need for new therapeutic options that are more effective, less invasive, and associated with fewer side effects. Recent scientific interest has focused on the pharmacological properties of essential oils, particularly bergamot essential oil (BEO), extracted from the peel of the bergamot fruit (*Citrus bergamia*). BEO is renowned for its antibacterial, anti-inflammatory, and antioxidant properties, which are primarily attributed to its natural compounds, including monoterpenes and sesquiterpenes. These compounds may offer synergistic health benefits, complementing existing drug therapies and potentially enhancing treatment outcomes. This study aimed to investigate the potential therapeutic effects of BEO in experimental models of IBD. Phytochemical analysis of BEO, using high-performance liquid chromatography with ultraviolet detection (HPLC-UV), identified several bioactive compounds. Notably, resveratrol (2.6 µg/mL), known for its antioxidant properties, was detected. Linalool (6.55 µg/mL), linalyl acetate (32.1 µg/mL), and limonene (26.1 µg/mL) were also present, recognized for their anxiolytic, sedative, antinociceptive, and anti-inflammatory effects. These compounds contribute to BEO's therapeutic potential, providing a multifaceted approach to disease management. In allelopathic assays, BEO exhibited a concentration-dependent effect on seed germination and seedling growth. High concentrations (125 to 1000 µg/mL for *Cichorium intybus* and 500 to 1000 µg/mL for *Raphanus sativus*) significantly inhibited germination and growth. Conversely, lower concentrations enhanced germination and growth, indicating minimal allelopathic effects and good biocompatibility. Toxicity assessments providing contrasting results. The brine shrimp lethality assay classified BEO as toxic to highly toxic against *Artemia salina*. However, the *Daphnia magna* toxicity test revealed a contrasting biocompatibility profile, with BEO exhibiting non-cardiotoxic effects under normal conditions. Under ethanol-induced cardiotoxic stress induced (10% ethanol), BEO did not offer protective effects. Cell viability assays (MTT test on C2C12 cells) revealed a dose-dependent cytotoxic response. At concentrations up to 50 µg/mL, viability remained comparable to the control group, indicating high biocompatibility. However, at 100 µg/mL, viability dropped just below the 70% biocompatibility threshold, signaling the onset of cytotoxic effects, which became more pronounced at 150 µg/mL and significantly decreased at concentrations of 200 µg/mL and above. These findings confirm the dose-dependent cytotoxic properties of BEO. *Ex vivo* studies on cortex and colon tissues revealed BEO's protective effects, particularly in the colon. BEO treatment increased the expression of the anti-inflammatory cytokine IL-10 while reducing pro-inflammatory markers IL-6 and TNF-α. The main compounds of BEO, limonene, linalool, and linalyl acetate were specifically tested, supporting their roles in mediating these anti-inflammatory effects. Preliminary findings suggest that bergamot essential oil holds promise as a therapeutic option for managing inflammatory bowel disease. Its anti-inflammatory, antioxidant, and protective properties warrant further investigation to elucidate the underlying molecular mechanisms. Future research should focus on evaluating the efficacy and safety of BEO in advanced preclinical models and clinical trials to confirm its potential role in human IBD treatment.

Attenuation of glucose-induced microglial neuroinflammation by a *Melissa officinalis* L. phytocomplex from *in vitro* cultured cells.

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Diabetes mellitus is a metabolic disorder characterized by impaired glucose homeostasis, leading to elevated blood glucose levels. Hyperglycaemic events, frequently observed in diabetic patients, are well-recognized contributors to progressive neurodegeneration (1). Chronic hyperglycaemia can affect multiple organs, including the brain, resulting in debilitating neurological complications (2). In particular, hyperglycaemia plays a pivotal role in driving neuroinflammation, the most common complication in diabetes, mainly through the activation of microglial cells (3). Modulating hyperglycaemia-induced neuroinflammation may therefore help reduce the neurological comorbidities associated with diabetes.

Melissa officinalis L., commonly known as lemon balm, is a medicinal plant renowned for its calming, anti-inflammatory, and antioxidant properties. It contains bioactive compounds, including flavonoids, phenolic acids, and rosmarinic acid (RA), which have demonstrated significant therapeutic potential in modulating various diseases, including those associated with diabetes (4). Such natural compounds have proven effective in alleviating diabetes-associated comorbidities, demonstrating their capacity to modulate inflammatory pathways and oxidative stress.

This study aimed to investigate the potential of a *Melissa officinalis* L. (MO) phytocomplex, derived from plant cell cultures and enriched with its principal polyphenolic component, RA, in attenuating hyperglycaemia-induced neuroinflammation in microglial cells.

BV2 murine microglial cells were exposed to high glucose (HG; 25 mM) to establish an in vitro model of diabetic neuropathy. Microglial cells were treated with the MO phytocomplex and compared with RA. The antioxidant and anti-inflammatory effects of the phytocomplex were assessed using Western blotting and immunofluorescence to analyze key markers of neuroinflammation (NF-kB, CD11, iNOS, ERK, SIRT1). Additionally, the impact of MO treatment on ROS production induced by hyperglycaemic conditions was evaluated.

MO treatment effectively prevented abnormal increases in NF-kB, CD11, iNOS, and ERK1/2 phosphorylation, while also restoring SIRT1 levels. Moreover, ROS production induced by high glucose levels was significantly reduced following MO treatment. HG-conditioned medium from BV2 cells significantly decreased neuronal SH-SY5Y cell viability, while MO treatment exhibited neuroprotective effects by counteracting the negative impact of HG. RA, the principal bioactive component of MO, demonstrated comparable efficacy.

Treatment with MO and RA significantly reduced microglia-mediated neuroinflammation induced by hyperglycaemia and oxidative imbalance through the attenuation of ROS production. These findings suggest that *Melissa officinalis* L. may serve as a potential therapeutic intervention to alleviate neuroinflammation associated with diabetic neuropathy.

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Comunicazioni orali - Sessione 4

Phytochemical investigation of *Withania somnifera* L. and evaluation of acetylcholinesterase inhibitory activity by spectrophotometric assay and STD-NMR analysis.

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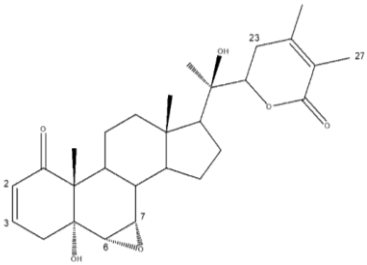
Withania somnifera L., commonly known as Ashwagandha, winter cherry or poisonous gooseberry, is a plant belonging to the Solanaceae family. The species name *somnifera* derives from Latin and means “inducing sleep” justifying its extensive use as sedative [1]. In the Ayurvedic system, *W. somnifera* is classified as "Rasayana" which means "tonic" as it acts mainly for body rejuvenation, defence against diseases, slowing down ageing and improving memory[2]. The roots represent the part of the plant most used in traditional preparations for the presence of bioactive compounds, mainly belonging to steroidal lactones called withanolides[3].

In this work, a green extract of *W. somnifera* L. roots was obtained using a Solid Liquid Dynamic Extractor (SLDE) with 50:50 EtOH: H_2O as solvent. The extract was purified by different chromatographic steps, yielding 24 steroids, whose structures were characterized by 1D-(^{1}H and ^{13}C) and 2D-NMR experiments as well as MS analysis. The isolated compounds mainly belong to the class of withanolides. Chemically, these are C27 oxygenated ergostane-type steroids, having a γ-lactone in the side chain. Among the isolated compounds, 1α,3β,20α_F-trihydroxy-20,22-witha-5,24-dienolide 3-*O*-β-glucopyranoside has been isolated for the first time from a natural source.

Ashwagandha, over the last three decades, has gained interest for its potential in brain-related disorders. Therefore, the acetylcholinesterase inhibitory activity of the green extract and all isolated steroids was tested by a spectrophotometric assay. Among the tested compounds, the most active compound resulted withanolide A which showed an IC_{50} value of 12.36 µM, higher than galantamine used as positive control (51.47 µM).

Subsequently, to obtain more information about the ligand-protein binding and highlight the protons of withanolide A that were in closest contact with the acetylcholinesterase protein, an STD-NMR experiment was performed. The results showed that the “pharmacophoric protons” regarded the C-2 and C-3, the epoxide (C-6 and C-7), the C-23 and the protons of the methyl group at C-27.



Withanolide A

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Metabolic profiling of *Polyporus umbellatus* (Pers.) Fr.

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Polyporus umbellatus (Pers.) Fr. (Sin. *Dendropolyporus umbellatus* (Pers.) Jülich) is an edible and medicinal mushroom in the family Polyporaceae (phylum Basidiomycota). Widely distributed in the Northern hemisphere [1], it is reported for the first time its presence in central Italy, Lazio. Even though Polyporus umbellatus has a well-documented history of use in traditional medicine, the complete phytochemical profile of both its aqueous and organic extracts has not yet been fully analyzed, particularly in samples of non-Asian origin. In this perspective, it is important to evaluate the presence of other possible bioactive compounds and investigate the quantities of metabolites with a nutraceutical impact. Pharmacologically active compounds have been identified as *Polyporus umbellatus* polysaccharides [2], steroids such as ergosterol and its derivatives, polyusterones, anthraquinones and other chemical compounds like nicotinic acid, mannitol and succinic acid [3]. High resolution NMR spectroscopy with ^{1}H-NMR, ^{1}H-^{1}H TOCSY, and ^{1}H-^{13}C HSQC was employed, finding 45 metabolites in both aqueous and organic extract, identifying and quantifying molecules belonging to different classes, such as amino acids (AA), organic acids (OA) carbohydrates, lipids and sterols among other metabolites of interest in nutrition and human health.

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Revealing the phytochemistry of the Mediterranean orchid *Himantoglossum robertianum* (Loisel.) P. Delforge sampled in different ecological habitats in Sardinia Island.

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Himantoglossum robertianum (Loisel.) P. Delforge (Orchidaceae) is a species widely distributed in the Mediterranean basin and known for its adaptability for colonizing grasslands and degraded urban lots.

Also referred to as the "giant orchid", this species stands as the earliest blooming orchid in Sardinia (Italy), and represents one of the 64 wild-occurring orchids in the island.

H. robertianum has been investigated for its essential oil, particularly for volatile organic compounds (VOCs) emitted by its inflorescences, however there is a lack of knowledge regarding leaves' chemical profiles.

The aim of this work was to explore the phytochemistry of *H. robertianum* leaves by ^{1}H NMR fingerprinting (elucidated with pre-purification of the compounds and structure elucidation performed by 2D NMR and UHPLC-MS experiments). Furthermore, the samples were harvested from six different populations growing in Sardinia, in order to estimate how the concentration of these compounds can vary in response to different growth environments.

By ^{1}H NMR profiling several primary metabolites were detected and semi-quantified such as sugars (*α*-glucose, *β*-glucose, and sucrose), amino acids (valine, alanine, trigonelline) and organic acids (malic acid, acetic acid, formic acid, citric acid).

The pre-purification procedure led to the elucidation of the aromatic region of the ^{1}H NMR profiles, finding the presence of secondary metabolites such as bis(4-hydroxybenzyl) ether, gastrodigenin and parishin A, which are all renowned for their biological activities. Among these, parishin A (72.5-31.6 µg of metabolite / mg of dried plant) and bis(4-hydroxybenzyl) ether (23.8-16.4 µg of metabolite / mg of dried plant) were the most abundant compounds semi-quantified. Flavonoids (quercetin trihexoside and kaempferol trihexoside) and other compounds of the parishin class, even if less abundant, were also detected. Notably, these metabolites are typical of *Gastrodia elata*, another orchid species, used in Chinese traditional medicine for its neuroprotective effects.

Furthermore, metabolomic analysis revealed site-specific variability, while no correlation between metabolites concentration and environmental variables was found, leading us to hypothesize the importance of microecological variability as well as biotic components such as symbiotic fungi, in determining the *H. robertianum* metabolic profile.

In conclusion, this study suggests that *H. robertianum* could be a source of bioactive compounds, particularly parishin A, gastrodigenin, and bis(4-hydroxybenzyl) ether, which were detected for the first time in this species. Moreover, these valuable therapeutic compounds were found in the leaves paving the way for a sustainable use of this plant.

Studying the biological activities of *Roccella tinctoria* DC. lichen and its main phenolic compounds.

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Lichens are fascinating organisms resulting from the symbiotic association between a fungus (mycobiont, usually an ascomycete) and an alga or cyanobacterium (photobiont) [1]. The genus *Roccella* boasts over 20 species of fruticose lichens, which contain a rich pool of phytochemicals, including depsides, monoaromatic phenols, and aliphatic acids and have been found to possess promising bioactivities, including antimicrobial, antifungal and antiproliferative ones, making them potential sources of new bioactive compounds for pharmacological purposes. Based on this evidence, present study was aimed at characterizing the phytochemical composition and bioactivities of *Roccella tinctoria* DC., a common species in Italy, known for its use as a source of purple dye [2]. To this end, two extracts, namely DF3 and DF4, from the thallus of *R. tinctoria*, obtained through a Soxhlet extraction with solvents of increasing polarity, and maceration with methanol, were used. The extracts underwent spectrophotometric, chromatographic and NMR analysis in order to characterize and purify the characteristic polyphenols, in particular erythrin, methyl orsellinate and montagnetol. Moreover, a screening of bioactivities, including radical scavenging, chelating and reducing activities, as well as cytotoxicity in diverse human cancerous and noncancerous cell lines from airway (i.e. bronchial epithelial BEAS-2B and lung cancer A549 cells) and biliary tract (i.e. H9 cholangiocytes and Mz-CHA-1 cholangiocarcinoma cells), and cytoprotection against oxidative damage induced by tert-butyl hydroperoxide (tBOOH), were performed [3]. The cytoprotective effects of both extracts and pure compounds were measured in terms of cell viability and intracellular oxidative stress. DF3 was found to contain higher levels of tannins and polyphenols than DF4, with methyl orsellinate predominant in DF3 while erythrin in DF4. Both extracts were able to scavenge both DPPH and ABTS radicals and to chelate both ferrous and ferric ions, while the pure compounds were exhibited only scavenging properties towards ABTS radical, suggesting a possible contribution in the extract. Neither extracts nor the pure compounds possessed ferric reducing activity. In the cytotoxicity assay, the extracts showed a dose-dependent cytotoxicity in A549 and Mz-CHA-1 cancer cells, with DF4 being more potent than DF3, with a less toxicity profile in noncancerous cells, while the isolated compounds, especially erythrin, showed an opposite trend. At nontoxic concentrations, the tested samples, especially DF3 and DF4 extracts, were able to affect the oxidative damage induced by tBOOH in all the cell models, by restoring cell viability in a concentration-dependent manner and reducing the intracellular ROS levels. In non-cancerous cells, DF4 was slightly more potent than DF3 and its cytoprotection was similar to positive control. A mixture of pure compounds showed a stronger cytoprotection than DF4, thus suggesting the presence of antagonistic or interfering compounds in the whole extracts. These findings are consistent with the results of antioxidant activity assays and encourage future studies to clarify the interest in *R. tinctoria* as a possible source of nutraceuticals.

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Valorization of *Olea europaea* L. tree pruning waste: optimization of phytochemicals extraction by D-optimal design approach

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Olea europaea L. is a tree species cultivated in the countries of the Mediterranean basin since ancient times, but climate change and pests are seriously hindering its cultivation. Consistently, new projects are now ongoing to reintroduce this species in new territories. Particularly, there is evidence of the presence of old olive trees in Oltrepò Pavese, a territory in Northen Italy where the climate is now favourable for its cultivation. Besides the significant efforts related to the reintroduction of the olive tree in Oltrepò Pavese, the management of these cultivations should also be deeply studied with a view to sustainable development and circular economy, which are the most important challenges of the coming years.[1]
To guarantee a sustainable management of *O. europaea* cultivations, in the present work waste as pruning wood was studied for their potential application in the pharmaceutical or cosmetic fields. Wood is a matrix particularly rich in secondary metabolites, thus representing a valuable source of phytochemicals with potential biological interest. The main limitation in using this matrix is often related to the separation of leaves and branches. For this reason, in the present work we proposed the direct use of pruned twigs as starting material for the preparation of extracts potentially endowed with biological properties, considering also the scalability of the methodology. For this purpose, we adopted a Design of Experiment (DoE) approach, and particularly a D-optimal model, that allowed us to compare the effect of three factors (temperature, time, and solvent) on three different matrices (branches, leaves, and their combination) simultaneously, employing 13 experiments. The DoE model was applied to both ultrasound-assisted extraction (USAE) and microwave-assisted extraction (MAE) with the aim of comparing the different energy sources. The responses considered were the free radical scavenging (FRS) capacity of the extracts and their content of oleuropein (HPLC-UV/PDA). Experimental conditions to maximise FRS activity and oleuropein content in all the starting biomass considered were identified. Results will be presented and discussed in due course.

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Phytochemical insights and biological activity of *Salvia karwinskii* Benth. exudate against phytopathogens and pests.

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This study investigates the phytochemical composition and biological activities of dichloromethane surface extracts from the aerial parts of *Salvia karwinskii*. The objective was to explore sustainable agricultural applications by evaluating the biological activity of the extract against bacterial, fungal phytopathogens and pests. Phytochemical analysis revealed the presence of three clerodane-type diterpenoids: hardwickic acid, involucratin C, and 6-hydroxyannonene. The exudate's minimum inhibitory concentrations (MICs) were assessed against two phytopathogenic bacteria using the microdilution broth method. The extract demonstrated moderate activity against *Clavibacter michiganensis* subsp. *michiganensis* (MIC: 500 µg/mL) and limited efficacy against *Pectobacterium carotovorum* subsp. *carotovorum* (MIC > 1000 µg/mL). Antifungal activity was evaluated *in vitro* against nine phytopathogens, compared to synthetic fungicides. The extract at 1000 µg/mL completely inhibited the mycelial growth of *Phaemoniella chlamydospora*, comparable to the synthetic fungicide Score® 25 EC. Additionally, significant inhibition of *Pythium dissotocum* (86.7%) was observed at 750 µg/mL, statistically similar to Switch® fungicide. Other notable results included 94.3% inhibition of *Stemphylium* sp., comparable to Ridomil Gold® WG. The extract also exhibited activity against *Fusarium solani*, *Alternaria solani*, *Phoma betae*, *Colletotrichum lindemuthianum*, and moderate inhibition of *Fusarium oxysporum* fsp. *lactucae* race 1. Interestingly, the mycelial growth of *Botrytis cinerea* was inhibited by 97.2% at 1000 µg/mL comparable to Switch®. Furthermore, the extract's efficacy against gray mold disease caused by *B. cinerea* on tomato fruits was assessed in preventive and curative treatments. In curative trials, the exudate at concentrations as low as 100 µg/mL achieved complete decay control, comparable to peracetic acid. However, no preventive activity was observed.

In addition, the bioinsecticidal potential of the extract was tested against *Tuta absoluta*, a major invasive pest of tomato crops. The exudate exhibited strong antifeedant and insecticidal properties, with an LC_{50} value of 0.410 mg/mL, outperforming the commercial insecticide Spinosad.

These findings highlight the potential of *S. karwinskii* exudate as a sustainable source of bioactive compounds for agricultural pest and pathogen management.

Towards eco-friendly metabolomics: a NADES-guided, standard free semi-quantitative metabolomics for *Melissa officinalis* analysis.

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In recent years, there has been a growing emphasis on the development of green extraction techniques that minimize environmental impact while maximizing yield of the extracted compounds. To this aim, in this study we investigated the potential of green solvents for extracting bioactive compounds from Melissa officinalis (MO) leaves. Specifically, we focus on the application of 20 Natural Deep Eutectic Solvents (NADES) with a relative polarity ranging from 0.34 to 1.29.

Their extraction affinity against a set of 11 plant metabolites was predicted using COSMO-RS software and experimentally validated using quantitative LC-HRMS analysis. Subsequently, the same extracts were subjected to non-target metabolomics to uncover the NADES selectivity towards the wide spectrum of MO leaf metabolites. Data preprocessing and feature alignment were performed using MZmine, and aligned features were annotated using SIRIUS+CSI:FingerID.

Overall, 249 and 195, metabolites were annotated in positive and negative ionization ion mode, respectively. Additionally, to have a more accurate view of the different NADES extraction capacity, we adopted a semi-quantitative approach that enables the prediction of concentration for all the annotated metabolites (N=444).

The results highlighted the selectivity of some NADES in extracting very diverse biochemical classes, providing valuable insights into the composition and concentration of bioactive compounds. Interestingly, thymol-menthol NADES demonstrated the ability to efficiently extract a broad range of bioactive compounds, yielding a metabolome comparable to that obtained with conventional ethanolic maceration. Overall, the entire workflow facilitated the green extraction and annotation of known bioactive molecules that had never been described in MO.

Plant products as innovative tools for honeybee health in veterinary medicine.

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Honeybee populations are declining due to the numerous threats they face, among which *Varroa destructor* plays a central role. If parasitic activity is not kept below certain threshold levels, it can lead to colony collapse. This phenomenon manifests as a complex syndrome with multifaceted symptoms.

To control *Varroa* infestations, commercially available synthetic active compounds are widely used. However, the increasing development of resistance mechanisms in the parasite is reducing the effectiveness of these formulations. Several independent studies have demonstrated the efficacy of naturally derived substances, such as essential oils (EOs), which are less likely to trigger resistance in mite populations.

In this study, we evaluated the acaricidal efficacy of two EOs, *Citrus bergamia* and *Citrus limon*, both of which previously demonstrated activity against *Varroa* mites in our residual contact toxicity tests. The assessment was conducted through semi-field and field experiments.

For the semi-field tests, two-level cages were employed. The upper level contained 20 honeybees and 10 *Varroa* mites, while the lower level was lined with filter paper saturated with different concentrations of EOs. Mite detachment from honeybees was recorded at 24 and 48 hours.

In the field experiments, cardboard strips soaked in EO solutions at varying concentrations were placed inside experimental hives. These strips were replaced weekly, and the number of mites falling onto the diagnostic bottom board was recorded. In the semi-field tests, the highest concentrations of bergamot and lemon EOs resulted in an average acaricidal efficacy of 33% and 73%, respectively. However, in the field experiments, efficacy declined, with bergamot and lemon EOs achieving average acaricidal efficacies of 50.7% and 40.3%, respectively, at the highest concentrations tested.

This study highlights the differences in acaricidal efficacy between laboratory-controlled conditions and real-world field applications. This disparity was expected given the significant differences in test environments, such as the open versus closed systems and the ventilation provided by worker honeybees within the hive. The reduced efficacy observed in the field may be partially attributed to the presence of sealed brood, which limits the penetration of EOs.

To mitigate the decline in efficacy observed when transitioning from controlled laboratory conditions to field applications, EOs should be incorporated into pharmacological carriers that minimize environmental influences on their performance. The development of innovative formulations that allow for a gradual and controlled release of EOs in the field is mandatory to enhancing their effectiveness as acaricides.

Venerdì 11 aprile

Comunicazioni orali - Sessione 5

Investigation of the molecular immune mechanisms of *Baccharis dracunculifolia* DC. through the use of an integrated in silico/*in vitro* model.

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Ethnobotany and the traditional use of medicinal plants are key starting points for the discovery and development of new therapeutic agents. *Baccharis dracunculifolia* DC., a plant traditionally used in South America to treat various pathological conditions, is gaining increasing attention for its therapeutic properties. With the aim of investigating the molecular mechanisms underlying its activity at the immune level, an integrated *in silico/in vitro* model was developed, combining the Network Pharmacology (NP) approach with data from both traditional and novel *in vitro* assays.

The phytochemical profile of the dry leaf extract of *B. dracunculifolia* (BDE) was evaluated by colorimetric and HPLC-DAD analyses, revealing a significant presence of polysaccharides, terpenoids, and polyphenols, including cinnamic acid derivatives, pinobanksin, pinocembrin, and artepillin C. These data were further extended using databases such as LOTUS, which allowed the identification of additional phytochemical constituents such as baccharin, drupanin, isosakuranetin, naringenin, and dracunculiphosides. These data were then used as input during computational analyses of the NP, which led to predictions about pharmacodynamic and pharmacokinetic processes, providing crucial information for planning subsequent experiments.

Using a platform such as SwissADME, the ability of individual molecules of the EDL phytocomplex to pass, in particular, the intestinal wall was evaluated in advance, and with the aim of assessing the bioaccessibility of the phytocomplex, these results were interfaced with those from the simulated digestion model.

Cellular biology analyses, in relation to the predictions made by NP, were initially performed on the THP-1 monocyte cell line. Using innovative equipment such as flow cytometry, cell viability, upregulation of membrane receptor expression (TLR-2 and TLR-4), and activation of major intracellular signaling pathways (p38, ERK1/2, and NF-κB) were analyzed. Both on THP-1 cells and PBMCs, biological activity was further evaluated through ELISA cytokine assays, both under basal conditions and after co-treatment with an inflammatory stimulus (LPS). Notably, a significant upregulation of cytokines such as IL-1β, IL-6, IL-2, and IL-10 was observed under basal conditions, along with an interesting downregulation of TNFα, particularly in the inflammatory stress model.

Overall, a good correlation was observed between *in vitro* studies and NP analysis, demonstrating that the integrated *in silico/in vitro* model can be an excellent approach for evaluating the biological activity of underexplored species.

Erucin ameliorates skeletal muscle dysfunction in Duchenne Muscular Dystrophy.

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Eruca sativa (arugula or cultivated rocket) is a rocket plant belonging to the Brassicaceae family, popular for its edible green leaves commonly used for salad and food dish recipes. It contains a wide range of compounds with nutraceutical and organoleptic properties, including isothiocyanates, phenolic compounds, and carotenoids. Among them, Erucin (4-methylthiobutyl isothiocyanate) is one of the major components of leaves obtained from the enzymatic hydrolysis of glucoerucin[1]. Several *in vitro* and *in vivo* studies demonstrated that Erucin exerts protective effects against several types of human cancers[2,3] as well as vasorelaxing and antihypertensive properties[4,5] due to its intrinsic capacity to slowly release H_2S[6]. Recently, it has been demonstrated that endogenous production of H_2S is impaired in mdx mice[7], a well-known genetic model of Duchenne muscular dystrophy (DMD). DMD is the most frequent X chromosome-linked disease caused by mutations in the gene encoding for dystrophin, leading to progressive and unstoppable degeneration of skeletal muscle (SKM) tissues. Based on this evidence and considering the latest guidelines for managing DMD[8] which emphasize the importance of nutrition in DMD, this study aims to evaluate the potential benefits of Erucin in addressing SKM dysfunction in mdx mice.

In vivo studies were performed on mdx mice and their littermates (WT). Mice were treated with Erucin (3mg/kg) or vehicle for 2 weeks and locomotory activity was performed. At the age of 7 weeks, the mice were sacrificed, and the quadriceps were collected. We evaluated oxidative stress and assessed mRNA levels using qPCR. Additionally, we conducted western blot analysis and performed proteomic analysis using LC-MS. Statistical analysis was carried out using either a T-test or one-way ANOVA. PEAKS Studio was used to process the mass spectrometry data.

Erucin treatment fully recovered the impaired SKM performance observed in mdx mice evaluating by rotarod and weight tests. This beneficial effect was associated with reduced oxidative stress in the quadriceps of mdx treated mice. Proteomic analysis showed that Erucin treatment increases the expression of proteins associated with myofiber development and maintenance, mitochondrial biogenesis, and ATP transport compared to the control group. Notably, mice treated with Erucin displayed elevated levels of MYH7, a marker of slow muscle fibers, as well as an upregulation of PGC-1α, a crucial regulator of mitochondrial biogenesis and oxidative metabolism.

Erucin, by releasing H_2S, reduces oxidative stress and enhances the expression of slow fibers involved in SKM resistance. Consequently, Erucin treatment improves the impaired SKM performance observed in mdx mice.

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Protective effects induced by the association of vitamin D3, vitamin K2, resveratrol, and water extracts from *Equisentum arvense*, *Crataegus curvisepala*, *Vitex agnus-castus*, and *Glycine max*.

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Postmenopausal women experience a decline in estrogen levels, which contributes to oxidative stress and reduced osteoblasts activity. Alternative therapeutic options for postmenopausal disorders can be found in medicinal plants, which generally have lower efficacy than synthetic drugs but fewer severe side effects. The combination of herbal extracts and micronutrients can enhance effectiveness, supporting bone health and reducing inflammation and oxidative stress. Additionally, traditional water-based extraction methods, such as infusions and decoctions, are biocompatible and safer for long-term use. The aim of the present study was to investigate the phytochemical and pharmacological properties of an innovative formulation constituted by vitamin K2, vitamin D3, resveratrol, and water extracts from *Equisetum arvense*, *Crataegus curvisepala*, *Vitex agnus-castus*, and *Glycine max*. Phenolic composition and radical scavenger properties were evaluated through chromatographic (HPLC-UV) and colorimetric (ABTS) methods, respectively. Biocompatibility limits were determined via brine shrimp lethality test and *Daphnia magna* cardiotoxicity assay. Protective effects were evaluated on C2C12 cell line exposed to the pro-oxidant stimulus constituted by hydrogen peroxide, and the gene expression of estrogen (ESR1), also known as Erα, and prolactin (PRLR) receptors, interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and receptor activator of nuclear factor kappa-B ligand (RANKL) was measured. The colorimetric analysis of total phenols and flavonoids revealed that phenolic compounds are over four times more concentrated than total flavonoids. This finding aligns with the chromatographic analysis, which identified phenolic acids, such as gentisic acid and p-coumaric acid, along with hydrocinnamic acids, such as caftaric acid and rosmarinic acid, as the primary phytochemicals present in the formulation. Moreover, the formulation demonstrated effectiveness as antioxidants in the ABTS assay. The tested formulation can be classified as non-toxic against *Artemia salina*, with LC_{50} value of 2.783 mg/mL. In the *D. magna* cardiotoxicity assay, the formulation at the tested concentration of 2.783 mg/mL was found to be biocompatible and non-cardiotoxic under basal conditions, although, it did not demonstrate any protective effect following the cardiotoxic stimulus induced by 10% ethanol. Considering the results of *A. salina* and *D. magna* toxicity assays, a concentration almost 3-fold lower (1000 µg/mL) was selected in order to evaluate protective effects of the formulation on C2C12 cells. The formulation was well-tolerated by the cells, in the selected concentration range (200-1000 µg/mL), without any significant alteration on cell viability. In C2C12 cells the formulation also blunted the hydrogen peroxide-induced upregulation of TNFα, IL-6, RANKL, ESR1, and PRLR; thus, further corroborating the potential inhibitory role of the formulation against the osteoclastogenic process.

The content in vitamins and total phenolic compounds, particularly hydroxycinnamic acids and resveratrol, could explain, albeit partially the efficacy of the formulation. Overall, the results of this study support the use of this formulation in reducing oxidative stress and inflammation, both of which are key drivers of the osteoclastogenic process. However, further *in vivo* and clinical studies are needed to confirm these findings and to fully elucidate the mechanisms underlying its therapeutic potential.

Hydrogen sulfide enhances the therapeutic effects of resveratrol in managing asthma symptoms.

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Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural compound found in plants such as grapes and berries, widely recognized for its antioxidant and anti-inflammatory properties. Emerging evidence suggests that resveratrol may play a role in improving lung function in the general population. Lung function impairments are often associated with conditions like asthma, a chronic inflammatory disease characterized by airway inflammation and symptoms such as coughing, wheezing, and shortness of breath. The primary challenge in asthma treatment is finding a therapeutic option that effectively alleviates symptoms while minimizing side effects. Current asthma treatments, such as β2-agonists and glucocorticoids, can have variable efficacy across individuals and are often associated with undesirable side effects. In this context, resveratrol presents a promising alternative for managing or preventing asthma symptoms. However, the clinical use of resveratrol faces significant limitations. The molecule is rapidly metabolized in the liver and intestines, resulting in low plasma concentrations. Consequently, only a small fraction of orally ingested resveratrol reaches target tissues in therapeutically meaningful amounts. This pharmacokinetic limitation reduces the efficacy of conventional oral dosing. To overcome this challenge, hydrogen sulfide (H2S), an endogenous gasotransmitter with known physiological and pathophysiological roles in the respiratory system, may offer a solution. Recent studies have demonstrated the therapeutic potential of H2S in respiratory diseases, particularly in experimental airway inflammation models (Roviezzo et al.). This led us to investigate the combined therapeutic effect of resveratrol (Resv) and an H2S donor, 4-hydroxybenzamide (TBZ), in an experimental model of asthma. In our study, mice were sensitized to ovalbumin (OVA, 100 µg), a model allergen, and treated intraperitoneally with either Resv (3 mg/kg or 10 mg/kg), TBZ (3 mg/kg), or a combination of Resv and TBZ (Resv-TBZ, 5 mg/kg). *Ex vivo* experiments demonstrated that resveratrol effectively reduced the cholinergic tone of airway smooth muscle. In *in vivo* studies, resveratrol administered at 10 mg/kg significantly reduced OVA-induced bronchial hyperreactivity (AHR) and lung inflammation, although it had no effect on allergic sensitization. Notably, the combination of Resv and TBZ exhibited a synergistic effect, reducing the production of inflammatory mediators more effectively than the individual compounds in J774 cells. In a second set of experiments, BALB/c mice treated with the Resv-TBZ combination showed superior control of OVA-induced bronchial hyperreactivity and improved salbutamol-induced airway relaxation compared to the parent compounds. Immunohistochemical analysis further confirmed the enhanced efficacy of the Resv-TBZ combination in reducing α-smooth muscle actin expression. This finding supports the combination's role in mitigating airway remodeling. Additionally, Resv-TBZ demonstrated a protective effect on airway function, correlating with restored lung structure, reduced pulmonary inflammation, and decreased mucus production compared to individual treatments. In conclusion, our results demonstrate the synergistic effects of resveratrol and TBZ in preventing the key features of asthma-like disease, including bronchial hyperreactivity and Th2-mediated inflammatory responses. These findings highlight the potential of the Resv-TBZ as an innovative therapeutic strategy for asthma management.

Cytoprotective effects of a polyphenol-based extract from *Humulus lupulus* L. against damage induced by respiratory toxicants in airway cells.

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Respiratory system, owing to its extensive gas exchange surface, is highly exposed to various pollutants, which can trigger oxidative stress, inflammation, and cell activation [1]. Among them, cigarette smoke (CS), which contains a mixture of diverse toxicants, such as benzo[a]pyrene and nicotine, causes many airway ailments by directly affecting bronchial epithelial cells, and inducing oxidative damage, inflammation and activation of signallings involved in antioxidant and inflammatory response [2]. In this context, redox-modulating agents, like herbal extracts and polyphenols, may represent promising protective strategies. In our previous studies, a commercial HOP hydroalcoholic extract from *Humulus lupulus* L. cones (Fam. *Cannabaceae*) showed cytoprotective properties towards the oxidative damage induced by *tert-butyl* hydroperoxide and influenza virus in airways cell models [3,4]. In the present study, a polyphenol-enriched hydroalcoholic extract from hop cones (namely HOPE), provided by EPO S.r.l. company, was studied for its cytoprotective effects towards injury induced by cigarette smoke condensate (CSC) from standard 3R4F cigarettes and its major toxicants, benzo[a]pyrene (B[a]P) and nicotine (NIC) in bronchial epithelial BEAS-2B cells. Moreover, a screening of antioxidant mechanisms, including radical scavenging, chelating, reducing and antiglycative was performed through standardized methods [4]. The polyphenolic profile of the extract was characterized by spectrophotometric and chromatographic analysis. The cytoprotective effects of HOPE towards respiratory toxicants were evaluated after diverse protocols of short and extended exposure. Particularly, the cells were subjected to a co-treatment of nontoxic concentrations of HOPE, selected in preliminary cytotoxicity studies, and the pollutants CSC, B[a]P or NIC. Different parameters such as cytotoxicity, oxidative state, release of lactate dehydrogenase (LDH) and pro-inflammatory mediators in supernatants were measured, according to previously standardized methods [4]. Moreover, the modulated expression of transcription factors involved in antioxidant and inflammatory response, particularly Nrf2 (nuclear factor erythroid 2-related factor 2) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), was assessed by immunofluorescence and rtPCR analysis. HOPE was found to possess marked radical scavenging and chelating activities, showing greater potency than HOP, with similar reducing and antiglycative effects. In the cytoprotective studies, HOPE showed to protect towards the cytotoxicity of CSC especially at low concentrations, also lowering ROS levels and slightly affecting the LDH release. Similar cytoprotective effects, with a marked lowering in the LDH and ROS levels, were also found against B[a]P stimulation, under most experimental conditions, and high concentration NIC. Under our experimental condition, the levels of inflammatory mediator and the expression of key factors involved in inflammation and oxidative stress were modulated. Altogether, present findings confirm the protective role of HOPE in respiratory ailments and support further research to elucidate its cytoprotective mechanisms against air pollutant-induced injuries, as well as the contribution of its identified phytochemicals.

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Beneficial effects induced by a blend of a new bromelain-based polyenzymatic complex plus N-Acetylcysteine in urinary tract infections.

Leone S, Recinella L, Libero ML, Ciaramellano D, Centulio AP, Genovesi G, Acquaviva A, Orlando G, Chiavaroli A, Ferrante C, Menghini L, Di Simone S, Brunetti L

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Urinary tract infections (UTIs) are infections that involve the urethra, bladder, and, in much more severe cases, even kidneys. These infections represent one of the most common diseases worldwide. Various pathogens are responsible for this condition, the most common being Escherichia coli (E. coli). Bromelain is a proteolytic complex obtained from the stem of Ananas comosus (L.) Merr. showing several beneficial activities. In addition to bromelain, N-acetylcysteine (NAC) has also been used. The purpose of this study was to evaluate the antibacterial, anti-motility, and anti-biofilm effects of a new polyenzymatic complex of Bromelian (1850 GDU/g, proteolytic activity as bromelain) in combination with NAC (the Formulation) on various strains of E. coli isolated from patients with UTIs. Subsequently, the anti-inflammatory and antioxidant effects of the Formulation were studied in an *ex vivo* model of cystitis, using bladder samples from mice exposed to E. coli lipopolysaccharide (LPS). Our results showed that the Formulation significantly affects the capability of bacteria to form biofilm and reduces the bacteria amount in the mature biofilm. Moreover, it combines the interesting properties of NAC and a polyenzyme plant complex based on bromelain in the right dose to affect the E. coli adhesion capability. Finally, the Formulation exhibited protective effects, as confirmed by the inhibitory activities on multiple inflammatory and oxidative stress-related pathways on bladder specimens exposed to LPS. This blend of active compounds could represent a promising and versatile approach to use to overcome the limitations associated with conventional therapies.

Comunicazioni orali - Sessione 6

Echinacea angustifolia DC. root extract: phytochemicals and molecular mechanisms in wound healing.

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Wound healing is a complex process involving a series of dynamic physiological and biochemical interactions that aim to regenerate and replace damaged connective tissue. This process includes stages such as hemostasis, inflammation, oxidative stress, proliferation, migration, and maturation[1]. During the reparative phase, fibroblast cells play essential roles, contributing to the regeneration of the skin barrier and extracellular matrix (ECM)[2]. Successful tissue remodeling leads to complete skin recovery, often without noticeable scarring. However, the repair process can sometimes result in the formation of scar tissue. In recent years, there has been an increasing interest in natural compounds as complementary therapies for wound healing, inflammation, and other skin conditions. Despite numerous ethnopharmacological studies suggesting the potential of plant-based treatments, there remains limited scientific evidence regarding their efficacy, chemical composition, and mechanisms of action.

Therefore, the innovative approach of this study lies in its comprehensive evaluation of the biological effects of *Echinacea angustifolia* root extract, combining advanced techniques such as HPLC-DAD for chemical profiling, *in vitro* assays on fibroblast cells, and analysis of key molecular pathways to provide scientific validation for the traditional use of this plant in wound healing.

Specifically, the hydroalcoholic extract (85% ethanol) of *E. angustifolia* roots (EAR) showed a polysaccharide content of 17.32 ± 1.33 mg Glu/g DW and a polyphenolic content of 50.36 ± 3.99 mg GAE/g of dried extract. Phytochemical profile analysis confirmed the presence of polyphenols, mainly caffeic acid derivatives, and the key compound, echinacoside, which was the most abundant compound. The antioxidant activity of the hydroalcoholic extract was found to be significant with a lipid peroxidation inhibition of 50.24 ± 1.38% (at 250 µg/mL) and DPPH scavenging activity of 243.24 ± 4.33 mg TE/g (IC_{50} = 36.79 ± 0.66 µg/mL) of dried extract.

In vitro experiments on 3T3-L1 fibroblast cells demonstrated that EAR significantly enhanced both cell proliferation and migration in a dose-dependent manner. EAR also exhibited anti-inflammatory effects by reducing the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, suggesting its potential to mitigate inflammation during wound healing. Additionally, the extract was found to modulate the expression of matrix metalloproteinase-9 (MMP-9) through the NF-κB signaling pathway, which is crucial for ECM remodeling.

The study further revealed that the extract's wound healing properties may be attributed to its ability to regulate growth factors like TGF-β1. In conclusion, the hydroalcoholic extract of *E. angustifolia* root shows promising wound healing potential, supported by its antioxidant, anti-inflammatory, and fibroblast-stimulating activities. These findings provide a scientific basis for the traditional use of *E. angustifolia* in wound care and suggest its potential as a natural therapeutic agent for enhancing tissue repair and regeneration.

Aliophen-XP, a patented malt- and hop-based formulation, inhibits psoriasis-like inflammation in mice.

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Aliophen® is a formulation, based on barley malts and hops, obtained by a patented production process (PCT/IB2018/056283). It possesses a high total polyphenolic content and its potential beneficial effect in disease prevention has been investigated in different preclinical models. In particular, the chemopreventive and antioxidant effects of Aliophen® formulation have been demonstrated in a mouse model of azoxymethane-induced carcinogenesis. To enhance the original formulation, through a specialized process, a highly concentrated polyphenolic composition named Aliophen-XP has been obtained. The increased concentration of bioactive molecules in Aliophen-XP offers significant advantages in terms of biological efficacy, such as enhanced chemopreventive effects on in vitro tumoral cells, without detectable toxicity on normal cells, and a robust antioxidant activity in cellular models. Psoriasis is a chronic, immune-mediated inflammatory skin disease, marked by keratinocyte hyperproliferation and abnormal differentiation. Experimental studies show that natural compounds, including polyphenols, have antinflammatory and antiproliferative effects in psoriasis like inflammation [2,3]. Here, we have investigated the effect of Aliophen-XP on immortalized human keratinocyte cell line (HaCaT) and in a murine model of psoriasis like lesion.

HaCaT cells were treated with increasing concentrations of Aliophen-XP (0.2-0.8mg/ml) and the protective effect against an oxidizing agent (tert-butyl hydroperoxide, tBHP) was assessed measuring intracellular concentration of reactive oxygen species (ROS) by CM-H2DCFDA assay. C57BL6/J male mice (8–11 weeks of age), slightly anesthetized with enflurane, received a topical application of imiquimod cream (5% w/w, 62.5 mg/ear) or vaseline (vehicle) to the external surface of the right ear, for 4 consecutive days. Group of mice received a topical application of Aliophen-XP, at two different concentrations (40 µl/mouse, 0.8 or 1.6 mg/ml dissolved in ethanol 100%) 30 minutes before each imiquimod application. Ear thickness was measured daily with a caliber. Ear erythema and scaling were scored independently on a scale ranging from 0 to 4. At the day 5, animals were sacrificed, ears and spleen were excised for further analysis.

The pre-incubation of HaCaT cells with Aliophen-XP (0.2-0.8mg/ml) for 30 minutes significantly reduced the intracellular ROS increment induced by tBHP ($p<0.005$, N=5); demonstrating the strong antioxidant effect exerted by the enriched formulation on the in vitro keratinocyte model.

Imiquimod topical application caused a psoriasis-like inflammatory lesion, characterized by increased epidermal thickening, erythema and scaling. In inflamed tissues, levels of IL-17 were significantly increased compared to control tissues. Topical treatment with Aliophen-XP as preventive intervention, reduced both clinical signs of psoriasis–like lesion and splenomegaly (delta ear thickness from 0.038 ± 0.013 mm to 0.007 ± 0.005 mm at the concentration of 1.6 mg/ml, $p<0.001$, N=3); this reduction was paralleled by decreased tissue levels of IL-17 ($p<0.05$, N=3).

Our results provide evidence for a beneficial effect of Aliophen-XP in psoriasis-like lesion in mice, highlighting new therapeutic potential of bioactive compounds contained in malt and hop.

Targeting melanoma: exploring plant extracts for tyrosinase and RAGE inhibitors

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Melanoma is an extremely aggressive form of skin cancer that originates from the malignant transformation of melanocytes. In 2020, approximately 57000 deaths were attributable to this tumor globally and this number is expected to increase by 68% in 20 years. In this pathology, many pathways are dysregulated conferring aggressiveness and resistance to chemotherapy[1]. This last property is particularly due to abnormal melanin production caused by tyrosinase overactivation[2]. Furthermore, the Advanced Glycation End-products (AGEs) have been associated with the promotion of melanogenesis, making its receptor (RAGE) a suitable target to improve patient outcomes[3].

Therefore, the objective of this research is to identify extracts with anti-tyrosinase and anti-AGE activities as a promising candidate for the prevention of melanoma or an enhancer of the available treatments.

Accordingly, thanks to the collaboration with the National Biodiversity Future Center (NBFC, https://www.nbfc.it/), 27 plant species of the Italian flora belonging to different families have been selected and collected using a taxonomic approach. All taxa have been extracted with methanol and treated to remove chlorophyll. Next, their phytochemical profiles have been analyzed, and their inhibitory effects on tyrosinase and antioxidant activities have been evaluated.

Finally, *Acanthus mollis* L. was selected both because it was one of the most active extracts and because it was included in the BelFrIt list, thus guaranteeing its safety (https://foodcomplianceinternational.com). Additionally, a literature survey allowed the identification of the metabolites produced by *A. mollis* responsible for anti-tyrosinase activity as DIBOA and Verbascoside[4].

Prompted by these results, we moved on to identify optimal extraction conditions of leaves of *A. mollis* using a design of experiments (DoE) approach, aimed to maximize the extraction of two specific metabolites. The experimental plan considered solvent-to-matrix ratio, extraction temperature, number of extraction cycles, and the percentage of ethanol in water. These conditions were applied to both Microwave Assisted Extraction (MAE) and Ultrasound Assisted Extraction (UAE). Both the models were validated, and the best extraction conditions were identified.

The extracts enriched with DIBOA and Verbascoside, are currently under investigation for their anti-AGE and anti-tyrosinase activities.

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Polydatin reduces cardiotoxicity and enhances the anticancer effects of sunitinib by decreasing pro-oxidative stress, pro-inflammatory cytokines, and NLRP3 expression

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Renal cell carcinoma (RCC) represents the main renal tumors and are highly metastatic. Sunitinib, a recently-approved, multi-targeted Tyrosine Kinases Inhibitor (TKi), prolongs survival in patients with metastatic renal cell carcinoma and gastrointestinal stromal tumors, however a dose related cardiotoxicity was well described. Polydatin (3,4',5-trihydroxystilbene-3-β-d-glucoside) is a monocrystalline compound isolated from Polygonum cuspidatum with consolidated anti-oxidant and anti-inflammatory properties, however no studies investigated on its putative cardioprotective and chemosensitizing properties during incubation with sunitinib.

We investigated on the effects of polydatin on the oxidative stress, NLRP3 inflammasome and Myd88 expression, highlighting on the production of cytokines and chemokines (IL-1β, IL-6, IL-8, CXCL-12 and TGF-β) during treatment with sunitinib. Exposure of cardiomyocytes and cardiomyoblasts (AC-16 and H9C2 cell lines) and human renal adenocarcinoma cells (769-P and A498) to polydatin combined to plasma-relevant concentrations of sunitinib reduces significantly iROS, MDA and LTB4 compared to only sunitinib-treated cells ($P<0.001$).

In renal cancer cells and cardiomyocytes polydatin reduces expression of pro-inflammatory cytokines and chemokines involved in myocardial damages and chemoresistance and down-regulates the signaling pathway of NLRP3 inflammasome, MyD88 and NF-κB.

Data of the present study, although in vitro, indicate that polydatin, besides reducing oxidative stress, reduces key chemokines involved in cancer cell survival, chemoresistance and cardiac damages of sunitinib through downregulation of NLRP3-MyD88 pathway, applying as a potential nutraceutical agent in preclinical studies of preventive cardio-oncology.

Anti-inflammatory effects of a commercial *Eleutherococcus senticosus* root extract: *in vitro* and *in vivo* evidence.

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Eleutherococcus senticosus (ES) Maxim., commonly known as Siberian ginseng, is a member of the *Araliaceae* family. Its root is widely utilized in traditional Chinese medicine. ES contains a variety of bioactive compounds, including flavonoids, triterpenoid saponins, organic acids, lignans, phenols, and polysaccharides[1]. Due to these compounds, it has been reported to exhibit various biological activities such as antifatigue, antioxidant, antiviral, neuroprotective, anti-diabetic, and anti-obesity effects[2]. Consequently, in recent years, ES has increasingly been incorporated into food supplements. In this study, we evaluated the effects of a commercial extract of ES, used as an ingredient in food supplements according to Italian D.M 10 August 2018, on regulating the inflammatory response. This was assessed *in vitro* using lipopolysaccharide (LPS)-stimulated macrophages, as well as in allergic and non-allergic *in vivo* experimental models of inflammation, including murine allergen-induced sensitization and cerulein-induced pancreatitis.

Analysis through ultra-high-performance liquid chromatography and high-resolution mass spectrometry of the commercial ES root extract revealed a distinct composition of various polyphenols, flavonoids, and saponins. The most abundant compounds identified were chlorogenic acid, neochlorogenic acid, and protocatechuic acid. We observed a significant, concentration-dependent inhibition of nitrite production and iNOS expression in LPS-stimulated cells when exposed to increasing concentrations of the ES extract (0.1-1 mg/mL, 2 hours before LPS treatment). Additionally, the extract inhibited the production of cytokines (interleukin-1β and tumor necrosis factor-α) and prostaglandin E_2 (PGE_2), without affecting cell viability. The commercial ES extract also demonstrated anti-inflammatory effects *in vivo*. In a murine model of asthma induced by ovalbumin (OVA), pre-treatment of mice with the ES extract (400 mg/kg) significantly reversed OVA-induced bronchial hyperreactivity. These protective effects on airway reactivity were closely associated with improvements in lung injury (assessed via haematoxylin and eosin H&E staining) and airway remodeling (measured by α-smooth muscle actin expression). The effects of the ES extract were linked to the inhibition of the sensitization process, indicated by reduced levels of IgE in plasma and pulmonary tryptase (a marker of mast cell activation) following OVA injection. Furthermore, the extract exhibited antioxidant effects in the lungs by increasing Nrf2 levels. Similarly, in the cerulein-induced acute pancreatitis model, the ES extract showed beneficial effects. H&E staining of the pancreas revealed inter- and intralobular edema and immune cell infiltration (neutrophilia) following cerulein injections. The extract provided protective effects, as evidenced by reductions in pancreatic edema and cell infiltration, along with decreases in circulating amylase and pro-inflammatory PGE_2 levels.

