1° Congresso Intersocietà

sui prodotti vegetali per la salute:

il ruolo delle piante medicinali nella medicina moderna

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

SIF
SOCIETÀ ITALIANA DI FARMACOLOGIA

Viola eugenia Parl.
Società Botanica Italiana

PROGRAMMA

dsf
Dipartimento di Scienze del Farmaco

via Marzolo, 5 Padova 15-17 giugno 2023

1° Congresso intersocietà sui prodotti vegetali per la salute: il ruolo delle piante medicinali nella medicina moderna

Con il patrocinio di:

Università degli Studi di Padova

Università degli Studi di Napoli Federico II

Regione del Veneto

Provincia di Padova

Comune di Padova

Ordine dei Farmacisti della Provincia di Padova

Ordine dei Farmacisti della Provincia di Napoli

Federazione Erboristi Italiani

Confcommercio Imprese per l'Italia Ascom Padova

Federsalute

Confcommercio Imprese per l'Italia

Comitato organizzatore
Marco Biagi
Raffaele Capasso
Nunziatina De Tommasi
Monica Montopoli
Luisa Pistelli
Giancarlo Statti
Gianni Sacchetti
Annabella Vitalone

Comitato scientifico
Francesca Borrelli
Gioacchino Calapai
Vincenzo Calderone
Filomena Conforti
Carmela Fimognari
Luigi Milella
Giorgio A.M. Pintore
Federica Pollastro

Comitato organizzatore locale e segreteria scientifica
Donatella Cicia
Veronica Cocetta
Paola De Cicco
Isabella Giacomini
Paolo Governa
Mattia Tinazzi

E-mail: congressointersoc.segreteria@gmail.com

Comunicazioni orali

Giovedì 15 giugno

12.30-14.00
Registrazione dei Partecipanti

Aula 1

13.30-14.00
Saluti di Benvenuto (Prorettrice dell'Università degli Studi di Padova, Direttore del Dipartimento di Scienze del Farmaco, Univeristà degli Studi di Padova, Presidente Ordine dei Farmacisti della Provincia di Padova, Presidente ASCOM Padova)

Moderatore: Russo A (Catania)

14.00-14.30
Lettura magistrale **Botanicals: focus on safety issues**
Fimognari C (Bologna)

14.30- 14.45
Herbal medicines in Italy: regulatory aspects, critical issues and innovation
Assisi A. (AIFA, Roma)

14.45-16.15 Sessione 1 Aula 1

Moderatori: Fimognari C (Bologna) - Russo A (Catania)

14.45-15.00
Antimutagenic and chemopreventive properties of 6-(methylsulfinyl) hexyl isothiocyanate on TK6 human cells by flow cytometry
Lenzi M (Bologna)

15.00-15.15
Phenolic composition of a pomace water extract of *Vitis Vinifera* L. and its protective effect in a colorectal cancer *in vitro* and *ex vivo* experimental model
Di Simone SC, Acquaviva A, Libero ML, Chiavaroli A, Recinella L, Leone S, Brunetti L, Orlando G, Menghini L, Ferrante C (Chieti)

15.15-15.30
Essential oil of *Lippia alba* (Mill.) and breast cancer
Borromeo I, De Luca A, Domenici F, Giordani C, Rossi L, Forni C (Roma, Medellin)

15.30-15.45

Erucin isolated from *Eruca sativa* Mill. seeds, induces apoptosis of human melanoma cells and modulates their aggressiveness *in vitro*
Maresca DC, Conte L, Romano B, Ianaro A, Ercolano G (Napoli)

15.45-16.00
Synergistic effect of plant extracts and anticancer drugs to improve therapeutic efficacy and overcome drug-induced resistance in cancer models
Rigogliuso S, Poma P, Adamo G, Labbozzetta M, Notarbartolo M (Palermo)

16.00-16.15
The protective role of troxerutin (TX) to counteract anaplastic thyroid carcinoma (ATC) progression
Paterniti I, Bova V, Casili G, Filippone A, Repici A, Cuzzocrea S, Esposito E (Messina)

14.45-16.15 Sessione 2 Aula 2

Moderatori: Roviezzo F (Napoli) – Orso G (Padova)

14.45-15.00
The beneficial role of resveratrol in a murine model of lung allergic inflammation
Cerqua I, Granato E, Rossi A, D'Avino D, Capasso R, Cirino G, Roviezzo F (Napoli)

15.00-15.15
Anti-inflammatory and healing activity of *Origanum vulgar*e essential oil on human keratinocytes cultures
Avola R, Granata G, C. Geraci C, Napoli E, Graziano ACE, Russo A, Cardile V (Catania)

15.15-15.30
Protective effects of a commercial extract of *Astragalus membranaceus in vitro* and *in vivo* models of inflammation
D'Avino D, Cerqua I, Granato E, Di Matteo R, Ullah H, Ialenti A, Daglia M, Roviezzo F, Rossi A (Napoli)

15.30-15.45
Beneficial effects of a topical gel containing xyloglucan and pea protein to treat phenol-induced oral ulcers *in vivo*
Casili G, Cucinotta L, Campolo M, Paterniti I, Lanza M, Filippone A, Basilotta R, Cuzzocrea S, Esposito E. (Messina)

15.45-16.00
Immunomodulatory properties of *Ulva pertusa* extract in a mouse model of DNBS-induced colitis
Filippone A, Mannino D, Ardizzone A, Casili G, Lanza M, Cucinotta L, Campolo M, Esposito E. (Messina)
16.00-16.15

A geranium for the health of lungs: a multi-target herbal product against viral infections
Carloni D. (Siena)

16.15 16.45 *Coffee break*

16.45 -18.30 Sessione 3 Aula 1

Moderatori: Calderone V (Pisa) - Dall' Acqua S (Padova)

16.45-17.00
***Valeriana officinalis*, *Ziziphus juhuba* and *Humuls lupulus* with melatonin: studies about neuromodulatory and protective effects for counteracting sleep disorders**
Libero ML, Chiavaroli A, Di Simone SC, Acquaviva A, Nilofar, Brunetti L, Recinella L, Leone S, Orlando G, Menghini L, Ferrante C (Chieti)

17.00-17.15
Evaluation of the efficacy of honokiol, neolignan from *Magnolia Officinalis* Rehder & E.H Wilson, in an *in vitro* model of microglial cellular senescence
Sasia C, Borgonetti V, Galeotti N. (Firenze)

17.15-17.30
Gamma-oryzanol: a new drug repositioning as a neuroprotective compound
Abate G, Pucci M, Tirelli E, Bonini SA, Mastinu A, Maccarinelli G, Zizioli D, Pezzotta A, Grilli M, Memo M, Uberti D (Brescia, Milano, Novara)

17.30-17.45
Anti-hyperalgesic properties of *Echinacea purpurea* in a mouse model of chemotherapy-induced neuropathy: alkamides versus polyphenols efficacy
Lucarini E, Micheli L, Maggini V, Ciampi C, Gallo E, Bogani P, Fani R, Pistelli L, Di Cesare Mannelli L, De Leo M, Firenzuoli F, Ghelardini C (Firenze, Sesto Fiorentino, Pisa)

17.45-18.00
The transcription factor Nrf2 mediates the effects of *Antrodia camphorata* extract on neuropathological changes in a mouse model of Parkinson's disease
Capra AP, Lanza M, Cucinotta L, Casili G, Filippone A, Basilotta R, Campolo M, Paterniti I, Cuzzocrea S, Esposito E. (Messina)

18.00-18.15

***Cannabis sativa* L. essential oils: anti-inflammatory and neuroprotective potential in a microglia cell model**
Pieracci Y, Ascrizzi R, Russo L, Giacomelli C, Martini C, Trincavelli ML, Montanari M, Fulvio F, Bassolino L, Paris R, Pistelli L, Flamini G (Pisa, Bologna)

18.15-18.30
Effects of a chronic treatment with a microdose of the Psilocybe mushroom-derived alkaloid psilocybin on the behavior of C57BL/6J mice
Nasini S, Colognesi M, Tidei S, De Martin S, Banzato M, Mattarei A, Folli F, Pappagallo M, Manfredi PL, Comai S (Padova, Milano, Coral Gables, Montreal)

16.15 -18.30 Sessione 4 Aula 2

Moderatori: Braca A (Pisa) – Statti G (Rende)

16.45-17.00
Pharmacognostic study of *Artemisia arborescens* (Vaill.) L., essential oil composition and biological activity
Polito F, Papaianni M, Woo S, Malaspina P, Cornara L, De Feo V (Salerno, Napoli, Genova)

17.00-17.15
Anti-virulence activity of abietane diterpenoid from *S. rosmarinus* Spenn. against methicillin-resistant *Staphylococcus aureus*
Iobbi V, Parisi V, Bernabè G, De Tommasi N, Bisio A, Brun P (Genova, Salerno, Padova)

17.15-17.30
Isolation of diterpenes from *Salvia officinalis* L. and their antimicrobial activity and mechanism
Donadio G, Nocera R, Dal Piaz F, De Tommasi N (Salerno)

17.30-17.45
The phytobiome of the medicinal plant *Origanum vulgare*: linking the bacterial endophytic communities to the essential oil
Semenzato G, Del Duca S, Vassallo A, Ascrizzi R, De Leo M, Pistelli L, Mucci N, Greco C, Padula A, Palumbo Piccionello A, Puglia AM, Emiliani G, Maggini V, Firenzuoli F, Fani R (Firenze, Pisa, Palermo)

17.45-18.00
New sesquiterpenes from *Zanthoxylum rhoifolium* Lam. barks

Di Stasi M, De Tommasi N, Parisi V, Hernandez V, Braca A (Pisa, Salerno, Merida)

18.00-18.15
Metabolite profile of green extracts of *Schisandra chinensis* fruits and evaluation of Tyrosinase inhibitory activity
Polcaro L, Piacente S, Masullo M (Salerno)