In conclusion, our data demonstrate that this commercial extract of ES exerts anti-inflammatory effects both *in vitro* and *in vivo*, suggesting its potential use in managing inflammatory diseases.

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Erucin and Sulforaphane, natural sulfur compounds for the management of metabolic disorders: evaluation of antioxidant effects and modulation of *de-novo* browning process on 3T3-L1 preadipocyte cells.

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Metabolic disorders often identify adipose tissue (AT) as the main organ involved. AT has different morpho-functional phenotypes depending on the different locations and functions. Brown adipose tissue (BAT) and white adipose tissue (WAT) establish a dynamic balance between preadipocytes and mature adipocytes leading to the identification of a beige adipocyte phenotype with middle characteristics between WAT and BAT, whose formation is allowed through the browning process.

Recent experimental evidence suggests that the modulation of the browning process may represent an interesting preventive and therapeutic approach in metabolic diseases with qualitative/quantitative alterations of AT such as obesity. Plant-based products have gained increasing interest in the last decade in the prevention of the onset and progression of pathological scenarios. Among these, isothiocyanates (ITCs), natural sulfur compounds derived from the enzymatic hydrolysis of glucosinolates contained in *Brassicaceae* edible plants, have been described for their beneficial effects in the management of several pathological alterations. ITCs sparked interest in their ability to release hydrogen sulfide (H_2S), an endogenous gasotransmitter that, among other things, plays a key role in metabolic homeostasis. The clear overlap about the effects exerted by ITCs and H_2S, provided by experimental evidence, led the researchers to investigate whether H_2S could be the "hidden" player behind the beneficial effects attributed to the consumption of *Brassicaceae*.

The aim of this work was to evaluate the impact of erucin (ERU) and sulforaphane (SFN), two isothiocyanates mainly derived from rocket salad and broccoli respectively, in the containment of oxidative stress affecting preadipocyte in metabolic diseases and in the modulation of the browning process on mouse embryonic fibroblast (3T3-L1 cells). The effects of ERU and SFN against the hydrogen peroxide (H_2O_2)-induced oxidative damage and ROS production were evaluated in preadipocyte. Following a specific differentiation protocol ERU and SFN were investigated as possible modulators of the browning process through *de-novo* differentiation. The cytological evaluation of the adipocyte phenotype, the citrate synthase activity, and the presence of the mitochondrial uncoupling protein 1 (UCP1) were carried out. The results obtained showed significant release of H_2S for ERU (100 and 300µM) and SFN (300µM) in undifferentiated adipocytes. ERU (3µM) and SFN (1 and 3µM) were able to limit the (H_2O_2)-induced oxidative damage and to contain the ROS production with a concentration-dependent trend. Morphological analysis seemed to draw out a positive and concentration-dependent effect of ERU and SFN reducing lipid deposition during the *de-novo* differentiation process and promoting the development of a brown-like phenotype. Citrate synthase activity and UCP1 levels detected at the end of adipocyte differentiation showed a positive trend for both ERU and SFN reaching a statistically significant increase for ERU tested at the highest concentration of 3µM. In conclusion, the ability of ERU and SFN to release H_2S, to positively act on oxidative stress on preadipocyte, and, at least partially, to drive the *de-novo* browning process, pointed out these natural sulfur compounds as potential modulators of metabolic alterations. In particular, ERU was a step forward in the modulation of the browning process, improving mitochondrial metabolic activity and thermogenesis pathway.

Lettura magistrale

Mediterranean diet and medicinal plants as microbiota modulators

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The Mediterranean Diet is widely recognized for its health benefits, and recently, its role in gut microbiota modulation has garnered significant interest. This presentation explores the synergistic relationship between the Mediterranean Diet and medicinal plants, emphasizing their collective influence on gut microbial composition and overall health. Rich in polyphenols and bioactive compounds, Mediterranean herbs and spices such as rosemary, turmeric, basil, sage, and garlic contribute to gut microbiota diversity and function. Some of these compounds act as prebiotics, fostering the growth of beneficial bacteria while inhibiting pathogenic species. Additionally, medicinal plants enhance microbial metabolite production, influencing metabolic pathways and immune regulation. The discussion integrates recent research on the microbiome-mediated transformation of polyphenols, the bioavailability challenges of specific compounds, and their implications for disease prevention. By highlighting the dietary and therapeutic potential of Mediterranean herbs and medicinal plants, this presentation underscores their role in promoting gut health and overall well-being.

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Comunicazioni orali - Sessione 7

Proceeding from animal to organoid models of triple-negative breast cancer to explore the therapeutic potential of erucin, an H_2S-realising compound from *Eruca sativa* Mill.

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Breast cancer remains the most prevalent type of cancer affecting women, regardless of age. Among the various subtypes, triple-negative breast cancer (TNBC) is the most aggressive form with the highest mortality rates. Currently, no effective treatments are available for TNBC, underscoring the urgent need for new therapeutic strategies, including dietary interventions[1]. Epidemiological studies have revealed that the consumption of Brassicaceae, a plant family rich in biologically active isothiocyanates (ITCs), is significantly correlated with the reduced probability of developing cancer during life. In this study, we sought to explore the potential anti-cancer effects of erucin (ERU), the most abundant H_2S-releasing ITC found in arugula (*Eruca sativa* Mill.)[2] using 4T1-bearing mice and TNBC patient-derived organoids. Our findings showed that ERU significantly reduced the proliferation of 4T1 cells in 2D cultures and also inhibited the growth of 3D spheroids in a time- and concentration-dependent manner. To further explore the effect of ERU, we employed female BALB/c mice injected with 4T1 cells and treated for 28 days, daily with oral administration of ERU (10 mg/kg). After this time, in these mice, the tumor and spleen volume and weight were significantly reduced compared to ERU-untreated mice. The tumor mass was then dissected and analysed by RT-PCR and western blot analysis. The results obtained revealed that ERU was effective in inhibiting the NF-κB signaling pathway as well as the expression of various inflammatory factors, such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNFα), and interleukin-6 (IL-6). Additionally, ERU inhibited angiogenesis in 4T1 tumors, as evidenced by reduced expression of vascular endothelial growth factor (VEGF) and its related receptor (VEGFR). Finally, we demonstrated that ERU significantly reduced growth, stemness markers, and epithelial-to-mesenchymal transition (EMT) in human TNBC organoids. In conclusion, we demonstrate that leaves of *Eruca sativa* Mill. contain ERU which is a natural molecule effective against TNBC due to its ability to release H_2S and target multiple oncogenic pathways underlying the severity and drug resistance.

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Boswellia Serrata extract as a new therapeutic strategy to slow the progression of colon cancer.

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Colon adenocarcinoma is one of the most prevalent forms of colorectal cancer and represents a significant global healthcare burden due to its high incidence and mortality rates. Despite advances in treatment, research into new, less toxic therapeutic agents, remains critical.

Boswellia serrata is a traditional medicinal plant known for its anti-inflammatory and anticancer properties. In this work, we evaluated the potential effects of Boswellia on Caco-2 cells, a commonly used model for human colon cancer. Caco-2 cells were treated with various concentrations of Boswellia extracts (200 μg/mL, 300 μg/mL, 400 μg/mL) and cell viability, migration and proliferation were evaluated using respectively the MTT assay, colony formation assay, and wound healing assay. In addition, the antioxidant and anti-inflammatory properties of Boswellia on Caco-2 cells were evaluated by ELISA assays. ZO-1 immunofluorescence was also performed to evaluate the effects of Boswellia on the membrane integrity of Caco-2 cells. The results demonstrated that Boswellia treatment led to a concentration-dependent decrease in cell viability, along with a reduction in cell proliferation and migration after 24 hours of treatment. In addition, ELISA assays suggested that Boswellia modulated markers involved in oxidative stress and inflammation. Although further investigations are needed, these findings suggest that Boswellia could serve as a potential therapeutic agent for the management of colon adenocarcinoma.

Boosting curcumin analogs' cytotoxicity with light: an *in vitro* study.

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Phytochemicals comprise a broad class of bioactive compounds naturally present in fruits, vegetables, cereals, and plants. Curcumin is one of the most extensively examined phytochemicals for its potential as both a cancer chemopreventive and chemotherapeutic agent. Nevertheless, curcumin has recently been referred to as frequently hitter or pan-assay interfering compound (PAIN). Another key property of curcumin is its inherent photosensitizing properties, as it can absorb visible light in the 300-500 nm range. This suggests its potential as a natural photosensitizer (PS) in photodynamic therapy (PDT), an approved therapeutic approach based on light-activated compounds and specific wavelengths to treat different cancer types. In this regard, numerous *in vitro* and *in vivo* studies have demonstrated that light irradiation enhances the cytotoxic effects of curcumin. Thus, the aim of this study was to assess the photosensitizing properties of novel curcumin analogs (obtained by modification of curcumin backbone for achieving specificity while retaining appropriate reactivity), along with cytotoxicity and phototoxicity on acute promyelocytic leukemia (HL-60) and breast cancer (MCF-7) cells, and underlying the molecular mechanisms involved.

To compare the cytotoxicity of curcumin derivatives under light or dark conditions, cells were treated with increasing concentrations of AP2961, AP2962, or AP2975 for 4h. They were then either exposed to low-intensity white light LED or kept in the dark for 30min. Afterwards, cells were washed and cultured in drug-free medium. Light irradiation enhanced the cytotoxic effects of curcumin's derivatives AP2961 and AP2975 on both HL-60 and MCF-7 cells. AP2975 showed the greatest phototoxic activity on HL-60 cells, thus the underpinned molecular processes were investigated in this cell line. To delve deeper into the molecular mechanisms involved in the cytotoxicity of photoactivated AP2975, we found that AP2975 significantly increased the percentage of HL-60 cells undergoing both early stages of programmed cell death (PCD) and late stages of PCD or necrosis. To explore the PCD pathway triggered by photoactivated AP2975, HL-60 cells were pretreated with different pharmacological inhibitors to hinder specific PCD, namely apoptosis, ferroptosis, and necroptosis. Apoptosis emerged as the sole mechanism underlying photoactivated AP2975's cytotoxicity, as evidenced by the enhanced cell viability observed after pretreatment with the pan-caspase inhibitor Z-VAD-fmk. Lastly, given that the production of reactive oxygen species (ROS) is the primary driver of tumor cell death induced by PDT, we assessed ROS levels in both a cell-free model and within cells. Photoactivated AP2975 generated peroxides in a cell-free system and promoted oxidative stress mediated by glutathione depletion in HL-60 cells.

In summary, our findings suggest that the novel curcumin analogs possess photosensitizing properties like curcumin, making them potential PS for PDT. Moreover, light exposure notably increased the cytotoxic effects of AP2975 in cancer cells, which can be attributed to its pro-apoptotic activity and the induction of oxidative stress.

Genistein and curcumin inhibit proliferation and invasiveness in BRAF^{V600E} mutant and wild-type melanoma cells: insights of the anticancer effects.

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Melanoma is one of the most deadly form of malignant cancers; ultraviolet radiation exposure together with genetic mutations, such as the BRAF ones, represent important risk factors and are involved in melanoma onset as well as in metastatic dissemination. The treatment of melanoma improved with monoclonal antibodies, such as BRAF inhibitors, that fail to increase patient survival and cause adverse effects in addition to being expensive. The discovery of innovative therapies would guarantee better results and would increase survival rate: a growing interest was addressed to nutraceuticals thanks to their anti-emetic, anti-oxidant and anti-proliferative effects, such as genistein and curcumin. This study aimed at investigating the possible anticancer effects of curcumin, genistein and their association in melanoma cells.

Human A375 (BRAF-mut) and CHL-1 (BRAF wild-type) cell lines were cultured and then treated with curcumin (25 □M; purity ≥80%, Sigma-Aldrich, USA) dissolved in DMSO or genistein (100□□M; purity ≥98%, Primus Pharmaceuticals, USA) dissolved in DMSO or curcumin+genistein (25+100□□M) for 24 hours: concentrations were chosen in accordance with cell viability assays for IC_{50} calculation.

Genistein and curcumin induced cell death in BRAF- mut and wild-type cell lines, as demonstrated by MTT assay and FDA/PI staining. The anti-apoptotic protein Bcl-2 expression was significantly reduced after curcumin and curcumin+genistein treatment, but unexpectedly not with genistein alone. Curcumin and genistein significantly increased DNA fragmentation in both cell lines, thus indicating apoptosis induction. Moreover, comet Assay confirmed that curcumin and genistein significantly stimulated cell death, as quantified by measuring the displacement between the genetic material of the nucleus ('comet head') and the resulting 'tail'.

Focal adhesion kinase (FAK) protein expression, studied for its invasion potential, was significantly reduced by genistein and curcumin in CHL-1 cells and after the treatment with genistein+curcumin in the most aggressive A375 cell line. These anti-proliferative effects were confirmed by scratch assay and phospho-p38 reduction. In addition, both curcumin and genistein alone and in association inhibited cell adhesion, thus indicating that these nutraceuticals could reduce invasion and metastasis.

The results obtained so far are promising and provide new insights for the anticancer effects of genistein and curcumin which could be used to improve therapeutic adherence and traditional drug response. However, further studies could clarify their effects in order to set up clinical trials for a future use in the clinical practice.

Secoiridoid-enriched extra virgin olive oil extracts enhance mitochondrial activity and antioxidant response in colorectal cancer cells: the role of oleacein and oleocanthal in PPARγ interaction.

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The secoiridoid-enriched fraction of extra virgin olive oil (EVOO) provides health benefits, but its underlying mechanisms remain unclear. To investigate, we analyzed the transcriptome of HCT116 colorectal cancer cells treated with secoiridoid-enriched extracts and identified genes of mitochondrial pathways. In vitro validation showed increased mitochondrial mass, driven by enhanced biogenesis and fusion, alongside higher respiration and ATP production. This led to increased reactive oxygen species, activating AMPK, NRF2, and antioxidant genes, along with PGC-1α. We focused on Oleacein (OL) and Oleocanthal (OC), two components of the extracts. Molecular docking and dynamics simulations predicted both compounds bind to PPARγ, with OL showing stronger affinity. Isolated OL and OC replicated the extract's effects; chemical inhibition and PPARγ silencing reduced them, confirming PPARγ's role. This study reveals the AMPK-PGC-1α-PPARγ axis as a key regulator of OL and OC's mitochondrial and antioxidant effects, supporting their potential as nutraceuticals and PPARγ modulators in therapeutic strategies.

Comunicazioni orali - Sessione 8

Protective effects of oleoylethanolamide on kidney dysfunction associated with obesity and metabolic disorders.

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The well-established pathophysiological connection between obesity and chronic kidney disease (CKD) stems from metabolic alterations caused by obesity. These alterations drive kidney damage and dysfunction through mechanisms such as lipotoxicity, chronic inflammation, and fibrosis.Oleoylethanolamide (OEA) is a naturally occurring endogenous lipid mediator that belongs to the N-acylethanolamine (NAE) family. It is synthesized in living organisms, including humans and animals, from natural precursors found in the body and the diet. Its derivation can be attributed to biochemical pathways involving lipids and fatty acids, particularly oleic acid, a monounsaturated fatty acid abundant in various natural sources like olive oil, nuts, seeds, and avocados. OEA has been widely studied for its metabolic benefits. However, emerging evidence highlights its significant anti-inflammatory and anti-fibrotic properties. This study explored the impact of OEA on renal damage induced by a high-fat diet (HFD) in obese mice.

OEA treatment significantly improved kidney function by restoring urine output and reducing proteinuria and albuminuria. It normalized serum creatinine and blood urea nitrogen levels, which were disrupted by HFD feeding, and downregulated key renal injury markers, such as kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin. OEA also ameliorated renal glucose dysmetabolism by reducing glycogen accumulation and modulating glucose transporter protein expression. In addition to its metabolic effects, OEA exhibited potent anti-inflammatory and anti-fibrotic activities in renal tissues. These benefits were demonstrated by reduced transcription of pro-inflammatory and pro-fibrotic markers. OEA also mitigated renal steatosis, improving lipid metabolism by upregulating peroxisome proliferator-activated receptor-α (PPAR-α) and fibroblast growth factor 21 (FGF21). Furthermore, it decreased triglyceride trafficking via the downregulation of diacylglycerol O-acyltransferase 1 (DGAT1). To verify its direct effects on the kidney, we demonstrated that OEA protected renal tubular cells (HK-2) from palmitate-induced lipotoxicity, as evidenced by Oil Red O staining. Furthermore, we showed that OEA significantly enhanced mitochondrial bioenergetics using Mito Stress assay by Seahorse analyzer.

In summary, OEA demonstrates a multifaceted ability to counteract CKD-related alterations linked to obesity by improving kidney function, mitigating inflammation, reducing fibrosis, and restoring lipid and glucose metabolism. These findings suggest that OEA is a promising candidate for therapeutic intervention in obesity-associated CKD and related metabolic disorders.

Valorization of two endemic Italian plants by the bioaccessibility of the metabolites present in their hydroalcoholic extracts using an *in vitro* digestion model.

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Traditional knowledge of medicinal plants is invaluable, especially since it includes the use of endemic species located only in certain areas. Preserving this knowledge is crucial to ensure that their benefits are not lost over time, and the use of innovative techniques can help us to confirm their qualities to enhance them [1]. This study focuses on two endemic plants: *Ptilostemon casabonae* (L.) Greuter and *Achillea erba-rotta* subsp. *moschata* (Wulfen) I.Richardson.

P. casabonae is a thistle-like plant native to Sardinia [2], while *A. erba-rotta* subsp. *moschata* is an herbaceous perennial plant growing in the Alps above 1800 meters[3]. These two endemic species have in common their digestive properties, which have supported their traditional use in the first case by consuming the leaves raw or cooked[2], and in the second case in the form of alcoholic and non-alcoholic drinks[3]. Recently, *P. casabonae* hydroalcoholic extract exhibited an interesting inhibitory activity towards alpha-glucosidase higher than that of acarbose[4] while no information are present for *A. erba-rotta* subp.*moschata*.

The aim of this work is to study the stability and the bioaccessibility of the specialized metabolites present in the the hydroalcoholic extracts of these endemic plants. The extracts were therefore subjected to a simulation of *in vitro* digestion using INFOGEST protocol[5] to verify whether the extracts undergo quali-quantitative changes in the phytochemical composition and whether the properties are maintained after the simulated gastrointestinal digestion, such as the inhibition of alpha glucosidase activity.

HPLC-PDA-MS/MS analysis revealed a complex polyphenolic fraction for both species. Consistent with existing reports on related species, flavonoids, particularly quercetin *O*-glycosides, and caffeoylquinic acid derivatives were identified in the hydroalcoholic extract of *P. casabonae*[2]. Apigenin, luteolin, myricetin, and their *O*-glucosides derivatives were also present in the hydroalcoholic extract of *A. erba-rotta subsp. moschata*. During the intestinal phase of the extract of *P. casabonae* and *A. erba-rotta subsp. moschata*, significant differences were observed for chlorogenic and cryptochlorogenic acids, as well as dicaffeoylquinic acid, with the appearance of new compounds and changes in their abundances, likely due to isomerization and degradation[6]. Total phenolic compounds were measured by the Folin-Ciocâlteu spectrophotometric method before and after the simulated digestion process to calculate the bioaccessibility. The bioaccessibility index of the *P.casabonae* extract is 71.04%, while for *A.erba-rotta* subsp. *moschata* is 59.10%.

Alpha glucosidase inhibitory activity was tested for both indigested extracts. After testing different concentrations of the extracts and of acarbose, which is the positive control, the IC50s were calculated. The IC50 of acarbose was 67.95 µg/mL, for the extract of *A. erba-rotta* subsp. *moschata* and *P. casabonae* it was 173.32 µg/mL and 28.23 µg/mL respectively. Due to the lower IC50, compared to the reference drug, *P. casabonae* was selected for further testes. The digested extract of *P. casabonae*, after purification of the enzymatic and biliary component, mantained the inhibitory activity for alpha glucosidase.

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Fructooligosaccharides mitigate metabolic disruptions induced by chronic fructose or galactose consumption in rats by reducing the accumulation of advanced glycation end products.

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A diet high in fat and sugar may contribute to chronic low-grade inflammation, which can lead to various metabolic disorders. Additionally, a diet enriched in reducing sugar (such as glucose and galactose) promotes the formation of advanced glycation end products (AGEs), which further exacerbate oxidative stress and inflammation, thus contributing to metabolic dysfunction [2];[3]. This research focuses on the evaluation of the metabolic effects of two widely consumed simple sugars, fructose and galactose, when added to a high-fat diet. Furthermore, it explores whether plant- derived fructooligosaccharides (FOS), a type of fermentable dietary fiber, can mitigate potentially harmful effects [3].

Six groups of male Sprague-Dawley rats (six per group) were fed the following diets for 8 weeks : a control group consuming a diet with 5% fat (CNT), a high-fat diet group with 20% fat (FAT), a high-fat diet supplemented with 10% FOS (FAT+FOS), a high-fat diet combined with 25% galactose (FAT+GAL), a high-fat and galactose diet with 10% FOS (FAT+GAL+FOS), a high-fat diet including 25% fructose (FAT+FRU), and a high-fat plus fructose diet with 10% FOS (FAT+FRU+FOS). At the end of the in vivo protocol, rats were sacrificed and the organs collected. Statistical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test, with the significance set at $p < 0.05$.

None of the dietary interventions significantly impacted body weight gain, blood glucose, or systemic inflammation markers. However, rats consuming high-fat diets with added fructose or galactose exhibited considerable increases in plasma triacylglycerol, cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels compared to the control group. Furthermore, both fructose and galactose consumption led to excessive lipid accumulation and elevated levels of AGEs in the liver and skeletal muscle [2],[3]. Interestingly, supplementation with FOS effectively prevented these metabolic disruptions.

This research highlights the detrimental impact of chronic consumption of simple sugars, particularly fructose and galactose when paired with high-fat diets. The findings underscore the potential of FOS as a protective dietary intervention, as it helps counteract the metabolic impairments induced by these sugars. These results contribute to a broader understanding of how dietary components influence metabolic health and emphasize the importance of dietary fibers in mitigating the harmful consequences of modern high-fat, high-sugar diets.

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Effect of N-acylethanolamines mixture (Olaliamid®) on fat-to-heart crosstalk compromised by obesity: *in vivo* and *in vitro* evidence.

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Obesity has a profound impact on heart function, primarily due to the accumulation of fatty acids and resulting disturbances in cardiac energy metabolism. These detrimental changes compromise mitochondrial function, which is essential for energy production in the heart. In this context, adipose tissue plays a key role in regulating cardiac function due to its capability to release bioactive molecules and cytokines that can influence lipid deposition and promote cardiac inflammation. N-acylethanolamines (NAEs) are a class of endogenous lipids, which are involved in maintaining cellular homeostasis and exert a plethora of pharmacological effects, modulating inflammation, pain, food intake, and neuronal functions. Here, we investigated the cardioprotective effect of an olive oil-derived NAE mixture OLALIAMID® (OLA) in reducing tissue damage caused by high-fat diet (HFD) feeding in mice. Male C57Bl/6J mice (6-week-old) were divided into three groups: (i) control group receiving standard chow diet and vehicle; (ii) HFD group receiving vehicle; (iii) HFD mice treated with OLA (10 mg/kg /die per os). At the sacrifice, the heart tissue was collected for following molecular determinations.

First, we demonstrated that OLA treatment improved cardiac lipid metabolism altered by HFD, reducing fatty acid synthesis and influx as shown by the decreased mRNAs of FASN, CD36 and DGAT1 as well as PPARγ. This was confirmed by the increased phosphorylation of AMPK, a key sensor involved in fatty acid β oxidation, evaluated by Western blot analysis. Consistently with lipotoxicity reduction, we observed OLA anti-inflammatory and anti-fibrotic effects, reducing the transcription of Il-1β and fibronectin.

Moreover, given the connection between adipose tissue and heart, we explored OLA effect on metabolic and mitochondrial function of mature adipocytes. 3T3-L1 mature adipocytes were pre-treated with individual NAEs (3 µM) or their combination OLA, (1–3 µM), for 1 hour before the challenge with 100–200 µM palmitate (PA). Mitochondrial bioenergetics was assessed using the SeaHorse XFe24 metabolic analyzer. The obtained data showed that OLA treatment improved mitochondrial bioenergetics, leading to enhancements in both basal and maximal oxygen consumption, proton leak, and ATP-linked respiration. These latter analyses were assessed using oligomycin and FCCP, which differently target the complexes of mitochondrial respiratory chain. Consistently, OLA upregulated key genes involved in lipid metabolism and mitochondrial function of adipocytes, including PPARα, carnitine-palmitoyltransferase (CPT)1, and a marker of mitochondrial biogenesis such as the co-activator of PPAR-γ, PGC1α. Moreover, OLA mixture significantly enhanced adipocyte storing function, as evidenced by macroscopic evaluations and increased expression of PPAR-γ, a crucial marker of adipogenesis. Remarkably, it promoted the conversion of the white adipocyte phenotype into a brown-like one, inducing the expression of thermogenic genes.

Our results indicate OLA multitarget effects in improving fat-to-heart crosstalk altered by lipid overnutrition, suggesting its possible application in the treatment of cardiometabolic syndrome related to obesity.

Cyanidin-3-O-glucoside beneficial effects against intestinal barrier injury induced by indomethacin.

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of pain and inflammation, but high doses or chronic administration can trigger serious adverse effects affecting the gastrointestinal (GI) mucosa, such as erosions and ulcers. Proton pump inhibitors are effective drugs for NSAID-induced gastropathy, but not for correlated enteropathy. The intestinal cytotoxic effects of NSAIDs are related to mitochondrial dysfunction, oxidative stress, inflammation, and altered integrity of epithelial barrier, which can represent an important risk factor for the development of various intestinal disorders, including Crohn's disease, ulcerative colitis, enteritis, and infections. Current research is focused on natural products as protective agents for therapy and prevention of NSAID-induced intestinal injury. Among these substances, particular interest is addressed to anthocyanins, a class of flavonoids broadly distributed in the Mediterranean diet, responsible for blue, purple, and red colour of plants, flowers, and fruits, such as berries, grapes, and red cabbage. These molecules are widely studied for their antioxidant, anti-inflammatory, anti-cancer, antimicrobial, and anti-obesity properties, and for their potential application in the prevention of heart diseases, cognitive disorders, and intestinal inflammation disturbances, thanks also to their good bioavailability in human body.

In this study, we used a model of intestinal epithelial cells injury induced by the NSAID indomethacin (INDO), to investigate the *in vitro* protective effects of cyanidin-3-O-glucoside (C3G), one of the most widespread anthocyanins.

Caco-2 human intestinal epithelial cells were cultured for 18 days post confluence to obtain fully differentiated cells. Subsequently, cells were pre-treated with C3G (20 and 40 µM) for 24 hours and then exposed to INDO (1 mM) for 24 hours to simulate the cytotoxic events related to NSAID-induced intestinal damage. Monolayer integrity was evaluated by trans-epithelial electrical resistance (TEER) measurement and by fluorescein permeability analysis. Reactive oxygen species (ROS) and Total Antioxidant Activity (TAA) levels evaluation were used to clarify C3G protective effects on INDO-induced oxidative stress. Proteins and genes involved in epithelial barrier functionality and in inflammatory response were determined by Western blot and Real time PCR techniques.

Pre-treatment with C3G dose-dependently prevented INDO-induced intestinal epithelial barrier damage, as demonstrated by increased TEER values, decreased fluorescein permeability across the monolayer, and improved expression of tight junctions' proteins. Moreover, C3G showed beneficial effects on the intracellular redox status, reducing ROS production and increasing TAA values. Furthermore, C3G dose-dependently inhibited the pro-inflammatory cascade induced by exposure to INDO, as demonstrated by the inhibition of NF-κB pathway, with decreased p65 nuclear translocation, IKK phosphorylation, and IL-6 and TNF-α gene expression.

Finally, in this study we demonstrated the protective effects of C3G against INDO-induced intestinal epithelial barrier injury, maintaining intestinal membrane integrity and cellular redox homeostasis, and inhibiting inflammatory response. Therefore, these results suggest that this anthocyanin may represent a natural approach for preventing and treating GI side effects related to NSAID administration.

Comunicazioni orali - Sessione 9

Evaluation of the efficacy of *Zingiber officinale* Roscoe, and its main terpene component, zingiberene, in modulating microglial senescence.

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Zingiber officinale Roscoe is an herbaceous perennial plant belonging to the *Zingiberaceae* family, widely distributed in Southeast Asia. The main components are terpenes, such as sesquiterpenes, the principal is zingiberene, and gingerols and shogaols[1]. There are many studies in the literature demonstrating the therapeutic potential of *Zingiber officinale* Roscoe as an anti-inflammatory, antioxidant, analgesic and anti-senescence agent[2]. Inflammation and cellular senescence are closely related to age-related and neurogenerative disorders[3], and are significant modifier of microglial functions[4]. Microglia cells are considered resident macrophages of the central nervous system (CNS), where represent 10-15% of immune cells[5]. Senescent microglia are characterized by morphological changes ("dystrophic" morphology), DNA alteration, a major activity and expression of β-galactosidase and a massive release of SASP (secretory phenotype associated with senescence) factors including pro-inflammatory cytokines[6]. So, targeting microglial senescence could be a possible intervention to modulate the development of these diseases[7]. In this work we investigated the effect of *Zingiber officinale* Roscoe, and its main terpene component, zingiberene, in reducing microglial senescence in an *in vitro* model of senescence, and in an animal model of chronic multiple sclerosis, Experimental Autoimmune Encefalomielytis (EAE).

In this work we investigated the effect of *Zingiber officinale* Roscoe (ZOE, 10 µg/mL) and zingiberene (ZNG, 1 µg/mL, the concentration presents in the extract) in an *in vitro* model of microglial senescence, treating BV2 cells with LPS 500 ng/mL for 10 days (4h/day) every 72h. At the end, specific tests were performed to evaluate the development of senescence and neuroinflammation and the effectiveness of the treatments. Then we investigated their therapeutic potential in the Experimental Autoimmune Encefalomielytis (EAE) model in modulating cellular senescence along with the development of clinical symptoms.

In the *in vitro* model, treatment with ZOE and ZNG were able to modulate cellular senescence parameters by reducing the expression and activity of β-galactosidase, increasing cell viability and reducing cell branching, typical of dystrophic microglial cells. Evaluation of SASP factors, showed a positive effect of the treatments in reducing these markers. In the *in vivo* model, treatment with ZOE (200 mg/Kg) and ZNG (20 mg/Kg), was able to reduce some cellular senescence markers in the spinal cord (p16, β-galactosidase) along with the management of clinical symptoms, such as motor disability and pain hypersensitivity.

In conclusion, we can say that ZOE and ZNG possess senomorphic activites and are able to reduce microglial senescence both *in vivo* and in the *in vitro* model. Therefore, we hypothesize that these treatments may be promising candidates for the management of age-related disorders associate with neuroinflammation and cellular senescence.

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Neuroprotective effects of *Actaea racemosa* in an *in vivo* model of spinal cord injury.

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Spinal cord injury (SCI) is a traumatic event that leads to substantial and often permanent impairments in both motor and sensory functions which triggers an inflammatory response and glial cells activation. The resulting inflammation can exacerbate functional impairments by promoting the formation of scar tissue and causing necrosis or apoptosis of neurons and oligodendrocytes, which leads to rapid cell loss at the injury site. To date, there are no effective treatments for SCI, making it one of the most difficult research challenges.

In the last years, there has been increasing interest in natural substances as potential remedies to alleviate or reduce symptoms associated with central nervous system (CNS) injuries. One such substance is *Actaea racemosa* (also known as *Cimicifuga racemosa*), a medicinal plant commonly used as a herbal remedy and dietary supplement, well-known for its high content in phenols, flavonoids, and alkaloids. Given these findings, this study aims to evaluate the neuroprotective effects of *Actaea racemosa* in an *in vivo* model of SCI.

SCI was produced in mice by extradural compression at T6-T7 vertebrae using an aneurysm clip for 1 minute. After 24 hours, spinal cord tissues were collected to perform several analyses. One of the main consequences that occur following a SCI is the loss of neuromotor function. For this purpose, a Basso mouse scale (BMS) test was performed just before the sacrifice to assess the effect of *Actaea racemosa* on motor function, showing that *Actaea racemosa* at the dose of 100 mg/kg was able to significantly restore motor function post-injury. Later, we performed a hematoxylin and eosin (H&E) analysis in order to evaluate tissue morphology following trauma. *Actaea racemosa* at the dose of 100 mg/kg was able to reduce tissue alteration of the perilesional area, restoring tissue architecture post-injury. Post-traumatic inflammation is marked by the infiltration of various immune cells, particularly neutrophils, mast cells, and macrophages, which migrate to the area surrounding the injury site. Accordingly, we decided to evaluate the mast cell amount by using toluidine blue staining, showing that *Actaea racemosa* significantly reduced the number of mast cells in spinal cord tissues. Moreover, we assessed the effects of *Actaea racemosa* on oxidative stress (OS) process. Our findings demonstrated that *Actaea racemosa*, administered at a dose of 100 mg/kg, effectively reduced the levels of malondialdehyde (MDA), a key marker of OS, and enhanced the activity of important antioxidant enzymes, including Copper Zinc Superoxide Dismutase (Cu/ZnSOD), Catalase (CAT), and Nuclear Factor Erythroid 2 Related Factor 2 (Nrf2). Moreover, *Actaea racemosa* demonstrated to modulate the levels of two important pro-inflammatory enzymes like inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) as well as the levels of inflammatory cytokines as interleukin-1b (IL-1b) and tumor factor necrosis-a (TNF-a) in spinal cord tissues, counteracting inflammatory process. Therefore, based on these findings, we can assume that *Actaea racemosa* could be an alternative therapeutic strategy for reducing OS and inflammation in SCI patients, potentially improving their quality of life. However, further studies are required to better understand its molecular mechanisms in spinal trauma.

Photochemical behavior and degradation products of natural cannabinoids.

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Cannabinoids have recently garnered significant attention due to their potential applications, ranging from medical use to cosmetics [1, 2]. In this context, an impressive number of compounds have been discovered and, considering the widespread diffusion of preparations intended for human use containing cannabinoids, there is a pressing need for data about their stability, degradation processes and by-products.

While thermal and biological inspired transformations of cannabinoids have been investigated, available data on their photoreactivity are scarce, outdated and, in some cases, conflicting [3].

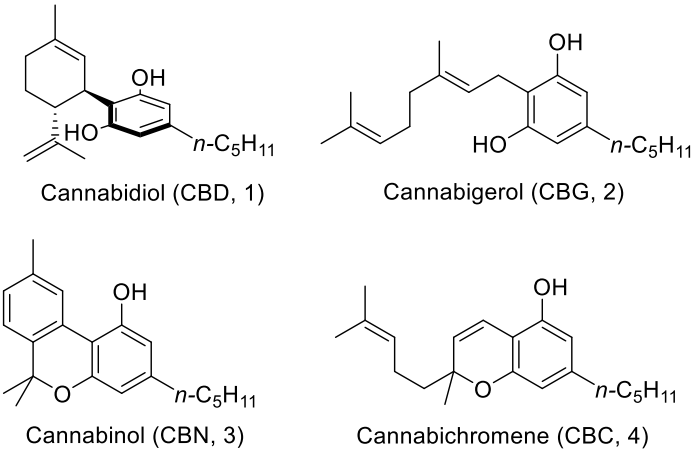

Fig. 1 *Structure of investigated cannabinoids*

In view of these premises, we focused on the photochemistry of cannabinoids in order to investigate the chemical paths involved in the photodegradation processes and the nature of the obtained products. Thus, irradiation of cannabidiol (CBD, **1**), cannabigerol (CBG, **2**), cannabinol (CBN, **3**) and cannabichromene (CBC, **4**) in different organic solvents has been performed. The photochemical behavior of these compounds in the chosen solvents has been thoroughly investigated by means of GC-MS analyses, products characterization, and the result compared with those obtained under thermal conditions or in the presence of acid catalysts [3, 4, 5]. The photochemical degradation of cannabinoids in solid matrix was also tackled by irradiating different types of decarboxylated vegetable *Cannabis*, the flowering plant historically acclaimed for being able to produce cannabinoids. Investigation of the photodegradation in the vegetable matrix allowed the assessment of the photodegradation profile of the strands examined, thus providing more data on the optimal storage conditions for *Cannabis* [6].

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Study on the neuroprotective activity of olive leaf extract from pruning waste against different types of stress in SH-SY5Y cells.

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Stress is a natural mechanism occurring in the body in response to extremely varied perturbations, with the ultimate aim of re-establishing homeostasis. The response to a stressful stimulus occurs at the whole-organism level, but also at the cellular level. In the last ten years, the study of substances effective in normalizing stress response – adaptogens – has provided interesting indications about herbal products with the peculiar ability to regulate stress response, but this is one of the topics where clinical outcomes are far to be fully elucidated.

This work aims to deepen these types of investigations and therefore models of cellular stress have been developed by studying for the first time vegetable substances derived from by-products, such as olive leaves. Olive leaf extracts (OLEs) were analysed in *in vitro* models of various cellular stresses on the neuronal cell line SH-SY5Y (human neuroblastoma).

Starting from pruning waste of *O. europaea* of different varieties from different Italian regions, two types of OLEs were produced: macerated in 70% v/v ethanol and decoctions. Their content in total polyphenols, total triterpenes and oleuropein have been characterized, through colorimetric assays and HPLC-DAD analysis of the phenolic fraction; OLEs were compared with standardized dry extract of the US Pharmacopoeia to verify and confirm their phytochemical potential. From these analyses, we found that extracts produced from pruning waste have an important percentage of oleuropein, polyphenols and triterpenes, and a phytocomplex that is perfectly comparable in terms of quality with that of the Pharmacopoeia extract. Considering that olive leaf antioxidant power is the most consolidated data from the literature, the anti-radical and antioxidant power of the extracts was quantified through DPPH and ORAC assays: it has been confirmed that even if produced from by-products, the extracts have an extraordinary antioxidant capacity; that of ethanolic extracts is slightly higher than that of decoctions, reflecting the respective quantities of polyphenols and oleuropein.

The pharmacokinetics of the main bioactive compounds of olive leaves were also examined: oleuropein is more than 70% stable after the digestive process, while hydroxytyrosol – the main product of the degradation of oleuropein, as well as the most antioxidant molecule contained in olive leaves – is more absorbed at the intestinal level and through the blood-brain barrier compared to oleuropein, being in fact more bioavailable.

Subsequently, OLEs were tested on SH-SY5Y cells to evaluate their cytotoxicity at 4h and 24h and it emerged that they are very well tolerated even at high concentrations. Through the development of a series of *in vitro* neuronal stress models, this study made it possible to demonstrate that olive leaf extract has notable neuroprotective properties against stress of different types, in particular: oxidative stress, cortisol, glutamate and serum deprivation. This was verified by cytotoxicity assays, quantification of intracellular ROS, and the study of ATP metabolism in relation to stressors. Furthermore, the molecular mechanisms linked to the regulation of the endocannabinoid system, heat shock proteins and neurotrophins were explored, leading to the awareness that olive extract modulates all these pathways, promoting a more efficient cellular response to stress.

Treatment with a mixture of standardized extracts obtained from *Centella asiatica*, *Echinacea purpurea* and *Zingiber officinale* prevents behavioural and neurochemical alterations induced by chronic social defeat stress in mice.

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Exposure to repeated social stress is a risk factor for the onset of several disorders, in particular psychiatric disorders such as anxiety and depression. Promoting resilience to stress is crucial to reduce stress-induced negative outcomes, and in this context, therapies based on natural products may acquire paramount importance.

Here we studied the preventive effects of a repeated treatment (p.o. daily, 3 weeks) with a combination of *Centella asiatica* (200 mg/kg), *Echinacea purpurea* (20 mg/kg) and *Zingiber officinale* (150 mg/kg) standardized extracts, on the chronic social defeat stress (CSDS) deleterious outcomes. After 10 days of CSDS exposure, male mice' performances were evaluated in paradigms relevant for social (social interaction test), emotional (tail suspension test), cognitive (novel object recognition) domains as well as for pain perception (cold plate and von Frey tests) and motor skills (rotarod). Mice were then sacrificed, the spinal cords, hippocampi and frontal cortices dissected and processed for RT-PCR analysis.

Extracts mix treatment prevented stress-induced social aversion, memory impairment, mechanical and thermal allodynia and reduced behavioural despair independently of stress exposure. The treatment stimulated hippocampal and cortical BDNF and TrkB mRNA levels and counteracted stress-induced alterations in pro- (TNF-α, IL-1β and IL-6) and anti-inflammatory (IL4, IL10) cytokines expression in the same areas. It also modulated expression of pain related genes (GFAP and Slc1a3) in the spinal cord.

The treatment with the extracts mix obtained from *C. asiatica*, *E. purpurea* and *Z. officinale* may represent a promising strategy to promote resilience and prevent the deleterious effects induced by extended exposure to psychosocial stress.

Cannabidiol-loaded cationic vesicles loaded in a thermosensitive hydrogel: an efficient nanoplatform for successful intranasal administration in a rat model of hydrocephalus.

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Cannabidiol (CBD) is the main non-psychoactive phytocannabinoid derived from Cannabis sativa L., acting indirectly on the endocannabinoid system, inhibiting FAAH, promoting anandamide accumulation, and interacts with key molecular targets such as PPARγ, 5-HT1A, GRP55, A2A, and TRPV1, contributing to anti-inflammatory, analgesic, and neuroprotective effects. However, its poor solubility, low bioavailability, instability, and significant first-pass metabolism limit its therapeutic efficacy [1].

To overcome these challenges, in this study CBD was encapsulated in nanovesicles and administered via the Nose-to-Brain route. Indeed, nanocarriers can overcome CBDchallenges [2] and intranasal drug delivery for CNS disorders offers advantages such as accessibility, non-invasiveness and high brain bioavailability, even if the limited delivery volume, mucus barriers and drug degradation remain as possible challenges.

Intranasal administration can ensure direct drug delivery to the CNS via olfactory and trigeminal pathways, enhancing therapeutic efficacy while avoiding systemic degradation. Although nasal drug delivery faces challenges such as mucociliary clearance and enzymatic degradation, the thermosensitive gel nanovesicles mitigate these issues by prolonging nasal retention, enhancing permeation. This innovative system represents a promising strategy for targeted CBD delivery to the brain, improving its therapeutic potential in neurological and inflammatory disorders, potentially maximizing brain bioavailability while bypassing hepatic metabolism. In the present study CBD-loaded cationic vesicle was developed. DOTAP Chloride (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride) is a widely used cationic lipid to impart the positive charge which can interact with mucin to further improve mucoadhesion and penetration across the mucus layer, prolong the CBD release from the formulation. Nanovesicles were fully characterised (97.0±2 nm, PdI 0.270±0.017, EE% 88.4±4.2%, and ζ-potential 49.0±3.2 mV). The vesicles were than loaded into a thermosensitive hydrogel (VS-CBD-TG) using poloxamer 407/188. Upon hydrogel formation, a 20% increase in size and a halving of the zeta potential were observed, while PdI remained stable and EE% decreased only by 10%. Based on Poloxamer 407 and Poloxamer 188, a thermosensitive hydrogel with transitions from liquid at room temperature to a gel at 34 °C, the nasal cavity temperature.

The pseudoplastic gel showed great mucoadhesion on mucin disks and syringability (Figure 1).

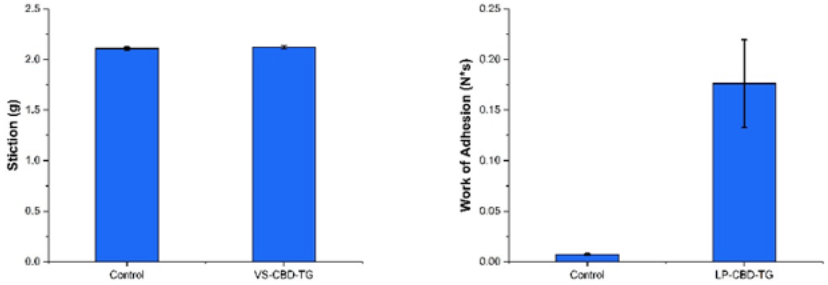

Figure 1. VS-CBD-TG siringability (A) compared to H 2 0 and mucoadhesion (B) compared to a P407/P188-TG.

When administered intranasally in a rat hydrocephalus model, characterized by neuroinflammation and intracranial hypertension, the formulation demonstrated potential for targeted CNS therapies, paving the way for innovative treatment strategies.

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Prosocial effect of non-psychotropic *Cannabis sativa* oil.

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Since ancient times, *Cannabis sativa* L. (*C. sativa)* has been used for the production of fabrics, baskets, and cords, as well as for recreational and pharmaceutical purposes. In particular, different ethnic groups traditionally burnt leaves and flowers from psychotropic cultivars containing high levels of Δ9-tetrahydrocannabinol (D9-THC) during religious or propitiatory rites to alter the state of consciousness. However, it is unknown if nonpsychotropic cultivars of *C. sativa* were also used and consequently there are no information about the effect of these varieties on human behavior. *C. sativa* metabolome comprises specific neuroactive compounds named cannabinoids. Cannabidiol (CBD), the major non psychoactive phytocannabinoid found in the plant, displays antioxidant, anti-inflammatory, neuroprotective, and pain-relieving properties. In recent years, there has been a growing interest in the use of CBD for the treatment of neurodevelopmental disorders and for its prosocial effects on behavior.

Considering these aspects, the present study aimed to evaluate the behavioral effects of an extract of nonpsychotropic *C. sativa* (NP-CS) in mice.

The phytocannabinoid content of the NP-CS extract was evaluated by reverse phase high-performance liquid chromatography coupled to UV detection (RP-HPLC-UV), while the volatile components were analyzed by gas chromatography-mass spectrometry (GC-MS). A wild-type mouse model (B6; 129P F2) was used to analyze the effect of NP-CS on social behavior. Specifically, animals were treated for 14 days (oral gavage) and motility, anxiety, and social effects were assessed.

The RP-HPLC-UV analysis demonstrated that D9-THC was present in lower concentration compared to other cannabinoids, such as CBD; GC-MS analysis showed also the presence of several terpenoids. According to the in vivo studies data, chronic treatment with NP-CS increased social interaction, without alteration of body weight, motility, and anxiety.

In conclusion, this study supports the prosocial activity of the NP-CS extract.

PEA-OXA restores cognitive impairments associated with vitamin D deficiency-dependent alterations of the gut microbiota.

Pagano S, Iannotta M, Perrone M, Infantino R, Giorgini G, Fusco A, Marabese I, Manzo I, Belardo C, Di Martino E, Boccella S, Silvestri C, Luongo L, Di Marzo V, Guida F, Maione S

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Vitamin D is a fat-soluble steroid hormone playing a pivotal role in bone metabolism and calcium homeostasis. Vitamin D is involved in many functions such as bone health, immune response in the periphery and in the central nervous system. In the gut, vitamin D regulates inflammatory pathways by potentially reducing the burden of gut diseases. Moreover, changes of Vitamin D status in the intestine have been linked with modifications in epithelial barrier functions and the gut microbiome ecosystem in clinical disorders, such as inflammatory bowel diseases. On the other hand, Vitamin D exerts a neuroprotective action potentially useful against neurodegenerative diseases. Interestingly, lower vitamin D levels have been detected in patients with cognitive impairments suggesting that Vitamin D deficiency (VDD) may represent a potential risk factor for pathologies such as dementia and Alzheimer's disease. Thus, in this study we evaluated the possible involvement of gut microbiota in the cognitive impairments mediated by VDD and investigated the effects of pharmacological treatment with 2-Pentadecyl-2-oxazoline (PEA-OXA) which is isolated and extracted from green and roasted coffee beans.

Both male and female mice were fed a diet with low vitamin D concentration or with normal vitamin D concentration for 6 weeks. Starting from week 4 animals were treated via oral gavage with PEA-OXA or vehicle up 2 weeks. Therefore, mice were submitted to behavioural, biochemical and electrophysiological analysis to assess whether their vitamin D status affected cognitive performance together with gut microbiota composition.

Both male and female VDD mice displayed recognition deficits in the novel object recognition test and pattern separation test and the administration of PEA-OXA completely reversed memory deficits in both tasks and genders. Consistent with behavioral data, PEA-OXA also restored the hippocampal neuroplasticity, in terms of recovery of LTP in the DG compared to VDD mice, where we didn't observe LTP. Furthermore, PEA-OXA reduced hippocampal neuroinflammation. The analysis indicated the elevation of the levels of pro-inflammatory cytokines and enzymes, as measured by ELISA, under VDD, which was significantly reduced in PEA-OXA-treated animals compared to VDD mice where pro-inflammatory mediators were high. Additionally, PEA-OXA treatment enhanced gut microbiota diversity, which tended to be decreased by VDD only in female mice, elevated the relative abundance of lactic and butyric acid-producing families, *Aerococcaceae* and *Butyricicoccaceae*, and reversed the VDD-induced decrease of butyrate-producing beneficial genera, such as *Blautia* in female mice, and *Roseburia* in male mice.

VDD provokes in mice neuroinflammation and reduced hippocampal synaptic plasticity and PEA-OXA treatment is effective at reducing such dysfunctions; and suggested a possible association between the abundance of specific gut bacteria population.

Comunicazioni orali - Sessione 10

Optimization of the management of olive leavels from Tuscan cultivars for their up-cycling as nutraceuticals.

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The leaves of the olive tree (*Olea europaea* L.) are produced in large quantities as a by-product of the mill and of the pruning operations. Due to their content in phenolic and secoridoid derivatives, they have been used for centuries in popular medicine and are nowadays listed in the European Pharamacopoeia and in the European Medicinal Agency monographs. Recent studies have proven that the extracts derived from the leaves are effective against diabetes and hypertension, with antioxidant compounds such as oleuropein and hydroxytyrosol being the main responsibles of the effects; the recovery of these bioactive substances from olive leaves with the aim of developing and producing food additives and supplements can therefore improve the sustainability of the olive oil production chain. In this context, the correct management of the biomass in terms of storage and drying plays a crucial role in the obtainment of high-quality extracts[1].
In this study, extracts from two of the main Tuscan olive cultivars (*Frantoio* and *Moraiolo*) were characterized by means of HPLC-DAD-MS, identifying and quantifying ca. 25 compounds. Quantitative data were used to compare the extracts and highlighted major differences among the two cultivars. The degradation kinetics of secondary metabolites in fresh and dried leaves were also analyzed, showing that in fresh leaves a 90% decrease occurs within 10 days from the harvest, while dry leaves maintain stable phenolic concentrations for up to 1 year. Different drying methods such as lyophilization and oven drying at different temperatures were tested: the best results in terms of oleuropein and total phenolics concentration were obtained with high-temperature oven drying. Finally, environmental factors such as seasonal variations and watering conditions were taken into account, showing that the highest phenolic concentrations in the extracts are obtained from plants in drought conditions.

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Optimization of secondary metabolites production in plant cell cultures.

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During their evolution plants developed many biosynthetic pathways to synthetize a wide array of molecules, known as secondary metabolites. Plants' biodiversity is still today a huge resource for drug discovery, but also for secondary metabolites uses in nutraceutical, cosmetic and agricultural applications. The demand of these molecules is growing as, besides their use as health and wellbeing promoting compounds, they can be used as natural dyes in the food, cosmetic, pharmaceutical and textile industries. Recent breeding programs in both annual and perennial crops are trying to increase their contents, but quality and quantity of these valuable molecules cannot rely only on agricultural products, also because they can hamper plant growth. Indeed, generally in plants only specialized cells, such as those in petals, or those exposed to environmental stresses, such as UV light, produce these metabolites.

In the recent years the biosynthetic pathway of many secondary metabolites, as well as the main players in their regulation, e.g. MYB and bHLH and other transcription factors (TFs) genes, have been elucidated thus allowing the generation of biotech plants and cell cultures accumulating increased amounts of these molecules. The development of reliable productive processes that exploit plant cell cultures, either genetically modified or not, as biofactories could constitute a sustainable source of fine chemicals for pharmaceutical, cosmetic and food industries.

We have developed tobacco cell lines in which the ectopic expression of peach *PpMYB10.1* and *PpbHLH3* genes was responsible for anthocyanins accumulation. Pigment production was the result of an up-regulation of the expression of key genes of the flavonoid biosynthetic pathway, such as *NtCHS*, *NtCHI*, *NtF3H*, *NtDFR*, *NtANS*, and *NtUFGT*, as well as of the proanthocyanidin biosynthetic pathway such as *NtLAR*. Repeated selection of the darker cells on solid medium allowed the isolation of the best clones from which liquid cultures were developed. Pilot scale-up experiments up to 25 L bioreactors allowed to reach optimal results after 2 weeks of culture allowing to recover up to 20 grams/bioreactor of freeze-dried material where total polyphenols concentration was about 400 mg/g.

Also, Cannabis cell suspension cultures have been explored for producing secondary metabolites. In this case we generated stably transformed cell lines expressing MYB and HD TFs involved in trichome differentiation, sites of secondary metabolites synthesis. These genes were expressed using a dexamethasone (DEX) inducible system. The effect of different DEX treatments on transcriptional activation was tested using the reporter genes GUS and RUBY, the latter causing the production of the red pigment betalain. The expression system was shown to be induced by DEX and in a concentration-dependent manner. Repeated induction also had a positive effect on the induction.

Both in tobacco and Cannabis cell suspension systems the manipulation of endogenous pathways has been achieved in order to channel metabolite production to the desired compound(s). If for Cannabis we are still far from a possible commercial exploitation, the production of flavonols from tobacco cells could be quickly scaled up to production scales.

Callus culture of *Mespilus germanica* L. (Rosaceae): a biofactory for bioactive compounds.

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The medlar (*Mespilus germanica* L., Rosaceae) is a fruit that has experienced a decline in consumption in favour of the more cultivated *Rhaphiolepis bibas*, despite having been a popular food in the past. Recent studies have shown a renewed interest in medlar due to its functional properties [1]. The *in vitro* culture of plant cells and tissues represents a promising and sustainable method for the production of bioactive compounds, which can be achieved while minimising the impact of factors such as pollution, drought, seasonal changes and plant safety. Among these *in vitro* techniques, callus culture can serve as a precursor to large-scale cell culture [2], on the other hand, callus elicitation (the stimulation of cells with elicitors), could enhance the expression of secondary metabolites. The aim of this work was to obtain in vitro the callus from explants of medlar fruit. As a second aim, the quali-quantitative analysis of specialised metabolites produced by both callus and callu elicitated with *Saccharomyces boulardii* was performed by high-resolution Orbitrap-Qexactive based electrospray ionization source mass spectrometer (HR-Orbitrap/ESI-MS) and MRM analysis (ABSCIEX API 6500 QTRAP® Mass Spectrometer) compared with the chemical profile of peel and pulp of the fruit. LC-MS/MS analyses of fruit and callus hydroalcoholic extracts allowed the identification of 66 compounds, including hydroxycinnamic acids, catechins, flavonoids, and several triterpenes. The quantitative study of the MRM data showed that the elicited callus profile was similar to that of the callus but richer in ursane and oleanane triterpenoids. The high concentration of specialised metabolites, especially pentacyclic triterpenes, highlights the bioactivity potential of callus and elicited callus and their promise as a bioreactor for the production of triterpenes. Based on the high number of triterpenoids detected and quantified in the callus culture, their antimicrobial activity was investigated by the disc diffusion method against some bacterial strains that are common food contaminants: *Acinetobacter baumannii*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella sp.*, *Shigella sp.*, *Staphylococcus aureus*. The callus showed interesting activity against *S. Aureus* and *A. baumannii*, while the elicited callus showed promising activity against *A. baumannii* and *L. monocytogenes*. Based on these results, biofilm formation, an important virulence factor of bacteria, was tested. The callus and elicited callus were able to inhibit biofilm formation induced by different concentrations of glucose and sucrose. These results suggest that *M. germanica* callus and elicitated callus may be future sources of bioactive triterpenoids.

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Circular biosolutions: compost teas as a new frontier in antiviral and antimicrobial therapy.

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The increasing demand for sustainable and eco-friendly solutions in medicine has led to a growing interest in the valorization of agro-industrial waste. Among these, compost teas (CTs) obtained from agrifood composted materials have emerged as promising sources of bioactive compounds with biological properties employed in human health. These bioactive extracts are rich in phenolic compounds, oxidized lignin derivatives, and other secondary metabolites, which can interfere with viral replication and reduce infectivity. In addition to their antiviral properties, CTs exhibit remarkable antioxidant and antimicrobial activities. Our study aims to characterize the biomolecular composition of CTs obtained from bell pepper (CT-BP) and citrus (CT-C) composted wastes, assessing their potential as natural bioactive agents. By investigating their antioxidant, antimicrobial, and antiviral properties, as well as their underlying structure-activity relationships, we provide valuable insights into the mechanisms through which compost-derived extracts exert their beneficial effects. Our results suggest that revealed that a higher abundance of aromatic compounds, particularly lignin derivatives, has been found in CT-BP compared to CT-C applying NMR spectroscopy. At the same time, Thermochemolysis analysis (TMAH-Pyr-GC–MS) confirmed these findings, showing that CT-BP had a higher content of oxidized lignin monomers, whereas CT-C contained more intact lignin structures. Additionally, both CTs displayed significant antimicrobial activity, particularly against multi drug resistant Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), with CT-BP demonstrating superior efficacy. Antimicrobial efficacy was attributed to the abundance of polyphenols and oxidized lignin derivatives, which may disrupt bacterial cell membranes. Conversely, Gram-negative microbial cells (*Escherichia coli* and *Klebsiella pneumoniae*) exhibited higher resistance, likely due to their complex outer membrane structure. Furthermore, CTs were tested against enveloped viruses (Herpes Simplex Virus type 1 (HSV-1) and Respiratory Syncytial Virus (RSV)). When virus was incubated together with the extract on the cell system (co-treatment assay), CT-BP exhibited potent inhibition of the infection, with IC50 values of 7.78 µg/mL (HSV-1) and 6.8 µg/mL (RSV), whereas CT-C showed weaker effects. Then, we tested the extract in virus pre-treatment assay by pre-treating the virus prior to the extract and secondarily inoculating the mixture on cells. Therefore, we observed the virucidal activity of CTs, with CT-BP demonstrating stronger inhibition than CT-C. However, no significant activity was observed against non-enveloped viruses, indicating that CTs specifically target the viral envelope. This study underscores the remarkable potential of compost teas derived from agro-industrial waste as multifunctional bioactive agents. Notably, the higher inhibition of enveloped viruses by CT-BP positions compost-derived extracts as promising, eco-friendly alternatives to conventional antiviral agents. By transforming plant waste into high value bioproducts, compost teas exemplify a sustainable approach to harnessing nature's biochemical arsenal. Their broad-spectrum bioactivity not only supports agricultural and biomedical applications but also aligns with global efforts toward circular economy and green biotechnology. Future research should explore the refinement and scalability of these extracts, paving the way for innovative, nature-inspired solutions to combat oxidative stress, microbial infections, and viral diseases.

Influence of wood distillate, a bio-stimulant, on the nutritional quality of fruits in four *Cucurbita* species.

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Bio-stimulants have garnered increasing attention as sustainable solutions in agriculture, offering an eco-friendly alternative to synthetic fertilizers. These natural products enhance plant growth, resilience, and productivity by stimulating physiological and biochemical processes. Unlike traditional fertilizers, bio-stimulants often improve nutrient use efficiency, enhance stress tolerance, and support biological soil health. Among the many bio-stimulants available, one of the most promising is wood distillate (WD), a byproduct of plant biomass pyrolysis for green energy production.

Wood distillate stands out due to its rich composition of biologically active compounds, including polyphenols, which are known to boost plant fitness and yield performance. Its sustainable origin and multifunctional properties make it an appealing tool for modern agriculture, particularly in improving the nutritional quality of crops.

This study evaluated the effects of WD, applied through fertigation at a concentration of 0.5% between June and September 2024, on the nutritional composition of four pumpkin varieties: *Cucurbita maxima* var. *Delica*, *Cucurbita moschata* var. *Napoletana*, *Cucurbita maxima* var. *Red Kuri*, and *Cucurbita maxima* var. *Red Hubbard*. At the end of the experiment, key nutritional parameters were analyzed, including total phenolic content (TPC), total flavonoid content (TFC), starch, vitamin C, and carotenoid levels. The results revealed significant enhancements in the nutritional profiles of WD-treated plants compared to untreated controls. Notably, the following improvements were observed:

- Antioxidant compounds: TPC levels increased in *Cucurbita maxima* var. *Delica*, *Cucurbita moschata* var. *Napoletana*, and *Cucurbita maxima* var. *Red Hubbard* (+32%; +134%; 56%, respectively); TFC levels rose in *Cucurbita moschata* var. *Napoletana* and *Cucurbita maxima* var. *Red Kuri*, (+114% and +97%, respectively); vitamin C content increased only in *Cucurbita maxima* var. *Red Hubbard* (+200%).
- Starch content: Both *Cucurbita maxima* var. *Delica* and *Cucurbita moschata* var. *Napoletana* showed higher starch levels compared to controls (+34% and 283%, respectively.
- Carotenoid content: *Cucurbita maxima* var. *Delica* and Cucurbita *maxima* var. *Red Kuri* displayed notable increases (+36% and +183%, respectively).

These findings highlight the potential of WD-fertigation to improve specific nutritional traits in pumpkins, with effects depending across variety. This suggests that WD may serve as a sustainable agricultural product for enhancing crop quality. Ongoing research aims to further investigate the antibacterial and antitumoral properties of these pumpkin varieties.

Chemical Profiling of Matcha Tea: Analysis of Principal Metabolites Variations Among Different Grades.

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Matcha, a finely ground green tea, has been highly valued in Japan for centuries for its numerous health benefits. Cultivated under the shade of latticed canopies made from bamboo poles and reed screens, Matcha is renowned for its vibrant green color and its high content of beneficial compounds such as catechins, theanine and caffeine. This study explores the chemical composition of three distinct grades of Matcha: ceremonial Grade 1 (G1), Grade 4 (G4), and culinary Grade (GA). The research analyzes the metabolic profile, with particular attention to the principal primary metabolites, such as amino acids (e.g., theanine and essential amino acids), and the principal secondary metabolites, such as phenolic compounds (catechins and flavonoids) and the typical xanthinic alkaloids found in tea. These metabolites are well recognized for their role in mitigating oxidative stress, supporting cellular homeostasis, and exhibiting anti-inflammatory, cardioprotective, and metabolic regulatory properties. Given that matcha is available in powdered form, it allows for convenient daily consumption, making it a promising candidate for nutraceutical and dietary applications.

The Bligh-Dyer extraction method was employed to separate the samples into organic and hydroalcoholic phases. The metabolite profiles were then analyzed using high-performance thin-layer chromatography (HPTLC) and NMR spectroscopy to ensure both qualitative and quantitative assessments of the key compounds. The complementary nature of these two analytical techniques allowed for a more comprehensive metabolite profile. HPTLC, being more sensitive, enabled the investigation of compounds potentially undetectable by NMR, such as aromatic amino acids, while NMR spectroscopy offered precise quantitative data. By combining these methods, the study achieved a more nuanced understanding of both primary and secondary metabolites.

The analysis revealed significant differences in metabolite concentrations among the three Matcha grades. These variations were influenced by factors such as seasonality, which determines the harvest period, and the maturity of the leaves, ranging from young buds to fully developed leaves. The amino acids analyzed exhibited a decreasing trend from G1 to GA, whereas secondary metabolites, such as polyphenols and xanthinic alkaloids, followed the opposite trend.

The combined use of HPTLC and NMR spectroscopy provided a deeper understanding of the chemical diversity of Matcha tea, highlighting the importance of integrating complementary techniques to achieve a more complete profile. Matcha is a highly prized and expensive product, and these analyses also allow for the verification of its quality. These findings shed light on the chemical diversity of Matcha tea, provide valuable insights into its composition, and suggest its potential for appropriate use also as a dietary supplement, given its rich content of bioactive compounds with health-promoting properties.

Citrus, Pomegranate, Conifers, and More: An Integral Green Extraction Technique Enabling the Sustainable Exploitation of Valuable Plant By-Products.

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Hydrodynamic cavitation (HC) is emerging as the most efficient, non-selective extraction technique of natural products, largely outperforming other techniques in terms of extraction and process yield. HC consists of a multiphase phenomenon including the generation, growth, and quasi-adiabatic collapse of vapor-filled bubbles under an oscillating pressure field, resulting in pressure shockwaves, hydraulic jets, extreme local temperatures and the generation of free radicals. In the most consolidated set-up, it is implemented by circulating a liquid or a liquid–solid mixture through static constrictions of various geometries. Extraction of natural products can be carried out using water as the only solvent.

HC-based extracts of citrus, pomegranate and conifer by-products showed interesting beneficial properties, evident even at lower dosages than reference extracts, thereby suggesting that this extraction method could be useful for improving the chemical-physical characteristics of the extracts and their bioaccessibility through biological barriers.

The possibility of using fresh biomass of relatively coarse size in high concentration, the fast extraction process (generally less than 20 minutes), the high extraction and process yield, and the unique structure and additional properties of the extracts allow the advantageous use of phytocomplexes, thus avoiding expensive purification processes and complying with the principles of green extraction of natural products. This innovation is especially important with natural products presenting a complex structure that hinders their extraction through conventional or other green techniques, thus enabling their exploitation and potential use in nutraceutics and medicine.

Diterpenes with potential anti-inflammatory properties from *Lavandula pubescens* Decne.

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Lavandula ssp are valuable medicinal plants belonging to the *Lamiaceae* family and are cultivated as an ornamental plant in many countries in Europe, north Africa, southwest Asia, western Iran, eastern India, China and Japan. Phytochemical studies on various *Lavandula* species have led to the identification of numerous secondary metabolites, including triterpenes, sesquiterpenes, diterpenes, and phenolic compounds [1-3]. Many of these species have been traditionally used for medicinal purposes. *Lavandula pubescens*, in particular, is recognized in Saudi Arabian traditional medicine for its antibacterial and anti-inflammatory properties. While preliminary research has been conducted on the essential oil of this plant, its surface exudate remains unexplored. In this study, the chloroform extract of the aerial parts of *L. pubescens* was separated by BIOTAGE, MPLC, and HPLC, then the isolates were characterised as diterpenoids by spectroscopic techniques including 1 and 2D-NMR and HRESIMS analyses. So far, the investigation led to the discovery of two never reported abietane diterpenes and three new isopimarane, as well as several previously reported pimarane, oleane and ursane derivatives. The diterpenes were identified as 7,16-dihydroxy-8,11,13-abietatriene, 16-hydroxy-8,11,13-abietatriene-7-one, 15,16-dihydroxy-isopimar-8(9)en-11-one, 8(14)-isopimaran-9,15,16-triol, 15,16-dihydroxy-9,10-isopimar-8 (14),10-dien-9-one. Based on the traditional use of this species for headache and cold and considering the already reported anti-inflammatory activity of structural- related compounds isolated from *L. multifida*[4], the anti-inflammatory potential of *L. pubescens* was evaluated. In detail, since proinflammatory cytokines displayed a key role in the inflammatory process, their modulation by surface exudate and isolates in THP-1 derived M0 macrophages stimulated with LPS was investigated. IL-6 secretion was inhibited by 90, 75, and 65% in cells exposed for 6 h to 50, 25 and 12.5 µg/mL, respectively, of the extract. Dihydroxy-iso-pimar-8(9)-ene and 15,16,17-trihydroxypimar-8(9)-ene were able to downregulateIl-6 in THP1 cell lines with EC50 of 40.8±0.03 and 19.4±0.4 µg/ml.

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Lettura magistrale

Herbal medicinal products in Italy: regulatory framework and tools to increase awareness on their role in the marketplace

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In order to be marketed in Italy, an herbal medicinal product (HMP) must be granted a Marketing Authorisation (MA) by the AIFA or the European Commission. The MA is regulated in Italy by the Legislative Decree 219/2006 which implemented the Community Directive 2001/83 and subsequent amendments and additions, including those introduced by the Community Directive 2004/24; the latter has established the simplified registration procedure ("Traditional Use Registration"), created to streamline the documentation to be presented to the regulatory authorities for the marketing of those medicines of herbal origin with a long tradition of medical use (for at least 30 years of which at least 15 years in the EU), intended to be used without medical supervision with a specific dosage and dosage schedule, limited to oral, external or inhalation use, defined as traditional herbal medicinal products.

Although the purpose of Directive 2004/24/EC was to prevent many medicinal herbal products from being withdrawn from the market due to the inability to meet the requirements established by Directive 2001/83/EC, the market for HMPs in Italy is still marginal compared to that of other non-medicinal products containing herbal substances or preparations. The most striking example is that of food supplements which are frequently marketed with usage "claims" that are very similar to the therapeutic indications authorized for HMPs. The consequence is that many pharmaceutical companies over the years have revoked or invalidated the MA of herbal medicinal products, ending up favoring the marketing of food supplements, which based on their different use, are subject to less stringent regulatory requirements and lower registration costs.

Consumers are shifting their preferences from synthetic drugs to herbal-based products considering their impact on health in the long term. In this context, HMPs may offer alternative or complementary therapeutic options for patients. However, the coexistence on the market of herbal medicines, food supplements and other products containing the same herbal substance or preparation at the same or similar dosages may confuse health professionals and consumers if they are not aware of the regulatory framework of each product category.

With this in mind, AIFA has set up a page on its website where information on the regulatory aspects and quality, efficacy and safety requirements for the marketing authorisation of HMPs can be found. In addition, the list of HMPs authorised in Italy is made available, which shows for each medicine the commercial name, the herbal substances/preparations included and the authorised therapeutic indications. From the webpage, through appropriate links, it is also possible to access the monographs on efficacy and safety of herbal substances/preparations prepared by the Committee on Herbal Medicinal Products (HMPC) and published on the EMA website, as well as the scientific and regulatory guidelines that underpin the authorisation processes for these medicinal products.

This initiative is part of a communication and educational endeavour to raise awareness of the role of HMPs with the aim of valorise these medicines in the interests of patients and the health professionals who prescribe and recommend them.

NOTE: the opinions expressed in this article reflect only the personal position of the authors and must not be understood or cited as formulated on behalf of the Italian Medicines Agency or reflect the position of the same or of its collegial bodies".

Comunicazioni orali - Sessione 11

Phytovigilance: Natural does not mean free of ADR.

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In recent years the consumption of remedies based on curative plants has become widespread, in the mistaken belief that these products are safer because of their natural origin. Phytotherapy is a field of science that uses plants either to treat diseases or as health-promoting agents. These are crude preparations of dried plants or any part thereof, such as leaf, stem, root, flower, or seed

The result of this belief is that plant products are sometimes proposed to the frail individuals such as children, pregnant or lactating women and elderly, without real awareness of the risk of adverse reactions, sometimes even serious, or the fact that they may interfere with pre-existing diseases or other ongoing drug therapies.

Reports from the various global surveillance systems show that adverse reactions (ADR) to medicinal plant preparations are not irrelevant.

In our study we offer an overview of the exposures to drugs, household or industrial products, pesticides, cosmetics, plants, fungi and poisonous animals treated by the Bergamo Poison Center in the last 5 years (2019-2023) with particular interest in herbal medicines such as supplements and nutraceuticals. Established in 1999 and recognized as a national Poison Center by the Ministry of Health since 2005, it has been included in the emergency medical service and it can be reached both by healthcare personnel and by the public, at free-toll number 800-883300. The activity has increased significantly over the years and the calls (42,197 in the period) for acute poisonings and ADRs came from everywhere in Italy. There were also 8,968 requests for generic toxicological or pharmacological information. In our observational retrospective study we analyzed the 736 ADRs case reports. Of them, 18 were about supplements and medical plants.

We collected personal data, geographical location, type of caller (patient, family member, physician), the plant that caused the reaction and the symptoms.

As expected, women are major users and develop adverse reactions more frequently than men; the age groups most affected are adults, newborns and infants. The respiratory, urogenital and cardiovascular tract were the most affected organ system, followed by the skin.

The data obtained show the fundamental role that poison control centers play in the management for acute poisonings and also for adverse drug reactions.

Adverse reactions to *Ginkgo biloba* medicinal products released in European countries.

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European Medicines Agency (EMA) approved *Ginkgo biloba* extracts as medicinal products "for the improvement of cognitive deterioration associated with age and quality of life in mild dementia" and for the "relief of the feeling of heaviness in the legs and circulatory disorders characterized by the sensation of cold in the hands and feet". No post-marketing study has yet been conducted in Europe on potential adverse reactions to Ginkgo-based products through the analysis of data from spontaneous adverse events reporting systems. Aim of this study was to contribute to defining the safety profile of Ginkgo by the analysis of real-world data represented by the suspected adverse reactions (SARs) to *Ginkgo biloba* traceable in the datasystem EudraVigilance.

A descriptive analysis of SARs to *Ginkgo biloba* collected in the years 2018-2024 has been performed, together with a disproportionality analysis of hemorrhagic events linked to Ginkgo biloba compared to hemorrhagic adverse events signaled for the platelet aggregation inhibitors ticlopidine and clopidogrel.

Analysis of SARs shows that women are more affected and those more frequently caused by *Ginkgo biloba* are hemorrhagic disorders. Disproportionality analysis conducted comparing hemorrhagic events with drugs ticlopidine and clopidogrel suggests a lower risk for hemorrhagic events caused by medicinal products based on *Ginkgo biloba*.

Our post-marketing analysis indicates that the occurrence of adverse reactions to Ginkgo-derived medicinal products is more frequent in women. Furthermore, the analysis confirms, although the risk is lower than synthetic anticoagulants and antiplatelet drugs, that *Ginkgo biloba* consumption by elders requires attention, because it is associated with the potential occurrence of hemorrhagic adverse events.

Role and impact of phytomedicines in the modulation of visceral hypersensitivity and mycobiome in DGBI

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Chronic visceral hypersensitivity associated with irritable bowel syndrome (IBS) and functional dyspepsia (FD) is characterized by abdominal wall distension and pain and is considered as a critic trigger in the pathophysiology of the disorders of the gut-brain interaction with significant impact in patient's quality of life. Finding effective solutions for visceral hypersensitivity leads to an overall improvement in well-being, and a better balance between gut disease and mental stress management.

On the other hand, in a visceral hypersensitivity model of IBS induced by water avoidance stress in mother-separated rats, it was demonstrated that visceral hypersensitivity is associated with both mycobiome and microbiome dysbiosis.[2]

A recent study (Omoloye et al. 2024) analyzed the effects of acute and long-term treatment with Menthacarin® (a complex of peppermint essential oil WS®1340 and cumin WS®1520) on markers of intestinal pain sensitivity in two rat models of visceral hypersensitivity, previously mentioned by Adam in 2006[1]. Hyperalgesia was induced through chronic corticosteroid administration and repeated acute mechanical hyperstimulation, characterized by increased excitatory electrophysiological responses of ACC neurons to colorectal distension (CRD) and an increase in the proportion of neurons responding to otherwise subthreshold stimulation. This model reproduces what patients with IBS show: a higher metabolic activity and functional differences compared to normal in the anterior cingulate cortex (ACC) in response to visceral pain stimulation.

Menthacarin® may also modulate microbiota and has shown in vitro antimicrobial effects on pathogenic intestinal species without affecting beneficial species, in line with literature showing both menthol and carvone have antibacterial and antifungal activities.

It is also shown how visceral hypersensitivity associated with mycobiome dysbiosis in a rat model is reduced by Menthacarin® due to its ability to rebalance associated fungal dysbiosis[2]. It is interesting to note that the reversal of visceral hypersensitivity in this animal model through treatment with Menthacarin® was associated with a change in mycobiome composition towards characteristics similar to those of control animals. While perhaps less studied than the intestinal microbiota, the intestinal mycobiota likely has a significant impact on health.[4]

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GINKGO BILOBA: FROM TRADITION TO MODERN APPROACHES IN FAVOUR OF EFFICACY AND SAFETY

Tongiani S
Indena S.p.A.

Ginkgo biloba extract has been traditionally used as adjuvant to vascular and cognitive health. The long-standing tradition of use combined with new clinical trials represent an important contribution to the evaluation of the tolerability profile of this botanical ingredient, which is still very popular in today's modern world where stress, anxiety, poor diet, lack of sleep, and exposure to artificial light significantly impact cognitive performance and mental well-being. Addressing these issues requires targeted nutritional and physiological interventions that combine support to microcirculation and neuroprotection. Specifically, microcirculation consists of small blood vessels that regulate the exchange of oxygen, nutrients, and hormones in the brain, and its disfunction may lead to vascular dysfunction, negatively impacting cognitive health. Neuroprotection, on the other hand, focuses on reducing oxidative stress, a major factor contributing to neurodegeneration.

VIRTIVA™ PLUS, a modern formulation of *Ginkgo biloba* combined with the ingredient phosphatidylserine from sunflower lecithin, has been developed and recently tested in preclinical and clinical studies. This combination enhances ginkgo benefits on blood circulation and nootropic properties of phosphatidylserine supporting neuroprotection and microcirculation, thus contributing to better cognitive performance.

Preclinical tests showed that VIRTIVA™ PLUS has dose-dependent antioxidant properties protecting neuronal cell from oxidative stress: MTT test on SH-SY5Y human neuroblastoma cells measured cell viability and damage caused by oxidative stress, whereas cell-free assays, such as the DPPH (radical scavenging) test, proved the ability of VIRTIVA™ PLUS to counteract free radical formation and oxidative stress.

In addition, a recent human study conducted in the U.S. explored the effects of VIRTIVA™ PLUS on cognitive performance. This double-blind, randomized, placebo-controlled trial involved healthy adults and demonstrated enhanced memory performance, improved focus and concentration and faster reaction time with a 4-week supplementation.

This combination of preclinical and human studies meets the need to support the safety profile of this ginkgo-based nutraceutical ingredient, ensuring it can be used safely and effectively. Ensuring the safety of botanical ingredients is a complex process that requires a systematic approach, and Regulatory Agencies including EFSA emphasize the need for an evaluation strategy. Companies operating in nutraceutical space should adopt a reliable Systemic Safety Assessment for botanical ingredients, focusing on a standardized methodology for evaluating the safety of botanical extracts used in health and wellness applications, integrating toxicology, pharmacology, historical usage data, and new clinical studies, ensuring a multi-disciplinary evaluation process.

Pharmaco-toxicological profile of "Aglianico del Vulture" red wine polyphenolic extract (RWP) as dietary supplement for preventing obesity-associated cardiometabolic complications

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Mounting evidence suggest that nutraceuticals containing polyphenols help to prevent obesity and cardiometabolic disease (CMD) by directly modulating signaling pathways in endothelial and inflammatory cells of the vascular compartment (*Potenza M.A. et al. Am J Physiol Endocrinol Metab. 2007*). Recently, the *Aglianico del Vulture* red wine (RWP) extract has demonstrated significant *in vitro* effects on polarization, inhibition of lipid peroxidation, histone acetylation levels and proinflammatory ROS, NO·, PGE2 production from human macrophages (*Santarsiero A. et al. Oxid Med Cell Longev. 2021*), suggesting its potential protective role on immunometabolic processes underlying atherosclerosis. In the present study, the RWP pharmaco-toxicological activities and immunometabolic profile have been evaluated on endothelial cells (ECs) from patients with CMD (obesity, dyslipidemia, and insulin resistance), as well as on spontaneously hypertensive rats (SHR), an animal model of metabolic syndrome.

ECs isolated from CMD patients (n=12) were incubated with RWP (dose-response: 0, 100, 200, 400, 800, 1600 and 3200 µg/mL; time-course: 0, 24, 48 or 72 h) to measure toxicological (apoptosis, by flow cytometry) and functional (migration, by wound healing) effects., SHR 9 week (wk) old (n=20) were randomized into 4 groups and treated by gavage with vehicle alone or RWP (200, 400, or 800 mg/Kg/day for a 3 wk period). Body weight and food intake were measured daily, systolic blood pressure (SBP) every 3 days (tail cuff). At the end of the 3 weeks treatment, plasma glucose concentrations and pro-inflammatory cytokine levels were measured in 12-wk old SHR. Vasodilation in response to acetylcholine (ACh, 10nM to 3µM/30 sec) and RWP (5 to 20 µg/ml/30 sec) was evaluated before and after N-nitro-L-arginine methyl ester preincubation (L-NAME, 100 µM/20 min) on mesenteric arteries (MVA) isolated and pre-constricted with noradrenaline 5µM (NA).

On human ECs, RWP induced apoptosis only at the highest concentrations (800 to 3200 µg/mL/72 h) and did not affect ECs migration up to 400 µg/mL concentration (*vs* untreated ECs). In *in vivo* studies on SHR, at all concentrations used 3-wk treatment with RWP significantly reduced SBP levels ($p < 0.001$ RWP-SHR *vs* vehicle treated-SHR), with no associated signs of organ toxicity, or significant changes in body weight and fasting blood glucose levels. Conversely, pro-inflammatory cytokines (CCL5, CXCL7) as well as adhesion molecules (TIMP1) levels showed a trend to reduction. On *ex vivo* experiments, ACh-mediated MVA vasorelaxation was significantly increased in RWP-SHR treated with 400 to 800 mg/Kg/day (*vs* vehicle-treated SHR, $p<0.05$). In addition, direct administration of RWP on MVA from 12-wk-old vehicle-treated SHR produced a reversible and dose-dependent vasorelaxation abolished by L-NAME preincubation ($p<0.001$ *vs* respective control), suggesting that RWP-mediated vasodilation is NO-dependent.

Findings obtained so far suggest that RWP may significantly contribute to the protection from cardiometabolic risk. Current experiments in co-coltured ECs and macrophages from CMD patients will help to clarify cellular mechanisms by which RWP administration may protect from initial atherosclerotic process.

Benzyl isothiocyanate suppresses development of thyroid carcinoma by regulating both autophagy and apoptosis pathway.

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Anaplastic thyroid carcinoma (ATC) is the most aggressive type of thyroid cancer, characterized by rapid growth and invasion and poor prognosis. Due to its rarity and aggressive nature, ATC is a difficult condition to treat, thus knowledge of the mechanisms underlying its progression represent important research challenges. Benzyl isothiocyanate (BITC) is a natural compound that has shown promising anticancer properties. The aim of this study was to evaluate the antitumor effect of BITC in ATC, highlighting signaling pathways involved in BITC mechanism of action. This work included *in vitro* and *in vivo* studies. The *in vitro* model was performed using different ATC cell lines to study the effect of BITC on the modulation of autophagy, apoptosis, cell migration and proliferation. To confirm the *in vitro* findings and better mimic the complex tumor microenvironment, an in *vivo* orthotopic model of ATC was used. This involved the in situ inoculation of ATC cells in mice, followed by treatment with BITC. Histological analysis of the mouse thyroids was conducted to evaluate the effects of BITC on tumor growth and progression and Western blot analysis was used to examine markers related to autophagy, apoptosis, and epithelial-mesenchymal transition (EMT). Results obtained indicate that BITC, both *in vitro* and *in vivo*, has the potential to slow the progression of ATC through interactions with autophagy, reduction in EMT and attenuation of inflammation. In conclusion, this study identifies BITC as a compound worth further investigation for the development of new treatment strategies for this aggressive form of thyroid cancer.

Sabato 12 aprile

Comunicazioni orali - Sessione 12

Neuroprotective effect of nutritional supplements in animal models of glaucoma.

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Glaucoma is a heterogeneous group of optic neuropathies characterized by typical alterations of the optic nerve head and the progressive loss of retinal ganglion cells (RGCs). Age and high intraocular pressure (IOP) have been identified as main risk factors; however, normal tension glaucoma occurs in patients with normal IOP values and a portion of patients shows progression even if IOP is pharmacologically maintained in the physiological range. Nevertheless, IOP-lowering drugs or surgical procedures remains the only therapeutic approach currently available.

Therefore, alternative IOP-independent neuroprotective strategies able to slow down or prevent RGC degeneration is the current therapeutic challenge for glaucoma management.

Over the years we have tested the neuroprotective role of natural compounds in in vivo models of glaucoma. In a first study we reported that the association of homotaurine, carnosine and forskolin (7 beta-acetoxy-8,13-epoxy-1 alpha, 6 beta, 9 alpha-trihydroxy-labd-14-ene-11-one) administered by intravitreal injection, prevented RGC loss in an experimental model of acute glaucoma developed in rats. In a more recent study we tested the efficacy of a dietary supplement containing forskolin, homotaurine, vitamins of the B group and spearmint extract, formulated with the commercial name of Gangliomix, in preventing RGC loss in a mouse model of acute glaucoma induced by transient ocular hypertension.

Altogether, our data suggest that the tested dietary supplements support RGC survival and may offer beneficial effects in glaucoma patients in combination with the currently used IOP-lowering therapy.

Eremurus species as new sources of metabolites with neuroprotective activity.

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Eremurus persicus (Jaub & Spach) Boiss. is a perennal herbaceous plant belonging to *Asphodelaceae* family also known as Hasan aloo or Zereshk. It is mainly diffused from Iran to West Himalaya and it grows in arid and semi-arid rocky mountains. This plant is well-known by the local people for its benefic effects on human health, like diuretic, anti-infection and hepatoprotective effects or it is used against atherosclerosis and skin inflammation. During the years, our research group isolated and characterized its main metabolite, (*R*)-aloesaponol-III-8-methyl-ether [(*R*)-ASME]. This metabolite was next subjected to ligand-based drug discovery campaigns and emerged virtually able to bind two innovative targets, both involved in the fight against neurodegeneration: HuD and proteasome [1]. HuD is a member of the human ELAV protein family and plays a crucial role in neuronal plasticity and differentiation [2]. Proteasome is a multicatalytic complex responsible for protein degradation. Despite proteasome inhibitors are well known anticancer agents, its activators are still poorly investigated, but preliminary results suggest that they may play a role to contrast neurodegeneration [3]. To confirm the activity of (*R*)-ASME, we optimized its extraction procedure comparing different sources (roots of *E. persicus* and *E. spectabilis*) and different extraction techniques (maceration and microwave assisted extraction). Furthermore, a proper fractionation procedure was set up to minimize waste generation maximizing the yields. During this procedure a new ASME analogue was isolated and identified as (*R*)-Germichrysone (Figure 1).

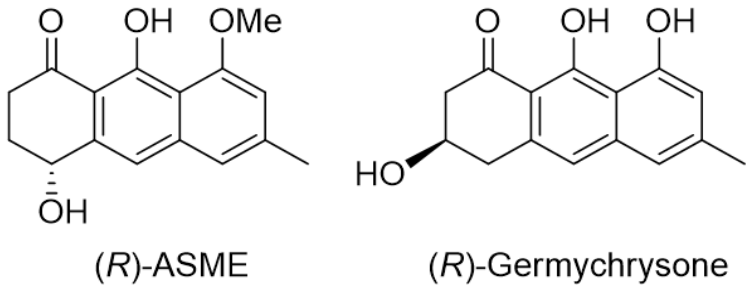

Figure 1. Chemical structure of (*R*)-ASME and (*R*)-germichrysone

The activities of both (*R*)-ASME and (*R*)-Germichrysone were evaluated on the HuD/BDNF cascade and on the activation of the ubiquitin-proteasome system. Results demonstrated that, despite structure similarity, only (*R*)-ASME was able to modulate the target in the low millimolar range. Considering the multifactorial nature of neurodegenerative diseases, (*R*)-ASME may be considered a promising candidate in this field. Further studies are now ongoing to optimize the newly identified hits to improve its potency, solubility and selectivity profile.

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Olive leaf extract reduces mast cell-mediated allergic inflammation.

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Mast cell-mediated reactions promote various allergic disease, including anaphylaxis, allergic rhinitis, asthma, and atopic dermatitis [1]. Different data demonstrated an intricate relationship between the use of antihistaminic drugs, the onset of side effects, and the development of resistance, underscoring the importance to find novel therapeutic approaches to treat allergic diseases [2]. Olive leaf extract (OLE), is a by-product of the olive tree rich in bioactive compounds, known for its numerous therapeutic properties, including antioxidant, anti-tumoral and antidiabetic effects [3]. In this study, we investigated the effect of OLE on the mast-cell-mediated allergic inflammation using human mast cells HMC-1.2. OLE reduced histamine and β-Hexosaminidase release from HMC cells activated by phorbol 12-myristate 13-acetate and calcium ionophore A23187 (PMACI) through modulation of calcium signal. Moreover, OLE decreased the PMACI-stimulated gene expression of proinflammatory cytokines such as tumor necrosis factor-a (TNF-α), interleukin-8 (IL-8) and interleukin-6 (IL-6) in human mast cells. This result was confirmed by multiplex assay in which the pre-treatment with OLE reduced the effective secretion of TNF-α, IL-6 and IL-8. These effects were correlated to ROS reduction and modulation of both mitochondrial mass and membrane potential. Finally, the inhibitory effect of OLE was nuclear factor (NF)-kB dependent as demonstrated by both activity assay and Western Blot analysis. Taken together, our results demonstrated that OLE inhibits mast-cell-derived allergic inflammation modulating mast cells degranulation, proinflammatory cytokines release and NF-kB activation. Therefore, OLE could represent a novel potential therapeutic approach for the treatment of mast cell–associated disorders.

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Exploring the anti-inflammatory and antioxidant properties of *Vitis vinifera* L. leaves extract: a sustainable approach to wine byproducts utilization.

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The wine business is one of the most significant and productive agro-industry worldwide, however it generates millions of tons of biological residues, carrying a huge negative impact related to the disposal of these materials into the environment. In the last few years the byproducts of the wine industry have attracted the attention as they represent a high potential to the development of new products thanks to their content rich in several bioactive compounds.

In particular, *Vitis vinifera* L. (*Vv*) leaves are well known in literature for their high content of bioactive molecules such as phenolic acids, flavonols, tannins and many other compounds of nutraceutical interest which are responsible of the diverse positive effects they exert on human health. Among them, *Vv* extract antioxidant and anti-inflammatory properties have been widely studied, together with their beneficial effects on the cardiovascular and gastrointestinal systems.

Vv extract was obtained through Naviglio® extraction system, then mass spectrometry analysis was conducted in order to characterise its major components. Target prediction analysis was carried out to identify possible molecular targets, then the antiradical activity of the extract was evaluated through DPPH assay together with Folin-Ciocalteu polyphenol quantification. We also performed a permeability assay using a PermeaPad® plate that mimics passive transfer through the intestinal barrier and then quantified the amount of polyphenols absorbed in a 24 hours-time period. The cytotoxicity of different concentrations of the *Vv* extract was evaluated through MTT assay on RAW 264.7 mouse macrophage cell models, then, the anti-inflammatory potential of the extract was investigated through MTT assay on the same cell line treated with lipopolysaccharide (LPS) and Real Time PCR was performed to assess the expression of pro-inflammatory markers. The mass spectrometry analysis evidenced the presence of numerous compounds belonging to different polyphenol groups, such as epicatechins and procyanidins.

The target prediction analysis revealed that metalloproteases 2 and 9 are the molecules most likely to bind to the extract. Although polyphenol quantification indicates that their content increases with the rising concentration of the extract, the DPPH assay demonstrated that the antiradical activity is highest between 0,1mg/mL and 0,5mg/mL, subsequently declining at higher concentrations. Conversely, the quantification of polyphenols absorbed through the PermeaPad® membrane shows a low absorption rate in the initial hours, followed by a rapid increase. MTT assay showed that every concentration tested of *Vv* extract is cytocompatible and that the highest concentrations are able to restore cellular viability after 24-hours LPS treatment. Real Time PCR showed that pre-treatment with *Vv* extract is able to decrease the expression IL-1ß, TNF-α and IL-6 genes after LPS insult.

To sum up, our results show that the *Vv* extract contains numerous bioactive molecules that exert anti-inflammatory activity, highlighting how the residues of the wine industry can represent an interesting source of active biological compounds that could exert beneficial positive effects on human health.

Ulva pertusa modulated colonic oxidative stress markers and clinical parameters: a potential adjuvant therapy to manage side effects during 5-FUrRegimen.

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One of the most used chemotherapy agents in clinical practice is 5-Fluorouracil (5-FU), a fluorinated pyrimidine in the category of antimetabolite agents. 5-FU is used to treat a variety of cancers, including colon, breast, pancreatic, and stomach cancers, and its efficacy lies in its direct impact on the patient's DNA and RNA. Specifically, its mechanism blocks the enzymes thymidylate synthetase and uracil phosphatase, inhibiting the synthesis of uracil, which cannot be incorporated into nuclear and cytoplasmic RNA. Despite being one of the most used drugs in oncology, it is associated with several significant side effects, including inflammation of the mouth, loss of appetite, and reduction in blood cells. In our study, we examined the reduction of side effects in a 5-FU regimen administered at doses of 15 mg/kg and 6 mg/kg for 14 days in 6-week-old male Sprague-Dawley rats. On the 14th day, the rats were treated orally for 2 weeks with 100 mg/kg of Ulva pertusa, a well-known seaweed from the Ulvaceae family, which has demonstrated powerful biological properties. The administration of this green alga alleviated the side effects of 5-FU, improving several parameters including body weight, food intake, and diarrhea index. It also helped reduce side effects in the blood, kidneys, and liver. Histological and molecular analyses were conducted on serum and colon tissues from the rats, examining changes in colon structure and the release of oxidative stress markers such as iNOS, COX-2, and nitrotyrosine. Several biochemical indicators, including SOD, CAT, GSH, MDA, and ascorbic acid, were also evaluated. Overall, our data indicated Ulva pertusa to be a promising therapeutic against 5-FU's adverse effects, therefore, it could be worthwhile to investigate the possibility of using this alga in safer cancer treatment formulations. Certainly, future preclinical and clinical studies could assess the alga's efficacy in diverse cancer treatment regimens, exploring its role as an adjuvant therapy that may reduce chemotherapy-related toxicity without compromising therapeutic outcomes.

Roots and aerial parts: a comparative study on the biological activity and composition of *Cistus monspeliensis* L.

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This study examines the anti-inflammatory and antioxidant properties of extracts from the aerial parts and roots of *Cistus monspeliensis* L., with a particular focus on the roots, which have not been previously studied. It evaluates their biological activity while characterizing their metabolite composition and inorganic profile. Various methodologies were employed to assess their effects. Cell viability was tested using the MTT assay on RAW 264.7 macrophages and SH-SY5Y neuroblastoma cells. The anti-inflammatory potential was analyzed by treating RAW 264.7 cells with lipopolysaccharide (LPS) and measuring the extracts' ability to protect against inflammation. The expression levels of key pro-inflammatory cytokines, specifically IL-1β and IL-6, were quantified using real-time PCR to determine the extracts' effectiveness in reducing inflammatory markers. The antioxidant activity was assessed by exposing SH-SY5Y cells to hydrogen peroxide (H_2O_2), which induces oxidative stress, and subsequently evaluating the extracts' ability to enhance cell survival. Mitochondrial integrity was examined using MitoTracker Green fluorescent dye, ATP production was measured to assess cellular energy metabolism, and catalase activity was analyzed to determine the extracts' role in neutralizing oxidative damage. The metabolite composition was characterized using ^{1}H NMR profiling and UHPLC-MS, while TXRF spectroscopy was used to determine their elemental composition.

The results revealed that both the aerial parts and root extracts demonstrated protective effects against LPS-induced inflammation, improving cell viability at concentrations between 1.56 and 6.25 µg/mL. However, only the root extract significantly reduced the expression of IL-1β and IL-6, indicating a stronger anti-inflammatory effect compared to the aerial parts. When assessing antioxidant activity, both extracts increased the survival rate of SH-SY5Y cells exposed to oxidative stress, but once again, the root extract showed superior effects. It notably enhanced mitochondrial survival, significantly increased ATP production, and elevated catalase enzyme activity, indicating its critical role in mitigating oxidative damage. The chemical analysis of the extracts elucidated these biological differences; both extracts contained 1-O-methyl-epi-inositol, but the aerial parts were particularly enriched in methoxylated flavonoids and labdane compounds, whereas the roots were predominantly composed of catechins, gallic acid, and pyrogallol derivatives. The inorganic analysis confirmed the absence of toxic elements, such as lead, in both extracts, thereby supporting their safety for potential therapeutic applications. These findings highlight the biological properties of the roots of *C. monspeliensis*, revealing their potential to be more effective than the aerial parts in combating inflammation and oxidative stress.

Comunicazioni orali - Sessione 13

Protective effect of *Salvia officinalis* L. hydrodistillation wastewater against *E. coli*-induced damage in Caco-2 cells.

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Essential oils are the most marketable product obtained from medicinal plants. However, the biomass used in the distillation process is returned as a solid residue together with variable amounts of wastewater containing high-value water-soluble compounds, which currently are not addressed to any further application. For this reason, we aimed to evaluate the phytochemical composition and biological activities of wastewater coming from hydrodistillation of *Salvia officinalis* L., and to assess the *in vitro* antioxidant and anti-inflammatory activities.

The phenolic HPLC profiles of the extract allowed the identification of more than 20 phenolic compounds. Among these, we detected phenolic acids such as cinnamic, rosmarinic and salvianolic acids, flavonols (quercetin derivatives) and flavons (luteolin and apigenin derivatives). The extract exhibited also good antioxidant activity as free radical scavenging ability (DPPH and TEAC assays) and superoxide dismutase mimetic activity. Additionally, using an *in vitro* model of intestinal inflammation exposing human intestinal epithelial Caco-2 cells to *Escherichia coli*, a well-known pathogenic bacterium that compromises the intestinal barrier, we evaluated the anti-inflammatory activity of the extract. In particular, Caco-2 human intestinal epithelial cells were cultured for 18 days post confluence to obtain fully differentiated cells. Then, cells were pre-treated with the extract (100 and 200 µg/mL) for 24 hours and subsequently exposed to *E. coli* for 2 hours to simulate *in vitro* pro-inflammatory events. The results obtained have shown how pre-treatment with the extract was able to prevent intestinal epithelial barrier damage induced by *E. coli* as demonstrated by a significant increase in delta TEER values compared to control cells in a dose-dependent way. Furthermore, our data demonstrated that the extract improved intestinal barrier function through the modulation of typical tight junctions inducing claudin-1 and occludin protein levels altered by *E. coli*. Additionally, the extract downregulated the protein expression of the inducible enzyme COX-2 in *E. coli*-challenged Caco-2 cells.

Similarly, we tested the anti-inflammatory activity using an *in vitro* model of murine macrophages (Raw 264.7) exposed to LPS. In particular, we demonstrated that the extract was able to prevent the nuclear translocation of the proinflammatory NF-κB transcription factor and the IL-6 gene expression induced by LPS.

In conclusion, herein we demonstrate the presence of compounds with established biological activity in wastewater coming from hydrodistillation of *Salvia officinalis* L.. The antioxidant activity and the *in vitro* protective effects against *E. coli*-induced intestinal epithelial cells injury and LPS-induced macrophages inflammation, support the potential of valorization of this kind of agro-industrial residues in the pharmaceutical and nutraceutical fields with positive effects both in environmental and economic terms of the aromatic plants' supply chain.

Effects of N-acylethanolamine association on metabolic dysfunction and cellular stress responses driven by obesity.

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N-acylethanolamines (NAEs) are endogenous lipids belonging to the class of non-canonical endocannabinoids, which play important roles in inflammation and energy metabolism regulation. NAE signaling is disrupted in obesity, but the pharmacological effects of NAE-based formulations in metabolic disorders remain poorly understood. Excessive nutrient intake activates stress-related mechanisms such as autophagy dysregulation, mitochondrial dysfunction, oxidative stress, and cellular senescence, all of which contribute to obesity-related dysfunctions. Targeting these cellular responses may be beneficial for obesity management. Recently, we investigated the beneficial effects of a formulation containing a mixture of NAEs derived from olive oil, with a higher content of oleoylethanolamide (OEA) and lesser of linoleoylethanolamide (LEA), palmitoylethanolamide (PEA), and stearoylethanolamide (SEA), in improving the metabolic damage (insulin resistance, lipid and glucose dysfunction, and adipose tissue reprogramming) in high-fat diet fed obese mice. Here, we assessed the effects of single NAEs on metabolic stress-related responses such as autophagy and cellular senescence, and associated mitochondrial oxidative damage, using different cell types involved in tissue metabolism (hepatocytes, adipocytes, and primary murine fibroblasts). *In vitro* studies on HepG2 cells showed that each NAEs induced the protective mechanisms of autophagy and mitophagy, as evidenced by the modulation of LC3-II/p62 pathway, the upregulation of ATG3 and ATG7 transcription and the increase of mKeima protein expression (evaluated by flow cytometry). Furthermore, NAE treatment further increased the autophagic pathway in presence of palmitic acid (PA) as shown by the phosphorylation of AMPK and reduced its cytotoxicity. Then, to mimic obesity-associated cellular stress, we exposed mouse ear fibroblasts to PA, one of the major components of fat-enriched diets, for 7 days. Notably, PA increased senescence markers, including SA-β-galactosidase-positive cells and p21/p16 expression. In contrast, NAE treatment enhanced mitochondrial respiration and downregulated p21 and IL-6 mRNAs, two tumor suppressors associated with the senescence-associated secretory phenotype (SASP). Furthermore, flow cytometry analysis demonstrated the senomorphic activity of NAEs in reducing oxidative stress related to doxorubicin-induced DNA damage.