18.15-18.30
^{1}H NMR-based metabolomic analysis of the lichen *Cladonia foliacea*.
Nozella AH, Debiasi L, Gheza G, Trincia S, Vallese C, Nascimbene, Poli F, Mandrone M (Bologna)

18.45 Cocktail di benvenuto

Venerdì 16 giugno

Aula 1

Moderatore: Conforti F (Rende)

9.00-9.30
Lettura magistrale: **Plant biodiversity to promote health and nutrition: re-discovering ancient varieties inside the *Prunus* genus**
De Leo M (Pisa)

9.30-10.45 Sessione 5 Aula 1

Moderatori: Froldi G (Padova) - Conforti F (Rende)

9.30-9.45
Application of wood distillate boosts antioxidant properties of *Solanum lycopersicum* L. (red tomato)
Fedeli R, Marotta L, Panti A, Carullo G, Fusi F, Gemma G, Butini B, Saponara S, Vannini A, Campiani G, Loppi S (Siena, Napoli)

9.45-10.00
Wild roses of the Apennines: metabolomic analysis and antioxidant activity
Trincia S, Tarozzi C, Nozella HA, Mandrone M, Chiocchio I, Mossetti U, Poli F (Bologna)

10.00-10.15
Chemical composition and biological activity of essential oils and crude extract from apical shoots and resin of *Pinus nigra* subsp. *laricio* Poiret
Fucile M, Perri M, Marrelli M, Zicarelli L, Lupia C, Statti G, Conforti F (Rende, Castelluccio Superiore)

10.15-10.30
Pharmacognostic evaluation of *Monarda didyma* L. "summitas cum floribus" growing in Trentino, Northern Italy
Smeriglio A, Trombetta M, Ingegneri M, Germanò MP, Miori L, Battistini G, Malaspina P, Cornara L (Messina, Baselga di Pinè, Genova)

10.30-10.45
A microemulsion approach to address the solubility and cutaneous bioavailability issues of *Stachys parolinii* extract
Vanti G, Milonaki E, Anagnostou M, Tomou E.-M, Grifoni L, Bergonzi MC, Karioti A, Skaltsa H, Bilia AR (Firenze, Atene, Thessaloniki)

9.30-10.45 Sessione 6 — Aula 2

Moderatori: Martelli A (Pisa) – Esposito E (Messina)

9.30-9.45
Effects of an oral solution containing xyloglucan and pea proteins on a murine model of gastroesophageal reflux disease
Ardizzone A, Mannino D, Casili G, Campolo M, Paterniti I, Lanza M, Filippone A, Repici A, Bova V, Cuzzocrea S, Esposito E (Messina)

9.45-10.00
Glucoerucin, a glucosinolate contained in *Eruca sativa* Mill., prevents the metabolic syndrome in mice fed a high-fat diet: modulation of the browning process and the irisin pathway
Flori L, Testai L, Miragliotta V, Lazzarini G, Piragine E, Citi V, Pagnotta E, Matteo, Righetti L, Di Cesare Mannelli L, Ghelardini C, Martelli A, Calderone V (Pisa, Bologna, Firenze)

10.00-10.15
A *Cynara cardunculus* L. leaves standardized extract promotes *in vitro* intestinal epithelial differentiation and barrier function through activation of AMPK/SIRT1 pathway.
Muscarà C, Salamone FL, Molonia MS, Saija A, Cimino F, Speciale A (Messina)

10.15-10.30
***Glycyrrhiza glabra* L. extracts: phytochemical characterization and inhibition of pro-inflammatory cytokines and JAK/STAT signalling pathway in LPS-stimulated RAW 264.7 cells**
Perri MR, Marrelli M, Zicarelli L, Conforti F, Statti G (Rende)

10.30-10.45
Investigation of olive leaf extracts from pruning wastes
Vaccaro F, Baini G, Cappellucci G, Miraldi E, Biagi M (Siena)

10.45-11.45 Coffee break e sessione poster

11.45-13.15 Sessione 7 — Aula 1

Moderatori: Borrelli F (Napoli) – Milella L (Potenza)

11.30-11.45
Sulforaphane promotes apoptotic and ferroptotic cell death in leukemia cells
Greco G, Schnekenburger M, Catanzaro E, Turrini E, Ferrini F, Sestili P, Diederich M, Fimognari C (Bologna, Lussemburgo, Ghent, Rimini, Urbino, Seul)

11.45-12.00

Cannabidiol loaded in liposomial formulation enanches antigenotoxic effect against camptothecin

Grifoni L, Brunha C, Sendão T, Dias A, Oliveira R, Bilia AR (Firenze, Portogallo)

12.00-12.15

Zosterabisphenone C, a new diarylheptanoid heterodimer from the seagrass *Zostera marina,* shows cytotoxic effects in colon cancer

De Cicco P, Cacciola NA, Amico R, Grauso L, Scarpato S, Mangoni A, Borrelli F (Napoli)

12.15-12.30

Perillaldehyde from *Ammodaucus leucotrichus* as a new ferroptosis inducer with relevant clinical potential

Turrini E, Catanzaro E, Maffei F, Guerrini A, Krysko DV, Fimognari C (Rimini, Ghent, Ferrara),

12.30-12.45

***Acmella oleracea* extracts from *in vitro* seedlings: chemical characterization and a novel preclinical application on the acute treatment of chemotherapy-induced neuropathy**

Zonfrillo B, Bellumori M, Firenzuoli F, Maggini V, Di Cesare Mannelli L, Ghelardini C, Mulinacci N, Innocenti M (Firenze)

12.45-13.00

Evaluation and characterization of the cytotoxic profile of two compounds extracted from the Caribbean sponge *Smenospongia aurea* in a panel of human cancer cell lines

Pellicioni V, Greco G, Costantino V, Teta R, Esposito G, Fimognari C (Bologna, Napoli)

13.00-13.15

Antiproliferative effects of Cannabis with low content of tetrahydrocannabinol: potential in the treatment of psoriasis

Rizzi L, Pellati F, Bresciani E, Molteni L, Meanti R, Corsi L, Torsello A (Milano)

11.45-13.15 Sessione 8 Aula 2

Moderatori: Rossi A (Napoli) – Sacchetti G (Ferrara)

11.30-11.45

Phytochemical and pharmacological investigation of industrial hemp inflorescences by-products

Acquaviva A, Di Simone SC, Libero ML, Nilofar, Chiavaroli A, Recinella L, Leone S, Brunetti L, Orlando G, Menghini L, Ferrante C (Chieti, Granada, Pescara)

11.45-12.00

***Gynostemma pentaphyllum* (var. Ginpent) protects against acute peripheral inflammation and motor alteration**
Mac Sweeney E, Bonini SA, Premoli M, Maccarinelli , Zhang L, Lucini L, Memo M, Mastinu A (Brescia, Piacenza)

12.00-12.15
***Artocarpus tonkinensis* protects mice against collagen-induced arthritis: decreases Th17 cell function and suppresses osteoclastogenesis**
Adorisio S, Ayroldi E, Delfino DV (Perugia)

12.15-12.30
Anti-oxidant and anti-inflammatory effects of ellagic and punicic acid in an *in vitro* model of cardiac fibrosis
Pallio G, Minutoli L, Irrera N, Squadrito F (Messina)

12.30-12.45
Essential Oil Composition, antioxidant activity and leaf micromorphology of five Tunisian *Eucalyptus* species
Romano B, Maresca DC, Polito F, Cornara L, De Feo V, Ercolano G, Ianaro A (Napoli, Salerno Genova)

12.45-13.00
Bergamot polyphenols improve hepatic inflammation, and reverse altered thalamus metabolism and brain structure in a mouse model of non-alcoholic fatty liver disease
Musolino V, Cardamone A, Coppoletta AR, Nucera S, Ruga S, Bosco F, Guarnieri L, Macrì R, Scarano F, Bava I, Caminiti R, Lorenzo F, Tucci L, Lupia C, Serra M, Mollace R, Maiuolo J, Carresi C, Muscoli C, Palma E, Gliozzi M, Mollace V (Catanzaro)

13.00-13.15
Identification of an induced-neurodegeneration preclinical model for the study of plant extracts with anti-glycantion activity
Pucci M, Abate G, Tirelli E, Mastinu A, Memo M, Uberti D (Brescia)

13.15-14.30 Light lunch

Aula 1

Moderatore: Calapai G (Messina)

14.30-15.00

Lettura magistrale: **EU Regulatory framework on food supplements**
Di Giorgi V (Ministero della Salute, Roma)

15.00-16.45 Sessione 9 Aula 1

Moderatori: Biagi M (Siena) – Taglialatela O (Napoli)

15.00-15.15
Phytotherapy in the treatment of chronic low-grade inflammation
Santagà D (A.V.D. Reform)

15.15-15.30
Tested to reach the highest quality: Labomar's approach for ensuring quality, safety and efficacy of nutraceutical products
Amadio E, Bassetto R, Perin S, Zanatta S (Labomar SPA)

15.30-15.45
Valorization of by-products from agro-foods for pharmaceutical, cosmetic, nutraceutical applications
Terenzi C, Medri F, Davani L, Montanari S, De Simone A, Andrisano V (Rimini, Torino)

15.45-16.00
Nutraceutical and nutritional composition of an Italian wild pears (*Pyrus communis* var. Zingaro) fruits at different maturation stages
Alderotti F, Dos Santos Nascimento LB, Centritto M, Sobolev AP, Gori A, Brunetti C (Firenze, Monterotondo)

16.00-16.15
Pharmacovigilance of cannabidiol for medicinal use and as a food supplement
Calapai F, Ammendolia I, Cardia L, Currò M, Cacciola A, Esposito E, Calapai G, Mannucci C (Messina)

16.15-16.30
Potential efficacy of Alliaceae and Brassicaceae edible plants in patients with type 2 diabetes: a systematic review and meta-analysis of clinical trials
Piragine E, Petri D, Martelli A, Lucenteforte E, Calderone V (Pisa)