NAEs and their combination may represent a nutritional strategy to counteract metabolic alterations linked to obesity and cellular dysmetabolism by activating protective mechanisms and reducing the senescent phenotype and associated oxidative stress.

Erucin, a natural hydrogen sulfide (H_2S) donor, ameliorates vascular dysfunctions associated with metabolic syndrome.

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H_2S is a gasotransmitter endogenously produced within the body by the action of three enzymes: cystathionine-γ lyase (CSE), cystathionine-β synthase (CBS), and 3-mercapto pyruvate sulfurtransferase (3-MST) [1]. H_2S contributes to vascular homeostasis and its impairment has been demonstrated in several cardiovascular diseases [2]. This study evaluates the possible beneficial effect of Erucin, a natural H_2S donor, in Metabolic Syndrome (MetS)-associated vascular complications.

In vivo studies were performed on db/db mice (n=6), a genetic model of MetS, and their littermates (WT). Animals were treated with Erucin (3mg/kg) for 4 weeks. At 10 weeks of age, mice were sacrificed, and aortas were harvested and used for *ex vivo* and molecular studies. *In vitro* experiments were performed on Chinase Hamster Ovary cells overexpressing subunit α1β1 of soluble Guanylyl Cyclase (sGC). Cells were treated with Erucin (1μM) or vehicle for 2h then the sGC persulfidation levels were assessed. Statistical analysis was evaluated using one or two-way ANOVA.

Ex vivo experiments using aorta harvested from db/db mice demonstrated decreased expression of CBS and CSE, which led to reduced vasorelaxation in response to L-cysteine. Molecular analysis indicated alterations in eNOS/NO signaling, evidenced by changes in the eNOS/Caveolin-1 (Cav-1) ratio, along with diminished vasorelaxation responses to acetylcholine (Ach) and isoproterenol (Iso). Treatment with Erucin improved the Ach-induced vasorelaxation but failed to ameliorate Iso-induced vasorelaxation suggesting that the beneficial effect of Erucin did not affect eNOS/NO signaling, therefore Erucin acts specifically on the smooth muscle rather than the endothelium. Additionally, Erucin administration restored cGMP levels, observed reduced in db/db aortas, indicating a defective soluble guanylate cyclase (sGC)/cGMP signaling pathway. sGC overexpressing cells treated with Erucin displayed a significant increase in cGMP levels due to its capacity to persulfidate sGC, enhancing its activity. Overall, these findings demonstrate a pivotal role of reduced cGMP levels in impaired vasorelaxation in a murine model of MetS involving an impairment of both H_2S and NO signaling. Exogenous H_2S supplementation through Erucin represents a promising alternative in MetS therapy, targeting smooth muscle cells and supporting the importance of lifestyle and nutrition in managing MetS.

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In vitro test to evaluate the anthelmintic efficacy of hazelnut and pomegranate by-products on _Trichostrongylus colubriformis_ and _Haemonchus contortus_ in sheep.

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The nematodes residing in the abomasum (i.e. Haemonchus contortus) and the small intestine (i.e. Trichostrongylus colubriformis) reduce voluntary feed intake and nutrient absorption, impacting the production of small ruminants drastically (Hoste et al., 2016. Adv Parasitol, 93:239-351). The control of this helminth is traditionally achieved with the use of anthelmintic drugs, however due to regulations in organic farming and the rise in anthelmintic resistance (AR), alternatives are sought after. The aim of the present study was to evaluate the in vitro anthelmintic effects of pomegranate (Punica granatum) and hazelnut (Corylus avellana) by-products extracts on T. colubriformis and H. contortus of sheep.

Extracts were tested in vitro on two development stages (eggs and infective larvae) of T. colubriformis and H. contortus using the Egg Hatch Assay (EHA) and the Larval Exsheathment Inhibition Assay (LEIA). The egg hatching rate was measured after incubation with each by-product extract for 48 h at 26 °C. Ensheathed infective larvae were incubated for 3h at 20 °C with each by-product extract.

The inhibition of egg hatching of T. colubriformis and H. contortus, assessed with the EHA, was 48.1% and 12.9% for hazelnut extract and 42.4% 21.6% for pomegranate extract, respectively. The inhibition of outer sheath removal, assessed with the LEIA, was 98.7% and 96.7% for hazelnut and 100% and 95.5% for pomegranate.

The results show that the tested extracts, whit their tannin and polyphenolic fraction, are rich in active molecules against T. colubriformis and H. contortus, and can be used to manage the risk of GIN infestations in sheep flocks, reducing the risk of AR progression.

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Effect of *Fabiana imbricata* Ruiz et Pav. essential oil in prostate cancer cells.

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Prostate cancer is the most common malignancy and the second leading cause of death among men, with over 1.2 million new cases diagnosed worldwide annually [1, 2]. Therefore, new therapeutic strategies are needed for this tumor, which still has a high mortality [1, 2]. Essential oils, characterized by their structural diversity, wide-ranging sources, and biological activities, offer substantial promise for chemotherapeutic drug development [3]. In view of these considerations, our interest was focused on a sample of essential oil from fresh leaves of *Fabiana imbricate* (Solanaceae), a Patagonian medicinal plant widely used in traditional medicine to treat different diseases, including genito-urinary disorders [4]. Chemical characterization was performed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The biological activity was tested in an *in vitro* model using androgen-sensitive (LNCaP) and androgen-insensitive (DU-145) human prostate cancer cells. The cell viability and cell membrane integrity were evaluated by MTT assay and LDH release, respectively. Genomic DNA and the activity of caspase-3 was tested to confirm the cell death for apoptosis. Western blot analysis was employed to evaluate the expression of Bcl-2, Bax, Hsp70, caspase-9, caspase-3, STAT-3, and superoxide dismutase (SOD) proteins. Assays to evaluate reactive oxygen species (ROS) and glutathione (GSH) levels were also performed. It was observed that for its active main components (oxygenated sesquiterpenes, 77.58%), the growth rate of both cancer cell lines was in inverse variation with the different concentrations of the natural product (25-200 □g/ml). In the same experimental condition, the sample showed an insignificant cytotoxic effect on normal cells. The results obtained, permit us also to hypothesize that this essential oil (25-100 □g/ml) is able to induce mitochondrial stress, leading to changes in the expression of Bcl-2/Bax genes and increasing the caspase-3 enzyme activity, DNA fragmentation, and the expression of cleaved caspase-9 and cleaved caspase-3. On the other hand, we observed a significant LDH release only at a higher concentration (200 □g/ml). Interestingly, this essential oil was able to increase intracellular ROS levels, probably by inducing apoptosis in part through the involvement of an oxidative stress process. Consistent with this hypothesis, both cells exposed to our product also resulted in a reduction of the expression of SOD enzyme, correlated with the depletion of GSH. On the other hand, ROS inhibitor N-acetyl cysteine (NAC) in part suppressed the capacity of essential oil to generate ROS production in both prostate cancer cells, and interfered with its capacity to attenuate cell viability, and to increase caspase-3 activity and DNA damage, suggesting the pivotal role of ROS in the apoptotic effect of our sample. Intriguingly, our sample also induced a down-regulation of Hsp70 expression that probably is involvement in the apoptotic process essential oil - evoked. In addition, in the same conditions, the active form expression of STAT-3 proteins resulted increasing. In summary, our study provides a further contribution to the hypothesis of essential oil use for the managing of tumors and suggests that the combination of our sample with other anti-cancer therapies could be used to affect prostate cancer.

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Neuroprotective effects of a novel plant-based formulation with magnesium and vitamin B6.

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The objective of this study was to explore the phenolic composition and the therapeutic potential of a novel formulation containing magnesium, vitamin B6, and aqueous extracts from *Vitex agnus-castus*, *Crocus sativus*, *Melissa officinalis*, *Betula pendula*, and *Betula pubescens*. This formulation was specifically developed to counteract neuroinflammation and depressive symptoms associated with premenstrual syndrome (PMS). The phenolic and flavonoid content was assessed using colorimetric and liquid chromatography techniques. Furthermore, antioxidant and reducing properties were evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and horseradish peroxidase assays.

To determine biocompatibility, the formulation was subjected to ecotoxicological tests, including allelopathy, the brine shrimp lethality assay, and *Daphnia magna* cardiotoxicity evaluation. The formulation was then tested in an experimental model using isolated mouse cortex samples exposed to a 60 mM K^+ Krebs–Ringer buffer, a toxic depolarizing stimulus mimicking inflammation, oxidative stress, and serotonin (5-hydroxytryptamine, 5-HT) depletion—key pathological features of neurological and psychiatric disorders, including depression.

Phytochemical analysis revealed that the formulation is particularly rich in benzoic acids, notably gentisic acid (155.31 µg/mL), and phenylethanoid compounds such as hydroxytyrosol (39.79 µg/mL), which support its antioxidant properties as demonstrated by DPPH (IC50: 1.48 mg/mL), ABTS (IC50: 0.42 mg/mL), and horseradish peroxidase (IC50: 2.02 mg/mL) assays. Ecotoxicological assessments classified the formulation as non-toxic, allowing for the identification of a biocompatible concentration (1000 µg/mL) suitable for application in isolated mouse cortex samples exposed to the K^+ 60 mM Krebs–Ringer buffer. In this experimental setup, real-time PCR was employed to analyze the expression of genes associated with inflammation, neurotransmitter regulation, and hormonal signaling, including *cyclooxygenase-2* (COX-2), *interleukin-6* (IL-6), *estrogen receptor-1* (ESR1), *prolactin receptor* (PRLR), brain-derived neurotrophic factor (BDNF), and *serotonin transporter* (SERT). The results demonstrated that the formulation downregulated *COX-2*, *IL-6*, *SERT*, *ESR1*, and *PRLR* gene expression while upregulating *BDNF* and *IL-10* expression.

In summary, this study provides evidence supporting the use of this novel formulation as a promising therapeutic approach to mitigate inflammation, oxidative stress, and neurotransmitter dysregulation associated with PMS.

Comunicazioni orali - Sessione 14

Novel gram-scale production of dietary isothiocyanate moringin, a slow H_2S donor.

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Moringa oleifera Lam. (*Moringaceae*), often referred to as the miracle tree, contains high amounts of bioactive nutrients and dietary antioxidants, which help in ameliorating oxidative stress and degenerating diseases. Native to northern India, *M. oleifera* is now widely distributed and cultivated across tropical and subtropical climates worldwide. Current research has revealed that *M. oleifera* is a significant tree with multifunctional applications. All parts of the plant, especially leaves and seeds, are used in human and animal nutrition added to food preparation as a supplement, and in the traditional medicine, exhibiting numerous nutraceutical and pharmacological properties including anti-inflammatory, antioxidant, anti-cancer, hepatoprotective, neuroprotective, hypoglycemic, and blood lipid-reducing functions. Most of the aforementioned health promoting properties are related to the presence of glucosinolates (GSLs) and isothiocyanates (ITCs). The major GSL of the plant, namely 4(α-L-rhamnosyloxy)-benzyl GSL (glucomoringin) is the precursor of bioactive 4(α-L-rhamnosyloxy)benzyl ITC (moringin), released upon hydrolysis catalyzed by the enzyme myrosinase (β-thioglucoside glucohydrolase; EC 3.2.1.147), at neutral pH value. Many beneficial effects of moringin have been especially ascribed to the activation of various detoxification enzymes and the reduction of certain inflammatory markers. More recently, the H_2S releasing capacity of different *M. oleifera* tissues were evaluated and compared. *M. oleifera* seeds showed the highest H_2S releasing capacity, followed by roots, leaves and stems. This result was expected as *M. oleifera* seed is a remarkable source of ITC moringin, an emerging slow H_2S donor. Noteworthy, moringa seed is largely exploited for its edible oil with nutritional value similar to that of olive oil, producing a seed cake as a byproduct of oil extraction.

In this work, we developed a novel efficient and green production of ITC moringin. Our straightforward approach combined the valorization of commercially available *M. oleifera* defatted seed cake and the sustainable use of food grade and bio-renewable solvents to boost circular economy, with positive environmental and economic impacts.

First, the defatted seed cake (100 g) was extracted in boiling water. After lyophilization, a fine powder enriched in glucomoringin (35.6%, w/w) was obtained, with an extraction efficiency of 85% on the starting cake. The freeze-dried extract was then hydrolyzed in a biphasic phosphate buffer/2-methyltetrahydrofuran medium in the presence of commercial myrosinase enzyme. In this biotransformation process, moringin was directly isolated in the organic phase as a single pure product, and then obtained as a solid, odorless and stable compound, after evaporation of the solvent. Hydrolysis of the enriched extract (30.2 g) provided 4.1 g of pure moringin with a yield of 74.5% on the expected value. It is worth mentioning that the novel method disclosed herein conveniently allows gram-scale production of the target compound without requiring any chromatographic steps of purification. In conclusion, *M. oleifera* defatted seed cake is an appropriate source for the production of pure moringin on the multigram scale intended for further bioactivity investigations. Particularly, emerging evidence suggests that moringin is endowed with biological properties mediated by H_2S-releasing or -inducing properties that require further elucidation.

Circular Economy and valorization of waste products from agri-food production: Calabrian liquorice leaves as a functional component in baked products.

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Calabria has a very significant biodiversity heritage due to its geographical, morphological and climatic conditions. Many plant species grow spontaneously and are rich in active compounds with specific beneficial activities on human health. Among these plants, licorice (Glycyrrhizza glabra L.) is particularly important also from an industrial point of view. Calabrian licorice is characterized by having a lower content of glycyrrhetic acid, an active ingredient that seems to be responsible for raising blood pressure, so it is a candidate as a source of phytocomplexes that have historically shown to have many beneficial activities on human health. Similar beneficial activities have also been found in the leaves, but these are not used at all, they are discarded and not used except as biomass. English: However, considering that the leaves, to promote robust growth and higher root yield, are pruned annually in late spring, before flowering and therefore during the balsamic time of the drug, it becomes important, therefore, to find an alternative use for these "wastes" that are rich in active components, especially polyphenolic ones. This work, carried out in collaboration with the Consortium for the Protection of Liquorice of Calabria DOP", aims to exploit production waste, in this case the leaves, for the search for bioactive components to be used in the nutraceutical field. The leaves were collected in three different areas of Calabria, in particular in the province of Cosenza. They were extracted with polar solvents (methanol, 50% methanol and water) using two methods (maceration and Soxhlet). All the extracts showed a high content of total polyphenols (about 500 mg/g of dry extract) and total flavonoids (about 20 mg/g of dry extract), highlighting a significant nutraceutical potential. One of the three types of leaves, moreover, was used to enrich a soft wheat flour, obtaining four formulations of biscuits with different percentages of leaf powder. This step, carried out in collaboration with the University of Life Sciences "King Mihai I" from Timisoara, Faculty of Food Engineering, allowed to evaluate the stability of the polyphenolic component during the baking process, in fact, the analysis of total polyphenols showed a quantitative non-variability. A sensory analysis revealed that the enrichment with 2.5% of licorice leaf powder did not change the taste compared to the control product. These results highlight how licorice leaves can be usefully used as a source of bioactive compounds for the production of functional foods, enhancing a by-product of the supply chain and promoting a circular economy.

White mulberry *in vitro* cultures for healthy product development.

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Plant species of the Moraceae family are known for their versatile applications in many fields, including agriculture, nutraceutical, cosmetic and pharmaceutical. Within this family, *Morus alba* L. (white mulberry) is the predominant species among over 150 species of the *Morus* genus which has an enormous economic value beyond sericulture. White mulberry is regarded as a treasure due to the richness in active ingredients and several related activities of its different parts; extracts and isolated compounds from leaf, fruit, root, bark, root bark, and twig have been investigated from both phytochemical and pharmacological perspectives [1]. Known the several activities recognised to white mulberry, and since, often, the extraction leads to the plant death, especially regarding the compound at the root bark level, alternative biotechnologies could be needed for the production of these valuable compounds.

In this scenario, the culture of plants, cells, tissues, and organs under aseptic conditions, generally called plant cell culture, could represent a sustainable alternative to the conventional methods. The *in vitro* cultivation offers several advantages compared to the *in vivo* material, among which, the continuous year-round production independent of geographical and environmental constraints, the uniform quality material productions free of pesticides, the safeguard of natural resources and biodiversity, the possibility of high product concentration produced by rapidly [2].

In light of the issues to overcome, the present study aimed to evaluate *in vitro* plant cell cultures of *M. alba*, for the obtainment of a new plant-derived material. Based on the possibility of less expensive and easier to control marketable materials [3], the growing conditions under totally darkness were pursued both on calli and cell suspensions. Following a more sustainable way of extraction, the juices were obtained by squeezing the cells and analysed; seventeen stilbenoid compounds were identified and quantified by LC-MS-DAD in the juices of calli under photoperiod, and calli and suspensions in the dark.

In addition, seen the driven demand for food products that can meet nutritional needs while also providing health benefits [4], in which context *Morus* trees perfectly fit, the antioxidant and anti-inflammatory activities of the juices were investigated on Caco-2 cells.

Together with the data obtained by the nutritional analysis on calli, the results show that plant cell cultures have the potential to be utilized for the production of innovative healthy food materials, bridging the gap between the even-increasing natural-based product demand and the need for more environmental, social and economic development.

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Ellagic and Punicic acid reduce oxidative stress and neuroinflammation in a kainic acid rat model of epilepsy.

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Epilepsy affects over 65 million people worldwide, placing a substantial burden on patients, caregivers, and society. Oxidative stress and neuroinflammation are increasingly recognized as key contributors to epilepsy pathogenesis. With approximately 30% of epilepsy cases being drug-resistant, there is an urgent need for novel therapeutic strategies. Medicinal plants offer a promising source of bioactive compounds with potential anti-seizure properties. Ellagic acid (EA) and punicic acid (PA), the primary bioactive components of pomegranate fruit and seed oil, respectively, exhibit well-documented antioxidant, anti-inflammatory, and anti-apoptotic effects. This study aimed to investigate the neuroprotective effects of EA, PA, and their combination in a kainic acid (KA)-induced rat model of epilepsy.

Sprague Dawley male rats (n=32) were randomly assigned to four treatment groups (n=8 per group) receiving oral administration of (i) saline solution (1 mL/kg), (ii) EA (50 mg/kg), (iii) PA (150 mg/kg), or (iv) EA (50 mg/kg) + PA (150 mg/kg) 30 minutes before KA injection (15 mg/kg, i.p.). A sham group (n=8) received saline solution both orally and intraperitoneally.

EA, PA, and their combination significantly reduced KA-induced seizures ($p<0.001$ vs. KA). Additionally, both compounds and even more their combination decreased oxidative stress and inflammatory mediators involved in epilepsy, including NF-κB, TNF-α, IL-1, and IL-6 ($p<0.001$ vs. KA), as well as the number of GFAP- and CD11b-positive cells. Histological analysis further revealed that EA and PA ameliorated neuronal damage and reduced neuronal loss by downregulating caspase-3 and Bax and upregulating Bcl-2 and NeuN ($p<0.001$ vs. KA).

This study provides the first evidence that EA and PA, particularly in combination, offer neuroprotective effects against KA-induced epilepsy. Their ability to mitigate seizures, oxidative stress, inflammation, and neuronal death suggests that these bioactive compounds could be valuable as dietary supplements for brain health and as potential nutraceutical options for epilepsy management.

From trauma to tranquility: how myrcene helps the brain bounce back.

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Mild traumatic brain injury (mTBI) is a significant public health concern, often leading to long-term cognitive, emotional, and behavioral impairments. Despite its prevalence, effective therapeutic strategies for mitigating mTBI-related consequences remain limited. Myrcene, a monoterpene abundant in various plant species, possesses notable anti-inflammatory, antioxidant, and neuroprotective properties. Preclinical studies have demonstrated its efficacy in reducing neuroinflammation, oxidative stress, and neuronal damage, suggesting its potential as a therapeutic agent for mTBI.

Among the various consequences of mTBI, alterations in pain sensitivity, aggression, and depression-like behaviors are particularly prominent. Given the lack of effective treatment options and the promising neuroprotective attributes of Myrcene, this study aims to investigate its efficacy in alleviating mTBI-induced behavioral and neurochemical alterations in the C57 mouse model.

Our results highlight the significant therapeutic potential of Myrcene in mitigating the behavioral and neurochemical effects of mTBI. Specifically, Myrcene improved pain sensitivity, social behavior, cognitive function, and emotional regulation in mTBI-affected mice through its anti-inflammatory, antioxidant, and analgesic properties. These findings underscore Myrcene's ability to restore neurotransmitter balance and reduce neuroinflammation, making it a promising natural treatment for TBI-related symptoms.

Aegonychon calabrum (Ten.) Holub: a source of bioactive compounds with antioxidant, anti-inflammatory, and antidiabetic potential

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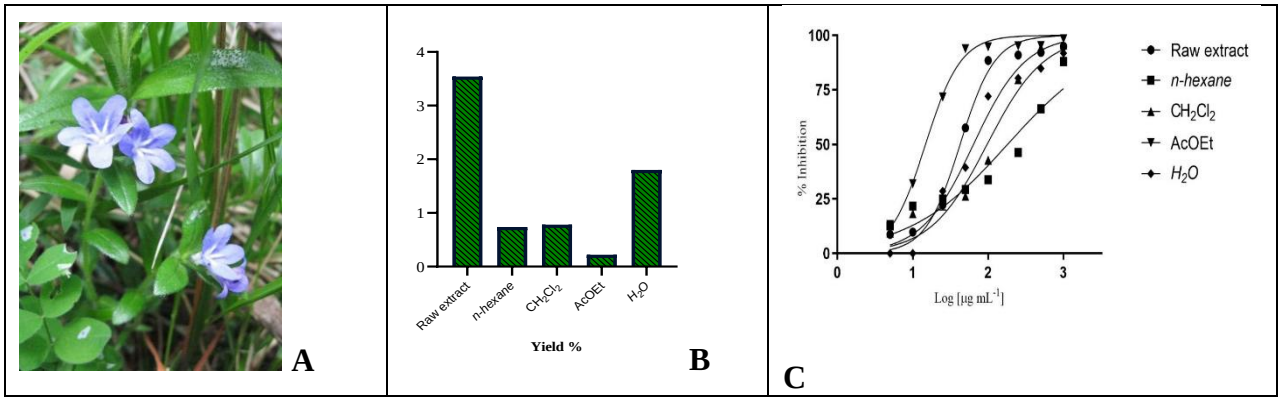
Medicinal plants have always been a valuable resource of bioactive compounds, with broad potential for pharmaceutical applications. The Boraginaceae family includes species with remarkable therapeutic and cosmetic properties. Among them, *Aegonychon calabrum*, a species endemic to the flora of Calabria and southern Italy, has been the subject of limited studies so far.



A: Flowers of *A. calabrum*, Italy, Villaggio Mancuso, Sila Piccola - Lorenzo Cecchi; **B:** Extraction yields; **C:** Radical scavenging activity induced by extracts.

The present research aims to enrich knowledge on this species by assessing the presence of phenolic acids by HPTLC analysis, and studying its biological activities, particularly its antioxidant, anti-inflammatory and antidiabetic activities. The material was subjected to maceration in methanol. The extract was portioned between n-hexane, dichloromethane and ethyl acetate, successively. Antioxidant activity was evaluated through DPPH radical scavenging ability and β-carotene bleaching protection. The potential anti-inflammatory effect through inhibition of nitric oxide production in the RAW 264.7 cell line was evaluated. Finally, the activity on alpha-amylase enzyme inhibition by hydrolysis of glycosidic bonds of digestible carbohydrates was studied. The results obtained revealed that *A. calabrum* is a rich source of phenolic compounds, including caffeic acid, ferulic acid, and chlorogenic acid. The methanolic extract of the plant demonstrated significant antioxidant activity in both the DPPH assay and β-carotene bleaching test. Specifically, an IC50 value of 14.77 ± 0.53 µg/ml was calculated for the ethyl acetate fraction in the DPPH test. In addition, the extract and some of its fractions appear to be promising anti-inflammatory agents, as they have been shown to inhibit nitroxide production in murine macrophage cells. Finally, the extract in ethyl acetate showed significant ability to inhibit α-amylase enzyme, with IC50 value of 19.51 ± 1.43 µg/ml. These results confirm the importance of *A. calabrum* as a source of bioactive compounds with potential applications in pharmaceutical and nutraceutical fields. In particular, the antidiabetic activity observed opens new perspectives for the development of complementary or adjuvant therapies for the management of diabetes, exploiting the potential of this plant endemic to southern Italy.

Elenco abstract poster

Venerdì 11 aprile

Poster - Sessione 1

P1. **Beneficial effects induced by a blend of biologically active compounds in an *ex vivo* model of prostatitis.**

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Prostatitis is an inflammatory condition of the prostate gland, which includes pelvic pain, urinary problems, and sometimes fever and chills. Bromelain, an enzyme complex extracted from *Ananas comosus* (L.) Merr., is well known for its antinflammatory activity. Some studies suggest that bromelain may improve blood circulation and alleviate pain associated with prostatitis. On the other hand, the direct impact of β-sitosterol, a plant sterol, on prostatitis is limited. In addition, different studies suggest that N-acetylcysteine (NAC) may be beneficial when used as an adjunct therapy alongside conventional treatments for prostatitis. The present study aims to evaluate the antinflammatory and antioxidant effects of a vegetal blend based on bromelain (1850 GDU/g), β-sitosterol and NAC in an ex vivo model of prostatitis consisting of samples from mouse prostate treated with lipopolysaccharide (LPS), a well-known proinflammatory agent. The tolerability of bromelain, β-sitosterol, and NAC (1, 5, 10, 20, 40 mg/ml) was evaluated on human fibroblast cells (HFF-1) by MTT assay. In mouse prostate specimens, gene expression of pro-inflammatory and pro-oxidant mediators, such as cyclooxygenase-2 (COX-2), nuclear factor-κB (NF-kB), and tumor necrosis factor- α (TNF-α) was analysed by real-time PCR. We tested three different mixtures based on bromelain, β-sitosterol, and NAC: mixture 1 (bromelain 57 µg/ml + β-sitosterol 62.5 µg/ml + NAC 200 µg/ml), mixture 2 (bromelain 570 µg/ml + β-sitosterol 625 µg/ml + NAC 2 mg/ml) and mixture 3 (bromelain 5.7 mg/ml + β-sitosterol 6.25 mg/ml + NAC 20 mg/ml). Our results showed that mixtures 1, 2 and 3 were well tolerated in HFF-1 cells, without showing toxic effects. Moreover, all three mixtures significantly reduced COX-2, NF-kB, and TNF-α gene expression. In conclusion, the mixtures showed antinflammatory and antioxidant effects, which could help overcome conventional therapies' limits.

P2. **Cannabidiol and beta-caryophyllene alone or in combination in an *in vitro* model of inflammation: a possible synergic effect.**

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Cannabis contains more than 500 distinct compounds, which include cannabinoids, terpenoids, and flavonoids. Cannabidiol (CBD) is the major non-psychoactive cannabinoid, whereas Beta-Caryophyllene (BCP) is one of well know terpenoids of *Cannabis sativa*. In the last few years, with the continuously increasing use of botanical components in dermatology, many studies have found different natural substances both effective and safe. The research has been focused on the therapeutic applications of *Cannabis sativa* (*C. sativa*) constituents for the modulation of the inflammatory process, related to the activation of the endocannabinoid system (ECS). CBD has become a new ingredient for topical application due to its function in alleviating skin inflammation. In addition, BCP has demonstrated effective anti-inflammatory activity in skin diseases such as atopic dermatitis (AD). BCP and CBD are natural compounds that have valid therapeutic properties with good safety profiles and minimal side effects. Recently, there is an emerging idea that the beneficial activities of CBD and BCP were better when they are in combination. The aim of this study was to evaluate the anti-inflammatory effect of the association of CBD and BCP using the *in vitro* model of lipopolysaccharide (LPS)-stimulated human keratinocytes (HaCaT cells).

We investigated the effects of CBD and BCP in human HaCaT cells exposed to LPS, an *in vitro* model of inflammation. The vitality of the cells was quantified by LDH and MTT assays. The levels of pro-inflammatory proteins: IL-1β, COX-2 and Phospho-NF-κB p65 (p-p65) were quantified by Western blotting (WB); Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) were quantified by quantitative real-time polymerase chain reaction (RT-qPCR).

When present in the incubation medium, CBD and BCP reduced the increased levels of pro-inflammatory proteins (IL-1β; COX-2 and p-NF-κB p65) induced by LPS. The anti-inflammatory effects of CBD were blocked by PPARγ antagonist whereas the CB2 antagonist is able to revert the effects of BCP. Selected concentrations of CBD and BCP were able to revert the increased levels of pro-inflammatory genes (IL-1β, IL-6 and TNFα) and these effects are major when the drugs are in combination.

Our results suggest that CBD in combination with BCP works in concert to produce a major anti-inflammatory effect with safety profiles.

P3. ***In Vitro* Anti-Inflammatory effects of ethyl acetate and n-butanol extracts from Aglianico Grape Pomace (*Vitis vinifera* L.).**

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Grape pomace is a major by-product of the winemaking industry, consisting of residual pulp, pressed skins, seeds, stem fragments, and yeast cells used during the pressing and fermentation stages. It is particularly rich in bioactive compounds, including catechin, gallic acid, syringic acid, caffeoyl tartaric acid, oleanolic acid, anthocyanins, and flavanols, which are well known for their antioxidant, anti-inflammatory, antitumor, and antimicrobial properties. In recent years, there has been increasing interest in valorizing viticulture by-products to mitigate their environmental impact and explore sustainable applications. Moreover, the promotion of food products with bioactive compounds that contribute to health benefits has gained attention. In this study, we investigated the *in vitro* anti-inflammatory effects of extracts from Aglianico grape pomace (Vitis vinifera L.), a grape variety cultivated throughout southern Italy and used to produce a prestigious DOCG wine, Taurasi. Two solvents with different polarity were chosen, namely Ethyl acetate (A) and butanol (B). The former selectively extracted oleanolic acid and conjugated linoleic acid along with small amounts of flavan-3-ols; the butanol extract turned out to contain anthocyanins and hydrophilic molecules such as carbohydrates and proline, along with oleanolic acid. *In vitro* experiments were conducted using the murine macrophage cell line J774, stimulated with lipopolysaccharides (LPS) (10 µg/mL) for 24 hours, to assess the effects of grape pomace extracts (A and B) at increasing concentrations (0.1–1 mg/mL, administered 2 hours prior to LPS stimulation). Pre-treatment with A and B extracts did not affect cell viability. However, a concentration-dependent and significant inhibition of nitrite production and inducible nitric oxide synthase expression was observed in LPS-stimulated macrophages treated with increasing concentrations of the extracts. Both extracts significantly reduced pro-inflammatory interleukin-1 beta and NLR family pyrin domain containing 3 expressions at all tested concentrations and increased anti-inflammatory interleukin 10 levels in LPS-stimulated macrophages. No significant effects of extracts A and B were observed on prostaglandin E_2 and tumor necrosis factor alpha production in LPS-stimulated cells.

In conclusion, A and B extracts from Aglianico grape pomace show *in vitro* anti-inflammatory effects, suggesting their potential for managing inflammatory diseases.

P4. **Discovery of a marine-derived Natural Matrix from the Indonesian ascidian *Polycarpa aurata* with anti-inflammatory properties as a source of hydrogen sulfide.**

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Marine natural products are considered as a promising source for new drug discovery, owing to the wide array of chemical bioactive compounds present in marine organisms [1]. In this context, we have characterized the metabolic profile of a fraction from the ascidian *Polycarpa aurata* extract, identifying it as a source of hydrogen sulfide (H_2S) with anti-inflammatory properties. The ascidian *Polycarpa aurata*, collected from the Indonesian coast, was extracted with methanol and, subsequently, with chloroform. The combined extracts were concentrated in vacuo, and the resulting residue was partitioned between water and butanol. The butanol soluble portion was subjected to chromatography resulting in five fractions (PAB1-PAB5). All fractions were capable of releasing H_2S in a cell-free assay, with PAB2 selected as the best candidate for further investigation as an H_2S donor. Indeed, the exposure of PAB2 (30-100-300 µg/mL) to murine macrophages J774 caused a significant increase in the intracellular amount of H_2S compared to the vehicle (°°°°p<0.0001). In another set of experiments, the cytotoxicity of PAB-2 (5, 10, and 50 µg/ml) in J774 cells was evaluated by MTT assay. PAB-2 at the different concentrations used did not affect cell viability. In order to evaluate the anti-inflammatory effect of PAB-2, J774 cells were stimulated with lipopolysaccharide of *Escherichia coli* (LPS 1 µg/ml for 18 h). PAB-2, at all concentrations tested significantly reduced LPS-induced increase in nitric oxide (NO) levels in a concentration-dependent manner and interleukin-6 (IL-6) expression (°p <0.05; °°°p <0.001 vs LPS), coupled with a reduction of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) expression as mRNA (°p <0.05; °°°p<0.001 vs LPS). The anti-inflammatory effect of PAB-2 was also proved in vivo in carrageenan-induced paw edema in mice. Paw edema was monitored for 2, 4, and 6 hours after carrageenan injection. Vehicle and PAB-2 were administered by intra-plantar injection at doses of 0.5, 1, and 5 mg/kg, 30 minutes before carrageenan challange. PAB-2 significantly reduced carrageenan-induced paw edema at all doses tested compared to the vehicle (*p<0.01, ***p<0.001, and ****p<0.0001). Based on these results, the dose of 1 mg/kg was selected for subsequent molecular studies. PAB-2 treatment significantly reduced the expression of iNOS and COX2 as mRNA (°°°p<0.01 and °°p<0.001) and protein (°°p<0.001) compared to the vehicle. In addition, PAB-2 treatment significantly reduced NO production (°°°°p<0.0001) and IL-6 expression as mRNA (°°°p<0.01) compared to the vehicle. These results well fit with the in vitro data. Therefore, for the first time, we have identified a marine-derived natural matrix as a source of H_2S with anti-inflammatory properties. This study paves the way for new possibilities in this field, offering promising therapeutic approaches.

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P5. **Exploring the anti-inflammatory and immunomodulatory properties of a *Mangifera indica* L. extract on murine and human macrophages.**

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The intestinal tract is the largest internal surface of the human body, which forms the barrier between the gut lumen and the host connective tissue. Constantly exposed to dietary and environmental antigens, the intestine also harbours a complex community of commensal bacteria contributing to intestinal tract immunological development. It is in this unique, complex, and vulnerable setting that inflammatory bowel diseases (IBDs) occur in genetically predisposed individuals (1,2). Intestinal mononuclear phagocytes, mainly macrophages, are crucial for maintaining gut homeostasis and immune defence at both innate and, subsequently, adaptative compartments. We recently described a novel molecular pathway that regulates this inflammatory response and immunological perturbance based on a novel dietary polyphenol (mangiferin) from Mangifera indica L. extract (MIE, commonly known as mango) (3,4).

In this study, we evaluated the effects of MIE on both murine and human macrophage function in vitro. Using various experimental techniques, we explored the effects of MIE on i) murine Toll-Like-Receptors (TLRs)-related trained immunity, ii) human macrophages polarisation, gene expression, phagocytosis, and finally iii) investigating the protective effect of MIE on CD14+-monocyte-derived macrophages from an adult IBD inception cohort.

Our results demonstrated that MIE, on peritoneal murine macrophages, was able to downregulate TLR2, TLR4, and TLR6 activation through a significant reduction of both IL-6 and TNF-α release. On human macrophages from healthy donors, MIE displayed protective effects on “M1-like” pro-inflammatory (and to a lesser extent on “M2-like”) macrophage phenotypes, also affecting the phagocytosis process and inducing functional changes in gene expression. Of clinical relevance, MIE was able to modulate TNF-α and IL-10 levels in M1/M2-polarised macrophages from IBD patients, suggesting a therapeutic role in IBDs.

Understanding how intestinal macrophages maintain the peaceful steady state and shape, when needed, intestinal immune responses are indeed crucial to identifying alternative and/or innovative therapeutic molecular/cellular targets and related Crohn's disease and ulcerative colitis (5). Our previous investigations and current findings on MIE in the context of trained immunity support the rationale for its clinical use as nutraceutical and/or functional food in IBDs.

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P6. **Exploring the anti-inflammatory and protective properties of *Achillea erba-rotta* subsp. *moschata* (Wulfen) I.Richardson in brain endothelial cells**

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Achillea erba-rotta subsp. *moschata* (Asteraceae), an alpine endemic plant is traditionally used to treat gastrointestinal diseases. An ethnobotanical survey conducted in Chiesa in Valmalenco (Sondrio, Lombardy, Northern Italy) between 2019 and 2023 (Bottoni et al, 2022; Bottoni et al., 2024), highlighted its traditional uses, including digestive, anti-inflammatory and pain-relieving properties, as well as hypotensive characteristics.

Despite its widespread use, limited studies have been conducted to validate its bioactivity. In literature, the anti-inflammatory activity of this species is demonstrate on the gastrointestinal tract (Bottoni et al., 2024); furthermore, different *in vivo* studies have shown beneficial effects of congeneric species in neuroinflammation (Mozafari et al, 2021; Elmann et al, 2011). This study aims to investigate the effects of *A. erba-rotta* subsp. *moschata* extract and its potential role in mitigating inflammation and restoring blood-brain barrier (BBB) integrity.

The decoction was prepared as documented during the ethnobotanical fieldwork, following the dosages and indications provided by the informants as previously described by Bottoni et al., 2022. For the laboratory analysis, the filtered decoction was freeze-dried. For *in vitro* experiments, immortalized human brain microvascular endothelial cells (ihBMEC) were treated with the extract at different doses (20 and 200 µg/ml) under inflammatory conditions induced by lipopolysaccharide stimulation (LPS, 200 µg/ml). Potential toxicity of the extract was assessed using XTT assay. Expression of inflammatory cytokines, matrix metalloproteinase and adhesion molecules were evaluated using real-time PCR. Lastly, tight junction protein levels were analyzed through western blot analysis.

The *A. erba-rotta* subsp. *moschata* extract at 200 µg/ml significantly reduced the expression of pro-inflammatory cytokines IL-6 and IL-1β after 6 hours of treatment compared to the LPS-treated group, in ihBMEC cells. Both concentrations, 20 and 200 µg/ml, have been shown to significantly reverse the increased permeability of BBB, caused by LPS stimulus, by recovery Occludin levels. Additionally, the extract at both doses effectively restores matrix metalloproteinase 2 (MMP-2) and intercellular ashesion molecule 1 (ICAM-1) levels, following their LPS-induces increase. Lastly, no cytotoxic effects were observed at any concentration.

These findings provide the first evidence that an aqueous extract of *A. erba-rotta* subsp. *moschata*, prepared according to traditional use, exerts anti-inflammatory effects and promotes BBB integrity. These effects are partially mediated through the modulation of inflammatory molecules, including ICAM-1 and MMP-2, which contribute to protection and restoration of tight junctions. This highlights its potential therapeutic application in conditions associated with BBB dysfunction and neuroinflammation.

Poster - Sessione 2

P7. **Brassicaceae-derived Erucin, an H_2S-releasing isothiocyanate, exerts anticancer effects on human triple negative breast cancer cells.**

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Triple-negative breast cancer (TNBC) represents an aggressive form of breast cancer, so called due to the absent expression of the three major prognostic receptors of human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) by tumor cells. For this reason, the therapeutic options remain limited and the mortality rate is still high [1]. Recently, biologically active plant-derived agents, such as isothiocyanates (ITCs) and polysulfides, gained significant interest due to their chemopreventive and anticancer activity. In this regard, it has been shown that the consumption of *Brassicaceae* family vegetables, such as broccoli, cauliflower, cabbage, or rocket salad, is associated with a lower risk of developing cancer. The anticancer propriety of these plants is attributed to the ITCs, which are produced during the hydrolysis of glucosinolates. Evidence from both in vitro and in vivo studies have demonstrated that the protective mechanism of ITCs involves the inhibition of phase I carcinogen-activating enzymes, the induction of phase II detoxification enzymes (such as quinone reductase and glutathione S-transferase), cell cycle arrest, and activation of apoptosis in cancer cells. Moreover, it has been shown that dietary ITCs are well absorbed in the body and hence possess good bioavailability, further rendering them promising candidates for novel anticancer therapies [2]. In the current study, we show that Erucin (ERU), an isothiocyanate releasing H2S, abundantly present in rocket salad (*Eruca sativa Mill.*), significantly decreased the viability of MDAMB231 cells, a validated in vitro model triple-negative breast cancer cells in a time (24-72h) and concentration (1–100 µM) -dependent manner by promoting a) apoptosis, through a pathway depending on the activation of caspase-3 and PARP; and b) autophagy via the induction of ULK1, ATG13, BECN1, and BNIP3 genes. Additionally, ERU prevented intracellular ROS generation while promoting the expression of key antioxidant genes (GCLC, GCLM, SOD1 and HMOX-1) and additionally inhibited MDA-MB-231 cell migration, invasion, and colony formation. Therefore, the natural H2S donor, ERU, modulates multiple protumorigenic signaling pathways, suggesting that Brassicaceae consumption could represent a novel and promising strategy for intervention against TNBC.

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P8. ***In vitro* study of the enhancement of anthracycline cytotoxic activity through photoactivation.**

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Photodynamic therapy (PDT) is a clinically approved therapeutic approach for the treatment of various types of cancer. In PDT, a photosensitizer is activated by light, generating reactive oxygen species (ROS) responsible for its cytotoxic effects on tumor cells. The combination of PDT with chemotherapy (photochemotherapy, PCT) leads to additive or synergistic antitumor effects, potentially improving the treatment efficacy and overcoming chemoresistance. The main limitations of PCT are the need to administer two different molecules, with distinct pharmacokinetic and/or pharmacodynamic profiles. Several chemotherapeutic agents, such as anthracyclines and anthraquinones, are chromophores, making them suitable candidates as photosensitizers for PDT. The aim of this study was to exploit the intrinsic photosensitizing properties of two anthracyclines, daunorubicin (dauno) and epirubicin (epi), to enhance their antitumor activity following photactivation. The cytotoxic activity of dauno and epi was investigated following irradiation in breast cancer cell lines (MCF-7, MDA-MB-231). In particular, the cells were incubated with increasing concentrations of dauno or epi (0.5 - 50 µM), then washed with 1x PBS and irradiated with low-intensity white LED light for 30 minutes. After 24 hours of recovery in drug-free complete medium, cell viability was analyzed using the MTT colorimetric assay. To further investigate the mechanism of cell death triggered by PCT with dauno and epi, the cells were treated with various inhibitors specific to certain cell death mechanisms. Specifically, apoptosis was inhibited using zVAD-fmk, a pan-caspase inhibitor; necroptosis was inhibited using necrostatin 1s, which blocks RIPK1, responsible for activating the necroptotic cascade; and ferroptosis was inhibited using deferoxamine (an iron chelator), vitamin E (a potent lipid ROS scavenger), or ferrostatin 1 (an inhibitor of ROS and lipid peroxidation). In both cell lines, photactivation reduced the half maximal inhibitory concentration values (IC50) of dauno, with an 11.8-fold decrease in MCF-7 cells and an 11.6-fold decrease in MDA-MB-231 cells compared to non-irradiated cells. For epi, the IC50 values in irradiated cells were 11.2 and 5.3 times lower than those obtained under dark incubation in MCF-7 and MDA-MB-231 cells, respectively. Following pre-treatment with inhibitors of ferroptosis or necroptosis, no significant recovery of cell viability was observed compared to anthracyclines alone. In contrast, pre-treatment with z-VAD partially counteracted the cytotoxic activity of dauno and epi, indicating the involvement of apoptosis in the induced cell death. However, no complete recovery of cell viability was observed, suggesting that both apoptotic and necrotic cell death are involved in the phototoxic activity of the two anthracyclines. Photactivation enhances the cytotoxic activity of epi and dauno by triggering both apoptotic and necrotic cell death mechanisms. Therefore, dauno and epi could be used in PCT as both photosensitizers and antitumor agents.

P9. **Identification and validation of antisenescence activity of *Salvia haenkei* extract using a high throughput screening.**

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Cellular senescence is defined as an irreversible cell cycle arrest that occurs in proliferating cells subjected to exogenous stimuli. As a vital cellular defence response, senescence plays a pivotal role in the regulation of both physiological and pathological processes, including tissue repair, aging, and cancer. By testing a library of more than 3000 natural and chemical compounds using a novel screening assay, we have found that an extract from Salvia haenkei (SH), a native plant of Bolivia, is a potent inhibitor of PTEN-loss-induced cellular senescence (PICS). The efficacy of SH was demonstrated with the decrease of replicative and UV-mediated senescence in human primary fibroblasts and in a model of in vitro reconstructed human epidermis. Specifically, senescent cells treated with SH have been shown to modulate paracrine senescence by interfering with the release of IL1α, a proinflammatory cytokine that is an essential regulator of paracrine senescence since it can control the senescence-associated secretory phenotype (SASP).

Furthermore, results have also shown the protective effects on photo/chronological skin aging of Salvia haenkei. Human immortalized keratinocytes treated with SH exhibited enhanced activity under both basal and stressful conditions induced by H202 and/or UVB irradiation. Treatment with the extract promoted wound healing, prevented the formation of reactive oxygen species (ROS) and delayed common aging phenotypes by upregulating several factors, such as occludin, filaggrin, and SIRT1.

In light of these promising results and the favorable safety profile of the extract, Salvia haenkei presents a compelling option for clinical use as an anti-aging skin treatment. Its multifaceted mechanisms of action suggest that it not only mitigates the effects of cellular senescence but also enhances skin resilience against oxidative stress and aging. Future studies should aim to further elucidate the underlying pathways involved in its protective effects, potentially leading to the development of innovative therapeutic strategies for age-related skin.

P10. **Mediterranean extract of *Sidnyum elegans*: a potential novel therapeutic agent against the triple-negative breast cancer.**

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Marine-derived substances from organisms such as sponges, mollusks, and ascidians are known for their diverse biological activities, ranging from cancer-fighting and antioxidant properties to antiviral, antibacterial, as well as neuroprotective effects to varying extents. [1]. However, the properties of the Mediterranean ascidian *Sidnyum elegans* remain largely unexplored. [2]. Thus, our research was designed to study the properties and the potential therapeutic effects of *Sidnyum elegans* in MDA-MB-231, a validated cell model of human triple-negative breast cancer. Toward this goal, the extract of the ascidian *Sidnyum elegans*, collected in the Gulf of Naples (Pozzuoli), were separated using water, ethyl acetate and then butanol (n-BuOH) that further divided the aqueous layer with different grade of polarity. The fraction dissolved in n-BuOH was then processed using medium-pressure liquid chromatography (MPLC) with an increasing gradient elution 100 % H_2O → 100 % MeOH that shifted from pure water to pure methanol, resulting in nine distinct fractions based on polarity, named SEB1-9. The different fractions were then tested for their ability to inhibit MDA-MB-231 cell proliferation in a range of 10-50µg/mL. Among them, the SEB5 fraction, eluted with MeOH/H_2O 7:3, was found to be the most active one, able to induce apoptosis in MDA-MB-231 cells as found in FACS analysis using AnnexinV/PI double staining and cleavage of Caspase-3 in western blot analysis. Additionally, FACS analysis revealed that SEB5 caused a time-dependent arrest of the MDA-MB-231 cell cycle in the G0/G1 phase, accompanied by a decrease in the S phase population. These results were confirmed by the reduced expression of different genes involved in cell cycle such as cyclin D1, cyclin B1, CDC25A, and CDK1 we found in SEB5-treated cells. Finally, SEB5 was also found effective in preventing the migration capacity of MDA-MB-231, which is one of the major malignant hallmarks of cancer progression. In summary, our results demonstrate that *Sidnyum elegans* extract SEB5, could pave the way to a novel therapeutic approach to treat triple-negative breast cancer.

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P11. ***C. cardunculus* L. subsp. *cardunculus*: antiangiogenic activity and formulation strategies.**

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C. cardunculus L. subsp. *cardunculus* [*Cynara cardunculus* L. var. *sylvestris* (Lam.) Fiori], known as wild artichoke, is a promising source of bioactive compounds with potential applications in the pharmaceutical industries. The leaves of wild artichoke were collected and extracted with an ethanol/water mixture (EtOH:H_2O 80:20% v/v, SyEt80) to examine their chemical composition and biological activities.

Analytical techniques, liquid chromatography coupled with mass spectrometry (LC-ESI-QTOF MS/MS) and photometric detection (LC-PDA), were employed to identify in SyEt80 many bioactive compounds belonging to various chemical classes like flavonoids (e.g., luteolin and apigenin derivatives), hydroxycinnamic acids (notably dicaffeoylquinic acids), and sesquiterpene lactones. Among the most abundant ones, there are dicaffeoyl-succinoylquinic acid (20.50 mg/g dry residue) and luteolin 7-rutinoside (31.56 mg/g dry residue).

Quantitative analyses revealed high total phenolic and flavonoid content (133.4 mg Gallic Acid Equivalents/g and 122 mg Quercetin Equivalents/g dry weight, respectively), highlighting the richness of bioactive compounds in this extract.

Additionaly, *in vivo* tests were performed on SyEt80 with the aim to evaluate its ability to modulate angiogenic processes, using two experimental models: the zebrafish embryo and the chick chorioallantoic membrane (CAM). Furthermore, since the ability of cyclodextrins to act as drug absorption promoters, experimental studies were also performed on SyEt80 complexed with hydroxypropyl-β-cyclodextrin (HP-β-CD, 2% w/w). Results showed a mild antiangiogenic activity of SyEt80 in both *in vivo* experimental models. Conversely, the same extract complexed with HP-β-CD, induced marked antiangiogenic effects. Notably, in zebrafish embryos, the HP-β-CD-complexed extract exhibited dose-dependent inhibitory effects (9.80%, 18.15% and 45.75% at 50, 100 and 200 µg/mL, respectively) on the release of endogenous alkaline phosphatase activity as a marker of vessel growth. Similarly, in the CAM model, the complexed extract determined a marked reduction of the vascular network development with a significant decrease of the number of blood vessels (33.33%, 37.89% and 59.45% inhibition at 25, 50 and 100 µg/egg, respectively) as compared with the control.

In conclusion, this study underlines that *C. sylvestris* leaves may represent a valuable source of bioactive compounds. Moreover, these findings also emphasize the importance of innovative formulation strategies, such as cyclodextrin complexation to improve the efficacy of the extract and its applicability in the development of pharmaceutical products.

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P12. ***Castanea sativa*** **Mill. bark extract: a chemopreventing agent inducing cytodifferentiation of a leukemic promyelocytic cell line.**

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Castanea sativa Mill. (CSM), a plant traditionally employed in nutrition and to treat various respiratory and gastrointestinal affections, has shown to possess cancer chemopreventive characteristics. In particular, CSM bark extract previously demonstrated antiproliferative and pro-apoptotic activities against a leukemic lymphoblastic cell line and antioxidant effect on TK6 cells. Starting from this evidence, the aim of the paper was to investigate the possibility of CSM bark extracts to induce cytodifferentiation of the leukemic promyeloblastic cell line HL60, evaluating the expression of two myeloid cytodifferentiation markers, CD11b and CD14. For this purpose, the first step was to analyze the extract's cytotoxicity in order to check that the treatment concentrations selected were adequate. Afterwards, the induction of differentiation of the extract was evaluated. All the analyses were performed by flow cytometry. Our results indicate that CSM bark extract was able to induce HL60 cytodifferentiation to monocytic/macrophage and granulocytic lineage. Overall, our findings suggest that CSM bark extract holds promise as a chemopreventive agent with the ability to induce differentiation of leukemic cells.

P13. **Biological investigation of ferulenol and prenilated coumarin from toxic giant fennel (*Ferula communis* L.).**

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The toxic giant fennel (*Ferula communis* L.) responsible of ferulosis, a hemorrhagic of livestock pasturing in areas of Sardinia is an excellent source of o ferulenol, a prenylated coumarin that shows also paclitaxel (Taxol) mimicry [1].

The phytochemical investigation of a sample of *F. communis* collected in July 2024 near Cagliari let to the isolation of ferulenol as the major constituent together with other derivatives represented respectively by the *E*-ω-hydroxyferulenol, *E*- and *Z*-ω-acetoxyferulenol and the *E*-ω-benzoyiloxyferulenol.

Despite ferulenol shows in vivo anticoagulant activity, its acute toxicity is low compared to warfarin. At the light of this, the natural compound and its derivatives have been tested in a cancer cell line (HeLa cells) and a normal cell line (3T3 fibroblasts) to evaluate their ability to affect viability and induce cell morphological alterations and apoptosis.

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Poster - Sessione 3

P14. *Helichrysum microphyllum* subsp. *tyrrhenicum*: a possible correlation between volatile compounds and phloroglucinols.

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Helichrysum microphyllum subsp. *tyrrhenicum* is one of the endemic species growing in Sardinia known for its essential oil. In this study the chemical composition of the flowered aerial parts of four samples of the plant collected in South-West Sardinia was investigated with a combined focus on volatile constituents and phloroglucinols to find a possible correlation with the presence of arzanol, the major anti-inflammatory compound. The volatile constituents were analysed by GC-MS as EO-HD and with HS-SPME identifying a total of 95 compounds of which 70 and 77 by EO-HD and HS-SPME respectively. The profile of the non-volatile phloroglucinols was investigated by HPLC-MS/MS. Arzanol concentrations ranged from 2.79 to 21.87mg/g, helipyrone showed the same trend but in lower concentration. Surprisingly, leaves and stems contain higher concentration of phloroglucinols than the flowers. The concentration of arzanol was positively correlated to the one of γ-curcumene and ethylpyrone in the EO, while a negative correlation was observed with the monoterpene limonene and linalool as well as with the sesquiterpene 5-eudesmen-11-ol [1].

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P15. **Aronia melanocarpa fruits: preliminary studies on chemical composition and biological activity.**

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Aronia melanocarpa is a shrub from the Rosaceae family, native to Eastern North America and Canada. Introduced in Europe in 1900 for commercial activities, it is now widespread in Russia and the rest of Eastern Europe. In recent decades, *A. melanocarpa* has become popular thanks to properties associated with its consumption, such as antioxidant, antitumor and anti-inflammatory activity, as well as for its protective activity against cardiovascular diseases and metabolic disorders [Rodríguez-Werner et al, 2019]. Furthermore, previous research on extracts derived from *A. melanocarpa* berries, demonstrates that if applied to the skin, after exposure to UV radiation, they can attenuate the serious histopathological damage caused by these.

In this work, a preliminary investigation was conducted on the possible inhibitory activity of *A. melanocarpa* berry extracts. These extracts were obtained using different solvents and extraction methods. The phytochemical profile was obtained by Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS). The inhibitory activity of the extracts was evaluated on three important enzymes involved in skin ageing processes: tyrosinase, elastase and collagenase.

The choice to test the extracts on these enzymes derives from some scientific evidence that documents the biological effect of the natural extracts of the plant on skin ageing and related morphophysiological alterations.

Extracts obtained from *A. melanocarpa* fruits were evaluated for antioxidant activity using ABTS and DPPH assays. The EC_{50} value and the total content of polyphenols and flavonoids were determined, for extracts with good antioxidant activity.

P16. **From olive mill processing waste to plant-based complexes for nutraceutical applications.**

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Olive cultivation in Sicily is a deeply ingrained tradition, with over 160,000 hectares of land dedicated to olive groves. This long-standing agricultural practice has been passed down through generations, playing a crucial role in the region's economy and solidifying its reputation for high-quality olive oil production. Sicily's distinctive climate and soil conditions create an optimal environment for olive growth, particularly for the Tonda Iblea variety, which is highly valued for its rich flavor and nutritional properties. The primary products derived from this cultivation are table olives and extra virgin olive oil. However, olive mills generate substantial amounts of by-products, including vegetation water (VW) and virgin pomace (VP). VW is considered highly polluting due to its high organic load and low biodegradability, while VP consists of woody fragments, pulp, and residual oil. Initially regarded as waste, these by-products have gained renewed interest in light of environmental sustainability efforts. They are currently repurposed for pellet production, fertilizers, animal feed, and water purification, yet their full potential remains largely unexplored.

This study aims to unlock the value of VW and VP, obtained from the milling of organic olives (Tonda Iblea cultivar), by developing plant-based complexes for use in the nutraceutical sector within a circular economy framework. Food-grade dry extracts were obtained through maceration, yielding polyphenol-rich extracts (PE). The resulting plant complexes were first analyzed using colorimetric assays to quantify key bioactive compound classes, followed by LC-DAD-ESI-MS analysis to characterize secondary metabolites in detail. Antioxidant and anti-inflammatory properties were evaluated using various spectrophotometric and spectrofluorimetric assays. Both VW and VP PE exhibited high phenolic content (5.87 and 4.92 g/100 g, respectively), with phenylethanoids and secoiridoids as the dominant compounds, followed by flavonoids (1.15 and 1.69 g/100 g, respectively). Additionally, both VW and VP demonstrated significant in vitro antioxidant activity (IC_{50} 1.92–696.71 µg/mL and 2.77–929.86 µg/mL, respectively), with VW exhibiting the strongest free-radical scavenging capacity. A similar trend was observed in anti-inflammatory tests, where VW displayed superior protease inhibitory activity (IC50 90.90 µg/mL vs. 139.29 µg/mL for VP). However, no anti-inflammatory activity was detected for VW in the bovine serum albumin denaturation assay, whereas VP showed strong anti-peroxidase activity (IC_{50} 0.98 µg/mL).

Based on these findings, VW was selected for further cell-based investigations. After assessing its cytotoxicity on Caco-2 cells across a wide concentration range (1–400 µg/mL), VW was tested at 24 and 48 hours in a wound-healing model to simulate a leaky gut condition. Hyaluronic acid (1 mg/mL) served as the reference standard. VW exhibited potent, concentration-dependent wound-healing activity within the range 6.25–200 µg/mL, achieving 50% wound closure at 24 and 48 hours at concentrations of 71.18 and 21.65 µg/mL, respectively. Given its promising biological activity, further studies are currently underway to investigate the antioxidant and anti-inflammatory effects of VW using a transwell model of Caco-2 cells to mimic the intestinal epithelial barrier in order to support its potential use in the field of inflammatory bowel diseases

P17. **Chemical markers in Italian propolis: chrysin, galangin and CAPE as indicators of geographic origin.**

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Bee propolis, a natural resinous material gathered by honeybees from plant sources, is widely known for its medicinal and biological benefits. Despite variations in its botanical and geographical origin, propolis consistently shows antioxidant, immunomodulatory, and antimicrobial properties.
It has a universal role in beehives as a sanitizing agent, composed of terpenes, wax and phenolic compounds, mainly made of leaf buds, but also of other plant secretions. However, these general properties are insufficient for its rational application in modern medicine, highlighting the need for further research.
Comprehensive research is essential to explore the chemical and pharmacological properties of propolis, focusing on its mechanisms of action, specific targets, and the unique phytocomplexes that vary with its geographical origin.
The aim of this work, focused on Italian propolis, is to better investigate and to clarify the chemical composition of samples collected in different geographical areas, providing an update of and an insight into the current literature. Moreover, this study aims to develop methods for the identification of chemical markers in Italian propolis and to determine their geographic origin in order to ensure the quality of propolis product.
By analyzing 27 samples collected in the same year from various regions, covering Northern, Central, and Southern Italy, as well as Capraia Island and Sicily, the total polyphenol (TP) and total flavonoid (TF) content, alongside the quantification of pinocembrin, chrysin, galangin, and caffeic acid phenethyl ester (CAPE), were investigated. Additionally, DPPH assays were conducted to evaluate the antiradical activity of propolis samples. Our results revealed that total polyphenols (TP) and total flavonoids (TF) vary significantly in propolis from different regions, particularly in samples from islands, and that pinocembrin is almost always present in Italian poplar propolis, although at different levels.
However, a more precise differentiation of the geographical origin was achieved through the quantification of the sum of chrysin and galangin, and CAPE. Conversely, the DPPH assay proved ineffective for this purpose, as the results were largely uniform and lacked statistical significance.This study contributes to the growing body of research on propolis by providing a more structured approach to characterizing its chemical composition by geographic origin. The results offer valuable insights into the development of more refined methods for distinguishing the origin of propolis, which may be useful for quality control, regulatory frameworks, and commercial evaluation of this natural product.
Building on this preliminary study, future research should aim to expand the scope by comparing sub-areas such as river regions, Apennine and Alpine propolis, as well as samples from inland hives in Sicily and Sardinia. Additionally, significant efforts should focus on enhancing the analytical depth beyond the examination of major constituents.

P18. **Non-volatile terpenoids and lipophilic flavonoids from *Achillea erba-rotta* Subsp. *moschata* (Wulfen) I. Richardson.**

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Achillea erba-rotta subsp. *moschata* is a perennial herb endemic to the Central Alps. Because of its digestive properties and its aroma, the plant is used in folk medicine and to aromatize beverages. Various studies have confirmed the gastroprotective and digestive activity of the plant, only evidencing, however, the presence of widespread volatile terpenoids and phenolics, leaving its phytochemical profile marginally investigated and mainly focused on antioxidant and antibacterial aspects. The closely related *A. erba-rotta* subsp. *erba-rotta* is a prolific producer of sesquiterpene lactones and lipophilic flavonoids, two classes of compounds associated with the functionality of the gastrointestinal system and anti-ulcer activity. Given the biological profile of the latter taxon, the importance of providing correct identification, and the lack in information in biomarkers to identify unique characteristics, we therefore investigated the occurrence of compounds from these two classes also in the subsp. *moschata*, with the twofold aim of identifying their active constituent and assessing their suitability for chemotaxonomic studies and the authentication of the plant in finished products [1].

1. Salamone, S., Aiello, N., Fusani, P., Rosa, A., Nieddu, M., Appendino, G., & Pollastro, F. (2023). DOI: 10.3390/plants12020402

P19. **Preliminary phytochemical investigations on *Prunus domestica* L. fruit.**

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Plums, the fruits of *Prunus domestica* L., have been recognized and used traditionally for centuries for their effects on the intestine. Thanks mainly to the presence of soluble and insoluble fibers, their consumption promotes evacuation and intestinal regularity. It is also necessary to highlight the presence of several organic acids, phenolic and polyphenolic compounds, vitamins, and mineral salts, which are fundamental additions to the daily diet.
The aim of this work was to evaluate the phytochemical profile of three brands of dried plums, whose corresponding fresh materials come from either Montalcino (Siena), Italy (unspecified) or abroad. Also, subsequent studies were carried out to asses the variations of some of the main components following the digestion process.
The extracts of the three samples, also examined on the basis of the extraction solvent used, were characterized by means of specific spectrophotometric assay. In particular, the main constituents that were quantified were polyphenols (adapted Folin-Ciocâlteu assay), flavonoids (direct reading), anthocyanosides (reading in acidic environment), and polysaccharides (adapted phenol-sulfuric acid assay). The results obtained by these tests are in line with data from other studies, confirming polysaccharides as the major component (> 20%). The phenolic composition was also examined by HPLC-DAD, which showed that it is mainly made up of cinnamic and hydroxycinnamic acids and derivatives.
Based on the traditional use for the effects at intestinal level, the study then moved on to the determination of the composition of the samples after having undergone a treatment that mimics the digestion process. This was possible through a simulated-digestion protocol, which uses variations in pH and enzymes characteristic of the physiological process. What was observed was a substantial maintenance of the sugar content, with residual percentages of polysaccharides generally higher than 60%. Furthermore, as regards the composition in phenolic compounds (analyzed using HPLC-DAD), approximately half of the quantity of cinnamic and hydroxycinnamic compounds was maintained, also considering possible intermediate partial modifications.
In conclusion, after evaluating the phytochemical profile of the plums under examination, it was possible to observe how a simulated digestive process does not lead to the complete degradation of the two classes of compounds (polysaccharides and phenolic compounds) underlying the intestinal effect for which they are consumed. Subsequent studies should focus on a finer molecular characterization of the sugar and phenolic components and the possible effects of each of them at the intestinal level.

Poster - Sessione 4

P20. **Recovery of bioactive compounds from the biomass of aromatic plants after distillation Using NADES: a sustainable alternative extraction method.**

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The increasing demand for EOs and the relatively low recovery from the plant cause a not negligible disposal problem linked to the by-products. The residual biomass deprived of the volatile compounds following steam distillation are rich in secondary metabolites of aromatic plants, such polyphenols. Currently, the biomass is recycled for bioenergy production; however, this is the less preferable procedure to manage biomass, according to the "waste hierarchy" proposed by the Environmental Protection Agency (EPA). According to the EPA's scheme, waste reuse and recycling are favored over energy recovery. Moreover, the reuse of the by-product would make the production of EOs more sustainable being a highly energy-intensive process.

Polyphenols are usually extracted via conventional methods that employ inflammable, toxic, and contaminant organic solvents. In recent years, the research effort focused on the development of more sustainable strategies by using green technologies with higher process performances and solvents with a lower environmental impact. In this context, Natural Deep Eutectic Solvents (NADES) have been proposed as safe and environmentally friendly alternatives to classic solvents. The NADES are eutectic mixtures composed of a hydrogen bond acceptor and a hydrogen bond donor capable of efficiently solubilizing lipophilic compounds and protecting thermolabile compounds. In the last years, several studies have successfully demonstrated the potentiality of NADES in extracting polyphenols from aromatic plants; however, the employment of NADES in the recovery of bioactive compounds of aromatic plant by-products has not been considered so far.

In the present study, the NADES were considered for the extraction of polyphenolic compounds from the biomasses of several *Lamiaceae* and *Asteraceae* plants widely distributed and cropped in the North of Italy for their characteristic aroma and therapeutic properties. Specifically, *Artemisia dracunculus* L., *Echinacea purpurea* (L.) Moench, *Helichrysum italicum* (Roth) G. Don, *Lavandula angustifolia* Mill., *Lavandula x intermedia* Emeric ex Loisel, *Melissa officinalis* L., *Salvia officinalis* L., *Salvia rosmarinus* Spenn., and *Salvia sclarea* L. were selected.

Different NADES formulations were prepared, and their extraction capability was compared to that of ethanol, a commonly extractive organic solvent to develop a sustainable extraction procedure for the recovery of bioactive compounds from oil-exhausted biomasses derived from the distillation of aromatic plants.

The chemical characterization of the extracted compounds was performed using advanced analytical techniques, including HPLC-DAD and UHPLC-HRMS.

The NADES composed of choline chloride and lactic acid at 1:1 molar ratio showed a superior extraction efficiency for biomasses from both the *Asteraceae* and *Lamiaceae* families compared to ethanol. Moreover, the NADES extracts contained a greater number of metabolites than the EtOH extracts, due to the higher extractive power of the eutectic solvent. Thus, NADES demonstrated to be a viable alternative system for the recovery of bioactive compounds from the EO exhausted biomasses considered in the study.

Looking ahead, future research should continue to explore NADES in the context of sustainability. Further investigations should focus on their environmental impact throughout their lifecycle and their potential to support circular economy principles.

P21. **Health benefits of traditional infusions based on alpine *Artemisia* species: Chemical profile and biological potential.**

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"Genepì" plants (*Artemisia genipi* Weber and *A. umbelliformis* Lam., Compositae family) are among the most sought-after species in the Alpine region, where they are traditionally used to prepare herbal teas and liqueurs with a unique flavour profile, as well as being appreciated for their healing properties, including digestive, diuretic, antiseptic and anti-cold effects (Vitalini et al. 2015; Sulaiman et al. 2023). Although phytochemical studies have mainly focused on essential oils, research on alcoholic beverages is still scarce and largely outdated (Vouillamoz et al. 2015). Even more surprisingly, no data are available regarding the chemical composition and biological activities of infusions.

The aim of this work was to determine the phenolic acid and flavonoid profile of the aqueous extracts of the two *Artemisia* species, obtained using the traditional infusion herbal tea recipe and subsequently freeze-dried. Analyses were carried out using HPLC-DAD and LC-MS, revealing that, while the extracts were qualitatively similar, they differed in the relative amounts of each compound. Flavonoids were more abundant than phenolic acids, with *A. genepì* showing a higher total flavonoid content than *A. umbelliformis* (34.95 µg/ml and 26.66 µg/ml respectively). Tricin, free or glycosylated, was the predominant flavonoid, in both samples. In *A. umbelliformis*, tricin-di-glucuronide was the most abundant derivative (11.77 µg/ml), followed by tricin-7-glucoside (5.60 µg/ml), whereas in *A. genepì*, an opposite trend was observed. Notably, both extracts contained a variety of flavonoid aglycones, including luteolin, tricin, and axillarin, in both free and conjugated forms.

Beyond the phytochemical characterization, some aspects of their bioactivity were investigated: (i) radical scavenger capacity using ABTS•+ and DPPH• assays and (ii) effects on cell viability in wild-type (HWT) and dystrophic (HDMD1) human muscle cell lines. *In vitro* results showed that *A. genepì* and *A. umbelliformis* inhibited the radical cation ABTS•+ by 76.2% and 68.4%, respectively, while acted by against DPPH• reducing it by 82.3% and 86.1%, with no significant difference between the two species, despite their distinct chemical profiles. In the second case, the aqueous extracts of *A. umbelliformis* (0.05 mg/mL) significantly enhanced cell viability by 25% in HWT and 30% in HDMD1 cells. Under differentiation conditions, 0.1 mg/mL of *A. genepì* aqueous extract notably increased cell viability in HDMD1 myotubes. No cytotoxic effects were observed for any aqueous extracts at lower concentrations.

Additionally, (iii) anti-inflammatory activity tests are underway to provide a more complete understanding of the potential benefits of these plant infusions.

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P22. **Health-Promoting effects, phytochemical constituents and molecular genetic profile of the Purple Carrot 'Purple Sun' (*Daucus carota* L.).**

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The purple carrot cultivar 'Purple Sun' (*Daucus carota* L.) is characterized by a relevant content of phenolic compounds and anthocyanins, which may play an important role in reducing the risk of chronic diseases and in the treatment of metabolic syndrome. In the present study, the genetic diversity, phytochemical composition, and bioactivities of this outstanding variety were studied for the first time. Genetic analysis by molecular markers estimated the level of genetic purity of this carrot cultivar, whose purple-pigmented roots were used for obtaining the purple carrot ethanol extract (PCE). With the aim to identify specialized metabolites potentially responsible for the bioactivities, the analysis of the metabolite profile of PCE by LC-ESI/LTQ Orbitrap/MS/MS was carried out. LC-ESI/HRMS analysis allowed the assignment of twenty-eight compounds, putatively identified as isocitric acid, phenolic acid derivatives, hydroxycinnamic acid derivatives, anthocyanins, flavanonols, flavonols, oxylipins, and the sesquiterpene 11-acetyloxytorilolone; compound, corresponding to the primary metabolite trihydroxyoctanoic acid (TriHOME), was the most abundant compound in the LC-ESI/HRMS analysis of the PCE, and hydroxycinnamic acid derivatives followed by anthocyanins were the two most represented groups. The antioxidant activity of PCE, expressed in terms of reactive oxygen species (ROS) level and antioxidant enzymes activity, and its pro-metabolic effect were evaluated. Moreover, the antibacterial activity on Gram (−) and (+) bacterial strains was investigated. An increase in the activity of antioxidant enzymes (SOD, CAT, and GPx), reaching a maximum at 0.5 mg/mL of PCE with a plateau at higher PCE concentrations (1.25, 2.5, and 5.0 mg/mL), was observed. PCE induced an initial decrease in ROS levels at 0.1 and 0.25 mg/mL concentrations, reaching the ROS levels of control at 0.5 mg/mL of PCE with a plateau at higher PCE concentrations (1.25, 2.5, and 5.0 mg/mL). Moreover, significant antioxidant and pro-metabolic effects of PCE on myoblasts were shown by a reduction in ROS content and an increase in ATP production linked to the promotion of mitochondrial respiration. Finally, the bacteriostatic activity of PCE was shown on the different bacterial strains tested, while the bactericidal action of PCE was exclusively observed against the Gram (+) *Staphylococcus aureus*. The bioactivities of PCE were also investigated from cellular and molecular points of view in colon and hematological cancer cells. The results showed that PCE induces proliferative arrest and modulates the expression of important cell-cycle regulators. For all these health-promoting effects, also supported by initial computational predictions, 'Purple Sun' is a promising functional food and an optimal candidate for pharmaceutical and/or nutraceutical preparations.

P23. **Valorization of shea butter from local production in Benin: a phytochemical and pharmacological study.**

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The shea butter tree or *Vitellaria paradoxa* (C.F. Gaertn.) is an endemic species belonging to Sapotaceae family, largely distributed in the sub-Saharan Africa [1]. plays a crucial role in both the ecosystem and the livelihoods of local populations, particularly through its kernels, which are processed to produce shea butter, also known as *butyrospermum parkii*, shea oleine or karite nut butter. It represents a multipurpose resource, being harnessed as a cooking oil, soap, and medicinal remedy. In traditional medicine, shea butter has been especially used to relieve dermatological disorders, such as burns, wounds, lesions and scars, and to counteract skin infections [1]. The extensive traditional use of sea butter has sparked growing scientific interest in its bioactive components and their potential therapeutic applications. Studies increasingly focus on its antioxidant, anti-inflammatory, and wound-healing properties [2,3], aiming to provide a scientific basis for traditional practices and to elucidate its mechanisms of action. In this context, and to promote the value of local production, the present study was aimed at characterizing the phytochemical profile and bioactivities of a shea butter sample produced by the TIKKONA Cooperative in the Republic of Benin (namely KAR-T), in comparison with a commercial product (namely KAR-K). To this end, the samples underwent gas chromatography/mass spectrometry (GC/MS) analysis to determine the phytochemical composition. Moreover, a screening of bioactivities, including cytotoxicity in human malignant melanoma A375 cells and HFF-1 noncancerous fibroblasts, wound healing and cytoprotection towards oxidative damage, in terms of cell viability restoring and oxidative stress inhibition, was performed according to previously reported methods [4]. The GC/MS analysis highlighted the samples were characterized by unique compositions in both saturated and unsaturated fatty acids, with palmitic and oleic acids the most abundant in KAR-T and palmitic and stearic acids in KAR-K. In addition, the presence of the triterpene lupeol was also detected in both samples but with a more significant quantity in KAR-T rather than in KAR-K. Both samples also resulted able to impair the cell viability of A375 cancer cells, although with a higher potency of KAR-K, which was cytotoxic starting from the concentration of 100 µg/ml, despite 250 µg/ml of KAR-T. Similarly, they reduced cell viability in HFF-1 fibroblasts, although at higher concentrations than those affecting A375 cells. When assessed for the wound healing, KAR-T and KAR-K progressively increased the migration of HFF-1 cells with respect to control over the time, inducing almost a complete disappearing of the wound area already after 24 h treatment. In the cytoprotective studies a modulation of the cell redox status was found too. The present findings support the traditional use of shea butter in skin disorders and highlight the need for additional studies to clarify the underlying mechanisms, investigate the role of specific phytochemicals for product standardization, and assess its bioactivity in other experimental models.

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P24. **Phytochemical profile and *in vitro* bioactivities of the Italian tomato landrace Riccio di Parma.**

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Solanum lycopersicum L. var. Riccio di Parma (Solanaceae family) is an Italian tomato landrace, first cultivated in 1867 in the Parma countryside, but gradually abandoned in the 1950s by the canning industry. This variety is characterized by its irregular shape, deep grooves, bright red color, green shoulder, and high resistance to drought and cryptogamic diseases; moreover, it is cultivated through dry farming techniques without irrigation, reducing the risk of contamination. In the attempt to valorize this local species and to highlight a possible interest in terms of safety and nutraceutical properties, a phytochemical and biological characterization of Riccio tomato and its byproducts, provided by La Sbecciatrice company (Caserta, Campania), was performed within the project "ON Foods – Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods", funded by the European Union – NextGenerationEU. To this end, whole tomatoes (T), tomato sauce (TS), and tomato waste (TW), collected over two harvesting years, i.e., 2022 and 2023, were freeze-dried and subjected to Bligh-Dyer extraction obtaining two different organic (O) and hydroalcoholic (HA) extracts for further analysis. The samples underwent a multimethodological phytochemical analysis, based on untargeted (NMR, ESI FT-ICR MS) and targeted (UV-Vis, HPLC-MS, ICP-OES) methodologies. Moreover, a screening of bioactivities associated to the polyphenol content, including antioxidant, chelating, reducing, and antiglycative as well as the cytotoxicity on human cell lines, both cancerous and noncancerous (e.g. pancreatic Bx-PC3 cancer cells and HFF-1 fibroblasts). Cytoprotection by nontoxic samples towards the oxidative damage induced by tert-butyl hydroperoxide (tBOOH), in term of cell viability and intracellular ROS (reactive oxygen species) levels, was evaluated too. HPLC-MS/MS analysis highlighted the presence of 11 polyphenols in HA extracts. Chlorogenic acid, rutin, and naringenin were identified and quantified as the most abundant polyphenols in T23 relative to T22, while the opposite trend was observed in tomato waste. Regarding hydroxycinnamoylquinic acids, they were found in similar quantities in TS22 and TS23, while flavonoids were more expressed in TS22 with respect to TS23. Moreover, the extracts were found endowed with chelating abilities towards ferrous and ferric ions, although with different potency and efficacy. T22 and T23 HA extracts were the most effective as ferric ion chelators, followed by sauce and waste, despite lower ferrous ion chelating abilities. O extracts from 2023 harvest exhibited marked ferrous ion chelating effects, whereas those from 2022 harvest, were more effective as ferrous ion chelators. All the samples were well tolerated by HFF-1 cells, except for T22 and TS22 O extracts and T23 HA extract, which significantly reduced cell viability at concentrations higher than 100 µg/ml. A marked cytotoxicity was also produced by the organic extracts in BxPC3 cancer cells; particularly, T22 O extract hindered the cell viability by about 45% at 50 µg/ml, although the effect was lower than that of doxorubicin. The extracts counteracted tBOOH-induced oxidative stress, with potency varying by extract type and product. Further studies are needed to confirm these findings, clarify the mechanisms, and verify the bioactivities of Riccio tomato extract in other experimental models.

P25. **Nutritional and bioactive potential of borlotto bean pod: a sustainable source for nutraceutical applications**

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Beans are the most widely consumed legumes worldwide, with an annual global production of 27.6 million tons, approximately 71% of which is allocated for human consumption (FAO, 2020). These legumes are rich in valuable nutritional and health-promoting compounds, particularly phenolic compounds, which are primarily concentrated in the seed coat and pod. Although these parts are often discarded during processing, they represent promising plant-based sources for the recovery of bioactive compounds with recognized health benefits. Notably, the pod constitutes approximately 40% of the total weight of *Phaseolus vulgaris* L. fruit.

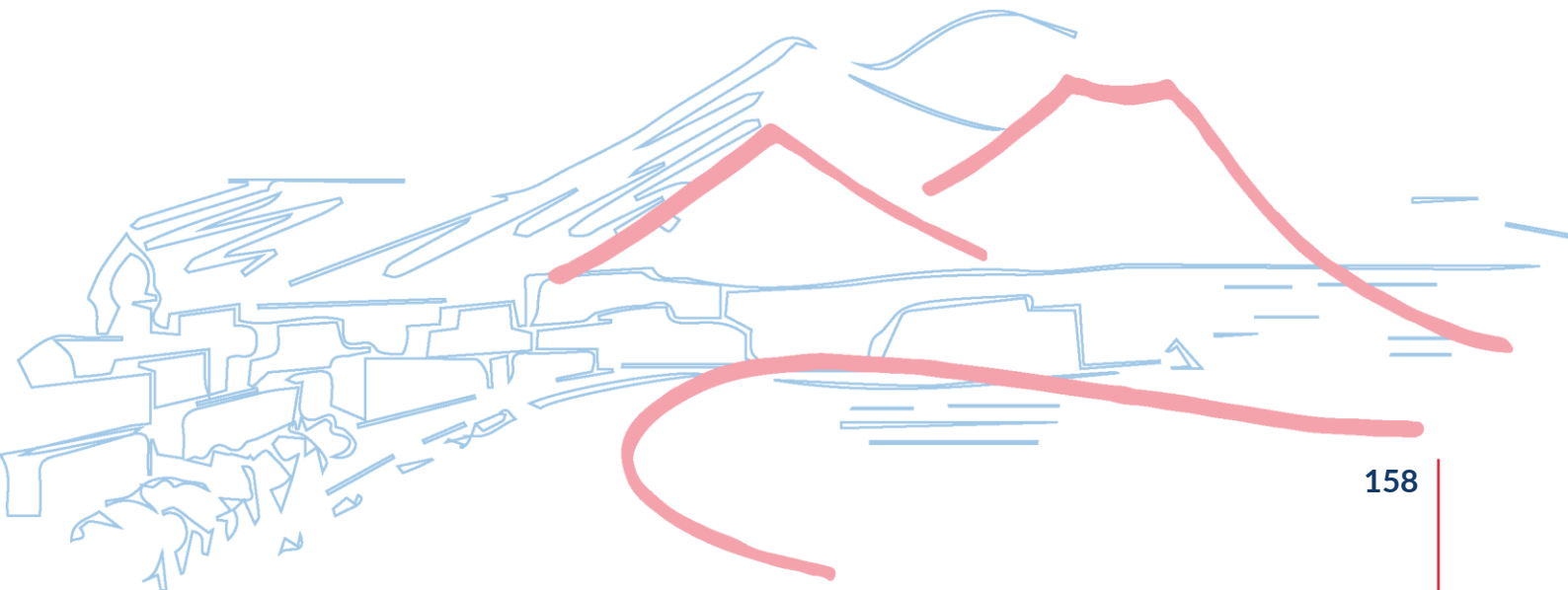
This study aimed to investigate the nutritional properties of raw Borlotto bean pod, to develop a food-grade extract (BPE), and to characterize its primary and secondary metabolites profile using ^{1}H-NMR and LC-DAD-ESI-MS analyses, respectively. Additionally, its antioxidant and anti-inflammatory properties were evaluated through various in vitro assays based on different reaction mechanisms and environments.

The analyzed matrix proved to be a valuable source of high-nutritional-value compounds, exhibiting a low caloric content, a substantial carbohydrate percentage (~20%), with half composed of fiber, and a very low lipid content, predominantly consisting of monounsaturated fatty acids. Moreover, it displayed a noteworthy sodium content. ^{1}H-NMR analysis identified the presence of α- and β-glucose, sucrose, and, most notably, inulin—a non-digestible carbohydrate with well-established prebiotic properties. Additionally, amino acids such as alanine, threonine, and gamma-aminobutyric acid (GABA), as well as organic acids including succinic, citric, and fumaric acids, were detected. This analysis also highlighted the presence of trigonelline, an alkaloid particularly abundant in legumes, known for its antioxidant, anti-inflammatory, hypoglycemic, galactagogue, antispasmodic, immunostimulant, and diuretic properties.

Beyond the primary metabolites identified, many of which already possess notable health-promoting effects, BPE was found to be a good source of polyphenols (556.89 ± 47.91 mg/100 g), with approximately one-fifth consisting of flavonoids (113.96 ± 8.55 mg/100 g). These flavonoids primarily belong to the flavan-3-ol class, including catechin, epicatechin, and their derivatives, while a modest amount of proanthocyanidins was also detected. BPE exhibited concentration-dependent antioxidant activity, particularly in hydrogen atom transfer-based assays (ORAC and β-carotene bleaching, with an average IC_{50} of 17.61 µg/mL), suggesting that its bioactive compounds primarily exert their effects through this mechanism. It also demonstrated excellent iron-chelating capacity (IC_{50} = 83.90 µg/mL), likely attributed to its significant flavonoid content.

Regarding anti-inflammatory activity, the relatively weaker effect observed in the bovine serum albumin denaturation assay suggests that the bioactive compounds within the plant-complex primarily act through an enzymatic mechanism, as evidenced by the protease inhibition test (IC_{50} = 370.56 µg/mL).

In conclusion, the Borlotto bean pod represents a valuable source of nutritional and health-promoting compounds, holding a significant potential for recovery and repurposing in the nutraceutical sector, aligning with the principles of a circular economy.



Poster - Sessione 5

P26. **Valorization of *Citrus bergamia* by-products: a sustainable approach for the prevention and treatment of metabolic diseases.**

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Metabolic diseases represent a crucial challenge to public health, with a steadily increasing incidence globally. These pathologies, characterized by a complex interplay of factors, including obesity, insulin resistance, hypertension, and dyslipidemia, lead to a significant increase in the risk of developing serious cardiovascular conditions and type 2 diabetes. This work aims to identify an innovative approach to address metabolic diseases, by exploiting a byproduct of bergamot processing as a potential source of effective phytotherapeutics for their treatment. For the analysis, a waste product derived from the production of Citrus bergamia essential oil and juice was used. The material was subjected to classic maceration in an alcoholic solution with ethanol and subsequently subjected to liquid-liquid extraction. Preliminary analyses were carried out on the inhibition of two enzymes involved in the digestion of lipids and carbohydrates, pancreatic lipase and alpha amylase. The antioxidant potential of the extracts was also evaluated to prevent the effects of oxidative stress which can cause increased insulin resistance and increase the risk of cardiovascular complications. Total phenolic and flavonoid content were evaluated for the ethanolic extract. The antioxidant potential activity was evaluated using the DPPH test and the β-carotene bleaching test. Inhibitory activity on lipase and amylase was evaluated by monitoring p-NPC hydrolysis and glycosidic bond hydrolysis in carbohydrate-digestible foods. From the analyses carried out, the ethanolic extract proved to be the most promising. In fact, it showed significant antioxidant activity: in the DPPH test it showed IC50 values of 371.20 ± 11.98 µg/mL; in the beta carotene bleaching test it showed an IC50 of 5.53 ± 0.13 µg/mL after 30 minutes of incubation and 18.09± 0.36 µg/mL after 60 minutes. The ethanolic extract also demonstrated significant inhibition of pancreatic lipase, with an IC50 of 0.399 ± 0.038 µg/mL. Regarding the inhibition of alpha amylase, the best result was obtained for the ethyl acetate fraction, with an IC50 value of 40.29 ± 4.43 µg/mL. The results of this study open new and interesting perspectives in the field of research on metabolic diseases. In particular, the use of an agro-industrial byproduct such as bergamot waste, from a circular economy perspective, represents an innovative and sustainable approach.

P27. **Effects of different cereals on diet-induced metabolic abnormalities: a focus on wheat and rye**

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A hypercaloric diet evokes chronic low-grade inflammation (metaflammation), fueles metabolic imbalances, whereas a shift to a normocaloric diet is recognized for alleviating these derangements [1,2].

Complex carbohydrates, found in whole grains, legumes, vegetables, and fruits, play a crucial role in maintaining metabolic health. Unlike simple carbohydrates, which are rapidly digested and absorbed, complex carbohydrates provide sustained energy release and regulate metabolic processes via different mechanisms [3,4]. Rye is a rich source of dietary fibres with different functionalities and bioactive compounds. Previous studies have found that fermentation of rye modulated some of the metabolic effects.

This study aimed to comparatively evaluate the potential additional benefits of supplementing a diet refined wheat, fermented rye, and unfermented rye following a dietary transition from a hypercaloric to a normocaloric intake.

Male Sprague-Dawley rats (*n*=9/group) were fed 24 weeks as follows: normocaloric rat diet (ND) or high fat diet (HFD). After 16 weeks of HFD intervention, 36 rats underwent a dietary shift for 8 weeks to normocaloric rat diet supplemented with refined wheat (HFD→ND+RW), normocaloric rat diet supplemented with fermented rye (HFD→ND+FR), normocaloric rat diet supplemented with unfermented rye (HFD→ND+UR) or normocaloric rat diet alone (HFD→ND). The ND-supplementations were supplied as crispbread. Blood and liver were collected at the end of the *in-vivo* protocol.

The consumption of a hypercaloric diet resulted in a marked increase in adipose tissue and a systemic elevation of leptin concentrations. These effects were reversed following the transition to a normocaloric diet. Additionally, the dietary shift led to a reduction in hepatic myeloperoxidase activity, suggesting a dampening of neutrophil activation. Furthermore, hepatic triacylglycerol concentrations were significantly elevated in the HFD group, but returned to physiological levels upon normalization of energy intake. However, the administration of crispbreads made from refined wheat, fermented rye, and unfermented rye did not exert any significant additional effects on these metabolic parameters.

All three types of crispbreads led to a significant reduction in hepatic cholesterol, which was not affected by the dietary shift alone.

Overall, this finding suggests a potential influence of macro- and/or micronutrients from the three different crispbreads on cholesterol metabolism, possibly through modulation of cholesterol synthesis pathways. Further investigation is warranted to elucidate the specific components responsible for this effect and their underlying mechanisms.

[1] "High-Fat, Western-Style Diet, Systemic Inflammation, and Gut Microbiota: A Narrative Review" Malesza IJ et al., (Cells. 2021)
[2] "Diet Change Improves Obesity and Lipid Deposition in High-Fat Diet-Induced Mice" Ji, T et al., Nutrients, 2023
[3] "Whole grain, bran and cereal fibre consumption and CVD: a systematic review" Barrett EM et al., Br J Nutr. 2019
[4] "The Effect of Rye-Based Foods on Postprandial Plasma Insulin Concentration: The Rye Factor" Iversen KN et al., Frontiers in Nutrition, 2022

P28. **Investigating the bioactive potential of rice-bran-derived gamma Oryzanol in gut health.**

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γ-Oryzanol (ORY), found in rice (*Oryza sativa* L.), is a mixture of ferulic acid esters with triterpene alcohols, well-known for its antioxidant and anti-inflammatory properties.

At first a comprehensive chemical characterisation of an ORY mixture was conducted using both HPLC and mass spectrometry analysis, which unveiled the presence of the four most predominant ORY components, which are cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate and β-sitosteryl ferulates.

In vitro studies were then carried out in order to assess the activity of ORY at intestinal level; in particular CACO-2 cells have been used as a model of the intestinal epithelial barrier. These cells were treated with different concentrations of ORY and then Real Time PCR was performed on target genes.

Moreover, the anti-inflammatory activity of ORY was also assessed in an *in vivo* mouse model. Intestinal tissues deriving from vehicle and ORY-treated mice were processed for RNA and protein extraction in order to carry out Real Time PCR on target genes and enzymatic assays.

Our results show that ORY is able to modulate the expression of different genes involved in the maintenance of the gut barrier integrity, like claudins, occludin and zonulin-1 and exerts an anti-inflammatory activity by modulating the expression of pro-inflammatory genes. The enzymatic assays revealed that ORY is also able to increase the activity of antioxidant enzymes in the gut.

In summary, our data suggest that ORY plays a role in modulating the gut barrier integrity, exerts potential immunomodulatory activities at intestinal level and also displays antioxidant properties.

Further investigations are needed in order to better understand the role of ORY in gut health and homeostasis.

P29. **Zn-Spirulina Platensis as strategy to counteract advanced glycation end products accumulation and metabolic imbalance in a murine model of hypercaloric diet.**

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Advanced Glycation End Products (AGEs) are harmful compounds formed through the non-enzymatic glycation of proteins, particularly in diets rich in sugars and fats. Excessive AGEs accumulation is linked to metabolic disorders, including obesity, metabolic syndrome, and diabetes [1]. *Arthrospira platensis* (Spirulina, SP), a blue-green microalga, has gained attention for its high nutritional value and abundance of antioxidant and bioactive compounds [2] and has been proposed as a nutritional supplement. However, its role in AGEs accumulation has not been investigated. This study aimed to explore the potential anti-glycation properties of Spirulina enriched with zinc (Zn-SP) and its ability to mitigate diet-induced metabolic derangements in a murine model.

Thirty 4-week old male C57BL/6OlaHsd mice were divided into three groups: a standard diet group (SD - 10% fat, n=10), a high-fat, high-sugar diet group (HFHS - 58% fat, 26% sugar n=10), and an HFHS group supplemented with Zn-SP 350 mg/kg (HFHS+Zn-SP, n=10) orally three times per week from week 5 onward. The intervention lasted 12 weeks, after which fasting blood glucose levels and glucose tolerance were assessed. Blood and liver samples were analyzed for metabolic and inflammatory markers. Bacterial community structure was investigated using metataxonomics on fecal and ileum samples collected at the end of the protocol. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post-hoc test, with significance set at $p<0.05$. Mice fed the HFHS diet exhibited increased body weight, impaired glucose tolerance, and elevated fasting blood glucose. These metabolic disturbances were accompanied by heightened systemic inflammation, as evidenced by increased levels of pro-inflammatory cytokines and hepatic enzymes (ALT and AST). Hepatic accumulation of AGEs, particularly carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL), was significantly higher in HFHS-fed mice, alongside upregulation of the AGE receptor (RAGE) and depletion of the detoxifying enzyme glyoxalase-1. Zn-SP supplementation significantly mitigated these effects by restoring glucose homeostasis, reducing hepatic inflammation, and lowering systemic cytokine levels. Additionally, Zn-SP prevented the excessive accumulation of AGEs in the liver and downregulated RAGE expression while preserving glyoxalase-1 activity. Notably, Zn-SP also reversed gut microbiota dysbiosis caused by the HFHS diet, restoring a more balanced microbial composition and improving gut-liver axis function.

This study provides compelling evidence that Zn-SP supplementation exerts potent anti-glycation effects, preventing hepatic AGEs production and/or stimulating AGEs detoxification, thus reducing hepatic inflammation. Furthermore, by restoring metabolic balance and improving gut microbiota composition, Zn-SP emerges as a promising dietary supplementation against diet-induced metabolic disorders.

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P30. **Validation of the traditional use of aromatic plants from Valtellina (SO) by *in vitro* model of *H. pylori*-related gastritis.**

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Remedies for gastric disorders have emerged as the most frequently cited in ethnobotanical studies concerning the traditional medicine of the Italian Alps, including the geographic area of Valtellina (SO, Italy). Here, folk knowledge regarding the use of medicinal plants remains alive. However, this important cultural heritage is slowly disappearing, thus precluding access to a source of bioactive compounds potentially useful for therapeutic applications.

The most common gastric disorder is acute gastritis, which is often correlated with the infection of the bacterium *Helicobacter pylori (H. pylori)*. *H. pylori* colonization leads to the activation of inflammatory pathways and the release of cytokines by the gastric mucosa, such as IL-6 and IL-8.

The gold standard therapy, aiming at eradicating the bacteria to prevent the severe outcomes of gastritis, is compromised by side effects of the antibiotic treatment, such as dysbiosis and antibiotic resistance.

This project aims at validating the potential efficacy of several aromatic species employed in Valtellina's traditional medicine for the treatment of gastric ailments, using an in vitro model of human gastric epithelial cells (GES-1) infected by *Helicobacter pylori*.

By combining information from ethnobotanical studies, local farmers, and agricultural institutions (Fondazione Fojanini, Sondrio, Italy), we created a list of medicinal plants from Valtellina and selected several species with a promising scientific background for further pharmacological studies. Most of these plants belong to the genera *Achillea* and *Artemisia*.

During the balsamic time (summer), we proceeded with the collection of *Achillea millefolium* L. in Val di Sacco (2000 m. of altitude); *Artemisia verlotiorum* Lamotte and *Artemisia absinthium* L. in Val Grosina (1000 m), *Achillea moschata* W., and *Artemisia genipi* Weber ex Stechm. in Val d'Eita (1800 m).

Then, plants were dried and extracted thus obtaining two types of extracts for each sample: an infusion, chosen according with the traditional use, and a hydroalcoholic extract, which is a raw and food grade extract suitable for plant exhaustion. We then proceeded with a preliminary quantification of reducing compounds through the Folin-Ciocalteu assay, to estimate and compare the polyphenolic content. Furthermore, we related the amount of total phenols with the biological screening of their anti-inflammatory activity (50µg/mL) in GES-1 challenged with TNF-α or *H. pylori* infection. The release of cytokines (IL8 and IL6) and the anti-bacterial activity were measured, thus obtaining preliminary evidence of *Achillea moschata* W. and *Artemisia genipi* Weber ex Stechm as the most promising species.

Further studies are ongoing to investigate the phytochemical composition of the selected extracts, and the stability in the gastric environment, with the final aim of identifying the active components and their mechanisms of action. We will also evaluate the activity of the extracts against specific microbial features, such as urease activity and adhesion to gastric cells. Moreover, by considering the well-known bitter taste of aromatic plants, the interaction of bitter compounds with TAS-2R receptors involved in gastric digestion and acid secretion will be addressed.

P31. **Exploring the dual role of *Mangifera indica* L. in alleviating inflammation and abdominal pain in inflammatory bowel disease.**

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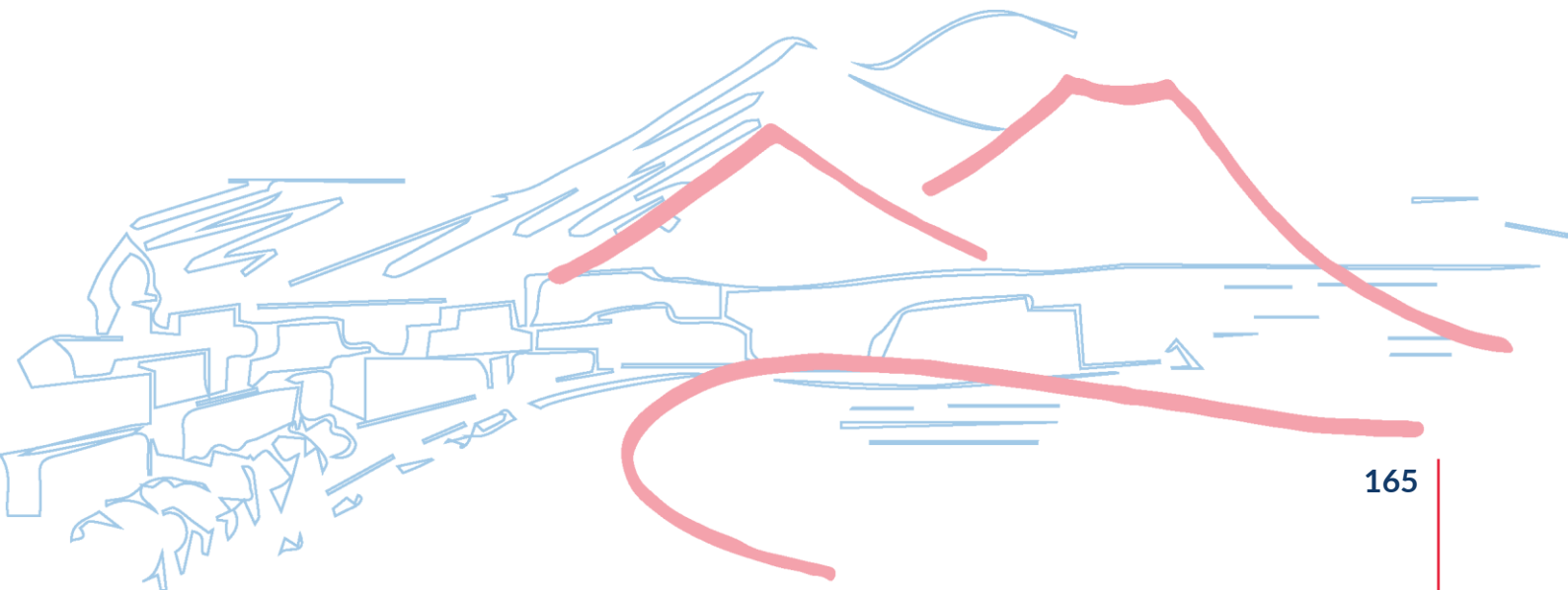
Inflammatory Bowel Disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD), is a chronic condition characterized by relapsing-remitting intestinal inflammation and immunological perturbation where the synergistic action of Th1 and Th17 cells plays a pivotal role in the onset and progression of pathology. This immune dysregulation is further sustained by the downstream release of a network of cyto-chemokines, which coordinate the recruitment and activation of leukocytes in the affected gut tissue (1). In this inflammatory milieu, chronic visceral pain emerges as a key symptom (2). Thus, addressing both gut inflammation and visceral pain is critical for improving patient outcomes in IBD therapy. In this context, the use of *Mangifera indica* L. extract (MIE, commonly known as mango), has recently attracted attention for its potential therapeutic properties in IBDs (3, 4). Therefore, the aim of this study was to evaluate the therapeutic potential of MIE, rich in mangiferin, in a DNBS-induced colitis model associated with visceral pain.