16.30-16.45
Development nutraceutical ingredients to slow biological aging
Campisi M, Cannella L, Baumann J, Pavanello S (Padova, Buchs)

16.45-17.15 *Coffee break*

17.15 -18.45 Tavola rotonda Editors in Chief

Moderatore: De Tommasi N (Salerno)

Phytotherapy Research Izzo AA
Fitoterapia Taglialatela Scafati O
Planta medica Braca A

20.30 Cena

Sabato 17 giugno

9.00-10.30 Sessione 11 — Aula 1

Moderatori: Pistelli L (Pisa) – Smeriglio A (Messina)

9.00-9.15
Enhancement of pomegranate peel by recovering the phenolic compounds: one-pot extraction with hydrolysis
Ugolini T, D'Agostino S, Cecchi L, Khatib M, Innocenti M, Mulinacci N (Firenze)

9.15-9.30
Green biotechnology for human feeding
Dalla Costa V, Piovan A, Filippini R (Padova)

9.30-9.45
The chemical assessment of the glucosinolate-myrosinase system: analytical challenges and technical pitfalls
De Nicola GR (Pescia)

9.45-10.00
Environmental impact of phytoextracts: the case of *Melia azedarach* L.
Popescu VS, Mac Sweeney E, Abate G, Pucci M, Tirelli E, Zhang L, Peron G, Gianoncelli A, Ribaudo G, Bulgari D, Gobbi E, Lucini L, Uberti D, Memo M, Mastinu A (Brescia, Piacenza, Milano)

10.00-10.15
***Acmella oleracea* ("jambù", Asteraceae): a spilanthol-rich source for the development of effective green insecticides and acaricides**
Ferrati M, Spinozzi E, Baldassarri C, Cappellaci L, Benelli G, Petrelli R, Maggi F (Camerino)

10.15-10.30
***Carlina acaulis*, a traditional medicinal plant for insect vectors management**
Spinozzi E, Ferrati M, Baldassarri C, Maggi F, Pavela R, Benelli G, Aguzzi C, Zeppa L, Cappellacci L, Palmieri A, Petrelli R (Camerino, Praga)

9.00-10.30 Sessione 12 — Aula 2

Moderatori: Chiavaroli A (Chieti) – Trombetta D (Messina)

9.00-9.15
***Cynara cardunculus* L. suppresses adipogenesis in 3T3-L1 adipocytes via AMPK signaling pathway activation**
Molonia MS, Salamone FL, Speciale A, Muscarà C, Saija A, Cimino F (Messina)

9.15-9.30
Walnut (*Juglans regia* L.) and chestnut (*Castanea sativa* Mill.) leaves as precious by-products for the treatment of skin aging induced by environmental pollution
Baini G, Cappellucci G, Biagi M, Miraldi E (Siena)

9.30-9.45
Assessment of antidiabetic, anti-obesity, and neuroprotective potential of two *Cannabis sativa* L. extracts
Mazzara E, Petrelli R, Mustafa AM, Caprioli G, Dall'Acqua S, Sut S, Nuñez S, López V, Cásedas G, Moliner C, Bonacucina G, Maggi F, Cespi M (Camerino, Padova, Zaragoza)

9.45-10.00
***Humulus lupulus* L. not only for beer! investigation of the bitter taste receptors involved in its mechanism of action**
Lela L, Ponticelli M, Russo D, Faraone I, Kioussi C, Stevens JF, Milella L (Potenza, Corvallis)

10.00-10.15
Addition of polyphenolic extracts of *Myrtus communis* and *Arbutus unedo* fruits to whey: valorization of a common dairy waste product as a functional food
Detti C, Nascimento LB, Gori A, Vanti G, Amato G, Nazzaro F, Bilia AR, Ferrini F, Mauro Centritto, Brunetti C (Firenze, Avellino)

10.15-10.30
Central sardinia's (Italy) native food plant, *Lactuca longidentata*: an exploration of its health benefits
Nilofar, Di Simone SC, Flores GA, Acquaviva A, Libero ML, Orlando G, Menghini L, Ferrante C (Chieti)

10.30-11.30 Coffee break e Sessione poster

11.30-13.15 Sessione 13 Aula 1

Moderatori: Montopoli M (Padova) - Capasso R (Napoli)

11.30-11.45
Safety and efficacy of red yeast rice phytocomplex and lovastatin: a comparative analysis
Rigillo G, Baini G, Miraldi E, Pani L, Tascedda F, Biagi M (Modena-Reggio Emilia, Siena)

11.45-12.00
The flavonoid luteolin attenuates colitis and M1 macrophage activation via TRPM8
Cicia D, Biscu F, Ferrante C, Iannotti FA, Nanì MF, Lucariello G, Amico R, Orlando G, Matteoli G, Capasso R, Izzo AA, Pagano E. (Napoli, Leuven, Chieti)

12.00-12.15

Naringenin promotes lysosomal regeneration: a potential therapeutic strategy for Hereditary Spastic Paraplegia

Guarato G, Feltrin S, Dianin F, Vantaggiato C, Napoli B, Gumeni S, Orso G (Padova, Lecco, Atene)

12.15-12.30

***In vivo* effect of sumac fruit (*Rhus coriaria* L.) on skin inflammation**

Sangiovanni E, Fumagalli M, Pelin M, Piazza S, Maranta N, Pozzoli C, Martinelli G, Sosa S, Dell'Agli M (Milano)

12.30-12.45

A novel integrated *in vitro/in silico* approach to investigate species of ethnobotanical interest with wound healing and immunomodulatory activities

Cappellucci G, Baini G, Vaccaro F, Miraldi E, Biagi M (Siena)

12.45-13.00

Modulation of neuroinflammation by essential oils obtained from different hemp varieties

Barbalace MC, Freschi M, Rinaldi I, Mazzarra E, Petrelli R, Maraldi T, Malaguti M, Maggi F, Hrelia S, Angeloni C (Bologna, Camerino, Modena)

13.00-13.15

Bio-pharmacological properties of *Pelargonium quercetorum* Agnew extracts: focus on potential application as agents against inflammatory bowel diseases

Chiavaroli A, Libero ML, Di Simone SC, Acquaviva A, Nilofar, Recinella L, Leone S, Brunetti L, Cicia D, Izzo AA, Orlando G, Zengin G., Menghini L, Ferrante C (Chieti, Napoli, Konya)

13.15

Premiazione e Chiusura del Convegno

SESSIONE POSTER VENERDI 16 GIUGNO ORE 10.45-11.45

Sessione 1

Moderatore: De Francia S (Torino)

P1. **Characterization of polyphenolic composition in *Leccinum Scabrum* and *Leccinum Versipelle* mushrooms**
Medri F, Terenzi C, Davani L, Montanari S, De Simone A, Andrisano V (Rimini, Torino)

P2. **Chemical characterization of the volatile and non-volatile profiles of *Achillea nana* L. aerial parts by SPME-GC/MS, GC/MS and HPLC/MS-MS, techniques**
Argentieri MP, Garzoli S, Meroni G, Martino PAM, Soggiu A, Bonizzi L, Zecconi AA, Iriti M, Vitalini S (Bari, Roma, Milano)

P3. **Chemical characterization of the volatile compounds of 12 species within the genus *Salvia* L. cultivated at the Botanic Garden of Pisa and study of the relationship with their geographical origin.**
Bozzini MF, Pieracci Y, Peruzzi L, D'Antraccoli M, Ciampi L, Pistelli L, Flamini G (Pisa)

P4. ***Calamintha nepeta* Savi: ethnobotany, phytochemistry and pharmacological properties**
Lupia C, Marrelli M, Perri MR, Zicarelli L, Conforti F, Duca A, De Biasio F, Canora G, Salamone E, Statti G (Rende, Lauria)

P5. **The synergistic inhibitory effect of fennel, lavender and oregano essential oils on nitric oxide production: an optimization by a mixture design methodology**
Marrelli M, De Luca M, Perri MR, Zicarelli L, Statti G, Conforti F (Rende)

P6. **Chemical composition and bioactivities of *Rosmarinus officinalis* L. cultivated and wild extracts in Campania region**
Francolino R, Martino M, Caputo L, Amato G, Chianese G, Formisano C, De Feo V, Ercolano G, Ianaro A, De Martino L (Salerno, Napoli)

Sessione 2

Moderatore: Testai L (Pisa)

P7. **Re-evaluation of Tuscan *Crocus sativus* L. wastes by metabolomic fingerprint and *in vitro* antiviral test against SARS-CoV-2**
Cioni E, Di Stasi M, Iacono E, Luminare AG, Gambineri F, Bazzichi A, Lai M, De Leo M, Braca A, Pistello M (Pisa, Siena)

P8. **Coumarins from ethyl acetate extract of gum ammoniacum**

Kharatha M, Alilou M, Donadio G, De Tommasi N, Moridi Farimani M (Tehran, Innsbruck, Salerno)

P9. **Moderate deficit irrigation can modulate the secondary metabolites of *Pistacia lentiscus* L. fruits during ripening**
Muti L, Vanacore D, Brunetti C, Dos Santos Nascimento L, Goracci G, Alderotti F, Detti C, Bilia AR, Ferrini F, Gori A (Firenze, Sesto Fiorentino)

P10. **Isolation and biological evaluation of *ent*-pimarane diterpenoids from *Lavandula pubescens* Decne**
Pouramin S, Parisi V, Bader A, Rosa E, De Tommasi N (Salerno, Makkah)

P11. **Phytochemical characterization and biological activities of *Stenomesson miniatum* bulb extract, a medicinal plant of the Andes**
Lianza M, Fimognari C, Nuzillard JM, Renault JH, Antognoni F., Bonvicini F, Poli F, Greco G, Turrini E (Rimini, Reims, Bologna)