The effects of MIE were evaluated in a 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis model in Sprague-Dawley rats. Two distinct experimental protocols for drug administration were employed: (I) MIE treatment commenced simultaneously with DNBS injection (Day 0) and continued for 7 days, and (II) MIE treatment began 7 days post-DNBS administration and was sustained for a further 14 days. To assess the ability of MIE to modulate the inflamed intestinal mucosal microenvironment, we performed an evaluation of the phenotypic profile of the cellular infiltrate, focusing on key pro-inflammatory cytochemokines, utilizing flow cytometry (FACS) analysis. Intestinal mucosal barrier integrity was assessed through histopathological examination of colon tissues. Additionally, alterations in microbiota-derived metabolites were quantified from both colon tissue and fecal samples using Gas Chromatography-Mass (GC-MS) spectrometry and Nuclear Magnetic Resonance (NMR) spectroscopy respectively. Finally, to evaluate the impact of MIE on visceral pain, we measured the viscero-motor response (VMR) and the abdominal withdrawal reflex (AWR) in response to colorectal distension, as indicators of hypersensitivity and pain perception.

Treatment with MIE resulted in a significant modulation of the immunological perturbations associated with DNBS-induced colitis, as evidenced by a marked reduction in the infiltration of pathogenic Th1 ($CD4^+/IFN\text{-}\gamma^+$) ($P \leq 0.01$; both Day 7 and Day 21; N=6), Th17 ($CD4^+/IL\text{-}17^+$) ($P \leq 0.01$, $P \leq 0.05$; Day 7 and Day 21 respectively; N=6) cells within the colonic lamina propria. These findings were consistent with a decrease in the extent of colonic inflammation, demonstrated by reduced expression of key pro-inflammatory cytokines (IL-1β, TNF-α) and chemokines (CXCL1, CXCL2). Histological analysis revealed a mild, yet statistically significant, improvement in intestinal barrier integrity following MIE treatment, with evidence of tissue healing from inflammation-induced damage. Furthermore, MIE administration restored the levels of microbiota-derived metabolites, such as acetate, butyrate and propionate, in both fecal content and colon tissue samples. Importantly, MIE treatment significantly alleviated visceral hypersensitivity and abdominal pain.

In conclusion, MIE effectively modulated the immune response and alleviated inflammation and visceral hypersensitivity in a DNBS-induced colitis model. MIE reduced the infiltration of Th1 and Th17 cells, decreased pro-inflammatory cytochemokines, and improved intestinal barrier integrity.



P32.**The impact of nutritional supplementation on dysbiosis: an *in vitro* evaluation of probiotics, vitamins, and plant extracts formulation in modulating intestinal barrier function.**

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The gut microbiota plays a fundamental role in individual health; its imbalance, known as "dysbiosis," can contribute to the development of gastrointestinal disorders and diseases, including Inflammatory Bowel Diseases (IBD), Irritable Bowel Syndrome (IBS), Ulcerative Colitis (UC), Crohn's Disease (CD), and Colorectal Cancer (CRC).

This study aims to evaluate the effects of a nutritional supplement (ENTERO-AD) containing probiotics (*Lactobacillus acidophilus* LA1, *Lactobacillus reuteri* LR92, *Bifidobacterium breve* Bbr8), *Matricaria chamomilla*, and B vitamins (B1, B2, B6) on intestinal inflammation. Using an *in vitro* model based on Caco-2 cell line, derived from human colorectal adenocarcinoma, inflammation was induced through two different stimuli: Lipopolysaccharide (LPS), and a combination of Tumor Necrosis Factor α/Interferon γ (TNF-α/IFN-γ).

ENTERO-AD positively affected Caco-2 cells in inflammatory conditions supporting junction proteins' integrity, Occludin and Zonula Occludens 1 (ZO-1). As a result, there was an increase in transepithelial electrical resistance (TEER) and a decrease in paracellular permeability.

The findings suggest that this formulation could contribute to mitigating the damage linked to intestinal mucosal inflammation in patients with dysbiosis associated with gastrointestinal disturbances and related pathologies, reducing the occurrence of severe symptoms and improving overall quality of life.

Poster - Sessione 6

P33. **Astaxanthin and environmental stress: a preliminary study in keratinocytes and *Caenorhabditis elegans*.**

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Climate change contributes to the intensification of environmental stress, such as heat waves and global temperature increase, with a direct impact on human health in terms of morbidity and mortality. Among the strategies to protect against risks associated with these environmental factors, which involve the use of plant components known to protect plants from abiotic and biotic stress, the marine carotenoid astaxanthin (AST) stands out. AST has antioxidant and anti-inflammatory properties and is found in the microalga *Haematococcus pluvialis*.

This research aimed to evaluate the potential protective effects of AST against environmental stresses such as hyperthermia, UVA and UVB radiation. The experiment was conducted through an integrated approach combining in vitro tests with human keratinocytes (HaCaT), which represent the epidermis and play a crucial role in the skin's barrier function against environmental stress, and the nematode *Caenorhabditis elegans*, a model organism sensitive to various types of environmental stress. Initially, the study involved treating HaCaT keratinocytes with non-cytotoxic concentrations of AST to assess its ability to prevent oxidative stress, in terms of the formation of reactive oxygen species (ROS), induced by tert-butyl hydroperoxide and hydrogen peroxide. The results showed a significant reduction in ROS production, highlighting the direct antioxidant effects of the carotenoid under study and providing a justification for investigating AST's ability to counteract oxidative stress induced by environmental factors.

HaCaT keratinocytes were then exposed to hyperthermia and high doses of UVA and UVB radiation, simulating acute environmental stress exposures. The treatment with AST showed thermoprotective and photoprotective effects, significantly reducing ROS formation induced by hyperthermia (44°C) and UVA radiation (5 J/cm^2), but it did not show antioxidant effects against UVB radiation. Under the same stress conditions, AST reduced the expression of genes encoding metalloproteinases and collagenases induced by hyperthermia and UVA radiation, counteracting the degradative processes triggered by oxidative stress.

The protective effects of AST were then confirmed in the nematode *C. elegans*, with a significant reduction in ROS production and mortality induced by both hyperthermia and high doses of UVA radiation.

These results demonstrate the ability of AST to protect against oxidative damage induced by acute environmental stress conditions, suggesting its potential use as a nutraceutical with both thermoprotective and photoprotective effects. However, further studies are needed to explore the mechanisms underlying these protective actions against environmental stress.

P34. **Resins and essential oils formulated in lipid nanovesicles for dermatological applications.**

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This study is a part of the European Project EthnoHERBS, which aims to develop an efficient platform for discovering novel therapeutic agents against skin disorders, relying on the great potential of traditional medicine and the rich biodiversity of the Flora of the Balkan peninsula. In this research, four plant species have been selected because of their great interest in the ethnomedicine related to skin diseases: *Origanum dictamnus* L., *Salvia fruticosa* L. Mill. [1], *Pistacia lentiscus* L. [2], and *Cistus creticus* L. [3]. Specifically, we used the essential oils (EOs) of *O. dictamnus* (OdEO) and *S. fruticose* (SfEO), and the resins of *P. lentiscus* (PlR) and *C. criticus* (CcR). These EOs and resins show antibacterial, antiviral, and antifungal activity, but also antioxidant and anti-inflammatory properties. Due to the high chemical instability and volatility of the constituents of both raw materials and the low solubility of the latter, a proper dosage form was necessary to preserve single components and optimize their skin bioavailability. For this purpose, we developed liposomal nanovesicles loading a combination of an EO and a resin to merge their biological activity.

The EOs were isolated by steam distillation, while the resins were collected directly from the plants. GC-MS and HPLC-DAD analyses were performed to obtain the fingerprints of EOs and resins. The main identified compounds were carvacrol for OdEO and eucalyptol for SfEO, used as markers for quantitative analyses.

Nanovesicles loading EOs and resins were prepared by the thin layer evaporation method using phosphatidylcholine and cholesterol [4,5] and including Tween 80 or Tween20 and Brij20 in the hydration medium as nanovesicles' stabilizers.

Size, Polydispersity Index (PdI), and δ-potential were measured by the Dynamic and Electrophoretic Light Scattering (DLS-ELS). Sizes were around 100 nm and the PdI was of about 0.2. Preliminary studies on the encapsulation efficiency revealed an essential oil entrapment of 96±2.5 %. Nanovesicles' morphology was observed by Scanning and Transmission Electron Microscopy, which revealed a spherical shape. All nanovesicles were stable over a month after storing at 4°C in the dark. Studies by the dialysis bag method showed a prolonged release over 72 h. The cytotoxicity of natural compounds and blank formulations was evaluated by MTT viability assay on normal human fibroblast cells. The natural compounds were nontoxic at 0.01 uL/mL, while the blanks were well tolerated at 0.1%. We also preliminarily evaluated the natural compounds' cytoprotective and repairing activity against oxidative stress produced by the oxidative agent H2O2 to investigate their potential applications in cosmetics. Merging this data, we will develop liposomes loaded with natural compounds at nontoxic and active concentrations for cells to evaluate their in vitro activity.

In conclusion, natural substances can be formulated in liposomes to stabilize their volatile and sensitive components and still maintain or improve their biological properties. In particular, liposomes loaded with natural compounds having antioxidant activity are promising for developing innovative products for skin delivery and cosmetic application.

Acknowledgments: This research was funded by the project EthnoHERBS (H2020-MSCA-RISE-2018, Grant Agreement No. 823973)

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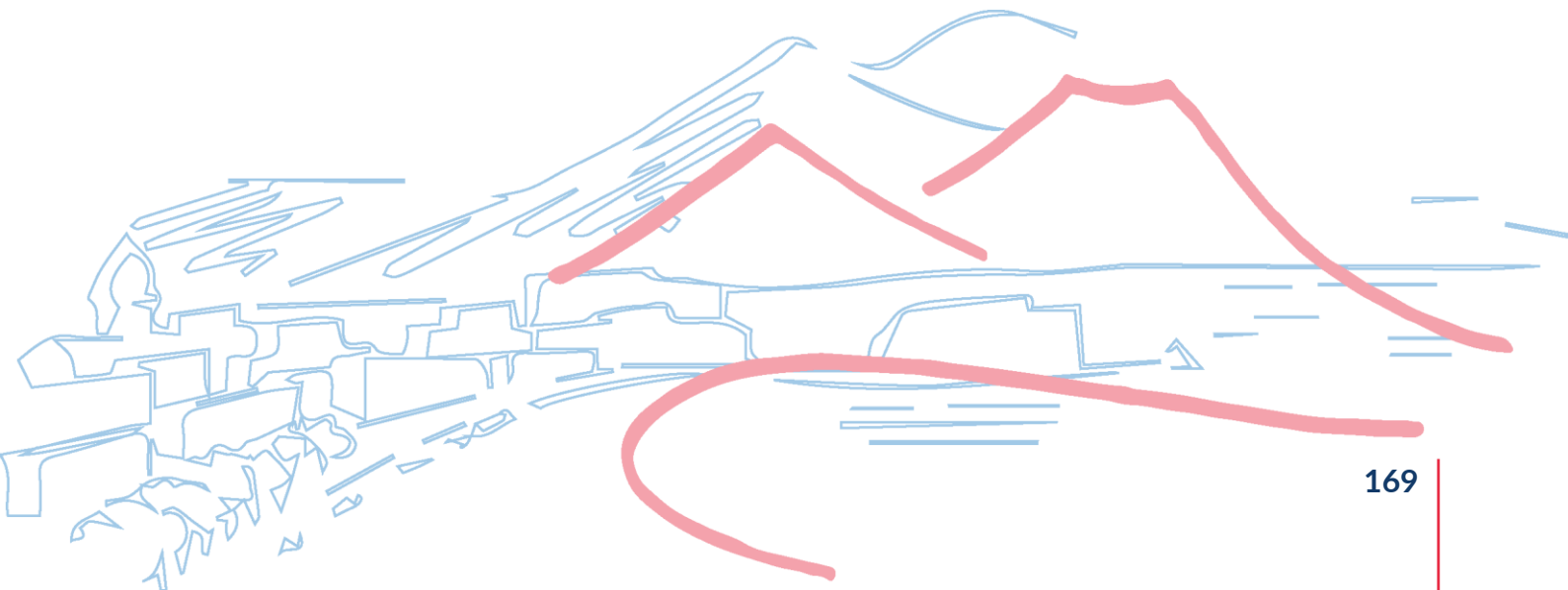

P35. **Officinal plants as new frontiers of cosmetic ingredients.**

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Nowadays, cosmetics are part of psychophysical well-being. They are considered as a multisensory experience that involves touch (rheology of the cosmetic), sight (colour) and smell (fragrances). The addition of functional plant extracts could satisfy these demands. Some medicinal plants have truly promising properties in the field of phytocosmetics. Below we list the most interesting ones.

Aesculus hippocastanum L. (seed), particularly rich in saponins (e.g., aescin) and flavonoids, displays strong anti-inflammatory and capillary-stabilizing properties, making it useful in reducing vascular fragility, oxidative stress, and improving skin texture, with potential applications in anti-aging skincare.

Camellia sinensis (L.) O. Kuntze (leaf) contains, among other substances, epigallocatechin-3-gallate, with important antioxidant, photoprotective and anti-inflammatory properties; these can be used as anti-aging and reduction of skin damage induced by UV rays. Due to the presence of tannins, tea also has sebum-regulating effects.

Drosera ramentacea Burch. (aerial part), due to its content in bioactive naphthoquinones (plumbagine), is a promising natural depigmenting agent, as it acts as a melanin inhibitor, also useful in acne for its antimicrobial, anti-inflammatory and antioxidant properties.

Eucalyptus globulus Labill. (essential oil), with its high 1,8-cineole content, offers antimicrobial, anti-inflammatory, and collagen-boosting effects, supporting its application in anti-aging, skin-brightening, and acne treatments by reducing inflammation and bacterial proliferation.

Harpagophytum procumbens (Burch.) DC. ex Meisn. (tuber) could also be a good anti-aging and anti-acne treatment, due to its anti-inflammatory, antioxidant and antimicrobial properties. The content of iridoid glycosides is responsible for COX-2 inhibition.

Kigelia Africana (Lam.) Benth. (fruit and bark), thanks to the same pharmacological properties, could also be a useful anti-wrinkle treatment as its phytoconstituents (iridoids, flavonoids) improve collagen production and, consequently, skin elasticity and wound healing.

Mentha piperita L. (essential oil), rich in menthol and menthone, soothes irritation, combats oxidative stress, promotes wound healing, and enhances circulation, making it beneficial for skincare oral care, and hair growth formulations (seems to promote scalp health and follicular proliferation).

Panax ginseng Meyer (root) is abundant in ginsenosides, polysaccharides, and peptides, known (among other activities) for their anti-aging, skin-whitening, and UV-protective properties. It enhances collagen synthesis, reduces wrinkles, and improves hydration and pigmentation control. Recent innovations, such as fermented extracts, increase its bioavailability in cosmetic applications.

Ribes nigrum L. (leaf extracts), is rich in anthocyanins, and flavonols, contributing to its antioxidant, and anti-inflammatory effects. It supports skin brightening, collagen synthesis, and UV protection while enhancing wound healing and hydration.

Ruscus aculeatus L. (hypogeal organs), protects the capillaries and reduces redness, due to ruscogenins and flavonoids, which provide anti-inflammatory and vasoprotective effects, potentially ideal for formulas aimed for sensitive skin.

Vitis vinifera L. (leaf) is a potent source of polyphenols, flavonoids, and stilbenes like resveratrol, making it suitable for anti-aging (protection from UV damage, skin hydration), and pigmentation control, as it exhibiting strong antioxidant, anti-inflammatory, and antimicrobial effects.

According to Regulation (EC) n.1223/2009, products boasting therapeutic or prophylactic properties for pathologies cannot be classified and marketed as cosmetics. However, the previously mentioned officinal plants, offering multifunctional properties, can be considered as valid innovative ingredients in cosmetic formulations.

P36. ***In vitro* and in silico study of *Sedum telephium* (L.) leaf juice: skin absorption and wound healing ability.**

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Sedum telephium L. is a succulent herbaceous species belonging to the Crassulaceae family. Endemic to Eastern Europe, it grows spontaneously in Italy, where its traditional use as a healing agent is well documented. Chemically, the main constituents of *S. telephium* L. are polysaccharides, in particular rhamnogalacturonans, and flavonoids, including glycosides of kaempferol and quercetin. In this work, starting from the phytochemical analyses of the juice obtained by squeezing the leaves (SED), performed by colorimetric assays and HPLC-DAD, we aimed to evaluate the skin absorption of the constituents of the phytocomplex and then, assess its skin healing activity. To accomplish this, we used innovative equipment such as MIVO®, and compared the obtained data with *in silico* prediction platforms. Additionally, the wound healing potential of SED and its isolated polysaccharides (SEDPOL) was evaluated both on a line of keratinocytes (HaCaT) and on human fibroblasts (HFF). The phytochemical analysis, in line with the literature, highlighted an abundant presence of polysaccharides accompanied by a pool of flavonoids, among which glycosylated forms of kaempferol and quercetin such as hyperoside, isoquercitrin and kaemperitrin, stood out. Using the software FiniteDoseSkinPerm, an *in silico* prediction of SED flavonoids absorption through skin was calculated. A comparison of these data with those extrapolated from the MIVO® platform revealed a positive correlation between the two techniques, although, in general, lower values were found for the individual constituents with MIVO®, plausibly due to the matrix effect of SED. Overall, *in silico* and *in vitro* analyses confirmed kaempferitrin as the most absorbable molecule in SED. The cell-based evaluation of the healing properties of SED allowed to observe a progressive migration over time of both keratinocytes and fibroblasts, reaching the maximum percentage increase 24 hours after treatment for both cell lines. Similar, but still lower, increases in cell migration were also highlighted for the isolated polysaccharide fraction, proving it is precisely the latter that imparts most of the healing activity and how, at the same time, the entire phytocomplex is endowed with greater activity.

P37. **Non-volatile secondary metabolites from *Araucaria* Juss. species: exploring their potential for medicinal applications.**

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Araucaria Juss is a genus of evergreen coniferous trees belonging to the Araucariaceae family widespread in the Southern Hemisphere especially South America, Australia, and New Guinea. Known for their distinctive appearance, *Araucaria* species are characterized by their large, scale-like leaves and cone-shaped reproductive structures [1]. Besides their ornamental value, *Araucaria* genus offers a fascinating area of study for their potential therapeutic applications given that many are used for ethnobotanical purposes [2]. In this communication, we present an in-depth phytochemical analysis, for what concerns their non-volatile content, of three *Araucaria* species leaves (*A. araucana* (Molina) K.Koch, *A. columnaris* (G.Forst.) Hook. and *A. cunninghamii* Mudie) collected in the Botanical Gqarden of Rome with the final aim to highlight their physiological properties and eventually support their use as medicinal resources. To date, phytochemical research of the *Araucaria* genus has mainly focused on the essential oil composition, while the non-volatile compounds have received relatively little attention. In this context, our study revealed the presence of different classes of non-volatile secondary metabolites such as triterpenoids, diterpenoids, biflavonoids, simple flavonoids and cinnamoyl derivatives. Each species presented a peculiar phytochemical pattern [3-5] but some compounds were found in common thus evidencing a certain phytochemical correlation. All the compounds identified in these three species are endowed with interesting biological effects which suggest their potential use as sources of natural therapeutics. These aspects will be widely presented and discussed during the communication.

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P38. **Hydrogen sulphide in erucin for the development of new treatments of psoriasis.**

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Although the phytochemical profile of cruciferous vegetables is eterogeneous, it is known that they are characterized by a high content of glucosinolates. Modern research is increasingly aimed at highlighting the biological properties of these true "functional foods". Rocket seeds contain the glucosinolate glucoerucin, from which, through hydrolysis by the endogenous enzyme myrosinase, the isothiocyanate erucin is obtained. Erucin is characterized by bitter taste and pungent odor; it's a recognized H_2S-donor and it could have antioxidant, vasorelaxant, antihypertensive and antitumor properties.

In our research we analyzed the pharmacological activity of erucin, obtained from seeds of *Eruca sativa* Mill., in an *in vitro* model mimicking the inflammatory component of psoriatic skin. This model is based on the use of HaCaT, an immortalized human keratinocyte cell line, which are treated with TNFα induce the inflammatory state.

Psoriasis, currently considered one of the global health problems, is a systemic, inflammatory and autoimmune disease for which there is no cure, but only therapeutic approaches with various long-term side effects. The prevalence of psoriasis varies widely among different populations affecting more than 60 million children and adults worldwide. There is no significant difference in the prevalence of psoriasis between men and women, and it occurs mainly in adults between 20 and 60 years of age. Psoriasis is characterized by skin hyperplasia caused by the imbalance of proliferation and differentiation of keratinocytes. The therapeutic strategies in the treatment of psoriasis are based on the severity of injuries and encompass topic or systemic therapies but they have several relevant side effects.

The inflammatory state was induced by treating the HaCaT cells with TNFα (50 ng/ml). After 24 hours, the activity of erucin (0.3uM- 3uM) on cell viability and gene expression of cytokines and factors involved in inflammatory pathways was tested. Cell viability was measured by MTT assays, while apoptosis and necrosis were evaluated by the Annexin V tests. In order to test the effects of erucin, Real Time PCR was used to assess the changes in specific mRNA levels of cytokines and factors involved in the psoriasis pathway.

Following treatment with erucin 0.3 uM, we observed a reduction in the number of keratinocytes and activation of the apoptotic process; Bax levels increased, Bcl-2 levels decreased and Caspases 3 and 7 slightly increase. Erucin induced significant changes in the levels of IL-23A, IL-1β, IL-6 and STAT3. Although preliminary, the data indicate that treatment with erucin might reduce cell proliferation and/or inhibits the synthesis and release of inflammatory cytokines.

Further studies are needed to confirm that erucin exerts antiproliferative effects by reducing cell proliferation and/or inhibiting the synthesis and release of inflammatory cytokines

P39. **Isolation and characterization of exosome-like nanoparticles and their evaluation as agents/carriers in functional cosmetics**

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Exosomes are extracellular vesicles with a size range of 50-500nm. They are formed by a lipid bilayer membrane and secreted by cells, carrying lipids, nucleic acids, proteins, cellular metabolites and they have a role in cell-to-cell communication. The term Exosome-like refers to their plant origin. Some research groups isolated exosome-like nanoparticles (ELNs) from different fruits and vegetables (grapefruit, cabbage, carrot, broccoli, lemon, ginger, etc.). These nanoparticles represent an interesting carrier for bioactive compound delivery: they are natural and originated from plant cells, making them biocompatible and safer. Moreover, they show intrinsic healthy properties (anti-inflammation, anticancer, antioxidation, etc.). They also could have applications in cosmetics, by encapsulating ingredients as peptides, to improve skin penetration and due to their characteristics, they can protect sensitive compounds against degradation. Thanks to their composition and ability to carry bioactive molecules, ELNs represent a revolutionary innovation in skincare. By incorporating them into creams, serums and other topical applications, ELNs can address major skin concerns like aging, pigmentation and environmental damage caused by oxidative stress. However, while they represent a potential rising star in modern healthy diets and biomedical applications, further research is required, especially regarding the development of standardized protocols for their isolation, identification, and large-scale production. Once selected the best plant matrix (considering different parts of the plant, stages and stress conditions), the aim of this project is the isolation and characterization of these exosome-like nanoparticles using different techniques: for the isolation the differential ultracentrifugation will be combined with the density gradient ultracentrifugation or size-exclusion chromatography, while their physical-chemical characteristics will be investigated using electron microscopy, DLS/NTA, mass spectrometry. After that, there will be a next phase that include *in vitro* tests using keratinocytes (HaCat cell lines) to evaluate biocompatibility, cell uptake and eventually biological effects. The final step of the aim is the loading of these nanoparticles with some active compounds commonly used in cosmetics and integration into a cosmetic formulation and its evaluation.

Poster - Sessione 7

P40. **GABAA receptors are involved in the anti-epileptic effects of a polyphenol-rich extract of white grape juice.**

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Epilepsy affects around 70 million individuals globally, thus representing more than 0.5% of the total worldwide illness burden. Given the complexity of this pathology, novel pharmacological agents are constantly sought to find better treatment options. Natural products represent great allies in the management of neurological disorders, such as epilepsy. This is because they are endowed with multitarget capacity, which allow a simultaneous targeting of defective pathways. On this line, the aim of this study was to investigate the effects of a polyphenol-rich extract of white grape juice (WGJe) in different rodent models of epilepsy, exploring its putative mechanism of action. Our results showed that the intraperitoneal injection of WGJe exerted anticonvulsant effects in pentylenetetrazole (PTZ)-induced seizures in ICR-CD1 mice, in which only tonic seizures were significantly hindered ($p < 0.01$). In WAG/Rij rats, a genetic model of absence epilepsy, WGJe did not significantly alter the number and/or duration of the spike-wave discharges in comparison to untreated rats. In genetically audiogenic seizures (AGS)-susceptible DBA/2 mice, WGJe was able to significantly hamper both clonic and tonic seizures ($p<0.01$). Moreover, WGJe showed to possess anxiolytic effects, as assessed through the open field test. Interestingly, the co-administration of WGJe with flumazenil, a $GABA_A$ antagonist, restored both clonic and tonic seizures in DBA/2 mice, along with did not protect mice against wild running in comparison to WGJe administered alone, thus suggesting that $GABA_A$ receptor mediates the observed results. Indeed, docking simulations confirmed that the main polyphenols of WGJe are able to interact with the benzodiazepine sites located in both extracellular and transmembrane domains in the $GABA_A$ receptor. Our study suggests the antiepileptic activity of WGJe and supports its utilization in the management of epilepsy as a novel complementary therapy.

P41. **Evaluation of plant extracts on paclitaxel-treated C8-D1A mouse astrocytes, a potential new strategy against neuropathy.**

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The International Association for the Study of Pain (IASP) defines neuropathic pain (NP) as pain caused by a lesion or disease of the somatosensory nervous system, characterized as a burning, pricking, squeezing, or freezing sensation [1]. However, current pharmacological treatments including tricyclic antidepressants, serotonin, norepinephrine reuptake inhibitors, gabapentinoids and opioids, are partially ineffective and related to strong side effects. Therefore, the identification of the best treatment option in the help of controlling pain remains challenging for clinicians. Circadian dysregulation, neurotransmitter level alteration and maladaptive plasticity of the central nervous system represent key risk factors in the onset of NP. The recently discovered glymphatic system is a network of pseudolymphatic vessels deputed for brain waste clearance, that is under circadian regulation [2], and, when disrupted, contributes to the onset of NP [3].

The aim of this research was to investigate, *in vitro*, the effects of different plant extracts on paclitaxel-induced damage on C8-D1A mouse brain astrocytes. Paclitaxel is a chemotherapeutic agent widely used in the treatment of many malignant tumors, however, patients treated with paclitaxel commonly experience severe NP, strongly affecting their quality of life. The connection between paclitaxel induced stress and glymphatic system dysfunction was investigated through the quantification of aquaporin-4 (AQP-4) water channels, the most abundantly expressed aquaporin in the central nervous system [4] and key player in facilitating cerebrospinal fluid influx across the astrocytes vascular endfeet and waste clearance.

Moringa oleifera Lam., *Withania somnifera* Dunal. and *Lepidium sativum* L. plant extracts were selected for their ability to regulate the circadian rhythm. Plant extracts were tested on C8-D1A mouse astrocytes to evaluate the range of safe concentrations and/or the concentrations able to potentially induce cytotoxicity and determine reactive oxygen species (ROS) production. Then, selected safe concentrations (0.1-10µg/mL) were tested on paclitaxel-treated (1µM) C8-D1A cells to determine the protective effects against paclitaxel-induced cell viability reduction using WST-1, a cell viability colorimetric assays, and ROS production using the fluorescent dye DHE. *Moringa oleifera* Lam. and *Lepidium sativum* L. extracts were selected for their ability to release hydrogen sulfide (H_2S). H_2S is an endogenous gasotransmitter bestowed with pleiotropic properties involved in the regulation of circadian-clock genes and in protection against neuronal damage [5]. H_2S release was tested using the fluorescent dye WSP-1, a sensitive dye that specifically reacts with intracellular H_2S.

All the extracts exhibited low to non-toxic effects on C8-D1A mouse astrocytes at the highest concentration tested (100µg/mL) while inducing a significant restoration of the cell viability in paclitaxel-treated cells at the concentration of 10µg/mL. Moreover, the most effective extracts were able to release H_2S, highlighting that its protective effects could be due totally or partially to this gasotransmitter. The use of natural extracts could potentially represent a novel pharmacological strategy in the treatment of NP.

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[4] https://doi.org/10.1016/j.nbd.2023.106035
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P42. **PEA and DHA, two natural compounds in the autism spectrum disorder management.**

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by stereotyped behaviors and deficits in social communication and social interaction. Given the complexity of the disorder, and the fact that symptoms and severity vary, the molecular mechanisms remain elusive, which limits the available therapeutic option. Palmitoylethanolamide (PEA), is a natural substance found in various plants, such as soy and peanuts, and it is also produced by the human body. PEA is a long fatty acid used as a supplement for its anti-inflammatory, analgesic, and neuroprotective properties. PEA's increasing recognition as a plant-based product comes from the fact that it offers a natural alternative with minimal side effects compared to traditional medications for pain and inflammation. Indeed, it is known to be helpful in treating conditions like chronic pain, fibromyalgia, and several neurodegenerative and neurological diseases. Recently, several studies suggested its beneficial role in reducing ASD symptoms in both preclinical and clinical studies (1).

Together with PEA, Docosahexaenoic acid (DHA), is primarily found in fatty fish but it is also present in algae, which is a plant-based source of DHA. This makes algae-derived DHA an important supplement for those who follow a vegan or vegetarian diet. DHA is considered a serum biomarker of autism (2).

In this study, we explore the synergistic effects of PEA and DHA investigating how their combination may enhance ASD. Considering that both PEA and DHA are known to be PPAR agonists (3,4), and several studies indicate crosstalk between neurosteroids and PPARs (5), we investigated the role of neurosteroids in the PEA+DHA activity.

We administered for 10 days, low dose of these compounds to genetically modified BTBR T+tf/J (BTBR) mice, a widely used ASD model, in vivo and ex vivo studies were performed. On the behavioral test we observed a significant improvement in social behavior and a reduction in repetitive actions. Moreover, in ex vivo studies, the combination of PEA and DHA reduced neuroinflammation and increased the levels of endogenous allopregnanolone (ALLO), a neurosteroid that acts as a potent positive allosteric modulator of GABAA receptors, in key brain regions (such as the hippocampus and cortex). Finally, PEA + DHA were also able to restore the key enzymes involved in the ALLO biosynthesis.

Taking together, our data shows that a new approach for ASD management could be based on the use of functional foods, novel foods that have been formulated so that they contain substances that have a possible health-enhancing or disease-preventing value, and at a concentration that is both safe and sufficiently high to achieve the intended benefit.

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P43. **A combination of 5-(3′ ,4′ -dihydroxyphenyl)- γ -valerolactone and curcumin synergistically reduces neuroinflammation in microglia by targeting the NLRP3 inflammasome and the NOX2/Nrf2 signaling pathway**

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Microglia, the brain's resident macrophages, produce inflammatory and neurotoxic factors that contribute to neurodegenerative diseases. Therefore, inhibiting neurotoxic microglia activation and inflammation is a promising therapeutic strategy. 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (γ-VL) is a proanthocyanidin metabolite with antioxidant, anti-inflammatory, and neuroprotective properties, but its impact on neuroinflammation and microglial activation is largely unexplored. Conversely, curcumin, derived from *Curcuma longa* L., is known to reduce microglial activation by lowering pro-inflammatory mediator production. Despite the well-documented individual effects of γ-VL and curcumin, their combined effects have not been studied.

This study aimed to evaluate the potential synergistic effects of γ-VL and curcumin in reducing microglial activation induced by the bacterial toxin lipopolysaccharide (LPS).

Primary rat cortical microglial cells were pre-treated with increasing concentrations of γ-VL and curcumin, either alone or in combination, before LPS stimulation. Cell viability was assessed by the MTT assay. The production and release of pro-inflammatory mediators were quantified using real-time PCR and ELISA, respectively. Nitric oxide production was measured with Griess reagent. The synergistic effect of γ-VL and curcumin was evaluated using the SynergyFinder Plus software, an interactive tool designed to analyze dose-response data of drug combinations.

The combination of γ-VL and curcumin showed a synergistic anti-inflammatory effect in LPS-stimulated microglial cells. Specifically, this combination exhibited a significantly greater ability to inhibit the production and release of pro-inflammatory mediators, such as IL-1β, TNF-α, and NO, when compared to each compound used individually. The underlying mechanism of this anti-inflammatory effect involved the suppression of NLRP3 inflammasome expression. Furthermore, the observed reduction in microglial activation was linked to the modulation of the NOX2/Nrf2 signaling pathway.

Our findings indicate that the combination of γ-VL and curcumin synergistically down-regulates neuroinflammation in microglia, paving the way for promising therapeutic approaches in the treatment of neurodegenerative diseases.

P44. **Evaluation of the combined effect of sulforaphane and isoliquiritigenin in a cellular model of neuroinflammation.**

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Neurodegenerative diseases, such as Alzheimer's and Parkinson's, represent a growing public health challenge, as they are characterized by multifactorial processes, including oxidative stress, abnormal protein accumulation, and chronic neuroinflammation [1]. Microglia, the resident immune cells of the central nervous system, play a crucial role in regulating neuroinflammation [2]. Upon activation, these cells can exist in different intermediate states between two functional phenotypes: M1 and M2. The M1 phenotype promotes inflammation and neuronal damage, whereas the M2 phenotype regulates immune functions, supporting damage repair and creating an anti-inflammatory environment [3]. However, the chronic activation of microglia toward the pro-inflammatory M1 state contributes to disease progression by releasing pro-inflammatory cytokines and reactive oxygen species. Currently, available therapies remain insufficient, as they primarily focus on symptom management rather than counteracting or slowing neurodegeneration, highlighting the need for multi-target approaches. In this context, natural bioactive compounds offer a favourable safety profile and broad multi-target potential. Among these, sulforaphane (SFN), an isothiocyanate derived from glucoraphanin found in Brassicaceae, and isoliquiritigenin (ILQ), a natural flavonoid present in Glycyrrhiza uralensis root, emerge as promising therapeutic candidates due to their antioxidant and anti-inflammatory properties.

This study aims to evaluate whether the combined use of ILQ and SFN exhibits a synergic anti-inflammatory and neuroprotective activity in BV-2 microglial cells exposed to lipopolysaccharide (LPS) as an in vitro model of neuroinflammation. The cytotoxicity of both compounds was initially assessed using the MTT assay, confirming that ILQ (≤10 µM) and SFN (≤5 µM) do not affect cell viability. BV-2 cells were then pre-treated for 2 h with ILQ and SFN, followed by exposure to LPS (100 ng/mL) for 24 h. As expected, LPS exhibited high cytotoxicity, which was reduced by 90% when BV-2 cells were pre-treated with ILQ (10 µM) and completely abolished with SFN (0.5µM).

To further explore the anti-inflammatory potential of both compounds, nitric oxide (NO) levels in the culture medium were assessed using the Griess assay. As expected, LPS treatment significantly increased NO production, whereas treatment with ILQ and SFN resulted in a marked and significant reduction.

Once the effective concentrations for both compounds were identified, BV-2 cells were treated with a combination of ILQ and SFN to evaluate a potential synergistic anti-inflammatory effect. Interestingly, the co-treatment with ILQ and SFN was neither synergistic nor additive. The results demonstrate that the NO reduction observed in the co-treatment condition was equivalent to the greatest reduction obtained when each compound was used individually at the same concentration.

These findings suggest that while both ILQ and SFN independently exert anti-inflammatory effects, their combination does not synergistically enhance these properties. Further studies are required to elucidate the underlying mechanisms of these compounds and the individual contribution to the modulating of neuroinflammation. This work was supported by MUR-PRIN 2022 (Prot. 20222W7P7S) to Cristina Angeloni.

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P45. **Beneficial effects of a customized botanical dietary supplement produced by an innovative vertical farming system in an *in vitro* model of neuroinflammation.**

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In recent years, there has been a growing interest in the use of botanical dietary supplement (BDS) for their antioxidant and anti-inflammatory properties. Bioactive compounds such as parthenolide (PTL), palmitoylethanolamide (PEA), and C-phycocyanin (PCN) have been demonstrated to selectively interfere with inflammatory cascades, thus revealing beneficial role in different clinical settings. Here we investigated in a in vitro model the potential beneficial effects of a customized BDS based on botanicals grown through the innovative vertical farming system (Algem Dol Care®), focusing on its ability to counteract neuroinflammation and to improve blood-brain barrier (BBB) integrity.

The human immortalized HMC3 microglial cell line and TY-10 endothelial cell line were exposed to the inflammatory stimulus LPT (LPS 100 ng/ml + TNF-α 50 ng/ml) in the absence or presence of BDS for 24 hours. In 1.5 g of BDS, the following are present: 300 mg of *Tanacetum parthenium* (0.3% parthenolide) PTL; 432 mg of palmitoylethanolamide PEA; 360 mg of *Arthrospira platensis* extract (40% phycocyanin) PCN. MTT assay assessed toxicity on HMC3 to determine effective doses. Western blot evaluated NLRP3 and pIKb in HMC3, and ICAM-1 and Claudin-5 in TY-10. PCR measured IL-1β and IL-6 expression. ROS production in microglia was evaluated by DCFDA assay.

BDS reduced LPT-induced microglia mortality in a dose-dependent manner with highest effects at the BDS concentration corresponding to the 1:90 dilution (2 mM PTL; 5 µM PCN 200 µg/mL) and 1:120 (1,5 mM PTL; 3,75 µM PCN; 150 µg/mL). The most effective BDS concentration resulted in a significant downregulation of the LPT-induced overexpression of the inflammatory complexes NLRP3 and NFkB in the microglia and this effect was associated with reduced expression of the cytokines IL-6 and IL-1b and ROS production. Endothelium exposure to LPT resulted in increased expression of the adhesion molecule ICAM-1 and in the junctional protein claudin-5, only the latter was reversed by BDS exposure. Interestingly, when LPT-treated microglia were co-cultured with endothelial cells, significant increase in the adhesion molecule ICAM-1 and decrease in the junctional protein claudin-5 were recorded in the endothelium and these effects were counteracted by the microglia treatment with BDS.

In conclusion, our study highlights BDS effectiveness in reducing microglial inflammation, without any direct effect on endothelium. Notably, BDS microglia protective effects may ameliorate the endothelial dysfunction due to the inflammatory insult. Our findings suggest that the use of innovative farming system to effectively combine bioactive compounds could be a promising strategy for neuroinflammatory disorders. Further experiments are needed to elucidate the relative contribute of selective bioactive compounds to the described protective effects.

Sabato 12 aprile

Poster - Sessione 8

P46. **Brain boosters from cannabis: CBGA and CBDA show promise in beating memory loss.**
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Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder and the most common form of dementia in the elderly population. It is characterized by cognitive decline, memory loss, language impairments, and movement difficulties, often accompanied by behavioral disorders such as anxiety and depression. The main neuropathological hallmarks include the extracellular accumulation of β-amyloid plaques and alterations in cholinergic transmission, both of which contribute significantly to cognitive impairment. Current therapies are primarily symptomatic and aim to slow disease progression rather than halt it. Cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA) are naturally occurring compounds derived from the *Cannabis sativa* plant that have gained interest for their multitarget potential, combining neuroprotective and anti-inflammatory effects with the ability to modulate the cholinergic system. This study evaluates the efficacy of CBGA and CBDA as potential therapeutic agents for AD through a preclinical approach in murine models.
In this study, an AD model was induced in C57 mice via intracerebroventricular injection of 5 µL of soluble amyloid-β (1–42) peptide (sAβ) using a 10-µL Hamilton microsyringe. Following disease induction, mice underwent repeated treatment with CBGA and CBDA (10 mg/kg, i.p.) for seven days. In the subsequent days, behavioral tests were conducted to evaluate memory, cognition, and depressive-like symptoms associated with neurodegeneration. Additionally, electrophysiological recordings were performed to analyze long-term potentiation (LTP) in the dentate gyrus (DG) of the hippocampus, a neurophysiological parameter linked to synaptic plasticity and memory.
The results showed that treatment with CBGA and CBDA significantly improved cognitive deficits induced by β-amyloid peptide, restoring short-term memory. Notably, while both compounds contributed to mitigating cognitive impairment, only CBGA significantly reduced depressive-like behaviors, suggesting a specific effect on emotional regulation. Electrophysiological analysis revealed improved synaptic plasticity in the hippocampus, demonstrated by LTP recovery in the dentate gyrus, further supporting the neuroprotective effects of phytocannabinoids.
The collected data suggest that CBGA and CBDA exhibit a promising pharmacological profile for AD treatment due to their ability to modulate cholinergic transmission and inhibit the production and aggregation of β-amyloid peptide. Their efficacy in behavioral tests and synaptic plasticity recovery indicates a potential role in slowing disease progression and improving patients' quality of life. In particular, the antidepressant-like effect observed with CBGA could offer an additional therapeutic advantage for AD treatment.

P47. **Effect of cannabidiol in combination with alpha lipoic acid and resveratrol the psychitric changes associated with social isolation.**

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Social isolation is a significant stressor that can lead to severe psychiatric alterations, including anxiety, depression, and aggression, frequently observed in disorders like post-traumatic stress disorder (PTSD). This study evaluates the therapeutic effects of cannabidiol (CBD), a non-psychoactive compound of *Cannabis sativa*, alone and in combination with resveratrol (RES) or alpha-lipoic acid (ALA), on behavioral and cognitive dysfunctions induced by prolonged social isolation in mice. Male C57 mice were exposed to social isolation for 30 days and subsequently treated with CBD (2.5–10 mg/kg), RES (20 mg/kg), or ALA (10 mg/kg) via oral gavage, either as monotherapy or in combination.

Behavioral assessments included the Resident-Intruder Test for aggression, the Hole Board Test for anxiety, and the Tail Suspension Test for depressive-like behavior. Socially isolated mice exhibited increased aggression, reduced exploratory behavior, and prolonged immobility. Repeated administration of CBD significantly mitigated these effects in a dose-dependent manner, with higher doses (10 mg/kg) restoring behavioral parameters to levels comparable to socially housed controls. Notably, the combination of a low, inactive dose of CBD (2.5 mg/kg) with RES or ALA resulted in synergistic effects, effectively reducing aggression and depressive-like behaviors.

These findings highlight CBD's potential as a therapeutic agent for neuropsychiatric conditions linked to chronic social stress. Moreover, the synergistic effects of combining CBD with natural compounds like RES or ALA suggest an innovative strategy to enhance therapeutic efficacy while reducing the required dosage of CBD, thereby minimizing potential side effects.

This study underscores the value of exploring combination therapies for treating psychiatric disorders and provides compelling preclinical evidence for the use of CBD and natural compounds in addressing the emotional and behavioral consequences of social isolation.

P48. **Elucidating the molecular mechanisms of oleocanthal in neuroinflammation.**

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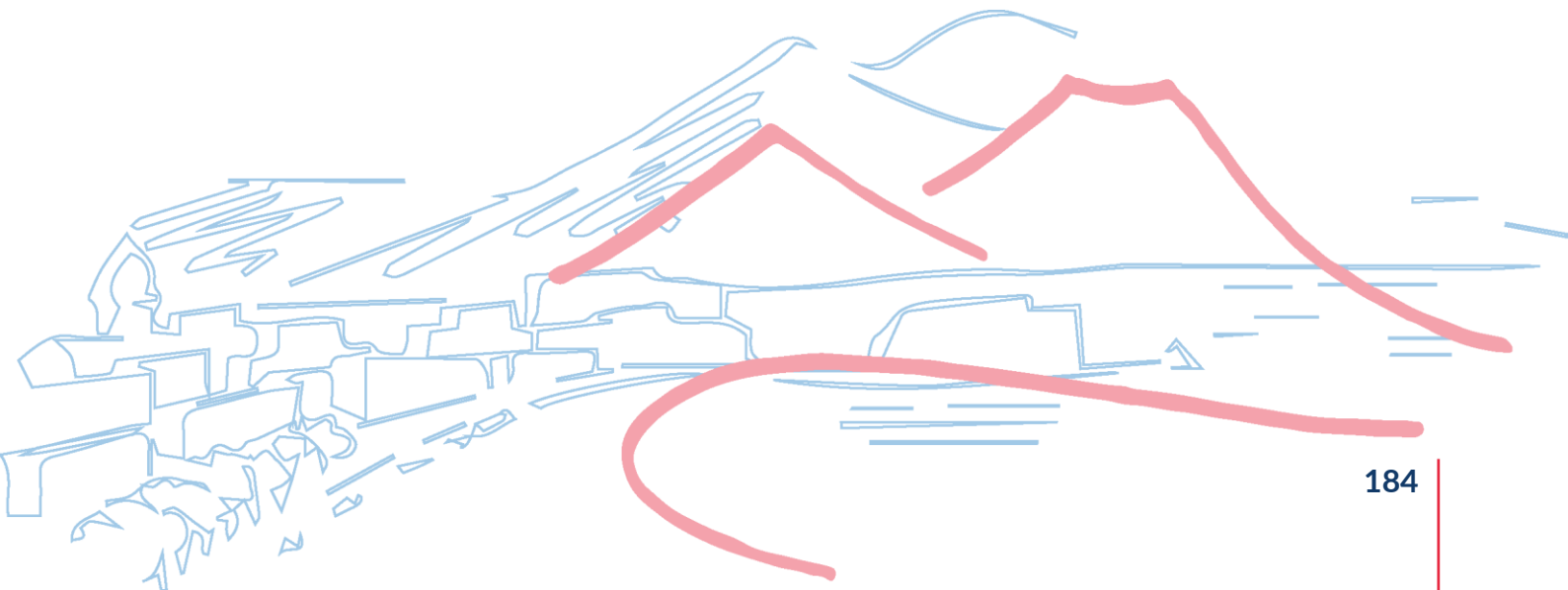
Neurodegenerative disorders are devastating conditions that affect millions of people worldwide, mainly within the elderly population. Their prevalence is increasing due to the rise in life expectancy, yet effective disease-modifying treatments remain elusive. Despite their distinct pathogenetic mechanisms, they share common hallmarks, including oxidative stress, mitochondrial dysfunction and chronic neuroinflammation. The latter is crucial for the pathogenesis of these diseases, and it entails the excessive pro-inflammatory activation of microglia, the resident immune cells of the brain, and consequent production of inflammatory mediators, ultimately leading to progressive neuronal damage. Therefore, targeting neuroinflammation could be a promising strategy for preventing and treating these disorders.
Our previous data have demonstrated that oleocanthal (OL), a compound found in extra virgin olive oil, exhibits significant anti-inflammatory activity in BV-2 microglial cells by counteracting the production of NO and other pro-inflammatory mediators. The aim of the present work is to further characterize the molecular mechanism underlying the *in vitro* anti-neuroinflammatory effect of OL. To do so, LPS-activated BV-2 cells, pre-exposed to 10 μM OL, were analyzed using multiple approaches.
We found that OL is able to completely rescue the LPS-induced reduction in phagocytic activity, analyzed by flow cytometry, suggesting that OL produces a shift towards the M2 phenotype, promoting the resolution of the inflammatory state.
Moreover, we evaluated the oxidative stress status of BV-2 cells by quantifying the intracellular ROS and GSH levels. While LPS impaired the redox equilibrium, significantly increasing ROS production and reducing GSH levels, OL restored them to the control values.
We then assessed the activation of NF-kB transcription factor by immunoblotting determination of both NF-kB phosphorylation and migration to the nucleus. Unexpectedly, OL only slightly inhibited the LPS-induced activation, which cannot fully explain the magnitude of its effect.
To further explore the mechanism of action of OL, we evaluated the activation of other key players in inflammation-related pathways, namely Akt, and the MAPKs ERK-1/2 and p38, by immunoblotting quantification of their phosphorylation. Our results suggest that OL strongly affects p38 signaling, likely by actively promoting its dephosphorylation and consequent inactivation.
We then investigated the interaction of OL with the TLR4 receptor system, quantifying TLR4 and its co-receptor CD-14 surface expression by flow cytometry. We hypothesize that OL promotes the reduction of CD-14, thus impairing endocytosis of TLR4, which therefore accumulates on the cell membrane.
Using a molecular docking approach, we further evaluated the potential interaction between OL and the LPS receptor system (specifically, CD-14 and the TLR4-MD2 complex) and found that OL could interfere with the LPS binding and internalization process, consequently blocking its signaling cascade.
In conclusion, this study provides insights into the molecular mechanism underlying the outstanding anti-neuroinflammatory activity of OL *in vitro*. While more research is necessary

to fully elucidate OL's complex mechanism of action, our results indicate that it could be a promising candidate in the context of neurodegenerative diseases.



P49. **Silybin overcomes doxorubicin resistance in colorectal cancer cells by targeting GLUT1**

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Despite significant advancements in cancer diagnosis and treatment, drug resistance remains the primary limiting factor in achieving successful patient outcomes. Drug resistance is a multifactorial phenomenon with molecular mechanisms that are still under investigation. It is well established that dysregulation of cancer metabolism contributes to tumor adaptability in hostile environments, promoting resistance and cell survival. Metabolic reprogramming can involve multiple pathways, including glycolysis, the pentose phosphate pathway, lipid metabolism, glutamine metabolism, and mitochondrial function.

Natural compounds represent a valuable source of biologically active molecules capable of modulating various metabolic pathways. Consequently, increasing attention has been directed toward exploring the potential use of natural compounds—characterized by good tolerability—in combination with conventional chemotherapeutic agents to enhance drug sensitivity by targeting specific metabolic alterations.

The primary objective of this study was to investigate new pharmacological strategies derived from natural sources to target cancer metabolic pathways and potentially prevent doxorubicin resistance. To achieve this, we employed an in vitro model using LoVo colorectal adenocarcinoma cells, both doxorubicin-sensitive (WT) and doxorubicin-resistant (-DOX). A comprehensive metabolic characterization of these cell lines was performed to determine which metabolic pathways were preferentially exploited by resistant cells for ATP production. Cell viability was assessed following exposure to different metabolic stressors. Mitochondrial dynamics were analyzed via confocal microscopy and flow cytometry. Additionally, qRT-PCR and Western blotting were conducted to evaluate the expression of key glycolytic factors, while glucose uptake was measured using the fluorescent glucose analog 6-NBDG.

Our findings revealed that LoVo-DOX cells undergo extensive mitochondrial remodeling, favoring a metabolic shift from oxidative phosphorylation (OXPHOS) toward glycolysis. These cells exhibited a heightened dependence on glucose for survival, as demonstrated by the overexpression of the glucose transporter GLUT1. Based on these metabolic profiling data, we investigated the effects of Silybin, a known modulator of glucose transporters. Silybin demonstrated increased cytotoxicity in LoVo-DOX cells compared to LoVo-WT cells, and its combination with doxorubicin exhibited a synergistic effect specifically in resistant cells. Additionally, Silybin was found to modulate glucose transporter expression and activity, inducing selective cytotoxicity in doxorubicin-resistant cells.

Overall, these results support the hypothesis that targeting GLUT transporters may represent a promising strategy to overcome doxorubicin resistance. Furthermore, the synergistic effects observed with Silybin suggest new potential avenues for combinatorial therapy in drug-resistant colorectal cancer.

P50. **Activity of a phytocomplex from in vitro cell culture of *Zanthoxylum piperitum* (L.) DC. on human fibroblasts.**

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In this study, we investigated the phytochemical and biological properties of a biotechnological phytocomplex derived from *Zanthoxylum piperitum* (L.) DC., an innovative product obtained via *in vitro* cultivation from small portions of the young leaves of the Japanese pepper mother plant. This sustainable biotechnological approach has gained significant attention in recent years, particularly in the cosmetics industry. The phytocomplex was designed to be rich in lignans while devoid of the characteristic pungent and spicy compounds found in traditional Japanese pepper. While the latter is primarily used for its anesthetic, skin-protective, and anti-allergic properties, the biotechnological phytocomplex was specifically developed to target the extracellular matrix.

Phytochemical analysis confirmed the presence of key compounds in the phytocomplex of *Z. piperitum*. Subsequent investigations were performed on human HFF fibroblasts, both under basal conditions and in an immunosuppressive model simulating the reduced reactivity typical of aged skin. Parameters assessed included, pro-collagen I production, expression levels of metalloproteinases (MMP-1) and tissue inhibitors of metalloproteinases (TIMP-1), as well as lysyl oxidase (LOX) expression. Additionally, a hydration test on HFF cells was conducted, alongside cell-free assays to evaluate collagenase inhibition activity and antioxidant potential (ORAC).

The biotechnological phytocomplex of *Z. piperitum* was found to be rich in lignans (>1.5% on a dry weight basis) and demonstrated a notable ability to stimulate pro-collagen I synthesis, both under normal and immunosuppressive conditions and to–modulate key molecular targets involved in collagen metabolism, especially LOX. Furthermore, the phytocomplex effectively protected human fibroblasts from osmotic damage, exhibited substantial collagenase inhibition, and displayed promising antioxidant activity, as indicated by its ORAC value.

Data obtained in this very first work on the biotechnological phytocomplex of *Z. piperitum* clearly revealed its potential as an innovative agent for functional cosmetics, with its specific and uncommon activity on the preservation of collagen metabolism.

P51. **Pinosylvin identified in *Pinus nigra* subsp. *laricio* inhibits LPS-induced inflammation in RAW 264.7 cells by suppressing pro-inflammatory cytokines and mediators and by downregulating the JAK/STAT Signaling Pathway**

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Stilbenoids, a group of naturally occurring phytoalexin polyphenols commonly produced by plants in response to stress conditions, exert a well-known anti-inflammatory activity. In particular, Pinosylvin (*trans*-3,5-dihydroxystilbene), an analogue of Resveratrol, was traditionally found in the heartwood of conifer trees belonging to *Pinus* species. *Pinus nigra* subsp. *laricio* var. *calabrica* knotwood, previously pounded, was extracted by Soxhlet apparatus through two consecutive extractions: the first in cyclohexane (110 °C, 6 h), the second in distilled water (110 °C, 6 h). The hydrophilic-obtained extract was investigated through HPLC analyses: thanks to retention time and UV-Vis spectra comparison with the commercial standard, Pinosylvin was identified in the knotwood of *Pinus nigra* subsp. *laricio* var. *calabrica*. Then, the anti-inflammatory potential of Pinosylvin commercial standard was evaluated by monitoring the inhibition of pro-inflammatory cytokines (IL-6, TNF-α) through ELISA kit and Nitric Oxide (NO) mediator in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Pinosylvin was also tested for its ability to inhibit the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) cascade, a Signaling Pathway involved in a wide range of biological processes connected with cell proliferation, differentiation and apoptosis. In addition, a molecular docking study on JAK2 binding site was performed. All these experiments were conducted and validated through a comparison with the well-established activity of Resveratrol commercial standard. Results showed that both Pinosylvin and Resveratrol at the sub-cytotoxic concentrations of 40 µM significantly inhibited the release of TNF-α if compared to control. Resveratrol showed the best inhibitory potential against IL-6, while Pinosylvin showed the highest percentage of NO inhibition (60%). As regards Western blot analyses, even if Pinosylvin significantly down-regulated both JAK2 and STAT-3 phosphorylated proteins, Resveratrol exerted the best inhibitory activity. This data was also confirmed by docking studies, which highlighted that both Pinosylvin and Resveratrol fitted into JAK2 receptor with favorable binding energy values (-7.9 kcal/mol vs -8.2 kcal/mol, respectively), even if, thanks to the additional -OH group on its molecular structure, Resveratrol was able to establish a third hydrogen bond with Leu932, probably responsible for the better binding energy value provided [1]. In conclusion, Pinosylvin could be considered a valid candidate as anti-antiflammatory agent, which properties require further investigations.

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Poster - Sessione 9

P52. **Antioxidant and wound repairing effect of protolichesterinic acid from *Cetraria islandica* in an *in vitro* model of intestinal inflammation.**

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The intestinal epithelium is responsible for the absorption of nutrients but also for protecting the intestine from harmful substances. In particular, it plays a pivotal role in inflammatory response at intestinal level [1] and just intestinal inflammation diseases management, such as inflammatory bowel diseases, are actually of great interest in the research. The vegetal reign is an important source of bioactive compounds useful for biomedical purposes and the study of their molecular pharmacology is a major challenge due to their great chemical diversity and often multi-pharmacological properties [2]. In this context, several lichen species are traditionally used for medicinal purposes in North-European countries [3]. One of these species is *Cetraria islandica*, commonly known as Iceland moss, which has been studied for its potential anti-inflammatory, antimicrobial and immunomodulatory properties [3-5]. In this study, one of the bioactive compounds isolated from *C. islandica*, protolichesterinic acid, was tested in an *in vitro* model of intestinal inflammation to evaluate its antioxidant and reparative properties. Protolichesterinic (10-1 µM) potential was evaluated in rat intestinal epithelial cells (IEC-6) treated with lipopolysaccharide from *E. coli* (10 µg/mL) plus interferon-γ (10 U/mL) as pro-inflammatory stimuli. Our results demonstrated that protolichesterinic acid was able to influence the oxidative stress related to inflammation that plays a pivotal role in IBD. The evaluated compound (10 and 5 µM) significantly reduced reactive oxygen release (ROS, $P<0.01$ vs LPS+IFN). Interestingly, the antioxidant potential was characterized also by a significantly increase of anti-oxidants factors including NAD(P)H quinone dehydrogenase 1 (NQO1) expression, at the concentration of 10 µM, and superoxide dismutase (SOD-2) expression at all concentrations tested ($P<0.0001$ vs LPS+IFN). Intestinal inflammation can lead to conditions that compromise the integrity of the intestinal barrier. To investigate the ability of protolichesterinic acid to restore intestinal barrier repair ability, a wound healing assay on IEC-6 cells was performed. All concentrations tested showed to increase the wound healing closure compared to LPS+IFN alone – treated cells and increased important adhesion molecule expression such as E-cadherin ($P<0.0001$ vs LPS+IFN). These findings could pave the way for further research into how *C. islandica* could help in the treatment of intestinal-related inflammatory conditions.

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P53. **Antibacterial, antioxidant and anti-inflammatory properties of essential oils in combination.**

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Essential Oils (EOs) have long been used in folk medicine for their several biological properties. The increasing prevalence of antimicrobial resistance has led to their renewed interest as complementary agents for the treatment of drug-resistant human and veterinary infections. This study aimed to investigate novel binary combinations of EOs to explore their potential therapeutic applications. The research focused on antibacterial, antioxidant and anti-inflammatory properties of EOs derived from *Origanum vulgare* L. (Oregano), *Juniperus communis* L. (Juniper) and *Cistus ladaniferus* L. (Rock rose). The *in vitro* antibacterial properties of these EOs, alone and in combination, were investigated by the minimum inhibitory concentration (MIC) and checkerboard test against *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), and *Escherichia coli*. The antioxidant capacity was evaluated using three redox-based assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power). The anti-inflammatory activity was assessed through the bovine serum albumin (BSA) denaturation inhibition assay.

The results revealed that Oregano possesses the most potent antibacterial activity against all the strains tested with MIC values ranging from 0.0312% to 0.125% (v/v), and superior antioxidant and anti-inflammatory properties compared to the other EOs. For the combinations of Oregano/Rock rose and Oregano/Juniper, the calculation of fractional inhibitory concentration (FIC) allowed to highlight synergic and additive effects against *S. aureus* and MRSA, respectively. Moreover, the binary associations exhibited additive interaction in antioxidant and anti-inflammatory activities. In conclusion, the results suggest that the combinations of EOs may offer a promising strategy for treating *S. aureus* infections, including those caused by drug-resistant strains and associated with oxidative stress and inflammation. Further research is warranted to explore the efficacy, safety, and optimal formulations of these EO combinations for practical applications.

Acknowledgments: Benedetta Galletta thanks the "Prof. Antonio Imbesi" Foundation for the fellowship.

P54. **Determination of phenolic content, antioxidant activity, and brine shrimp toxicity of the aerial part extracts from *Sinapis alba* and *Sinapis arvensis* (Brassicaceae) growing wild in Sicily (Italy).**

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As part of a project aimed at the valorization of taxa included in the Brassicaceae family belonging to the spontaneous flora of Sicily, our research team has recently focused on studying the taxa of *Sinapis* L., utilized as food plants and in traditional medicine as sources of bioactive compounds with application in pharmaceutical, nutraceutical and cosmetic fields. In Italy, the genus is represented by *Sinapis arvensis* L., *S. pubescens* L., and *S. alba* L., the latter consisting of three infra-specific subdivisions namely subsp. *alba*, subsp. *dissecta* (Lag.) Bonnier, subsp. *mairei* (H.Lindb.) Maire. All these specific and intraspecific taxa are present in Sicily.

Particularly, current research is focused on the aerial parts (leaves, flowers, and stems) of *Sinapis alba* (white mustard) and *Sinapis arvensis* (wild mustard).

The total phenolic and flavonoid content of the 70% methanol extracts from the aerial parts of the selected species has been spectrophotometrically quantified. Moreover, the antioxidant properties were determined by *in vitro* methods based on different mechanisms: the primary antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assays, while the ferrous ion chelating activity assay estimated the secondary antioxidant properties. Finally, *Artemia salina* lethality bioassay was performed for the preliminary toxicity assessment.

The total phenolic content was found to be higher in *S. alba* leaf, flower, and stem extracts (68.53 ± 2.57, 69.33 ± 1.83 and 47.01 ± 1.56 mg GAE/g extract, respectively) than those of *S. arvensis*. The same trend was also observed for the flavonoid content (32.94 ± 0.91, 29.93 ± 0.67 and 17.36 ± 0.41 mg QE/g extract, respectively).

Concerning the antioxidant properties, all the extracts of the two species showed mild to moderate DPPH radical scavenging activity compared to the standard butylated hydroxytoluene (BHT); among the extracts, *S. alba* flower extract displayed the best activity, reaching 95% inhibition at the highest concentration tested (IC_{50} = 1.00 ± 0.04 mg/mL). In the reducing power assay, both species showed mild to moderate reducing power, compared to the standard BHT being *S. alba* extracts more active than those from *S. arvensis*. In the ferrous ion chelating activity assay, *S. arvensis* extracts displayed good secondary antioxidant properties, higher than those from *S. alba*, with flower extract being the most active (IC_{50} = 0.15 ± 0.09 mg/mL) followed by stem and leaf extracts (IC_{50} = 0.22 ± 0.005 and 0.37 ± 0.08 mg/mL, respectively).

The results of the antioxidant tests indicate that the extracts of the two *Sinapis* taxa possess antioxidant properties based on different mechanisms: the best primary antioxidant activity was observed for *S. alba* extracts, whereas those from *S. arvensis* displayed better secondary antioxidant properties.

At last, the results of *A. salina* lethality bioassay showed the absence of toxicity against brine shrimp larvae for all the extracts.

Overall, the obtained results improve the knowledge of the *Sinapis* taxa, also indicating *S. alba* and *S. arvensis*, belonging to the spontaneous flora of Sicily, as safe sources of antioxidant compounds.

P55. **Exploring new lamiaceae extracts to counteract oxidative stress and cellular senescence in *in vitro* models.**

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Skin aging is a complex process marked by the development of wrinkles, a decrease in skin elasticity, and a reduction in turgidity. Moreover, this phenomenon is characterized by variations in cellular phenotype and the components of the extracellular matrix, such as collagen and elastin. This alteration in epidermal structure is strictly related to a loss of the integrity of the skin barrier that may increase the susceptibility to skin disorders like cancer. Skin aging is influenced by both internal and external factors. Ultraviolet radiation (UV) has been identified as one of the extrinsic factors that promote skin aging since it can cause DNA alteration and, therefore, enhance reactive species of oxygen (ROS) production.
Therefore, the use of natural compounds with antioxidant and anti-senescence activity could play a key role in treating skin aging-related disorders. For this reason, this project aims to evaluate the effect of new vegetal extracts obtained from plants of the Lamiaceae family named RR, PL, and VDL.
At first, the antioxidant and antisenescence activity of the extract was evaluated on epidermal keratinocytes (HaCaT). All three extracts reduced ROS levels in basal conditions, but only PL shows some effect in oxidative conditions. Moreover, PL and VDL affect the expression of some senescence markers like IL-6 and IL-18. Subsequently, these extracts were also tested on human fibroblast (WI38), and they confirmed their beneficial effects.
In conclusion, the extracts PL and VDL could be suitable candidates for developing cosmetic products useful for treating skin aging.

P56. **Correlation between sustainable agronomic practices and antioxidant potential: focus on leaves' extracts of three medicinal mediterranean wild species.**

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The objective of this study was to assess the impact of various agronomic factors on plant growth and the accumulation of secondary metabolites with antioxidant properties, a critical feature of herbal extracts due to their inherent pharmaceutical and nutraceutical significance. The three selected spontaneous herbaceous species for this investigation were *Silybum marianum* (milk thistle), *Achillea millefolium* (yarrow), and *Trifolium pratense* (red clover), whose extracts, enriched in phenolic compounds, are well-documented for their potential to alleviate a wide range of ailments. The plants were cultivated at the Terminillo Apennine Centre "Carlo Jucci" in the Province of Rieti, a University of Perugia agricultural experimental center.

A total of three agronomic factors were evaluated, each comprising two treatment options, resulting in eight distinct combinations. The factors included: 1) the presence or absence of plant growth-promoting rhizobacteria (PGPR) inoculation; 2) high versus low fertilization rates of potassium oxide (K_2O) and phosphorus pentoxide (P_2O_5), aimed at modulating nutrient availability; and 3) the imposition of water stress at 40% of field capacity in comparison to a control condition with full field capacity (no water stress).

The progression of plant growth was monitored throughout the designated growing season, employing the BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) scale to delineate key phenological phases, such as germination, vegetative development, and flowering. The eight treatment combinations were administered until the flowering stage was reached.

Only the leaves of the plants were collected, and hydroalcoholic extracts were prepared to evaluate the total antioxidant capacity (TAC). This assessment was conducted using the FRAP, DPPH, and ABTS assays. These three assay types were chosen because they offer complementary insights into the chemical mechanisms underlying TAC, as well as the physicochemical characteristics of the constituents within the phytocomplexes.

The results obtained revealed evident correlations between plant growth conditions and TAC. PGPR inoculation was observed to generally enhance plant growth and TAC across all three species, with the most notable effects observed in *Silybum marianum* and *Achillea millefolium*. Fertilization treatments, notably those with elevated levels of K_2O and P_2O_5, exhibited a stimulatory effect on plant growth, as evidenced by an increase in biomass production. However, the impact of these treatments on TAC was found to be species-specific. Water stress led to a reduction in plant growth for *Achillea millefolium* but increased the antioxidant properties in *Trifolium pratense*, which showed higher TAC under water stress conditions. The correlation between the presence of PGPR inoculation and moderate fertilization, alongside water stress, was particularly significant for *Silybum marianum* and *Achillea millefolium*. The importance of PGPR inoculation in these species, in conjunction with moderate fertilization and water stress, was notably emphasized. These findings underscore how targeted agronomic strategies can enhance the pharmaceutical and nutraceutical value of medicinal Mediterranean wild plants, thereby highlighting the necessity of developing sustainable agricultural practices, especially in regions vulnerable to climate change.

The present research was funded by the Project PRIN2022 Prot. 2022B8KE33 (Definition of a validated food supplement from controlled cultivation of Mediterranean plants to counteract osteosarcopenia in elderly (PhytoMuscleBone).

P57. **Role of purple corn anthocyanins against Doxorubicin-induced cardiotoxicity: an insight into NF-κB pathway.**

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Doxorubicin (Doxo) is a chemotherapeutic agent belonging to the anthracycline family. Despite its anticancer efficacy and its wide spectrum of action, its use is limited by a cumulative dose-dependent cardiotoxicity that can cause progressive cardiomyopathy and heart failure. Although there are a lot of effective primary and secondary prevention strategies, currently none of these seems to be relevant for all types of patients and cancers. Moreover, the majority of them shows side effects or interferes with Doxo anticancer activity reducing its effectiveness. Since inflammation has been also proposed to be involved in Doxorubicin-induced cardiomyopathy, a possible strategy to prevent cardiotoxicity would be to reduce Doxorubicin-induced inflammation.

Interestingly, anthocyanins are a class of flavonoids with multiple biological activities, including cardioprotective and anti-inflammatory properties. Moreover, anthocyanins from purple corn demonstrated a protective role in mice against Doxo-induced cardiotoxicity.

Given these premises, we aimed to study whether anthocyanins from purple corn could counteract Doxo-induced cardiotoxicity through the modulation of the pro-inflammatory NF-κB pathway. We used extracts from two *near-isogenic* maize lines (yellow and purple corn) which are genetically identical except for the gene regulators promoting the synthesis and accumulation of anthocyanins, allowing to discriminate the effect of anthocyanins, present only in Red extract, from the one of other flavonoids present in both extracts (Yellow and Red).

We tested the preventive effect of Yellow and Red extracts on HL-1 murine cardiomyocytes challenged with Doxo at two different concentrations which mimic the plasmatic doses of Doxo found in patients.

Our results showed that Red extract improved viability of HL-1 cardiomyocytes upon Doxo treatment and down-regulated the Doxo-induced inflammatory mediators analyzed. On the other hand, Yellow extract had always little or no effect. Thus, anthocyanins exerted this anti-inflammatory activity. Particularly, anthocyanins from purple corn showed cardioprotective activity, inhibited nuclear translocation of NF-κB and decreased levels of iNOS, COX-2, cytokines, nitric oxide and PGE_2 induced by Doxo.

In conclusion, supplementation with an anthocyanin-rich extract from purple corn may represent a strategy to prevent Doxo-induced inflammation and toxicity through NF-κB modulation in cancer patients.

Poster - Sessione 10

P58. **Exploring the chemical diversity of *Cannabis sativa* essential oils: a study on four selected genotypes.**

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Industrial hemp (*Cannabis sativa* L.) is a versatile crop with a wide range of applications, primarily in medical therapy, textiles, biofuel production, cosmetics, and construction (Nissen et al. 2010). *C. sativa* exhibits significant genetic and phenotypic variability, which, while challenging to manage, presents a valuable opportunity for breeders to develop new lineages with improved features for fiber and seed production. In the last years, hemp cultivation has been widely promoted for its favourable agronomic traits combined with a low environmental impact. Despite its benefits, the primary hemp supply chains generate substantial amounts of waste, the efficient reuse of which aligns with the principles of a zero-waste, circular economy. Among these waste products, hemp inflorescences stand out as a valuable resource, particularly for the extraction of both volatile and non-volatile bioactive compounds. Recently, an increasing number of studies have been focused on hemp essential oil (EO), due to its potential industrial applications (Ascrizzi et al. 2020; Benelli et al. 2018). This EO is a complex mixture of volatile compounds, predominantly terpenes and terpenoids, which are responsible for the distinctive aroma of various *Cannabis* strains. Their specific combinations define the unique aromatic bouquet of each strain, a crucial factor influencing consumer preferences. The chemical composition of hemp EO is influenced by multiple factors, including genotype, year of cultivation, and phenological stage (Pieracci et al. 2021, 2023). In turn, the chemical profile is closely linked to the EO biological activity, affecting its potential applications. Building on our previous findings, the present study aimed to chemically characterize four underexplored *C. sativa* genotypes selected by CREA-CI of Bologna. While all samples were rich in mono- and sesquiterpene hydrocarbons as well as oxygenated sesquiterpenes, they exhibited significant differences in relative abundance and key compounds. The analysed EOs were tested for their antimicrobial activity by MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) against foodborne bacterial strains. Moreover, a trained panel was conducted to evaluate EO sensorial profiles.

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P59. **Phytochemical analysis and biological activities of *Odontites vulgaris* Moench.**

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Odontites vulgaris Moench (Orobanchaceae) is an annual herbaceous plant, used for medicinal purposes since the ancient times [1]. It is used in Mongolian medicine to cool the blood, alleviate itching, promote detoxification and diuresis, and relieve symptoms of rheumatoid arthritis [1]. Iridoids, phenylethanoid glycosides, flavonoids, triterpenes, and organic acids represent the main identified phytochemicals, though its true bioactive compounds remain unidentified [2]. In this study, we examined an Italian species of *O. vulgaris* from the Maiella National Park in central Italy, in order to characterize its phytochemical and biological profile. To this end, an ethanolic dry extract from the aerial parts (20:1 drug/extract ratio) was prepared through 96 h maceration, followed by evaporation under vacuum. In order to identify the characteristic compounds, the extract was subjected to chromatographic separation, using an elution system composed of *n*-butanol and distilled water (82:18 *v/v*) and then a mixture of *n*-butanol, methanol, and distilled water (70:10:30 *v/v/v*). The isolated compounds were identified through NMR and MS analysis and identified as quinic acid and the iridoids aucubin, catalpol, shanzhiside methyl ester, melampyroside, 8-*epi*-loganin, and caryoptoside [3]. A screening of diverse bioactivities, including the scavenging activity against DPPH and ABTS radicals, ability to affect AGE (advanced glycation end-products) generation, cytotoxicity in H69 human cholangiocytes and RAW 264.7 murine macrophages, and cytoprotection towards oxidative damage induced by tert-butyl hydroperoxide (tBOOH), was performed according to standardized methods [3]. The extract exhibited radical scavenging properties against both $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals, without affecting the AGE formation, with an efficacy less potent than the positive control trolox. In the tested cell lines, the extract showed toxicity signs starting from the highest concentration of 600 µg/mL, where cell viability was reduced by about 40% of the control. The same concentration also increased the intracellular oxidative stress, increasing levels of reactive oxygen species more than three times in H69 cells and more than twice in RAW 264.7 cells ROS with respect to control. In both cell lines, nontoxic concentrations of the extract were able to protect the cells from the oxidative damage induced by tert-butyl hydroperoxide (tBOOH), by restoring the cell viability and lowering the oxidative stress. However, at non-toxic concentrations, the extract exhibited cytoprotective effects towards the tBOOH-induced oxidative damage in both cell lines, by restoring viability and reducing oxidative stress. Present findings highlight Italian *O. vulgaris* as a promising source of bioactive compounds, with some identified for the first time. Notably, the cytoprotective properties of the extract agree with previous evidence [2] and suggest a possible contribution of the identified compounds. However, further investigations to better characterize the bioactivities of the tested extract and its metabolites are needed.

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P60. **Characterization and quality control of herbal drugs.**

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Plants are a source of a broad range of natural bioactive molecules with diverse therapeutic properties, continually being explored to develop new medicines. Traditional medicine has always relied on these natural products to treat many illnesses; today, most of them represent the basis of pharmaceutical drugs. Based on this knowledge, characterization and quality control of plant sources and extracts to be used for medical purposes are essential to ensure their effectiveness, safety, and compliance with current regulations. The quality of plant-based drugs depends on several factors, including the botanical species, the harvesting method, storage conditions, and processing methods, which influence the chemical and biological composition of the final product.

Sveba Srl is one of the leading European contractors (CDMO) operating in the food supplement sector. It specializes in designing, developing, and producing high-quality food supplements. The present work aims to show an example of a dossier carried out by the company about controls that need to be performed on raw materials before food supplement development. Specifically, the dossier shown is based on the chemical characterization and quality control performed on Melissa (Melissa officinalis L.).

Through Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) analyses, the chemical profile of Melissa is characterized, focusing on the secondary metabolite of interest, rosmarinic acid. Subsequently, the quality control of the plant drug includes the assessment of contaminants such as pesticides, heavy metals, mycotoxins, and pathogenic microorganisms, which must comply with the limits set by international regulations. These preliminary controls are essential to ensure the success of a food supplement, primarily in terms of efficacy and safety for the end consumer.

P61. **Investigating the anti-aging benefits of *Salvia haenkei*: an *in vivo* approach.**

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Aging is a gradual and intricate process marked by the progressive decline of key bodily functions and is recognized as a significant risk factor for various diseases, including cancer, diabetes, and cardiovascular and neurodegenerative disorders. The accumulation of senescent cells in different tissues contributes to an age-related condition known as the senescence-associated secretory phenotype (SASP). To counteract senescence and promote a healthier tissue environment, recent research has focused on identifying longevity-enhancing compounds from botanical sources.

Previous studies conducted in our laboratory have demonstrated the anti-senescence properties of a standardized extract of *Salvia haenkei* (SH) *in vitro*, specifically in human epidermis and lung fibroblast models. This study aimed to investigate whether the extract also possesses anti-aging effects in an *in vivo* model of naturally aged mouse. The findings revealed that administering a low dose of SH extract prolonged the lifespan and improved the health of naturally aged mice by delaying senescence, reducing systemic inflammation, and decreasing fibrotic markers in multiple tissues, such as the skin, muscles, cartilage, and kidneys. Additionally, treated mice exhibited enhanced muscle strength, vitality, and thicker fur compared to age-matched controls.

Overall, these results confirm the safety of a low-dose SH extract treatment both *in vitro* and *in vivo*. The extract effectively improved key aging-related parameters, paving the way for future clinical trials in humans.

P62. **Innovative breeding system to develop new crop strategies to obtain pharmaceutical metabolites.**

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Microalgae are unicellular organisms that have attracted growing interest in the pharmaceutical field due to their versatility and ability to produce a wide range of bioactive compounds [1][2]. These microorganisms are able to synthesize complex and bioactive molecules that can be exploited for therapeutic, food and industrial purposes [2]. One of the key characteristics of microalgae is their highly active and reactive genome, which allows them to rapidly change and adapt their transcriptomic profile, consequently giving them high adaptability. Based on these genomic characteristics, innovative techniques that permit the combination of microalgae and higher plant cells were developed and applied to structure a new combined genome, opening new avenues in the production of high-yield bioactive compounds.

Applying these techniques, a combined genome was realized by fusing a cell of a microalgae (*C. vulgaris*) with a cell of *Cannabis sativa* L. These combined cells have a microalgae-like structure (unicellular) and are able to produce cannabidiol (CBD), known for its therapeutic potential.

CBD has shown promising effects in the treatment of neurological disorders, anxiety, chronic pain and inflammation, without significant psychoactive effects [4][5].

The use of a microalgae-like genotype to produce CBD represents an alternative and efficient way to traditional cannabis cultivation, reducing the costs and resources needed to produce this molecule. Furthermore, microalgae offer numerous advantages over higher plants, especially for crop management. In fact, their ability to be cultivated continuously overcomes the seasonality that limits the use of traditional plants. Microalgae can be cultivated in controlled systems, allowing for constant, high-quality, and sustainable production without the use of pesticides. Another significant benefit of microalgae is their positive environmental impact. During the photosynthesis process, they absorb a high quantity of carbon dioxide and release oxygen.

Finally, microalgae are an efficient "delivery system" for both CBD and other bioactive substances [6]. Microalgae, as a "delivery system," ensure superior metabolite bioavailability due to their ability to protect and transport molecules through the digestive system [3][6].

The innovative breeding system described could revolutionize the approach to the production of biodrugs and biosupplements, with a positive impact on the biopharmaceutical chain, the environment, and finally, on human health.

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P63. **Exploring the medicinal value of nature reserves: the case of Bosco Siro Negri**

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Historically, natural products are recognized as the primary source of compounds for medicines, supplements, cosmetics and foods. Even today, natural products obtained from plant species continue to be an important technological and socioeconomic resource, but their gain is often hindered by the impoverishment of biodiversity. Biodiversity is one of the Earth's most valuable and often underestimated resources, threatened by agricultural, construction, and tourism development, along with improper waste management with consequences for the loss of natural habitats and species.

To overcome this problem, various conservation efforts have been established, such as the International Union for Conservation of Nature's (IUCN) Red List, which tracks the risk status of over 37,400 species [1]. Another promising strategy for biodiversity protection is the institution of Nature Reserves. The IUCN defines these reserves as clearly defined areas dedicated to long-term conservation, and they also provide ecosystem services like food, water, and climate regulation. The subject of our work has been Bosco Siro Negri, located near the industrial cities of Pavia and Milano in Northern Italy, on the right bank of Ticino River, which covers an area of 1,352 hectares [2]. It was established as a Nature Reserve in 1973, managed by the University of Pavia with a mission to preserve its biodiversity for scientific research. While many studies have focused on plant and animal surveys, this work investigates the reserve's role in conserving medicinal species. The flora of Bosco Siro Negri includes 113 species, and a study was conducted to assess their presence in the European Pharmacopeia (EP), European Medicinal Agency (EMA) databases, and historical records. Notably, 44 species have been historically used for medicinal purposes, despite only a few appearing in official databases, indicating the reserve's unrecognized value in preserving pharmaceutical plants.

To deep our knowledge about Bosco Siro Negri and to highlight its value in the medicinal chemistry field, we exploited an already existing checklist of this area and built a database of about 1,500 secondary metabolites produced by the species growing there. Next, we implemented this data with their classification (e.g., tannins, flavonoids, terpenoids, etc) and associate them with their isomeric SMILES code and their IUPAC name. Preliminary results highlighted that most of the metabolites produced by the considered species belong to flavonoid class (38%), followed by organic acids (21%) and terpenoids (12%).

Metabolomic profiling of the flora suggests potential for future research, as 28 species have not yet been investigated for their metabolomic profiles.

This research emphasizes the crucial role of natural reserves like Bosco Siro Negri in preserving species with medicinal value, protecting them from external threats, and facilitating future pharmaceutical discoveries through the study of their metabolomic profiles. This integral nature reserve has demonstrated to be a hotspot of pharmaceutically interesting plants and the exploitation of the DB of secondary metabolites for computational screening could represent a powerful tool to identify new active metabolites.

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Poster – Sessione 11

P64. **Ecosystem services and phytotherapeutic valorization of resilient species.**

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In recent decades, climate change has increased the importance of identifying plant species capable of adapting to adverse environmental conditions. In this context, *Pistacia lentiscus* L., an evergreen shrub typical of the Mediterranean, stands out for its ability to tolerate prolonged periods of drought, while playing a fundamental role in soil stabilization and the formation of microenvironments favorable to biodiversity. Furthermore, *P. lentiscus* L. is recognized as having important health properties; in fact, it has been used since ancient times for the medicinal virtues of the gum resin called mastic, whose complex composition includes over 120 different components, especially tetracyclic and pentacyclic triterpenes. Already known in ancient Egypt, it was one of the main ingredients used for embalming, the Byzantine and medieval ages, mastic occupied a special place in folk medicine as a remedy for gastralgia, peptic ulcers and for oral hygiene, and later became part of several official pharmacopoeias for its anti-inflammatory and antibacterial properties, in particular against Helicobacter pylori. This work aimed to exploit the ecosystem services provided especially by resilient plant resources that, fitting into a broader research framework aimed at mitigating the effects of climate change through sustainable use of biodiversity, can address both environmental challenges and those related to human health according to the most recent canons of "one health". The study focused on drug extracts that can be considered by-products and therefore eco-sustainable, in particular on the leaves and bark of the mastic tree.
The main objective of the research was to characterize the phytochemical composition of the extracts, with particular attention to the total content of polyphenols and flavonoids, and to evaluate their potential biological activities in vitro. The results highlighted antioxidant, antidiabetic and antiobesity activities. In particular, the ability of the extracts to inhibit pancreatic lipase and α-amylase was determined: two therapeutic targets for the treatment of type 2 diabetes mellitus. The bark extracts showed a modest activity in inhibiting pancreatic lipase, however, the results on the inhibition of α-amylase highlighted that the leaf and bark extracts showed a marked activity of inhibition of the enzyme with IC50 values lower than the positive control (acarbose, IC50 of 20.36 ± 1.38 µg/ml). In particular, the leaf extract was found to inhibit α-amylase with an IC50 of 4.04 ± 1.26 µg/ml and the bark extract showed IC50 of 7.86 ± 1.50), suggesting a promising antidiabetic effect. In conclusion, this study highlighted the remarkable therapeutic and applicative potential of *P. lentiscus* L., demonstrating that a plant resource so closely linked to the Mediterranean territory can be valorized not only for its ecological role, but also as a valuable source of bioactive compounds with important therapeutic properties. The results obtained clearly highlight the value of *P. lentiscus* L. as a resource for the development of nutraceutical and pharmaceutical products. In particular, they outline new perspectives for the development of complementary or adjuvant therapeutic strategies for the management of chronic diseases such as type 2 diabetes mellitus and other pathologies related to oxidative stress.

P65. **Hemp byproducts: optimizing supercritical extraction and unveiling antimicrobial potential.**

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Hemp has been cultivated since ancient times, thanks to its wide range of applications – from nutraceutical to therapeutic, from abuse to textile and construction industries. In the last few years, because of the diffusion of non-psychotropic varieties, the hemp market has been skyrocketing, with an increase in hemp biomass and consequent byproducts (1).

The project PRIN 2022 PNRR "NOrCa - Not Ordinary Cannabis – (P2022TXJX8) aims at the valorisation of *Cannabis sativa* waste and by-products in view of a circular economy and environmental compatibility. Within this project, Whole Lotta Hemp Srl (Parma) provided a sample of aerial parts that are a byproduct of hemp seed cleaning.

A design of experiment (DoE) was followed to optimise a supercritical fluid extraction of major and minor cannabinoids with $scCO_2$. A total of 21 SFEs was performed at different conditions of time, temperature, pressure, CO_2 flow, and percentage of ethanol used as co-solvent. The highest yields (up to 10.48%) were obtained by adding 5% ethanol at a pressure of 150 bar. In contrast, extractions performed without any co-solvent gave lower quantitative yields.

While a preliminary chemical characterisation performed by GC-MS revealed little difference between extracts and a not surprising abundance of cannabinoids (mostly CBD), they showed promising results in terms of their antimicrobial activity, tested against a Gram+, *Staphylococcus aureus*, and a Gram-, *Escherichia coli*. It was evaluated through determination of the minimal inhibitory concentration (MIC) following CLSI's standard method of dilutions in microplate (2). None of the extracts demonstrated complete inhibition of *E. coli* growth at the highest concentrations tested, achieving a maximum inhibition of 30% at the highest concentration tested. Conversely, more interesting results were obtained against *S. aureus*: all tested extracts demonstrated a minimum inhibitory concentration (MIC) of less than 100 µg/mL, with some extracts achieving a MIC as low as 6.25 µg/mL, better than chloramphenicol used as a positive control in this study.

The considered by-products demonstrated an activity against *S. aureus* comparable to data reported in the literature regarding hemp inflorescences (3). The similarities in the results are not maintained against *E. coli*, likely attributable to the processing the sample underwent prior to being classified as waste material.

Extracts obtained without ethanol addition seem more active, but a statistical correlation between antimicrobial activity and extractive conditions is still unclear and under evaluation. At the same time, the current aim is to optimise a chromatographic separation of DoE extracts in order to identify and purify the main components responsible for the antimicrobial activity.

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P66. ***Erucastrum virgatum* subsp. virgatum (Brassicaceae) endemic to Sicily (Italy) as a new potential source of health-promoting phytochemicals: phenolic content and biological properties of leaf extracts.**

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Several species belonging to the *Brassicaceae* family have been recognized as rich sources of phytochemicals with healthy nutritional effects and a promising wide range of biological activities. *Erucastrum virgatum* C.Presl subsp. *virgatum* (Brassicaceae) is a perennial herb endemic to Southern Italy (Sicily, Calabria, and Basilicata). Ethnobotanical studies report the use of leaves and flowering shoots of this species in traditional Sicilian cuisine. Despite its food use, no studies regarding the phytochemical characterization or evaluation of the biological properties are available in the literature. Therefore, in continuation of our investigations into species included in the Brassicaceae family growing wild in Sicily, it seemed interesting to undertake a study on *E. virgatum* subsp. *virgatum*.

The present work aimed to establish a suitable method to obtain bioactive-rich extracts from the leaves of *E. virgatum* subsp. *virgatum* collected in the locality of Scaletta Zanclea (Messina, Sicily). The leaves were lyophilized and subjected to different extraction methods, using ethanol (EtOH) and water (H_2O) as green solvents: extraction in ultrasonic bath at 50 °C with 70% EtOH and H_2O (UB-70%EtOH and UB-H_2O), maceration with 70% EtOH and H_2O (Mac-70%EtOH and Mac-H_2O), decoction (Dec-H_2O), and Soxhlet extraction with 70% EtOH (Sox-70%EtOH).

The extracts were investigated for their phenolic content and biological properties. The quantitative determination of total polyphenols and flavonoids was attained by spectrophotometric methods, which highlighted higher contents for all the 70% EtOH extracts compared to the aqueous ones. In particular, Sox-70%EtOH showed the highest phenolic content and Mac-70%EtOH the greatest amount of flavonoids. The antioxidant properties of *E. virgatum* subsp. *virgatum* extracts were investigated by *in vitro* methods based on different mechanisms. All the extracts showed moderate "radical scavenging" activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test and mild reducing power. In both antioxidant assays the 70% EtOH extracts exhibited better activity than the aqueous ones, being the most active Sox-70%EtOH in the DPPH test and Mac-70%EtOH in the reducing power assay. Contrary to what was observed in the other antioxidant tests, in the ferrous ion chelating activity assay all the aqueous extracts showed strong activity, higher than that of the 70% EtOH ones, UB-H_2O being the most effective. The antimicrobial properties were investigated against selected Gram-positive and Gram-negative bacteria by standard methods. Weak inhibitory properties were observed only for the 70% EtOH extracts, and Sox-70%EtOH displayed the best activity. Furthermore, the extracts were found to be non-toxic after preliminary toxicity evaluation by the *Artemia salina* Leach lethality bioassay. The obtained results indicated Sox-70%EtOH and UB-H_2O as the most promising among the 70% EtOH and H_2O extracts, respectively. The qualitative-quantitative phenolic profile of these extracts was characterized by HPLC-PDA/ESI-MS analysis.

Our findings provide a significant contribution to the knowledge of the phytochemical composition and the biological activities of *E. virgatum* subsp. *virgatum*, not investigated so far, also indicating the leaves of this plant as potential safe sources of antioxidants.

P67. **Harnessing essential oils for sustainable agriculture: effects on germination and biochemical features of chickpea.**

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The agricultural sector is facing pressing challenges due to rapid global population growth, increasing food production demand, climate change, and restrictions on the use of agrochemicals. These challenges highlight the need for innovative and sustainable practices to enhance crop productivity and quality, while reducing environmental impact. Essential oils (EOs) have emerged as a promising *green* solution, offering bioactive properties that support plant growth and improve seed nutritional quality. This study investigates the effects of 8 EOs derived from aromatic and medicinal plants (*Eugenia aromatica* (L.) Merr. & L.M. Perry, *Origanum compactum* Benth., *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Lippia citriodora* Grisebach, *Rosmarinus officinalis* L., *Myrtus communis* L., *Thymus satureioides* L., and *Mentha pulegium* L.) on chickpea seeds germination, evaluating physiological [germination rate (GR)] and biochemical [i.e., total phenolic content (TPC), total flavonoid content (TFC), and total soluble protein content (TSPC)] features. EOs were extracted using hydrodistillation with a Clevenger apparatus, and their chemical composition was analyzed by GC-MS. Chickpea (*Cicer arietinum* L.) seeds, chosen as model species, were first sterilized with 5% sodium hypochlorite, and subsequently hydro-primed with varying EO concentrations (0.00%, 0.01%, 0.05%, 0.10%, and 0.25%). The results showed a dose-dependent and species-specific effect among the tested EOs. Specifically, at higher concentrations, *E. aromatica* (>0.1%) and *O. compactum* (0.25%) completely inhibited germination. In contrast, lower concentrations (0.01%) of *C. atlantica* and *L. citriodora* stimulated germination, with increases of +21% and +30%, respectively. Antioxidant levels varied across EOs, with *L. citriodora* showingthe the highest TPC increase (+21%) at 0.01%, while *T. satureioides* and *R. officinalis* showed the highest TFC increases (+91% and +76%, respectively) at 0,01%. The higher concentrations of *E. aromatica* increase TPC (+176,04%) and TFC (+12,5%) levels while higher concentration of *O. compactum* reduced both TPC (-32,51%) and TFC (-20,82%) levels. TSPC increased by 38% with *E. aromatica* at 0.01% and remained stable across concentrations for *L. citriodora*. These results revealed the dual role of EOs: biostimulants or phytotoxicants, depending on the species and their concentration. Specifically, *C. atlantica* and *L. citriodora*, improved both the germination and seed biochemical properties at 0,01% while higher doses of *E. aromatica* and *O. compactum* reduced both the germination and the antioxidant levels. This study emphasizes the importance of optimizing EOs applications in agriculture to maximize their potential as sustainable tools in modern farming practices for the replacement of agrochemicals.

P68. **LC-ESI/HRMS-guided isolation of alkylamides from *Echinacea angustifolia* roots.**

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Echinacea angustifolia DC., also known as purple coneflower, is a medicinal plant used for centuries. Interest in *Echinacea* spp. is mainly focused on their immunomodulatory effects, particularly in the prevention and treatment of common cold, coughs, bronchitis, upper respiratory infections, and some inflammatory conditions [1]. *E. angustifolia* contains several groups of bioactive metabolites, including alkylamides, considered important for activity [2]. Alkylamides are made up of an aliphatic chain deriving from a poly-unsaturated fatty acid connected to a short-chain amine [3].

E. angustifolia extracts have been reported to inhibit the LPS-induced production of TNF-α, IL-1, IL-6, and IL-8 cytokines and chemokines from human monocytes/macrophages and from macrophage-like cell lines. They appeared to mediate these effects through both cannabinoid receptor (CB)-dependent and CB-independent mechanisms [4-5]. In particular, mainly for their content in alkylamides, *E. angustifolia* extracts have been reported to be capable of activating the cannabinoid receptor type-2 (CB2), playing a role as anti-inflammatory and immune-modulatory principles. Bioactive compounds targeting the endocannabinoid system may be a valid approach for patients with symptoms associated with atopic eczema and other inflammatory skin diseases as well [4-5].

In the frame of a project aimed at investigating natural products with dermocosmetic properties, in the perspective of testing single compounds along with extracts, a SLDE-Naviglio extraction using as solvent a mixture of EtOH:H_2O (75:25) was performed for an in-depth knowledge of the chemical composition of *E. angustifolia* roots, with particular attention to alkylamides. The extract was submitted to liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-ESI/QOrbitrap/MS), in positive ion mode, allowing the identification of alkylamides which were isolated and successively characterized by 1D- and 2D-NMR experiments. The isolated alkylamides possessed an aliphatic chain containing from 15 to 16 carbon atoms, with one or more unsaturations and hydroxyl groups, and an isobutylamide moiety. As an example one of the isolated alkylamides is shown in Figure 1.

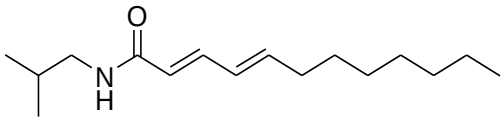

Figure 1. Alkylamide

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P69. **Mass spectrometry-based specialized metabolite profiling of three Salicornia species extracts with potential antidiabetic activity.**

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Soil salinization is an increasing environmental challenge that threatens agricultural productivity and food security worldwide. Staple crops like wheat, rice, and corn are highly sensitive to salinity, whereas a few salt-tolerant species, known as halophytes, are able to grow and thrive in saline conditions, and thus, are emerging for their ecological, nutritional, and healthy importance in the context of climate change [1]. Caryophyllales order contains the highest percentage of halophyte species (21% of the total). Within this order, the Amaranthaceae family comprises the highest number of halophyte genera, including *Salicornia* (syn. *Sarcocornia)* genus, which is widespread in the Mediterranean area [2]. In this study, three different *Salicornia* species were selected: *Salicornia europaea* L., *Salicornia fruticosa* (L.) L. and *Salicornia perennis* Mill. *S. europaea* is among the most extensively studied species within the genus, for its phytochemical composition and potential applications [3] while *S. fruticosa* and *S. perennis* remain relatively underexplored, with limited studies available on their phytochemistry, biological activities, and potential uses [4].

The aim of this work was to investigate the chemical quali-quantitative composition of the selected *Salicornia* species by UHPLC-HR-Orbitrap/ESI-MS^2 analysis, followed by antidiabetic properties through *in vitro* inhibition of *α*-amylase and *α*-glucosidase enzymes [5]. The aerial parts of *S. europaea*, *S. fruticosa*, and *S. perennis* were collected between July and September 2023 along the Italian coasts, and extracted with methanol, then partitioned with *n*-BuOH/H_2O (1:1 v/v). LC-MS^2 analyses of the *n*-butanol residues revealed phytocomplexes rich in phenolic acid derivatives, flavonoids (mainly quercetin, isorhamnetin, and kaempferol glycosides), saponins, and fatty acids. Flavonoids and saponins were quantified by using rutin and pharbitoside A as pure external standards, and the significant difference (p value < 0.05) between groups of values was evaluated by using a one-way ANOVA. *S. fruticosa* species resulted particularly rich in quercetin rutinoside (48 ± 4 mg/100 g fresh weight (FW) ± standard deviation (SD), while *S. europaea* in quercetin hexoside (20 ± 1 mg/100 g FW ± SD). Among the annotated saponins, oleanolic acid glucuronide hexoside was the most abundant in *S. fruticosa* extract (38 ± 3 mg/100 g FW ± SD). The *α*-amylase inhibitory activity of the *n*-butanol extracts was tested in a concentration range of 0.01-1.00 mg/mL using acarbose as control (IC_{50} = 0.06 ± 0.001 mg/mL). *Salicornia* species showed a dose-dependent effect. In particular, *S. europaea* showed a slightly higher inhibition (IC_{50} = 0.43 ± 0.05 mg/mL) compared to *S. perennis* (IC_{50} = 0.65 ± 0.09 mg/mL) and *S. fruticosa* (IC_{50} = 0.96 ± 0.14 mg/mL). In the *α*-glucosidase assays, all species exhibited mild activity, with enzyme inhibition ranging from 25% to 50% at 5 mg/mL.

In conclusion, thanks to their remarkable environment adaptability and chemical composition, *Salicornia* species are interesting plant sources from a botanical, ecological, agricultural, and nutraceutical point of view encouraging further future studies on their potential biological value.

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P70. **Evaluation of different analytical methods in the titration of plant extract-based products.**

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The complexity of plant matrices poses multiple challenges in determining the active ingredients that characterize the phytocomplex. The analysis and characterization of a finished nutraceutical product is a delicate compromise between analytical accuracy and precision on one side, and production and commercial aspects on the other, linked to the nature of the ingredients used and the specifications adopted by the producers. All of this is done to satisfy the customer's needs and meet their requests through the specifications displayed on the label.

In this work, we took as an example a product in tablets containing a dosage of green tea leaves dry extract (*Camellia sinensis* (L.) Kuntze) titrated in polyphenols and catechins. The finished product was analysed with various UV and HPLC methods, collecting and comparing the analytical results.

The values obtained were evaluated based on the selectivity of the methods, reasoning on their specificity and on the possible problems related to the nature of the instrumentation adopted.

UV-vis spectroscopy-based methods allow for non-specific quantification, expressing the content of a class of molecules characterized by a common reactivity. Specifically, the methods adopted are based on colorimetric reactions (Folin-Ciocâlteu, ferrous tartrate) or on absorption at a wavelength specific to an active ingredient (catechin).

HPLC determination allows for the precise quantification of individual components, avoiding interference from other substances present in the finished form (originating from components of the extract or from other actives and excipients).

The interaction with other molecules is useful for effectively monitoring the product over time, evaluating its behaviour and stability. All of this is aimed at achieving efficient and targeted process controls, which guarantee the quality and effectiveness of the final product.