P12. **Phytochemical profile, cytotoxicity and redox state modulatory activities of olive leaf (*Olea europaea* L.) enriched extract in prostate cancer cell lines**
Malfa GA, Luca T, Siracusa L, Di Giacomo C, Bianchi S, Sergi A, Castorina S, Acquaviva R (Catania)

P13. **Cytoprotective properties of the ethyl acetate extracts from *Anacyclus maroccanus* Ball. e *Anacyclus radiatus* Loisel aerial parts**
Percaccio E, Sissi S, Vitalone A, Mazzanti G, Markouk M, Di Giacomo S, Di Sotto A (Roma, Marrakech)

Sessione 3

Moderatore: Smeriglio A (Messina)

P14. **Use of phytotherapics and nutraceuticals in colorectal cancer patients undergoing chemotherapy.**
Di Grazia D, Giacchi D, Gaspari M, Allegra S, Armando T, Storto S, Chiara F, De Francia S, Mussa MV (Torino)

P15. **The beneficial effect of glucoraphanin in an *in vitro* model of sarcopenia**
Esposito E, Mitidieri E, Micheli L, Ciampi C, Cirino G, Ghelardini C, Di Cesare Mannelli L, Sorrentino R, d'Emmanuele di Villa Bianca R (Napoli, Firenze)

P16. **Phytotherapy use to treat chemotherapy-related side effects in lung cancer patients**
Gaspari M, Bettonte G, Di Grazia D, Allegra S, Armando T, Storto S, Chiara F, De Francia S, Mussa MV (Torino)

P17. **Anti-proliferative and anti-migratory potential of *Glycyrrhiza glabra* L. extracts on breast and gynecological cancer cell lines**
Perri MR, Marrelli M, Zicarelli L, Conforti F, Statti G (Rende)

P18. **Pigmented potatoes and their preventive effect against LPS-induced inflammation in THP-1 macrophages**
Toccaceli M, Marinelli A, Bassolino L, Pacifico D, Parisi B, Nicoletti F, Lo Scalzo R, Mandolino G, Petroni K (Milano, Bologna)

P19. ***In vitro* evaluation of the neuroprotective effect of *Achillea millefolium* L. flowering part extract**
Freschi M, Barbalace MC, Angeloni C, Malaguti M, Hrelia S (Bologna)

Sessione 4

Moderatore: Paterniti I (Messina)

P20. ***In vitro* protective effects of a standardized extract of *Opuntia ficus indica* L. and *Olea europaea* L. against indomethacin-induced intestinal epithelial cells injury**
Salamone FL, Muscarà C, Molonia MS, Saija A, Cimino F, Speciale A (Messina)

P21. **Focus on some herbal substances associated with safety concerns**
Carlucci V, Rodda M, Brunasso Cattarello L, Gillio Tos E, Baldone G, Conto A (Colleretto Giacosa)

P22. **Fatty Acid composition, Antioxidant, and *in vitro* Anti-inflammatory activity of five cold-pressed *Prunus* seed oils, and their Anti-biofilm effect against pathogenic bacteria**
Amato G, Fratianni F, d'Acierno A, Ombra MN, De Feo V, Ayala-Zavala J, Coppola R, Nazzaro F (Avellino, Salerno, Hermosillo, Campobasso)

P23. **Pomegranate by-products valorization: cell-based antioxidant activity of the hydroalcoholic leaf extract**
Marengo A, Sanna C, Foglietta F, Serpe L, Rubiolo P (Torino, Cagliari)

P24. **Study of an herbal preparation composed by a combination of *Ruscus aculeatus* L. and *Vitis vinifera* L. extracts, magnolol and diosmetin to address chronic venous diseases through an anti-inflammatory effect and AP-1 modulation**
Nocera R, Eletto D, Santoro V, Parisi V, Bellone ML, Izzo M, Tosco A, Dal Piaz F, Donadio G, De Tommasi N (Salerno, Ferrara)

SESSIONE POSTER SABATO 17 GIUGNO 10.30-11.30

Sessione 5

Moderatore: Di Sotto A (Roma)

P25. **Use of acupuncture in cancer patients undergoing chemotherapy, immunotherapy, and radiation therapy**
Allegra S, Di Nino A, Armando T, Storto S, Chiara F, De Francia S, Mussa MV (Torino)

P26. **Biotechnological strategies for enhanced production of antioxidant metabolites in *in vitro* cultures of *Isatis tinctoria* (Brassicaceae)**
Davì F, Taviano M.F, Nicosia N, Speranza J, Cavò E, Ragusa S, Arena P, Kwiecień I, Ekiert H, Miceli N (Messina, Milano, Leicester, Palermo, Krakow)

P27. **Shoots formation by indirect organogenesis and VOCs production in *Acmella oleracea* L. ("jambù", Asteraceae)**
Maggini V, Palagano E, Menicucci F, Cencetti G, Gallo E, Bettini PP, Michelozzi M, Bogani P, Firenzuoli F (Firenze, Sesto Fiorenitno)

P28. **The importance of (not) being a waste: the case study of *Cichorium endivia* L.**
Parisi V, Bellone M, Santoro V, Vitiello M, Dal Piaz F, De Tommasi N, Donadio G (Salerno)

P29. **Essential oils as potential acetylcholinesterase and butyrylcholinesterase inhibitor. A case study: *Elettaria cardamomum* (L.) Maton essential oil**
Pavarino M, Cagliero C, Marengo A, Bicchi C, Rubiolo P, Sgorbini B (Torino)

P30. ***Foeniculum vulgare* Miller: revalorization of a local food waste**
Santoro V, Rosa E, Donadio G, Polito F, Caputo L, De Feo V, De Tommasi N (Salerno)

Sessione 6

Moderatore: Trombetta D (Messina)

P31. **Spilanthol-rich extracts from *Acmella oleracea* (L.) R.K. Jansen and their efficacy on stored product pests**
Spinozzi E, Ferrati M, Piergentili A, Petrelli R, Cappellacci L, Maggi F, Angeloni S, Ricciutelli M, Drenaggi E, Boukouvala MC, Kavallieratos NG, Benelli G (Camerino, Atene, Pisa)

P32. **Development of a "foodomics" platform for monitoring the transformation process of aromatic plants for the food industry**
Evangelista AR, Nitride C, Andolfo G (Napoli)

P33. **Nutritional and health valorization of plants indigenous to Western Sahara**
Chiozzini C, Maietti A, Tacchini M, Abdi Bellau ML, Maresca I, Sacchetti G, Guerrini A (Ferrara, Tindouf)

P34. **Evaluating the effect of *in-vitro* digestion on the total polyphenol content of *Vitis vinifera* L. by-products**
Mastellone G, Gennaro G, Marengo A, Sgorbini B, Larrea V, Cirrincione S, Lamberti C, Hernando I, Gai F, Rubiolo P, Cagliero C (Torino, Valencia, Grugliasco)

P35. **Antidysmenorroic effects of the non-psychotropic *Cannabis sativa* L. phytocomplex on isolated human myometrium**
Occhiuto C, Giofrè S, Smeriglio A, Trozzi A, Tranchida PQ, Occhiuto F (Messina)

P36 ***Crepis vesicaria* L.: an underexploited herbal species with promising health-promoting effect.**
Mangieri C, Carlucci V, Benedetto N, Cafarelli A, Covino MA, Lupia C, Milella L (Potenza, Castelluccio Superiore)

Sessione 7

Moderatore: Acquaviva R (Catania)

P37. **Mosquitocidal and anti-inflammatory effects of hemp (*Cannabis sativa* L.) essential oils from monoecious, male, and female inflorescences and their encapsulation in nanoemulsions**
Rossi P, Cappelli A, Marinelli O, Valzano M, Pavoni L, Bonacucina G Petrelli R, Ianni M, Mazzara E, Ricci I, Maggi F, Nabissi M (Camerino)

P38. **Potential beneficial effect of grape seed extract on LPS-induced gut permeability damage and oxidative stress**
Nicolosi RM, Imperia E, Altomare AM, Guarino MPL, De Vita D, Pasqua G (Roma)

P39. **Preliminary investigation about morpho-anatomical and phytochemical features and antioxidant activity of leaves and flowers from Sicilian *Plumeria rubra* L. cv. "Classica palermitana"**
Ingegneri M, Malaspina P, Betuzzi F, Di Gristina E, Cornara L, Trombetta D, Smeriglio A (Messina, Palermo)

P40. **The use of nanovesicles to enhance the antioxidant activity of an extract of *Onopordum illyricum* leaves**
Sanna C, Caddeo C., Tuberoso CIG., Floris S, Masala V., Pintus F (Cagliari)

P41. **The Effect of *Olea europea* L. extract and its compound oleuropein, on cardiotoxicity induced by doxorubicin**

Maiuolo J, Musolino V, Gliozzi M, Mollace V (Catanzaro)

P42. **Antioxidant and neuroprotective properties of an aqueous dry extract from the *S. officinale* aerial parts**
Napolitano B, Percaccio E, Másedas G, Garzoli S, Nicotra G, Di Giacomo S, Les F, López V, Di Sotto A (Roma, Saragozza, Milano)

Sessione 8

Moderatore: Marcotullio MC (Perugia)

P43. **Essential oils bearing specialized metabolites with potential tyrosinase inhibitory activity: focus on dermal absorption from topical formulations**
Sgorbini B, Capetti F, Pavarino M, Cagliero C, Argenziano M, Cavalli R, Bicchi C, Rubiolo P (Torino)

P44. **Anti-aging activity of natural extract for phytocosmetic applications**
Sacilotto A, Tinazzi M, Giacomini I, Montopoli M, Cocetta V (Padova)

P45. **Screening of extracts from *Crataegus laciniata* Ucria (Rosaceae) for the treatment of skin hyperpigmentation**
Mirabile S, Cacciola A, Raimondo FM, D'Angelo V, Germanò MP (Messina, Palermo)

P46. **Phytochemical and biological characterization of *Fragaria x ananassa* Duch.**
Di Sotto A, Percaccio E, Di Giacomo S, Mezzanotte E, Milana MR, Cesa S, Ingallina C, Acciaro E, Cairone F, Vitalone A (Roma, Monterotondo)

P47. **Boosting *Coriandrum sativum* essential oil yield and quality using natural deep eutectic solvents (NADES)**
Scandar S, Zadra C, Lanari D, Marcotullio MC (Perugia)

P48. **Marine macroalgae as a promising source of natural product**
Armeli Minicante S, Spagnuolo D, Manghisi A, Morabito M, Genovese G (Venezia, Messina)

P49 **Optimization of the microwave-assisted hydrodistillation of *Carlina acaulis* essential oil by fractional factorial design study**
Spinozzi E, Ferrati M, Lo Giudice D, Felicioni E, Petrelli R, Benelli G, Maggi F, Cespi M (Camerino, Pisa)

Sessione 9

Moderatore: Turrini B (Bologna)

P50. **Anthraquinones: genotoxic until proven otherwise? A study on a substance-based medical device to implement available data for a correct risk assessment**
Gasperini S, Lenzi M (Bologna)

P51. **Erucin, a natural hydrogen sulfide (H_2S) donor, improves skeletal muscle (SKM) dysfunction related to obesity**
Casale V, Smimmo M, Casillo GM, Bello I, Panza E, Cirino G, Bucci M, Vellecco V (Napoli)

P52. **Phytochemical investigation and antibacterial activity of *Satureja bachtiarica* Bunge aerial parts**
Rahmani Samani M, D'Urso G, Masullo M, Piacente S (Salerno)

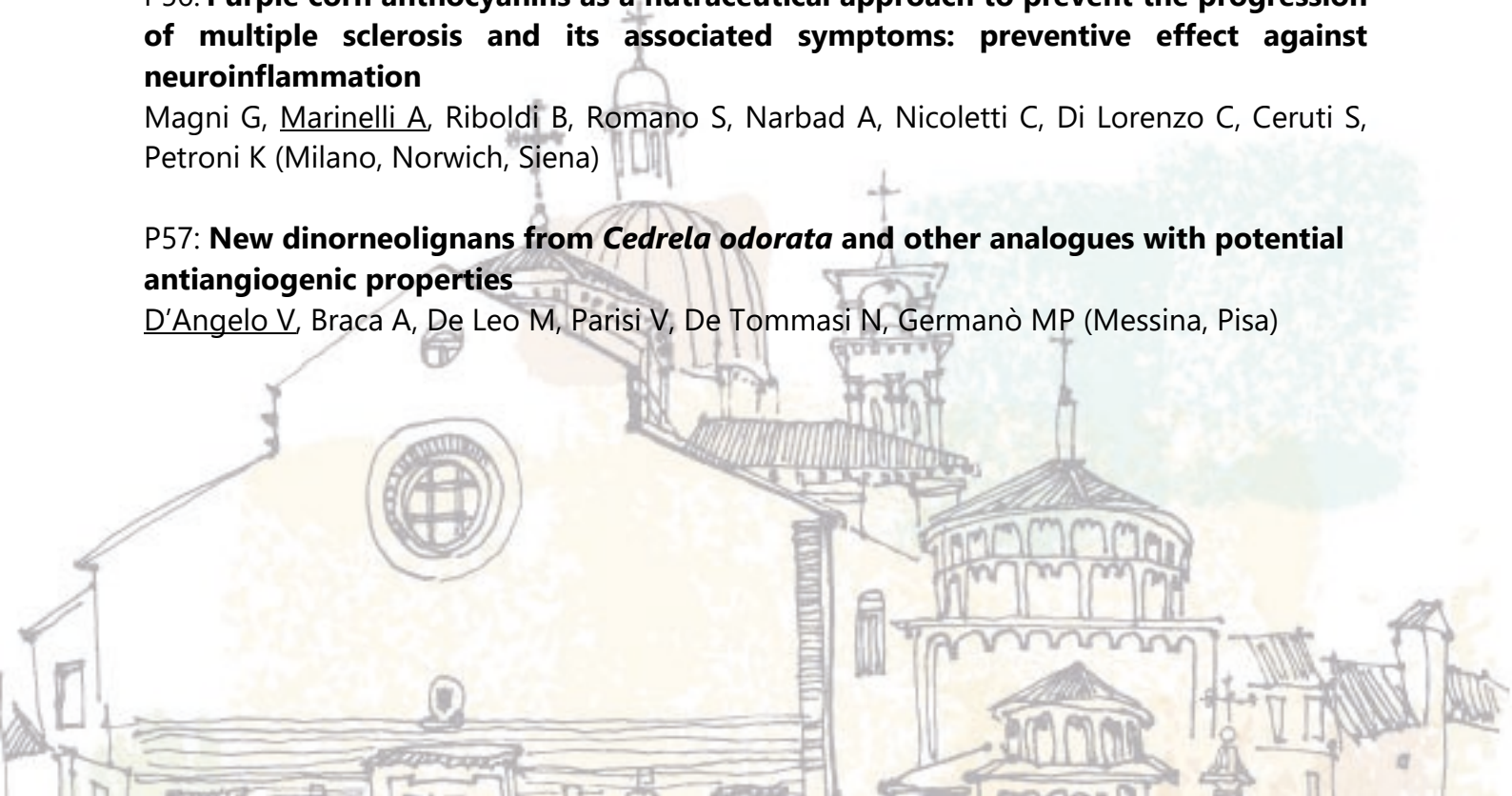
P53. **Phenolic profile and biological activities of hydroalcoholic extracts from leaves, flowers and stems of *Sinapis pubescens* L. subsp. *pubescens* (Brassicaceae) wild from Sicily (Italy)**
Arena P, Miceli N, Marino A, Davì F, Cavò E, Spadaro V, Raimondo FM, Cacciola F, Laganà Vinci R, Mondello L, Taviano MF (Messina, Palermo, Roma)

P54. **Investigation of the anti-inflammatory activity of oleocanthal and oleocanthalic acid in an *in vitro* model of neuroinflammation**
Rinaldi I, Barbalace MC, Freschi M, Malaguti M, Digiacomo M, Giusti L, Lucacchini A, Angeloni C, Hrelia S (Bologna, Pisa, Camerino)

P55. **Evaluation of the anti-adipogenic and lipolytic activity of *Ficus carica* L. and γ-oryzanol**
Tirelli E, Pucci M, Chiocchio I, Mandrone M, Mac Sweeney E, Mastinu A, Memo M, Poli F, Uberti D, Abate G (Brescia, Bologna)

P56. **Purple corn anthocyanins as a nutraceutical approach to prevent the progression of multiple sclerosis and its associated symptoms: preventive effect against neuroinflammation**
Magni G, Marinelli A, Riboldi B, Romano S, Narbad A, Nicoletti C, Di Lorenzo C, Ceruti S, Petroni K (Milano, Norwich, Siena)

P57: **New dinorneolignans from *Cedrela odorata* and other analogues with potential antiangiogenic properties**
D'Angelo V, Braca A, De Leo M, Parisi V, De Tommasi N, Germanò MP (Messina, Pisa)



Elenco abstract comunicazioni orali

Giovedì 15 giugno

Lettura Magistrale

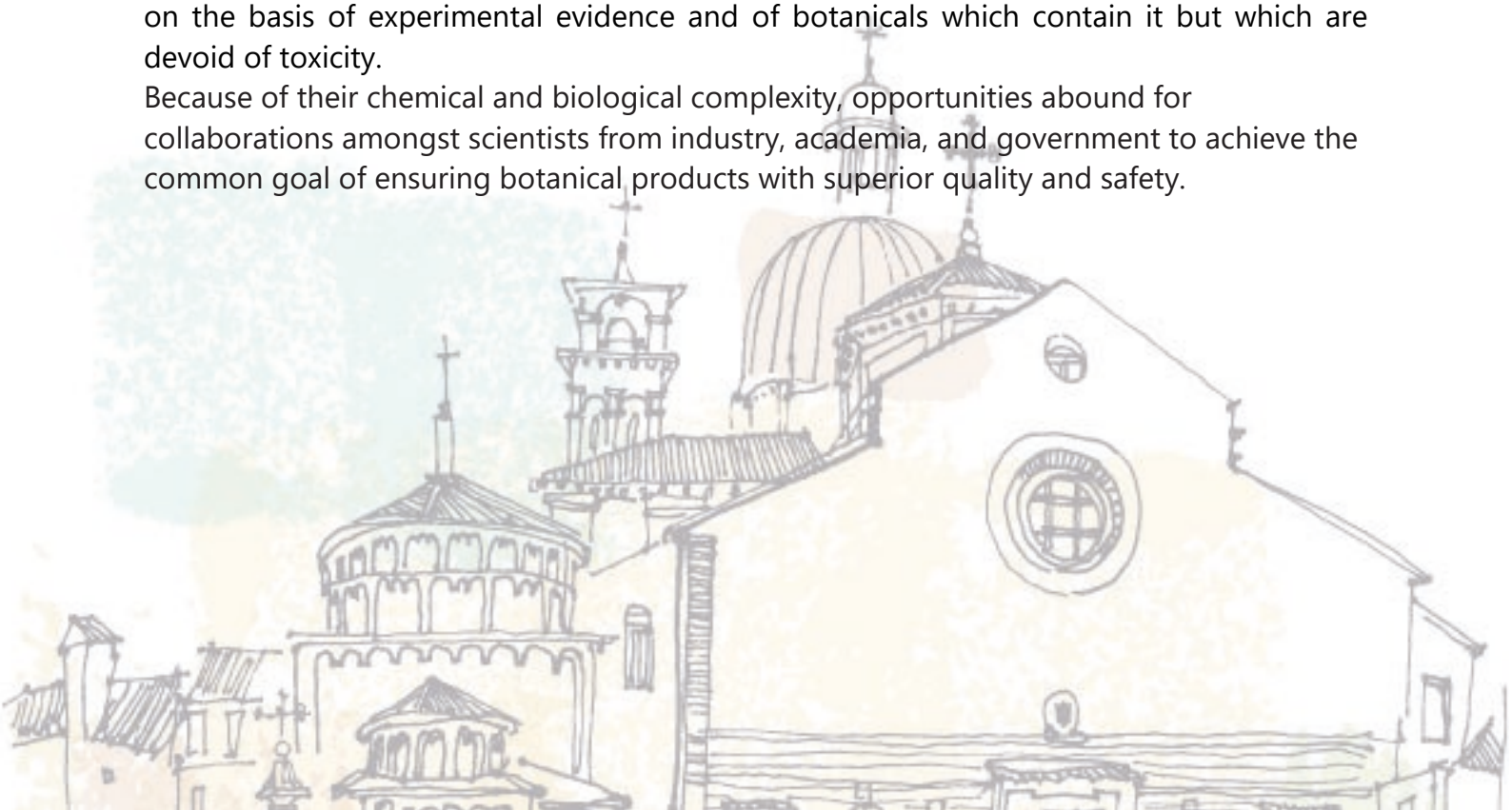
Botanicals: focus on safety issues

Fimognari C

Dipartimento di Scienze per la Qualità della Vita, Alma Mater Studiorum – Università di Bologna, 47921 Rimini, Italia