Poster - Sessione 12

P71. **Enhancement of the biological activity of plant extracts for phytotherapeutic use: the Bergafort® case.**

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The use of hypolipidemic natural extracts, therefore antiobesity, is a major research topic in the nutraceutical field. The control of lipid absorption occurs through the inhibition of water-soluble enzymes that catalyze the digestion of dietary lipids by splitting the ester bond that links the hydroxyl groups of glycerol to long-chain fatty acids: pancreatic lipases. Currently the only drug used for this purpose is orlistat, which despite significant activity, has side effects. These often limit compliance, thus leading to the search for plant extracts with pancreatic lipases inhibition activity. The University of Calabria, together with the nutraceutical company NaturextraLab, has developed the supplement Bergafort®, which is characterized by the exploitation of the synergy of action between two species that characterize the Calabrian agri-food production: bergamot (*Citrus bergamia* Risso & Poit.) and kumquat (*Fortunella margarita*). The Bergafort® supplement is therefore a hypolipidemic and anti-obesity product, useful for keeping lipid levels under control and free of side effects unlike Orlistat, one of the drugs currently most used in the treatment of obesity. The plant extracts used in Bergafort® come from *Citrus bergamia* Risso & Poit. and *Fortunella margarita*. The lipase test was conducted in vitro according to protocols validated by scientific literature and each test was performed in triplicate to obtain the mean value. The inhibition of enzymatic activity was evaluated spectrophotometrically, using p-nitrophenol octanoate (p-NPO), a chromogenic ester, as a substrate. An aqueous solution of enzyme was prepared from crude type II porcine pancreas. A solution of NPO was prepared and added to a solution of DMSO. The composition of the reaction mixture included: NPO, Tris-HCl buffer, extract tested at different concentrations and enzyme solution. The mixture was incubated at 37 °C. In the control, an equal volume of DMSO was added instead of the extract. Orlistat was used as a positive control. The inhibitory activity was assessed using the following formula: Lipase inhibition (%) = [1-(ABSNPO - ABSDMSO) / ABS0] x 100. Bergafort® is based on the concept of a synergistic mechanism, i.e. the principle that the interaction between two extracts results in a sum of the effects (sum synergism), i.e. an effect that is greater than the sum of the effects of the individual extracts (potentiation synergism). The IC50 was assessed in the extracts, which corresponds to the concentration of the compound to be tested that is required to reduce the activity of an enzyme by half in a defined period of time. The biological activity of inhibition of pancreatic lipase of the extracts taken individually, and standardized in the concentration of the fraction of total polyphenols and total flavonoids, is for *Citrus bergamia* Risso & Poit IC50 0.52 ± 0.13 mg/ml. For *Fortunella margarita*.: IC50 0.60 ± 0.07 mg/ml. Among the tested mixtures, the results obtained reveal that the 80/20 mixture (i.e. with 80% of *Citrus bergamia* Risso & Poit: and 20% of *Fortunella margarita*) shows an IC50 0.47 ± 0.05 mg/ml which represents the sought synergistic effect with high activity of inhibition of pancreatic lipase

P72. *Daucus carota* pulp calli genetic transformation for antioxidant bioactive compounds production

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The field of genetic engineering in biosynthetic pathways is expanding rapidly in botanical research, driven by the increasing global demand for plant-derived compounds with beneficial health effects. One key area of focus within this research involves the regulation of anthocyanin biosynthesis, which is controlled by the MYB-bHLH-WD40 transcriptional complex.

This complex plays a crucial role in regulating various stages of the biosynthetic pathway, with certain MYB transcription factors activating specific genes in response to signals derived from cell development and environmental stimuli[1].

Studies conducted with maize (*Zea mays* L., 1753) cell cultures has demonstrated that the expression of a bHLH transcription factor, in combination with MYB genes, both of which act as activators of anthocyanin production, results in a substantial increase in the accumulation of these antioxidant compounds[2].

Based on these findings, the present project proposes the genetic transformation of carrot (*Daucus carota* L., 1753) pulp calli to express the Sn gene from maize, a bHLH transcription factor, with the aim of establishing a biotechnological platform for the controlled, stable, and reproducible production of anthocyanins, which have a wide range of beneficial effects on human health. For this pourpose, carrot pulp calli were transformed using *Agrobacterium tumefaciens* with a gene cassette vector, which included the Sn gene in tandem with a kanamycin resistance marker gene (NPTII). Since anthocyanin production is often triggered by stress factors, such as pathogen attacks, the engineered tissues were exposed to continuous light within a growth chamber. This treatment was intended to serve as a stimulus to activate the biosynthetic pathway of anthocyanins. The results indicate that the expression of the Sn gene alone is sufficient to significantly promote the synthesis and accumulation of anthocyanins in the transformed tissues. Given that the Sn gene in maize, as well as in other species such as *Lotus corniculatus*[3-5], is capable of transactivating both early and late stages in the flavonoid biosynthetic pathway, further studies were carried out to investigate the expression of both early (CHS) and late (DFR and ANS) biosynthetic genes in the selected transformed lines. The expression of these genes, which are highly conserved across various plant species, was carefully monitored to determine which regions of the biosynthetic pathway were most influenced by the Sn transcription factor. This information is critical for understanding how the Sn factor regulates flavonoid synthesis and its potential applications in increasing anthocyanin production. The expression studies were conducted, alongside qualitative analysis of the same transformed samples, to gain deeper insights into the impact of Sn on specific anthocyanin accumulation and to evaluate the overall stability and efficiency of the engineered biosynthetic system.

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P73. **In vitro evaluation of the cytotoxic and genotoxic potential of organic cyanobacteria extracts from the Bagni San Filippo thermal waters**.

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Cyanobacteria are photosynthetic prokaryotic organisms present in multiple aquatic and terrestrial environments. They can multiply to high densities and form blooms in fresh and brackish water. Cyanobacteria can produce a wide range of secondary metabolites, including cyanotoxins, which can persist in water and bioaccumulate in aquatic plants. In recent decades, the problem of cyanobacterial blooms has intensified on a global scale in face of climate change and water eutrophication. It is becoming an emerging public health problem due to the toxic effects of cyanotoxins. More recently, several studies have shown that cyanobacteria also produce molecules with interesting biological properties, such as anticancer, anti-inflammatory, and antibiotic activities. The aim of this study was to investigate the cytotoxic and genotoxic potential of eleven organic extracts of cyanobacteria of various species collected from the Bagni San Filippo thermal waters (Tuscany). The extracts were tested on a human immortalized keratinocyte cell line (HaCaT) and a squamous epidermoid carcinoma cell line (A431). Cells were treated with increasing concentrations of extracts (from 10 μg/mL to 250 μg/mL) for 24 or 72 hours and cell viability assessed using the MTT colorimetric assay. The methanolic extract of cyanobacteria in the butanolic phase showed a significant cytotoxic activity on keratinocytes following 72 hours of treatment. The cytotoxic potential of this extract was also tested on A431 cells, showing partial selectivity for transformed cells compared to keratinocytes (percentage of viable cells at 100 μg/mL 14.7% and 57.5%, respectively). To determine whether the cytotoxicity depends on DNA damage, we performed the histone H2AX phosphorylation assay, which measures DNA double strand breaks. The results showed no genotoxic effect for the extract. In conclusion, the extract was cytotoxic, but not genotoxic. Further studies will be performe to investigate the mechanisms underlying its biological activity.

P74. **Anthocyanins as a possible strategy to protect against mycotoxin toxicity**.

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Mycotoxins are toxic secondary metabolites produced by certain fungi (molds), contaminant of agricultural crops. Although exposure to mycotoxins can occur through different routes as respiratory (inhalation of aerosolized particles), mucous, and cutaneous, the main one is gastrointestinal, caused by ingestion of contaminated food, beverages, and water. Following ingestion of mycotoxin-contaminated food or feed, intestinal epithelial cells could be exposed to a high concentration of toxins and an alteration of the intestinal barrier function can be produced. Epithelial integrity is critical in maintaining a physical, but selective barrier between external and internal environments.

Among the various fungal pathogens, the most well-known in temperate zones, such as northern Italy, are *Fusarium verticilloides* and *F. proliferatum* causing contamination of corn seeds with fumonisin B1 (FB1). In these areas the climatic conditions are also particularly favourable for the development of other *Fusarium* mycotoxins, such as deoxynivalenol (Don) and zearalenone (Zea). Increasing evidence suggests that ongoing climate changes will significantly affect the occurrence and the diversity of fungal diseases with an increase on the amount and variety of mycotoxins contamination on the environment. Furthermore, due to structural stability and insensitivity to high temperatures, mycotoxins are able to remain active for long periods while withstanding most agricultural decontamination processes. Consequently, they are able to enter into the food chain and constitute a serious health risk to both animals and humans, as well as significant economic losses. For this reason, several official agencies such as the FAO/WHO Expert Committee on Food Additives, U.S. Food and Drug Administration and the European Union, have established for human consumption the threshold mycotoxin content in maize flours and foods.

Despite the distinct mechanistic pathways of mycotoxins, the potential danger to human and animal health comes from chronic exposure to low doses of multiple mycotoxins in the diet. In fact, once combined these may lead to additive, synergistic or antagonist inter-actions (multi-mycotoxic effect).

Previous studies in our laboratory have demonstrated that pigmented maize, rich in anthocyanins (ACNs) and phlobaphenes, have a lower level of FB1. This is probably due to the fact that flavonoids reduce the attack by the pyralid, an insect that acts as the vector of fungal infection, and consequently mycotoxin accumulation. ACNs are also widely known for their antioxidant and anti-inflammatory activity, which can prevent several chronic diseases, including cancer.

Aim of this study was to examine whether ACNs are able to mitigate the cytotoxicity of mycotoxins.

Undifferentiated Caco-2 human intestinal cells were used to test the protective effect of ACNs against the intestinal toxicity induced by FB1 as well as other mycotoxins, such as Don and Zea. The ability of ACNs to reduce cytotoxicity, increase the expression antioxidative genes thus counteracting inflammation and apoptosis due to food contaminants, has been investigated.

These preliminary results seem to indicate that ACNs represent a promising protective approach for reducing the toxicity of mycotoxins and a possible strategy to dampen adverse effects of food-borne mycotoxins on human and animal health.

P75. ***Trifolium pratense* extract mitigates skeletal muscle atrophy: a nutraceutical approach for the prevention of sarcopenia.**

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Sarcopenia, the age-related loss of muscle mass and function, increases the risk of frailty, fractures, and loss of independence, ultimately reducing quality of life and contributing to higher morbidity and mortality. With life expectancy rising in Western countries, sarcopenia represents a growing yet unresolved public health challenge, as no pharmacological treatments are currently available. Although physical exercise and proper nutrition can help mitigate muscle loss, their effectiveness is often limited in elderly individuals due to declining mobility.

Recent interest in medicinal plants as potential therapeutic agents has highlighted their ability to counteract various pathological conditions, including muscle atrophy. This study explores the protective effects of native Mediterranean species to be used in nutraceutical formulations on skeletal muscle cells exposed to atrophic stimuli that mimic sarcopenia and disuse (e.g., dexamethasone, starvation). Additionally, we assess whether controlled cultivation protocols can influence the bioactive metabolite content of these plants, potentially enhancing their anti-sarcopenic properties.

Among the tested species, hydroalcoholic extract of *Trifolium pratense* demonstrates promising anti-atrophic effects, effectively preserving C2C12 myotube diameter against dexamethasone-induced muscle wasting. However, its protective efficacy varies depending on cultivation conditions, with some enhancing its bioactivity while others showing no significant effect. Furthermore, *Trifolium pratense* completely abolishes dexamethasone-induced reactive oxygen species production, underscoring its antioxidant properties.

These findings suggest that *Trifolium pratense* could serve as a promising nutraceutical intervention to counteract muscle atrophy associated with aging and disuse. Moreover, optimizing its cultivation conditions may enhance its therapeutic potential, paving the way for targeted agronomic strategies to maximize its bioactivity. Future research should focus on identifying the specific bioactive compounds responsible for its effects and evaluating their efficacy in preclinical and clinical settings. By integrating plant-based interventions into sarcopenia management, this study contributes to the development of accessible, non-pharmacological strategies aimed at improving muscle health and quality of life in aging populations.

P76. **A novel aromatherapy formulation utilizing *Pinus cembra* essential oil to enhance relaxation and sleep quality.**

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The pursuit of natural interventions to improve sleep quality and promote relaxation has garnered significant attention in recent years. Aromatherapy, the therapeutic use of essential oils, has been identified as a promising approach in this domain. Pinus cembra, also known as Swiss pine, Stone pine or Arolla pine, is indigenous to the Alpine regions of Europe and has been traditionally utilized for its aromatic properties. Historically, Pinus cembra wood has been used in the construction of beds and interior paneling in Alpine regions, with anecdotal reports of its calming effects and with bags containing wood chips being used to aid in a deeper, more relaxing night's sleep.

We have developed a formulation based on Pinus cembra essential oil intended for environmental diffusion to promote relaxation and enhance sleep quality. Optimal diffusion concentrations and aromatherapy-embedded packaging could ensure easy and efficient aromatherapy

The essential oil of Pinus cembra contains a complex profile of bioactive compounds. Gas chromatography and NMR spectroscopy analyses identified over 130 volatile constituents (Lis et al, 2017), with composition varying by plant part. Twigs with needles: α-pinene (36.3%), limonene (22.7%), β-phellandrene (12.0%); Needles: α-pinene (48.4%); Bark & twigs without needles: Limonene (36.2%, 33.6%), β-phellandrene (18.8%, 17.1%); Wood & cones: α-pinene (35.2%, 39.0%), β-pinene (10.4%, 18.9%). Wood and cone oils also contain high amounts of oxygenated diterpenes, which may contribute to their physiological effects. The dominant presence of α-pinene, limonene, and bornyl acetate is of particular interest due to their reported calming and autonomic-modulating properties.

Sensory and molecular characterization of Pinus cembra aroma has been performed (Ghadiriasli et al, 2020). The study identified 103 odorants, including monoterpenes, sesquiterpenes, organic acids, and unique aromatic compounds such as germacrene D, thymol, carvacrol, and cinnamaldehyde . Principal component analysis showed that terpenes and sesquiterpenes correlated with higher hedonic ratings, reinforcing the link between Pinus cembra's scent profile and its relaxing properties.

A randomized, blinded, cross-over clinical study involving 15 subjects over 253 nights investigated the effects of sleeping in beds made from solid Pinus cembra wood compared to melamine-faced chipboard beds. The study found a significant increase in vagal activity, a decrease in heart rate, and improved cardiorespiratory interactions during sleep in the Pinus cembra beds. Participants also reported enhanced well-being and intrapsychic stability upon waking. These observations align with previous findings that exposure to volatile phytochemicals of Pinus cembra (α-pinene, limonene, bornyl acetate) improves vagal activity. (Grote et al, 2021)

The essential oil of Pinus cembra offers a promising natural intervention for relaxation and sleep enhancement through environmental diffusion. Its complex aromatic profile, rich in bioactive terpenes, contributes to autonomic regulation and subjective well-being. Traditional use, sensory evaluations, and physiological studies all support its calming effects, highlighting its potential application in aromatherapy.

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Poster - Sessione 13

P77. **Safety and efficacy of Red Yeast Rice supplements: new evidences of the literature and regulatory implications**.

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Red yeast rice (RYR) is a product of rice fermentation by the yeast *Monascus purpureus*, originally used in China as a food coloring and flavor enhancer for centuries. Today, it is used as a dietary supplement to lower LDL cholesterol levels, reduce the risk of cardiovascular events, and decrease mortality related to coronary heart disease. The cholesterol-lowering effects of RYR are related to a mechanism similar to statins, namely the inhibition of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase by monacolins. Furthermore, the monacolins contained in red yeast rice extract are characterized by a significantly higher bioavailability than that of statins due to greater absorption. However, the use of this supplement has been the subject of debate due to potential side effects similar to those of statins, with adverse events mainly affecting the musculoskeletal and connective tissue with an intake of 3 mg/day.

This analysis aims to evaluate the evidence regarding the efficacy and safety of RYR and the analysis of the regulatory implications that have seen its use modified with significant changes over the years.

The analysis of European regulations and scientific literature relating to red yeast rice (RYR) reveals, in fact, a significant regulatory evolution. Starting in 2011, when EFSA recognized a causal link between the intake of 10 mg/day of monacolin K (active component of RYR) and the maintenance of physiological levels of LDL cholesterol. However, subsequent scientific evidence has correlated monacolin K to an adverse effect profile superimposable to that of statins, even at lower dosages. This issue led, in 2022, to a restriction of use to <3 mg/day. But the persistent concerns related to the heterogeneity of products and safety have finally led the European Commission (Regulation (EU) 2024-2041 of 29/07/2024) to revoke the health claim relating to monacolin K.

P78. **Safety profile of *Hypericum perforatum* medicinal products released in European countries.**

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European Medicines Agency (EMA) approved *Hypericum perforatum* extracts as medicinal products "for the treatment of mild to moderate depressive episodes" and "for the short-term treatment of symptoms in mild depressive disorders". Aim of this study is to contribute to defining an update safety profile of *Hypericum perforatum* by the analysis of suspected adverse reactions (SARs) traceable in the European datasystem EudraVigilance controlled by EMA. A descriptive analysis of SARs to *Hypericum perforatum* collected in the years 2006-2024 has been performed. In EudraVigilance 2001 cases were found, of which 72.3 % were about women. Aggregation of suspected adverse reactions (SARs), performed according to the System Organ Classification (SOC), shows as the more signaled group of adverse events potentially caused by *Hypericum perforatum* are the group of "General disorders and administration site conditions" followed by "Nervous system disorders", "Gastrointestinal disorders", "Skin and subcutaneous tissue disorders" and "Psychiatric disorders". Since *Hypericum perforatum* herbal preparations have been shown to interact with a number of drugs, in our analysis pharmacological interactions were also investigated. SARs regarding drug interactions related to *Hypericum perforatum* have been signaled starting from 2006 and the total number of them is 101. According to sex distribution, 65.3% of them are related to women, while in agreement with age distribution, adult age (18-64 years) is the more affected. Drugs more frequently involved in SARs caused by pharmacological interactions with *Hypericum perforatum* are estrogenic and progestinic drugs and synthetic antidepressants. In conclusion, analysis of SARs signaled in EudraVigilance indicates that more frequent SARs related to Hypericum use are "General disorders and administration site conditions", a class of disorders that encompasses conditions of a general kind that result from a disease, the treatment of disease. Moreover, results show that adverse reactions are more signaled for adult women. Data on pharmacological interactions suggest to avoid the concomitant use of *Hypericum perforatum* herbal preparations with estrogenic and progestinic drugs and synthetic antidepressants.

P79. **Analysis of suspected adverse reactions to *Whitania somnifera* containing products reported to the Italian Phytovigilance System.**

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Withania somnifera (L.) Dunal, commonly known as ashwagandha, has been used in the traditional Ayurvedic medicine systems to treat several disorders. Recently, interest has grown in its potential health benefits, particularly for stress management, cognitive function, and physical performance [1]. While several studies have explored its biological effects, few have focused on its safety. In addition to mild side effects (e.g., nausea, vomiting, and diarrhea), more serious reactions, such as liver toxicity, have been reported [2]. In this context, this study aims to enhance our understanding of the safety profile of *W. somnifera* by analyzing spontaneous reports of adverse reactions (ARs) related to products containing this ingredient, collected by the Italian Phytovigilance System (IPS).

To this end, all reports of ARs involving ashwagandha received between January 1, 2002, and November 1, 2024, were analyzed. The terms "Indian ginseng" and "Winter Indian Cherry" were also included in the data selection. Causality was assessed using the modified WHO–UMC scale [3]. Two reviewers independently reviewed the data, with a third resolving disagreements. Continuous data are presented as median and interquartile range (IQR), while categorical data are shown as counts or percentages.

A total of 15 spontaneous reports of adverse reactions (ARs) related to ashwagandha-containing products were collected, with 67% from health professionals and 60% submitted in the past five years. The median patient age was 59 years (IQR = 41.0–61.0). Men were affected in 53.3% of cases, and women in 46.7%. ARs were mainly related to gastrointestinal (37.9%), nervous system (14.8%), and vascular (10.3%) disorders. Serious reactions occurred in 26.7% of cases. The products involved were mostly food supplements (66.7%), herbal products (26.7%), and food (6.6%). The most common reason for use was asthenia/tonic (33.3%). Many products contained a combination of ingredients, mostly herbal extracts, with a median of 4 (range: 1–30). Concomitant products or diseases were specified in about 50% of cases. The median duration of use was 20 days (IQR = 1–30). The clinical condition was "recovered" in 60%, "in recovery" in 13%, and "not recovered" in 6.6%. Dechallenge was positive in 60% and rechallenge in 27%. Causality was assessed as probable/likely in 26.7%, possible in 40%, and unlikely in 33.3%.

Present findings suggest that ashwagandha-containing products can cause ARs, sometimes leading to more serious side effects, particularly on the liver, especially when used alongside other natural substances or drugs. However, since many products contained multiple ingredients, it is difficult to determine the specific role of ashwagandha in these reactions. Overall, this data highlights the importance of the IPS as an effective tool for monitoring food supplement risk signals, especially since safety studies are not mandatory.

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P80. **Design of a food supplement based on plant derivatives: from raw materials to laboratory prototype**

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The design of a food supplement is a complex process involving several stages, ranging from carefully selecting raw materials to creating a laboratory prototype. The primary objective of food supplement development is to create a high-quality, safe, and effective product.
This study describes the various stages involved in developing a liquid food supplement based on plant extracts and derivatives to improve microcirculation. Specifically, a product based on pomegranate juice and blueberry dry extract has been developed. The production process begins with the selection of raw materials, during which botanical and agronomic data, processing methods, and chemical-physical analytical controls on plant substances are examined. The selection of raw materials, in addition to quality, also focuses on plant safety, ensuring the absence of pathogens and chemical contaminants, such as pesticides and heavy metals. The next step involves product formulation, in which different active ingredients are combined to create a synergistic action. In addition to the active ingredients present in a formula, excipients are included, based on the pharmaceutical form chosen; these ingredients must comply with the limits established by current regulations. Once the formulation was defined, the laboratory prototype was developed to test the interaction between the various ingredients used and the organoleptic characteristics of the final product. The prototype underwent a stability protocol to assess chemical-physical and microbiological parameters over time.
In conclusion, developing a food supplement based on plant derivatives is a complex process requiring a multidisciplinary approach characterized by a close interaction between scientific, technological, and regulatory expertise to produce a high-quality, effective, and safe product.

P81. **Protective effects of *Olea europea*, *Scutellaria baicalensis* and policosanols on mouse cardiac tissue.**

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Cardiovascular diseases, such as atherosclerosis, heart failure and hypertension, are among the most common causes of death in the world. Many mechanisms are involved in their pathogenesis. One of them, which is still being studied, is oxidative stress as well as inflammatory mechanisms. *Olea europea*, a Mediterranean plant, is known for its beneficial effects, such as antibacterial, anticancer, antioxidant and antidiabetic activities. One of its most important compounds is oleuropein. *Scutellaria baicalensis*, from the Lamiaceae family, is a Chinese plant known for several beneficial effects. Through baicalin, a flavonoid compound, it can display therapeutic effects on cardiovascular and neurodegenerative diseases, as well as antimicrobial action. Policosanols are a mixture of octacosanol, hexacosanol, and triacontanol, three long-chain alcohols purified from sugar cane. Policosanols can also be found in beeswax. Policosanols showed an interesting activity in lowering cholesterol and reducing glycation and oxidation processes. The aim of our study is to evaluate the potential protective effects of the vegetal blend composed of *Olea europea*, *Scutellaria baicalensis* and policosanols at different concentrations. Firstly, we evaluated the viability of human fibroblast cells (HFF-1) exposed to our vegetal blend at different concentrations (1, 5, 50, 500 and 1000 µg/ml) for 48 hours, by MTT analysis. Then, we evaluated gene expression of proinflammatory and prooxidant markers, as well as troponin I and brain natriuretic peptide (BNP) in mouse cardiac tissue specimens, exposed to *E. coli* lipopolysaccharide (LPS). We tested three different mixtures: mixture 1 (*Olea Europaea* 5 µg/ml + *Scutellaria baicalensis* 10 µg/ml + Policosanols 1,5 µg/ml), mixture 2 (*Olea Europaea* 50 µg/ml + *Scutellaria baicalensis* 100 µg/ml + Policosanols 15 µg/ml), mixture 3 (*Olea Europaea* 500 µg/ml + *Scutellaria baicalensis* 1000 µg/ml + Policosanols 150 µg/ml). *In vitro* studies showed that our vegetal blend was well tolerated by cells without any significant toxicity. *Ex vivo* studies showed that mixture 3 significantly reduced gene expression of cyclooxygenase-2 (COX-2), nuclear factor-kB (NF-kB), tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS). Moreover, mixtures 1, 2 and 3 decreased troponin I and BNP and increased catalase (CAT) gene expression. No mixture affected glutathione peroxidase (GPX) gene expression. In conclusion, our evidences suggest that the combination of *Olea europea*, *Scutellaria baicalensis* and policosanols can exert protective effects on mouse heart specimens. Future studies are needed to confirm our results and to explore the potential use of this vegetal blend in cardiovascular diseases.

P82. **The role of perivascular adipose tissue in the vascular activity of the natural coumarin osthole.**

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Cnidium monnieri (L.) Cusson is a plant widely used in traditional Chinese and Vietnamese medicine as an antihypertensive remedy. One of the most representative compounds identified in *Cnidium monnieri* is the coumarin osthole, which possesses, among others, an interesting vasorelaxant activity. Vessel tone is finely regulated by the mechanical activity of smooth muscle and by factors released by both the endothelium and perivascular adipose tissue (PVAT), a specialized type of adipose tissue surrounding several vessels. As PVAT, nowadays considered an active endocrine and paracrine organ, plays a key role in health and disease, this study aimed to evaluate the functional interaction between PVAT and osthole in the regulation of vascular tone.

In vitro rat aorta rings were used as the experimental model: the mechanical activity was recorded under isometric conditions.

In a first series of experiments, the effect of PVAT on the vasorelaxant activity of osthole was evaluated in endothelium-deprived thoracic and abdominal aorta rings stimulated by KCl. In thoracic preparations, the presence of PVAT did not alter the potency of osthole, but significantly reduced its efficacy. In abdominal rings, however, PVAT markedly decreased both the potency and efficacy of the coumarin.

In endothelium-deprived abdominal rings stimulated by the α_1-adrenergic agonist phenylephrine, the presence of PVAT significantly reduced both potency and efficacy of osthole. No effect, however, was detected in thoracic rings.

When a functional endothelium was present, PVAT antagonised the vasorelaxant efficacy of osthole but only in thoracic preparations.

To elucidate the mechanisms underpinning the functional interaction among osthole, PVAT, and endothelium, aorta rings were pre-treated with specific pharmacological tools before the addition of the coumarin. In endothelium-deprived abdominal rings pre-contracted by KCl or phenylephrine, the unselective ET_A and ET_B receptor antagonist tezosentan and the mitochondria-targeted antioxidant mitotempol, respectively, caused a leftward shift of the concentration-response curve to osthole.

In endothelium-intact thoracic rings pre-contracted by phenylephrine, however, neither tezosentan nor mitotempol modified the antirelaxant effect of PVAT.

In conclusion, the results of this work highlight the existence of a functional interaction among PVAT, endothelium, and osthole in rat aorta rings, which differs between the thoracic and abdominal portions of the vessel. These preliminary experiments suggest that endothelin and radical species might be involved in this phenomenon.

P83. **Protective effect of extracts from *Origanum majorana* L. (Lamiaceae) against hemin-induced oxidative stress in human cardiomyocytes.**

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Origanum majorana, known as sweet marjoram, is a hemicryptophyte, perennial subshrub belonging to the Lamiaceae family, generally 20-60 cm high, widely distributed in the Mediterranean area. The stems are reddish, square, erect and pubescent. The leaves are grey-green, small, simple, ovate, opposite, petiolate and pubescent. The flowers are small, pentamer, zygomorphic, hermaphroditic, with a white or pale pink corolla and a grey-green calix. Thanks to the content of bioactive compounds, *O. majorana* has been used in traditional medicine as an antipyretic, analgesic, and antimicrobial.

The beneficial properties of *O. majorana* are ascribed to the elevated content of bioactive molecules, which include essential oils, responsible of its characteristic scent, and a variety of phenolic compounds. Among them, rosmarinic acid, typically one of the most abundant, is a phytochemical particularly interesting for its established biological activities useful for human health.

Based on these considerations, we decided to study the effects of two phytochemically characterised extracts obtained from different part of *O. majorana* (twigs and leaves&flowers) in an *in vitro* model constituted by human cardiomyocytes (AC16 cells) stressed with hemin, a well-known oxidative stress inducer in cells. With this model, we aimed to mimic the detrimental effects of free heme, which are linked with hearth failure.

The plant object of this study was collected in Siracuse (Sicily, Italy), during its flowering time, and the matrixes were dried in ventilated oven at 35±2°C before use. The extracts, called MF (leaves and flowers) and MT (twigs), were prepared by digestion under continuous stirring, with an hydroalcoholic solution (50% v/v), temperature of 40°C, time 4h and ratio 1:30. The extraction procedure was repeated 3 times. The obtained extracts were phytochemically characterised, at first by quantitative spectrophotometric assays for the determination of total phenolic, total flavonoid, total tannin, and non-tannin phenolic compounds. Subsequently, the complete characterisation of the extracts phenolic profiles was carried out by HPLC-MS/MS analysis, which revealed that the two extracts were identical from a qualitative point of view.

The antioxidant activity of the two extracts was then evaluated in *in vitro* cell-free systems, on DPPH, H_2O_2 and $O_2^{\bullet -}$, finding strong and similar antioxidant properties for MF and MT.

Once we have established the strong antioxidant activity of the extracts and therefore the rationale to apply the in the above discussed model, we moved to the *in vitro* cellular model. Our findings revealed that the pretreatment with the extracts was able to totally recover the hemin-induced ROS production in AC16 cells. Finally, in order to assess if other the already establish direct antioxidant activity of MF and MT there was also an indirect component in their mechanism of action, we screened by qPCR analysis their possible influence on the gene expression of four endogenous antioxidants (heme oxigenase 1, Catalase, superoxide dismutase, and ferritin), involved in the detoxification of heme and in counteracting the related oxidative stress. We didn't find any significant changes in gene expression following the treatment, concluding that the observed effect was mainly owed to the intrinsic antioxidant properties of the extracts.

Poster - Sessione 14

P84. **Hyaluronic acid-based nanoparticles loaded with rutin as vasculo-protective tools against anthracycline-induced cardiotoxicity.**

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Anthracycline-based therapies exert endothelial damages through peroxidation and the production of proinflammatory cytokines, resulting in a high risk of cardiovascular complications in cancer patients. Hyaluronic acid-based hybrid nanoparticles (LicpHA) are effective pharmacological tools that can target endothelial cells and deliver drugs or nutraceuticals.

This study aimed to prepared and characterized a novel LicpHA loaded with Rutin (LicpHA Rutin), a flavonoid with high antioxidant and anti-inflammatory properties, to protect endothelial cells against epirubicin-mediated endothelial damages. LicpHA Rutin was prepared using phosphatidylcholine, cholesterol, poloxamers, and hyaluronic acid by a modified nanoprecipitation technique. The chemical-physical characterization of the nanoparticles was carried out (size, zeta potential, morphology, stability, thermal analysis, and encapsulation efficiency). Cytotoxicity studies were performed in human endothelial cells exposed to epirubicin alone or in combination with Free-Rutin or LicpHA Rutin. Anti-inflammatory studies were performed through the intracellular quantification of NLRP-3, MyD-88, IL-1β, IL-6, IL17-α, TNF-α, IL-10, and IL-4 using selective ELISA methods. Morphological studies via TEM and image analysis highlighted a heterogeneous population of LicpHA particles with non-spherical shapes (circularity equal to 0.78 ± 0.14), and the particle size was slightly affected by Rutin entrapment (the mean diameter varied from 179 ± 4 nm to 209 ± 4 nm). Thermal analysis and zeta potential analyses confirmed the influence of Rutin on the chemical-physical properties of LicpHA Rutin, mainly indicated by the decrease in the surface negative charge (from -35 ± 1 mV to -30 ± 0.5 mV). Cellular studies demonstrated that LicpHA Rutin significantly reduced cell death and inflammation when compared to epirubicin alone. The levels of intracellular NLRP3, Myd-88, and proinflammatory cytokines were significantly lower in epirubicin + LicpHA Rutin-exposed cells when compared to epirubicin groups ($p < 0.001$).

Hyaluronic acid-based nanoparticles loaded with Rutin exerts significant vasculo-protective properties during exposure to anthracyclines. The overall picture of this study pushes towards preclinical and clinical studies in models of anthracycline-induced vascular damages.

P85. **Ellagic acid improves the cardiac contractility trough the intracellular calcium handling.**

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Ellagic acid (EA) is a hydrolysable tannin present in free form or glycosylated in pomegranate (Punica granatum) fruit [1]. EA exhibits a series of cardiovascular effects, mainly through an ACE enzyme inhibition, a vasorelaxation, mediated by NO and a blockage of L-type calcium channels, and a protection of the myocardial infarction area, due to the control of apoptosis and activation of the mitochondrial respiratory enzymes [2-4]. At the present, there are very few studies that highlight the effects and the underlying mechanisms of this tannin on the cardiac contractility [5]; therefore, the aim of this study is to deeper investigate them.
Following Langendorff procedure, an ex vivo model of isolated and perfused rat heart, heart rate (HR), left ventricular developed pressure (LVDP), dP/dt and the time to recovery of diastole, a useful index for evaluating and comparing contractility between multiple subjects, were continuously monitored.
Perfusion with EA didn't influence the coronary flow (CF) in the range tested (0.1-10 µM), while EA, at 1 and 10 µM, showed a significant increase in LVDP and dP/dt values. Interestingly, a more marked effect of EA was observed when it was perfused in the presence of a coronarospasm induced by AngII (0.1 µM), in which the tannin concentration-dependently increased - more than basal - both LVDP and dP/dt, reaching the maximum increase of LVDP and dP/dt at 10 µM, without observed effects on CF. These results suggest that EA plays a positive inotropic effect. Moreover, the effect of EA on speed of contraction (+dP/dt) was similar than the impact on speed of relaxation (-dP/dt), suggesting that this tannin might act on both processes. Finally, EA minimally influenced the time of recovery of diastole. To explore the contribution of EA in the intracellular calcium handling, we used specific antagonists of SERCA and RyRs, cyclopyazonic acid (CA) and red ruthenium (RR), respectively. Hearts perfused with CA, 150 nM, showed a significant reduction of functional parameters as well as CF. The subsequent perfusion with AngII caused additive reduction of functional parameters. Then, EA showed a very modest positive inotropism and a concentration-dependent increase in the time of recovery of diastole. The perfusion with RR significantly compromised the LVDP and in similar manner CF and dP/dt. At increasing concentrations, EA, perfused after the pre-treatment with AngII, did not improve CF, rather it was further concentration-dependently reduced, and the time of recovery of diastole saw an increasing concentration-independent trend.
In conclusion the results obtained in this study first demonstrate the positive inotropism of EA on isolated and perfused hearts. Future experiments will focus on a deeper comprehension of the intracellular pathways engaged, exploring the contribution of other putative intracellular actors.

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P86. **Erucin, a natural (H_2S) donor, improves multiorgan-damage in Heart Failure with preserved Ejection Fraction.**

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Heart Failure with preserved Ejection Fraction (HFpEF) is an increasingly prevalent form of heart failure, accounting for over half of all heart failure cases worldwide[1]. Among the different HFpEF phenotypes, the cardiometabolic/obese variant is one of the most common and has been linked to several metabolic alterations, including systemic inflammation, renal, skeletal muscle and endothelial dysfunction. While pharmacological treatments such as sodium-glucose cotransporter type 2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1RA) have shown some benefits, lifestyle interventions focusing on diet and physical activity have also emerged as adiuvant approach in HFpEF management. However, the underlying mechanisms driving the beneficial effects of these interventions remain poorly understood. In this scenario, a hydrogen sulfide (H_2S) role is emerging. H_2S is an endogenous gasotransmitter that plays a crucial role in regulating cardiovascular function and metabolic homeostasis [2]. It is produced from L-cysteine by the action of three enzymes: cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and 3-mercapto-pyruvate-sulfurtransferase (3MST). Impairment of H_2S signaling is associated with several inflammatory-based cardiovascular diseases[3,4], making it an attractive target for HFpEF therapeutic intervention. Erucin is an isothiocyanate derived from *Eruca sativa* (rocket plant) that exerts several beneficial effects in modulating inflammation and metabolic pathways, due to its ability to slowly release H_2S[5]. Given the diet-sensitive nature of HFpEF and the prevalence of comorbidities like hypertension, diabetes, and obesity in HFpEF patients, this study aims to explore the potential benefits of Erucin in an animal model of HFpEF.

In vivo experiments were performed on six-week-old male Dahl salt-sensitive rats. The animals were fed a chow containing 8% NaCl (high-salt diet; HS) or 0.3% NaCl (low-salt diet; LS) for five weeks. Then the animals fed the HS diet were randomized into two groups and treated with Erucin (3 mg/kg/day; HS+Eru) or vehicle for the following six weeks by oral gavage. At 17 weeks, the three experimental groups were sacrificed, and the kidney and the aorta were collected for assessing vascular reactivity, the protein expression of H_2S-generating enzymes, H_2S levels, and cyclic nucleotide content.

Aorta harvested from HS rats displayed an impaired L-cysteine-induced vasorelaxation compared to LS rats and H_2S content. These effects were coupled with a reduction of CSE and CBS protein expression. A similar dysregulation in CSE and CBS expression was observed in the kidneys of HS rats alongside H_2S levels. Furthermore, both aorta and kidney displayed a significant decrease in 3'-5'cyclic inosine-monosphate (cIMP) levels, a non-canonic cyclic nucleotide produced by soluble guanylate cyclases (sGC), in HS rats compared to LS. Erucin treatment increased the expression of $sGC_{\beta1}$ and cytochrome b5 reductase (CYB5R3), the sGC reduced form, thereby restoring the cIMP content in the kidneys. Additionally, in the aorta, Erucin-treatment restored cIMP levels and improved the Phenylephrine-induced contraction compared to the vehicle group.

Our results indicate a dysregulation of the CSE-CBS/H_2S pathway in multi-organ failure associated with HFpEF. Erucin, a natural slow H_2S donor, improves the extracardiac dysfunctions of HFpEF by targeting the sGC/cIMP pathway.

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P87. **Hyperxanthone G as a regulator of gut oxidative stress and inflammatory responses**.

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Gut homeostasis is essential for the maintenance of overall health, and its dysfunction is closely associated with the progression of many chronic diseases, including inflammatory bowel disease (IBD). Several factors, including inflammation and oxidative stress, can compromise this barrier, leading to impaired intestinal function and tissue damage [1]. When the intestinal epithelium is damaged, it triggers an inflammatory response that primarily activates macrophages. These immune cells release several pro-inflammatory mediators that contribute to further epithelial cell damage and dysfunction [2]. The resulting inflammatory cascade exacerbates the damage to the intestinal barrier, compromising its integrity and function. The dynamic interaction between intestinal epithelial cells (IECs) and immune cells plays a pivotal role in maintaining and amplifying this inflammatory response, which is a key driver of several intestinal diseases, such as IBD [3]. Recent studies have shown that chronic intestinal inflammation, often associated with oxidative stress and intestinal dysbiosis, can impair the ability of epithelial cells to maintain intestinal barrier integrity [4]. In this context, modulation of the inflammatory response and the restoration of intestinal barrier function are crucial therapeutic goals for developing targeted treatments for diseases like IBD and other gastrointestinal disorders. Research suggests that maintaining the integrity of the gut microbiota, which plays a key role in supporting epithelial cells and modulating immune responses, may represent a promising therapeutic strategy to limit chronic inflammation and improve intestinal function [5]. Recent advances in the search for natural compounds with antioxidant and anti-inflammatory properties have focused attention on plant extracts as promising therapeutic agents for inflammatory conditions. Among these, *Hyperxanthone G*, a compound isolated from the n-hexane/ethyl acetate extract of *Hypericum scabrum* roots, has shown potential in regulating oxidative stress and inflammation. The present study aimed to evaluate the antioxidant and anti-inflammatory effects of *Hyperxanthone G* in both IEC-6 cells and J774A.1 macrophages under inflammatory conditions induced by lipopolysaccharide (LPS) and interferon-gamma (IFN-γ). Our results show that *Hyperxanthone G* significantly reduces reactive oxygen species (ROS) levels in IEC-6 cells ($P < 0.05$ vs LPS+IFN) at concentrations of 10 and 5 µM, indicating its ability to mitigate oxidative stress. Furthermore, *Hyperxanthone G* (10-1 µM) significantly increased the expression of superoxide dismutase 2 (SOD-2), an important antioxidant enzyme, at concentrations of ($P < 0.05$ vs LPS+IFN). In addition, *Hyperxanthone G* was found to significantly promote cell migration in IEC-6 cells, suggesting that it could enhance the intestinal repair process, particularly in models of wound healing, that mimic epithelial barrier restoration. In macrophages J774A.1, *Hyperxanthone G* significantly reduced ROS and nitric oxide (NO) levels at concentrations of 20 and 10 µM ($P<0.05$ vs LPS). These findings highlight the dual effect of the compound on both intestinal epithelial cells and macrophages, with a significant impact on reducing oxidative stress and inflammatory signalling, also in IBD. Taken together, these results suggest that *Hyperxanthone G* has potential antioxidant and anti-inflammatory properties, acting not only on intestinal epithelial cells but also on immune-related cells such as macrophages.

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P.88 **Improving *Rosmarinus officinalis* L. extracts´ health potential with *Bacillus* G36 metabolites formulated in AgNP**

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Rosmarinus officinalis L. is a medicinal plant with a traditional use for healing several diseases ranging from inflammatory to neurological diseases to mention the most relevant. The healing potential relies on its antioxidant potential due to its bioactive components among which are monoterpenes, constituting the essential oil; diterpenes like carnosol and its derivatives; triterpenes like ursolic acid; and fenilpropanoids among which caffeic acid and rosmarinic acid and isoforms are the best known. All these components are secondary metabolites key for plant adaptation to environmental changes. As secondary metabolites, their synthesis is inducible so finding elements that trigger plant secondary metabolism is relevant to enhance healing potential of medicinal plants, as is the case in rosmarinus.

In addition to plant's genetic endowment to enhance adaptation, plants recruit soil bacterial strains to benefit growth. These strains are known as Plant growth promoting rhizobacteria (PGPR) and communicate with plants by specific chemical molecules termed elicitors. Some strains and specific elicitors are able to trigger secondary metabolism, increasing bioactive contents.

Nanotechnology appears as an innovative approach to increase effectiveness of specific molecules when a nanoparticle is synthetized in order to profit from NP's size, shape and characteristics to move through the system. So, we hypothetized that metabolites from *Bacillus* G36 would be able to reduce Ag+, biosynthetizing a functionalized AgNP able to trigger rosmarinus´ secondary metabolism, increasing bioactive contents on rosemary extracts, and therefore, its healing potential.

AgNP biological synthesis. Metabolites from *Bacillus* G36, a consolidated beneficial strain, were incubated with $AgNO_3^-$ solutions for 24h at 37 °C, in different pH conditions, resulting in the biosynthesis of AgNP.

AgNP characterization. Full characterization by UV spectrum, TEM, XRD and FTIR was carried out.

Experimental plant set up. Rosmarinus branches were detached from the plants and immediately sprayed with treatment solution (control, strain suspension, metabolites and AgNP, n=3). Plants were allowed to dry and ethanolic extracts were prepared. Extracts were characterized by total phenolic contents, total flavonol contents, antioxidant potential; secondary metabolites (carnosol and rosmarinic acid) were analyzed by HPLC.

Results. A brownish color after incubation indicative of reduced silver, confirmed AgNP synthesis with UV absorption at 410-420 nm, indicative of reduced silver. AgNP were spherical, with an average diameter of 7.5 nm showing a coating of organic matter from the bacterium. Hence, bacterial metabolites were able to reduce silver, creating a unique coating that confers special activity to the AgNP. NP under 10 nm were most suitable to penetrate to subcellular levels and therefore, cause changes in cell metabolism. When sprayed on rosmarinus, AgNP increased total phenols (40%), total flavonols (40%), antioxidant potential (60%), rosmarinc acid (30%), and carnosic acid (25%); bacterium and metabolites alone did not affect total phenols and total flavonols; interestingly, the bacterial metabolites enhanced antioxidant potential to similar levels than the NP. Finally, the unique characteristics of the AgNP coated with bacterial metabolites is evidenced.

P89. **Evaluation of the chemical profile and anti-viral potential of Jujube (*Ziziphus jujuba Mill.)* Drupes**

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Recently, the interest in bioactive compounds derived from natural sources has significantly increased. Food plant extracts represent a rich source of natural compounds with antimicrobial, antioxidant and anticancer properties that can inhibit the growth of pathogens. In this work, we evaluated the potential of extractive mixtures from fruits (and their waste) grown in the Campania Region (Italy), jujube drupes (*Zizyphus jujuba Mill.*). The drupes were dissected into their peel, pulp, and seed parts, each of which was extracted by ultrasound-assisted maceration and further fractionated, thus obtaining a polyphenolic and lipid fraction in addition to the sugar fraction.

Ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) tools highlighted that the polyphenolic component of the seed was strongly dissimilar from that of the edible parts, being constituted by swertisin and its derivatives. Moreover, the peel mostly accounted for triglycosylated flavonols, whereas the pulp was rich in volatile aromatic glycosides. Among lipids, p-coumaroyl triterpenes mainly characterized the peel.

The cytotoxicity effect of all fraction was evaluated by the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The antiviral activity was assessed against a broad spectrum of viruses. Several time of addition assays were performed to investigate the antiviral effect of compounds against emerging and re-emerging viruses. Non-cytotoxic concentrations of each extract, ranging from 3.1 µg/mL to 200 µg/mL, were used to perform *in vitro* test. Molecular tests and Western blot analyses were also carried out. The jujube mixtures, specifically the peel and pulp polyphenolic fractions and peel lipophilic fraction (the latter enriched mainly in ursane-type triterpenes), showed a remarkable inhibitory activity against a broad spectrum of viruses. In detail, the jujube drupes extracts displayed remarkable antiviral activity against enveloped viruses, acting in early stage of viral life cycle.

The acquired data suggest jujube-active mixtures as promising candidates for the prevention and treatment of clinical manifestations caused by viruses.

P90. **Medium-Term Effects of a Polyphenol-Rich Drink made from Red Grape Pomace Extract on Non-Alcoholic Fatty Liver Disease and Cardiometabolic Risk Profile in Individuals with Type 2 Diabetes Mellitus**

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Background: Red grapes are an important source of polyphenols, including anthocyanins and resveratrol. Acute studies have shown that grape polyphenols can reduce plasma glucose concentrations. In particular, a polyphenol-rich beverage, made from red grape pomace, has been shown to reduce postprandial insulin levels and improve insulin sensitivity in healthy volunteers. Recent evidence suggests that polyphenols could reduce liver fat, an independent factor associated with type 2 diabetes (T2DM) and cardiovascular risk.

Objective: This study aims to evaluate the effects of a polyphenol-rich beverage made from red grape pomace on liver fat, the cardiometabolic risk profile, and the gut microbiota in patients with T2DM, compared to a placebo (polyphenol-free) drink.

Materials and Methods: This randomized, placebo-controlled study is designed as a crossover experiment. Twenty patients with T2DM (ages 35-70, BMI 25-35 kg/m², HbA1c ≤7.5%), undergoing diet therapy or diet plus metformin, were recruited. After a 2-week run-in period, participants consumed 150 mL per day of a polyphenol-rich beverage (BPol: 1.562 g/150 mL total polyphenols, 28 g/150 mL soluble sugars, 105 kcal) or a placebo beverage (BCon: 28 g/150 mL soluble sugars, 105 kcal) for 6 weeks, with a 2-week wash-out period between treatments. At the end of the run-in and after each treatment, participants underwent anthropometric and metabolic assessments in a fasting state, and liver fat was assessed by MRI. After each treatment, a standard meal (960 kcal, 18% protein, 30% fat, 52% carbohydrates) with 150 mL of the polyphenol-rich or placebo beverage was administered for postprandial metabolic assessments.

Results: Thirteen participants completed the study (12 males/1 female, mean age 59±7 years). Before treatment, participants exhibited abdominal obesity (BMI 30±3 kg/m², waist circumference 105±8 cm) and atherogenic dyslipidemia (fasting triglycerides 163±75 mg/dL, HDL cholesterol 38±6 mg/dL). These parameters showed no significant changes after the treatments. Fasting plasma glucose levels were significantly lower with the polyphenol-rich beverage compared to the placebo (140±21 vs 148±27 mg/dL, p=0.030), while fasting insulin levels did not differ between treatments (14.5±6.9 vs 15±6.9 µU/mL, p=0.71). The postprandial glycemic response (iAUC) showed a trend toward improvement with the polyphenol-rich beverage compared to the placebo (5906±2611 vs 7525±2756 mg/dL·min, p=0.063), although the difference was not statistically significant. In contrast, the postprandial insulin response (iAUC) was significantly reduced (-37%) with the polyphenol-rich beverage compared to the placebo (12211±4126 vs 17691±2923 µU/mL, p=0.026).

Conclusions: Preliminary results from this study suggest that a polyphenol-rich beverage may improve fasting blood glucose levels and reduce postprandial insulin levels in patients with T2DM and high cardiometabolic risk.

P91. **Phytosterols and policosanols from sorghum extracts modulate NPC1L1 expression: a new frontier against hypercolestererolemia**

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Hypercholesterolemia represents the main risk factor in the pathogenesis of cardiovascular diseases, the currently leading cause of death worldwide. Along to pharmacological therapies the employment of nutraceuticals may represent an efficient strategy to reduce plasma cholesterol levels, and there is emerging particular interest in sorghum phytocompounds. The aim of this study is to evaluate the effects of phytosterols and polycosanols, extracted from sorghum, in modulating the expression of NPC1L1, a transporter responsible for the uptake of 70% of exogenous cholesterol in enterocytes. Here, an in vitro intestinal barrier model was developed consisting of Caco-2 and HT-29 cells, which in 14 days of co-culture differentiate into enterocytes and mucin-producing cells, respectively. Monolayer integrity was assessed by measuring trans-epithelial electrical resistance (TEER) before treating the cells for 24 hours with increasing doses of different fractions obtained from sorghum extract: that enriched in phytosterols (FAS), that enriched in polycosanols (FAP), and that without either (FNA). None of treatments influences the cell viability and the barrier integrity with respect to control. Protein levels of NPC1L1 and the phosphorylated form of its transcription factor STAT-3 were then assessed. Results showed that FAS fraction significantly reduced NPC1L1 protein levels by 40% similarly to the treatment with Ezetimibe, the drug currently used for NPC1L1 inhibition. Furthermore, unlike Ezetimibe, FAS results in a reduction in phosphorylation levels of STAT-3, suggesting a different mode of action between FAS and the drug that likely goes through regulation of NPC1L1 transcription. Finally, preliminary experiment on cholesterol transport in the different conditions are underway. The final goal should be the intake of supplements and/or functional foods enriched in sorghum extracts to support the ongoing therapies in the management of hypercholesterolemia.

Con il contributo non condizionante di:

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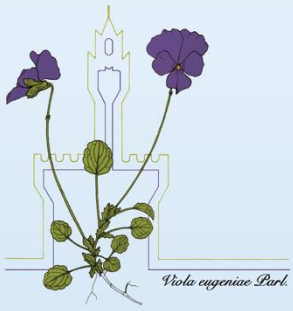

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