Today, the marketing and use of botanicals for health and wellness benefits continues to thrive worldwide, with consumers projected to spend more than $140 billion globally by 2024. However, research on the quality and safety of these products has lagged behind sales. Botanicals have peculiar characteristics as they can be made up of multiple phytochemicals, some of which are biologically active and many of which are chemically undefined. Their complex nature can pose a number of problems, among which compositional variability, toxicity, and phytochemicals interactions are the most relevant. Moreover, botanicals are characterized by a complex matrix. Various compounds present in the plant matrix, such as cellulose, heteropolysaccharides, pectins, etc., can reduce the bioavailability of a specific phytochemical, for example by slowing down its release or hindering its complete release, by inhibiting specific intestinal carriers involved in its uptake or by inhibiting it the bioactivation. On the other hand, other components of the botanical can improve bioavailability, synergize or antagonize the biological activity of a specific phytochemical, modify or alter the structure of the matrix. The matrix could also modulate the toxicity of the single substances that characterize the botanical product by reducing or increasing its toxicity under the dose, conditions of use and their metabolic pattern. It is therefore not surprising that there are numerous examples of a particular phytochemical resulting toxic on the basis of experimental evidence and of botanicals which contain it but which are devoid of toxicity.

Because of their chemical and biological complexity, opportunities abound for collaborations amongst scientists from industry, academia, and government to achieve the common goal of ensuring botanical products with superior quality and safety.



Herbal medicines in Italy: regulatory aspects, critical issues and innovation

Assisi A
Agenzia Italiana del Farmaco (AIFA)

In order to be marketed in Italy, an herbal medicinal product must be granted a Marketing Authorisation (MA) by 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 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 herbal medicinal products 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 herbal medicinal products, even though they are not subject to fulfil the same requirements of quality, efficacy and safety. 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. A further critical element is the coexistence on the market of the same herbal substances/preparations at the same dosages both as medicines and as food supplements and/or as ancillary substances present within a medical device.

The existence of these "*borderline*" products is the result of a lack of clarity in the legislation which lends itself easily to different interpretations, leading to considerable disharmonization between the various Member States of the European Union in the classification as a medicine or not of a product containing an herbal substance/preparation. This discrepancy has repercussions on safety and the protection of public health, as the use of non-medicinal products such as food supplements and medical devices is frequently perceived as completely risk-free by consumers and by physicians themselves.

The promotion and dissemination among healthcare professionals of the knowledge of the differences in the authorization processes in terms of quality, efficacy, safety requirements and of the regulatory framework that regulates the placing on the market between herbal medicinal products and other non-medicinal products containing herbal substances or preparations, food supplements and medical devices *in primis*, represent an essential aspect for identifying solutions aimed at promoting the market of herbal medicinal products and increasing awareness on the safe use, to which national and EU regulatory institutions, the academic world and the pharmaceutical companies themselves must necessarily contribute.

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".

Antimutagenic and chemopreventive properties of 6-(Methylsulfinyl) hexyl isothiocyanate on TK6 human cells by flow cytometry

Lenzi M

Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna

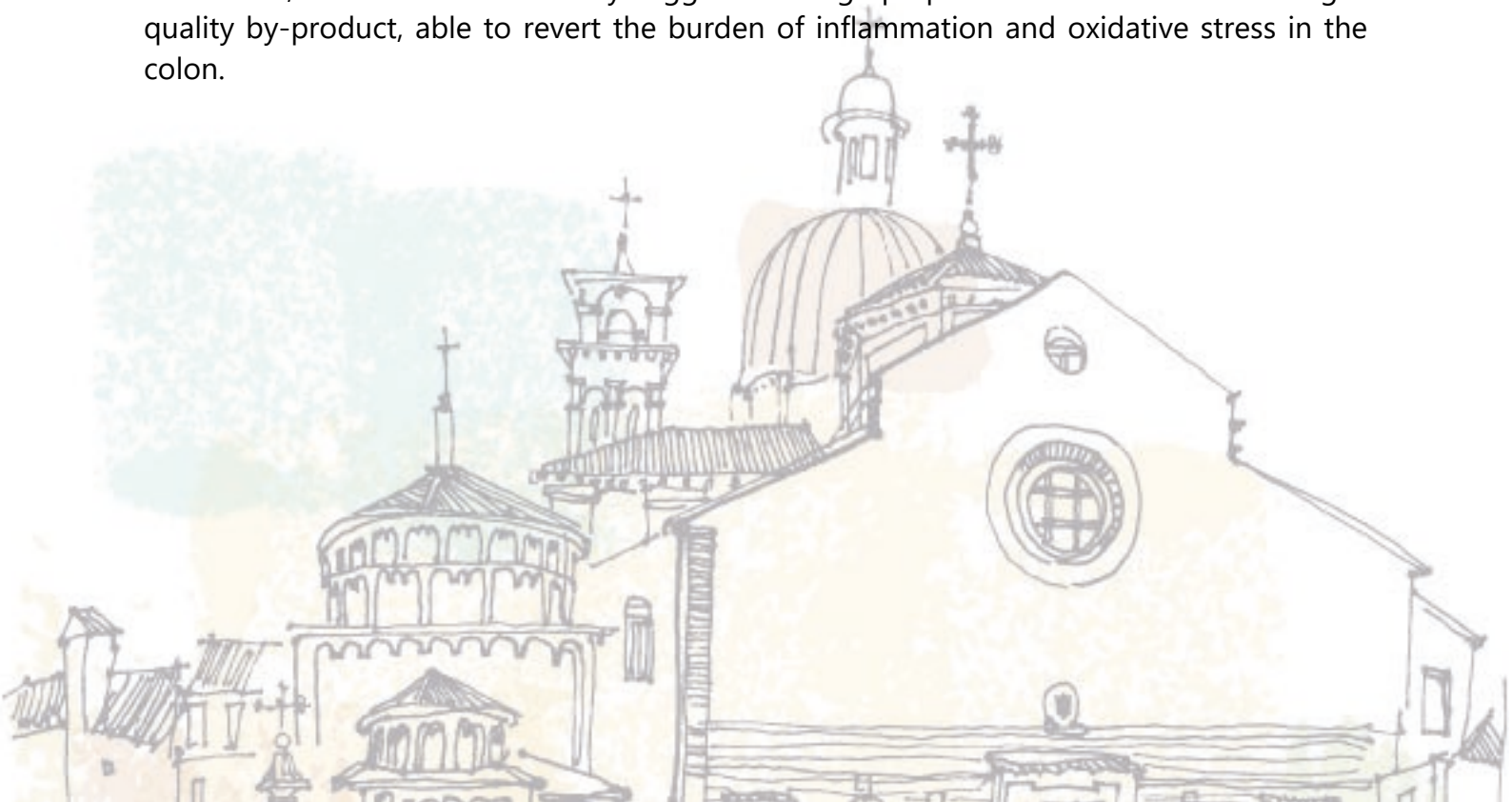
6-(methylsulfinyl) hexyl isothiocyanate (6-MITC), is the main bioactive compound present in Wasabia japonica rhizome. Several scientific studies have shown that 6-MITC possesses interesting antimicrobial, anti-inflammatory, antiplatelet and antioxidant properties which therefore suggested us it could have an interesting chemopreventive potential. In a previous study, we demonstrated, in two different leukemia cell lines, its ability to modulate several mechanisms supporting its antitumor activity. For this reason, we thought useful to continue the research, by investigating the potential antimutagenic activity of 6-MITC and thus better define its profile as a possible chemopreventive agent. 6-MITC antimutagenic effect against two known mutagenic agents: the clastogen Mitomycin C (MMC) and the aneuplodogen Vinblastine (VINB), was analyzed, in terms of micronuclei frequency decrease, after short- and long- time treatment on TK6 human cells, using a new automated protocol of the "In Vitro Mammalian Cell Micronucleous Test" by flow cytometry. The results showed a different behavior of the isothiocyante. In particular, 6-MITC was unable to counteract the MMC genotoxicity, but when it was associated with VINB a statistically significant decrease in the micronuclei frequency was registered. Overall, the results obtained suggest a potential antimutagenic activity of 6-MITC, in particular against the aneuploidogen agents. This ability, to inhibit or counteract the mutations at the cellular level has a great therapeutic value and it represents a mechanism through a chemopreventive agent can express its activity.

Phenolic composition of a pomace water extract of *Vitis Vinifera* L. and its protective effect in a colorectal cancer *in vitro* and *ex vivo* experimental model

Di Simone SC, Acquaviva A, Libero ML, Chiavaroli A, Recinella L, Leone S, Brunetti L, Orlando G, Menghini L, Ferrante C

Department of Pharmacy, Medicinal Plant Unit (MPU), Botanic Garden "Giardino dei Semplici", "G. d'Annunzio" University of Chieti-Pescara, Via dei Vestini, 66100 Chieti, Italy

Grape (*Vitis vinifera* L.) pomace is a solid by-product deriving from the winemaking process containing precious bioactive compounds which exert health-promoting properties, including antioxidant, cardioprotective, anticancer, antiinflammatory, antiaging, and antimicrobial properties. In particular, in the present study we analysed the phenolic composition of a water extract of grape pomace (WEGP) and we conducted in vitro studies on colorectal cancer cell line SW480 followed by an ex vivo investigation on isolated mouse colon tissue challenged with Escherichia coli lipopolysaccharide (LPS). Concerning the phenolic profile, 19 phytochemicals were identified through HPLC-DAD-MS and catechin was found to be the prominent flavonoid. The cell viability assay showed a significant reduction of SW-480 cell viability in both basal and LPS-induced inflammatory conditions. The grape pomace extract was also able to reduce vascular endothelial factor A (VEGFA), hypoxia-induced factor 1α (HIF1α), and transient receptor potential M8 (TRPM8) LPS-induced gene expression. Moreover, the extract inhibited mRNA levels of different pro-inflammatory bimarkers involved in colon inflammation, among which nuclear factor kB (NFkB), cyclooxygenase (COX)-2, tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-1β, IL-10, inducible nitric oxide synthase (iNOS), and interferon (IFN)γ, in isolated colon. Furthermore, WEGP increased the gene expression of antioxidant catalase (CAT) and superoxide dismutase (SOD), in the same model. All these modulatory effects exerted by the grape pomace extract under study, could be correlated - at least in part – to the phenolic content. In particular, the additional docking calculation we performed, confirmed the existance of interactions between catechin and TRPM8 receptor, deeply involved in colon cancer. In conclusion, the results of the study suggested the grape pomace water extract as a high-quality by-product, able to revert the burden of inflammation and oxidative stress in the colon.



Essential oil of *Lippia alba* (Mill.) and breast cancer

Borromeo I[1], De Luca A[1], Domenici F[2], Giordani C[3,4], Rossi L[1], Forni C[1]

[1] *Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica, 00133 Rome, Italy;*

[2] *Department of Chemical Science and Technologies, University of Rome "Tor Vergata", 00133 Rome, Italy;*

[3] *Instituto de Física, Universidad de Antioquia, Calle 70 No. 52-21, Medellìn 050010, Colombia;*

[4] *Grupo Productos Naturales Marinos, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Calle 70 No. 52-21, Medellín 050010, Colombia*

[a] *PhD School in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica, 00133 Rome, Italy*

Neoplastic diseases are the second cause of death worldwide. Although chemotherapy is the most common therapy against cancers, many pharmaceutical companies are focusing their efforts on the production and use of plant-based drugs, basing on their fewer side effects respect to the synthetic drugs currently available. Phytochemicals, commonly synthetized in fruits and vegetables have shown an influence on the development and progression of different tumors by inhibiting cancer cell proliferation and metastatic invasion. Such effects have been linked to their antioxidant and chemopreventive activities, which can be studied for possible future anti-cancer therapies.

Lippia alba (Mill.) N.E.Br. ex Britton & P. Wilson is an aromatic plant, which essential oil is used in folk medicine of Central and South America for its biological activities: i.e. cytotoxic, antifungal, antibacterial, antiviral and anti-inflammatory, but also antispasmodic, digestive, antiasthmatic, analgesic and sedative. The carvone/limonene chemotype is considered as the most important in South America. In the last years the interest on its antioxidant activity has increased and, nowadays, it is gaining economic importance going beyond the traditional uses in the tea and in the cosmetics industries. So far, few investigations in oncology on the possible use of this essential oil have been performed. The aim of this study was to test the antitumor activity of essential oil of *L. alba* on breast cancer lines.

Triple Negative Breast Cancer (TNBC) cell lines, which lacks the expression of the estrogen, progesterone and human epidermal growth factor receptors (ER^- PR^- $EGFR^-$), thus characterized by a great metastatic potential, were selected: SUM149 (a mesenchymal TNBC cells), MDA-MB-231 (a highly aggressive, invasive and poorly differentiated TNBC), MDA-MB-468 (a cell line with epithelial morphology that was isolated from a pleural effusion of a 51-year-old Black female patient with metastatic adenocarcinoma of the breast).

Preliminary tests were conducted on pure and diluted (1:500 in dimethyl sulfoxide) essential oil to determine the its best concentration, thus the cell lines were treated for 48 hours with plant essential oil. The results showed different responses of the cell lines to the same treatment.

In SUM149 and MDA-MB-231 cells the treatment with *L. alba* oil decreased proliferation, increased antioxidant activity and lipid peroxidation, and showed a high cytotoxic effect associated with the release of lactate dehydrogenase, compared with controls. While, no effect was observed in MDA-MB-468.

These results represent an excellent beginning for further studies to understand the beneficial and antineoplastic properties of *Lippia alba* essential oil and its possible future use in cancer therapy.

Erucin isolated from *Eruca sativa* Mill. seeds, induces apoptosis of human melanoma cells and modulates their aggressiveness *in vitro*

Maresca DC, Conte L, Romano B, Ianaro A, Ercolano G
Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, 80131 Naples, Italy

Melanoma is the most dangerous form of skin cancer and is characterized by chemotherapy resistance and recurrence despite the new promising therapeutic approaches. In the last years, erucin (ERU), the major isothiocyanate present in Eruca sativa, commonly known as rocket salads, has demonstrated great efficacy as an anticancer agent in different in vitro and in vivo models. More recently, the chemopreventive effects of ERU have been associated with its property of being a H2S donor in human pancreatic adenocarcinoma. Here, we investigated the effects of ERU in modulating proliferation and inducing human melanoma cell death by using multiple in vitro approaches. ERU significantly reduced the proliferation of different human melanoma cell lines. A flow cytometry analysis with annexin V/PI demonstrated that ERU was able to induce apoptosis and cell cycle arrest in A375 melanoma cells. The proapoptotic effect of ERU was associated with the modulation of the epithelial-to-mesenchymal transition (EMT)-related cadherins and transcription factors. Moreover, ERU thwarted the migration, invasiveness and clonogenic abilities of A375 melanoma cells. These effects were associated with melanogenesis impairment and mitochondrial fitness modulation. Therefore, we demonstrated that ERU plays an important role in inhibiting the progression of melanoma and could represent a novel add-on therapy for the treatment of human melanoma.

Synergistic effect of plant extracts and anticancer drugs to improve therapeutic efficacy and overcome drug-induced resistance in cancer models

Rigogliuso S[1], Poma P[1], Adamo G[2], Labbozzetta M[1], Notarbartolo M[1]

[1]*Department of Biological, Chemical and Pharmaceutical Science and Technology (STEBICEF), University of Palermo, 90128 Palermo, Italy.*

[2]*Cell-Tech HUB at Institute for Research and Biomedical Innovation, National Research Council of Italy (CNR), Palermo 90146, Italy.*

Therapeutic and pharmacological approaches in the treatment of cancer are constantly evolving, however, one of the main problems remains the development of multidrug resistance (MDR). This phenomenon is complex and multifactorial; constitutive activation of the NF-κB pathway has been observed in several solid and hematologic tumors and has been associated with oncogenesis, being involved in the control of several key mechanisms, including anti-apoptotic and resistance gene regulation. The MDR phenotype is often linked to the over-expression of MDR1 gene encoding for P-gp efflux pumps. (1) The main pharmacological strategy, object of preclinical and clinical research, to overcome MDR and revert the resistant phenotype, is represented by the co-administration of anticancer drugs and P-gp substrate-inhibitors, to restore the effective therapeutic concentration of the drug in the resistant tumor cells. The P-gp inhibitors used so far have not produced results that can be transferred to the clinic, due to the numerous toxic effects that characterize these molecules. (2) In fact, P-gp physiologically performs an important protective function in all cells, therefore the search for inhibitory molecules, free of toxicity for healthy tissues, is urgently needed. (3) The identification of peculiar mechanisms of natural molecules, such as terpenes, curcuminoids, flavonoids, both on the expression and on the function of P-gp, seems to have the advantage of a low toxicity with the same efficacy of other inhibitors. (1) We have identified several molecules, all present in natural foods, able to interfere with P-gp mediated MDR; in this context, we focused our attention on the effect of curcumin and heptacosane on two different resistant models of solid and hematological tumors. Our data show that curcumin can interfere on the P-gp expression through inhibition of NF-κB pathway and acts contextually as a direct inhibitor of its function, both in an acute myeloid leukemia (AML) cell line (HL60-R) and in breast cancer (BC) cell line (MCF-7R). Heptacosane, has an exclusive action on the pump function, characterized by a strong interaction with the drug-binding pocket site (DBP), whereby it assumes a conformation that could close the efflux pump and a binding site of the nucleotide-binding pocket (NBP), such as verapamil. Both compounds promote the accumulation of a pump substrate, doxorubicin, although it would appear that heptacosane has specific tumor action against AML. Our data underline the possibility to use non-toxic P-gp inhibitors, in combination with different anticancer chemotherapy drugs, to increase their efficacy in leukemia and breast cancer patients with MDR phenotype. Furthermore, since P-gp is physiologically expressed in healthy tissue, our aim will also be the specific delivery of inhibitory molecules and anticancer drugs, using extracellular vesicles (EV) isolated from plants (4) to protect the healthy tissue. EVs are currently considered one of the most promising therapeutic effectors of cellular origin. (5) We believe that this strategy could be useful to improve the therapeutic approach in different MDR tumor models without producing greater toxicity.

The protective role of troxerutin (TX) to counteract anaplastic thyroid carcinoma (ATC) progression

Paterniti I, Bova V, Casili G, Filippone A, Repici A, Cuzzocrea S, Esposito E
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences University of Messina

Anaplastic thyroid carcinoma (ATC) is a rare thyroid neoplasm characterized by high aggressiveness and a high mortality rate. Conventional therapy consisting of surgery, cytotoxic chemotherapy and radiation therapy is ineffective, so a large proportion of patients are often referred for palliative care. Due to poor survival outcomes, it is necessary to investigate new therapeutic strategies to counteract the progression of ATC. Troxerutin (TX) is a bioflavonoid widely found in various fruits and vegetables that has been shown to exhibit numerous pharmacological properties, including antineoplastic and anticancer activities. The aim of this study was to evaluate the antioxidant and anti-inflammatory properties of TX to counteract cancer progression in an in vitro model of ATC.

Human ATC 8305C cell lines were treated with increasing concentrations of TX (10 µg/ml, 30 µg/ml, 100 µg/ml, 300 µg/ml) to evaluate their effect on cell viability by MTT assay. After identifying the most cytotoxic concentrations, cell lysates were performed in order to evaluate the effects of TX on apoptosis, oxidative stress and inflammation markers. In addition, the effects of TX on cell migration were evaluated by the Wound Healing assay, while the involvement of the inflammatory response was tested by enzyme-linked immunosorbent assay (ELISA).

Our results revealed that TX at concentrations of 100 µg/ml and 300 µg/ml, was able to reduce ATC cell viability. TX significantly reduced the expression of anti-apoptotic factors such as BCL-2 and increased the expression of pro-apoptotic factors such as Caspase-3 and BID. Then, TX appears to be involved in the modulation of oxidative stress mediators, such as MnSOD, HO-1, GSH and ROMO-1. Furthermore, TX has been shown to reduce the migratory ability of tumor cells and to modulate non-canonical NF-κB pathway markers, such as NIK and TRAF-6.

Therefore, based on these results, the use of TX could be considered a promising strategy to counteract anaplastic thyroid cancer progression, thanks its antioxidant and anti-iflammatory effects and its ability to modulate apoptosis pathway.

The beneficial role of resveratrol in a murine model of lung allergic inflammation

Cerqua I[1], Granato E[1], Rossi A[1], D'Avino D[1], Capasso R[2], Cirino G[1], Roviezzo F[1]

[1]*Department of Pharmacy, University of Naples Federico II, Italy*

[2]*Department of Agricultural Sciences, University of Naples Federico II, Naples (Italy)*

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a non-flavonoid polyphenolic molecule found in several different plant species, such as grapes and berries. This molecule is known for its antioxidant and anti-inflammatory properties and a positive association of resveratrol intake with lung function has been observed in the general population. The impairment of lung function is the basis of pathologies like asthma. Asthma is a long-term inflammatory disease of the airway driven by a T-helper 2(Th2) immune response characterized by cough, wheezing, shortness of breath, and chest tightness. Finding an effective treatment that can reduce the concomitance of all these symptoms is a crucial issue for clinicians. Indeed, the current treatment requires β2-agonists and glucocorticoids as the standard therapy, but this provides not only the appearance of side effects but also interpersonal variability. In this contest, resveratrol could be a valid option in the prevention of asthmatic symptoms. Thus, we have investigated the role of resveratrol in an experimental model of asthma. Resveratrol efficacy was first evaluated in-vitro on bronchi harvested from naïve BALB/c mice. Then, an experimental model of asthma was reproduced by treating BALB/c mice with a subcutaneous administration of ovalbumin (OVA,100μg) at days 0 and 8. Part of the mice was intraperitoneally treated with resveratrol (OVA-RESV,10mg/kg, 30 minutes before OVA administration).

In-vitro experiments confirmed the ability of resveratrol in reducing the cholinergic tone of airway smooth muscle. Also, the in-vivo resveratrol treatment showed a beneficial effect on OVA sensitization. In fact, the OVA sensitization was confirmed by the increase of the plasmatic level of IgE ($p<0.05$), and by bronchial hyperreactivity ($p<0.0001$) compared to saline control mice. The resveratrol treatment affected the sensitization phase by reducing IgE in the plasma samples ($p<0.05$). Further, a significant effect was also observed in the reduction of OVA-induced bronchial hyperreactivity ($p<0.0001$) as well as the pulmonary level of the asthma-related Th2 cytokines, interleukin-4, and interleukin-13 ($p<0.05$). The protective effect on lung function well correlates with the restoration of lung structure and the reduction of pulmonary congestion evaluated by hematoxylin-eosin staining. Besides the anti-inflammatory effect, the immunohistochemical analysis confirmed the activity of resveratrol on airway smooth muscle by reducing the α-smooth muscle expression as well as the expression of procollagen type I N-propeptide (PINP) and procollagen type III N-propeptide (PIIINP).

In conclusion, these data confirmed the protective role of resveratrol in preventing the development of the cardinal features of asthma-like disease such as bronchial hyperreactivity and Th2 inflammatory response.

Anti-inflammatory and healing activity of *Origanum vulgar*e essential oil on human keratinocytes cultures

Avola R[1], Granata G[2], C. Geraci C[2], Napoli E[2], Graziano ACE[1], Russo A[3], Cardile V[1]

[1]*Department of Biomedical and Biotechnological Sciences, University of Catania, Via Santa Sofia, 97, 95123, Catania, Italy;*

[2]*Institute of Biomolecular Chemistry, National Research Council (C.N.R.), Via Gaifami, 18, 95026, Catania, Italy;*

[3]*Department of Drug Sciences, University of Catania, Via S. Sofia 64, 95125, Catania, Italy.*

The inflammatory skin diseases, especially if chronic, can have a negative influence on the individual physical, emotional and social well-being. Conventional treatments often involve the use of synthetic drugs that are sometimes ineffective and with side effects. Therefore, for the treatment of numerous inflammatory skin disorders, researchers have focused their attention on the use of natural compounds, both as oral food supplements and as topical formulations with high tolerability, which are gaining increasing popularity and which for their positive effects turn out to be an attractive approach.

Among them, the essential oil from *Origanum vulgare* (OEO), a pervasive aromatic plant of the *Lamiaceae* family typical of the Mediterranean flora, is widely used in folk medicine as a remedy against numerous diseases. Although these promising activities seem to result from the synergism of some major components, such as the phenolic compounds thymol and carvacrol, and other minor components, including monoterpene hydrocarbons such as p-cymene and γ-terpinene, the molecular mechanisms by which they exert their pharmaceutical effects are not well understood.

The aim of this study was to evaluate the antioxidant/anti-inflammatory and/or healing capacity of OEO on a human keratinocyte cell line (NCTC 2544), treated with IFN-γ and histamine reproducing an in vitro model of inflammation.

OEO characterization was performed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The viability of the treated and control cells was assessed by the tetrazolium salt metabolization test (MTT) and the crystal violet staining. Antioxidant activity was measured by determination of reactive oxygen species (ROS) and superoxide dismutase (SOD)-1 levels and 1,1-diphenyl-2-picril-hydractil (DPPH) assay. DNA damage was evaluated by analysis of 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels. The anti-inflammatory capacity of OEO has been verified through the measurement of some markers involved in inflammation, such as cyclooxygenase-2 (COX-2), intracellular adhesion molecules-1 (ICAM-1), inducible nitric oxide synthetase (iNOS), proliferating cell nuclear antigen (PCNA), by Western blot analysis and Reverse Polymerase Chain Reaction (RT-PCR). Extracellular matrix (ECM) conditions were examined by evaluation of gene expression and protein synthesis of metalloproteinases (MMPs)-1 and -12. Finally, the "scratch test" was used to evaluate the healing capacity of OEO.

Compared to the untreated control, OEO showed high antioxidant activity and radical oxygen scavenging capabilities. In IFN-γ and H treated cells, OEO displayed a significant reduction of ROS, ICAM-1, iNOS, COX-2, 8-OhdG. Moreover, modulated the expression of MMP-1 and MMP-12 and promoted cell proliferation in scratch test cultures.

This study made clearer some mechanisms involved in the ability of OEO to reduce some parameters of inflammation and to promote cell motility during the healing process.

Protective effects of a commercial extract of *Astragalus membranaceus* in *in vitro* and *in vivo* models of inflammation

D'Avino D, Cerqua I, Granato E, Di Matteo R, Ullah H, Ialenti A, Daglia M, Roviezzo F, Rossi A
Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy

Astragalus membranaceus (AM) (Fisch.) *Bunge* is one of the most well-known medicinal species from *Astragalus* genus and its root (Astragali Radix) is used as herbal medicine. AM contains various bioactive compounds including triterpenoid saponins, flavonoids, and flavonoid glycosides. It has numerous biological activities including tonic, hepatoprotective, diuretic and expectorant and has been shown to exhibit immunomodulating, antihyperglycemic, antioxidant, and antiviral activities[1]. Because of its abundant biological activities, in recent years, it has gradually been applied to food supplements. In this study, we evaluated the effects of a commercial extract of AM, used as ingredient of food supplements following the Italian D.M 10 August 2018, in regulating the immune-inflammatory response in *in vitro* (lipopolysaccharide (LPS)-stimulated J774 macrophages and A23187-stimulated murine peritoneal macrophages (PM)) and in non-allergic and allergic *in vivo* experimental models of inflammation (mouse zymosan-induced peritonitis and allergen-induced sensitization).

Ultra-high-performance liquid chromatography high-resolution mass spectrometry analysis of the commercial AM extract showed the presence of several flavonoids of which the most abundant were formonetin, glycitein and biochanin A. In the presence of increasing concentrations of the AM extract (0.25-1 mg/mL, 2 h before LPS) a concentration-dependent and significant inhibition of prostaglandin (PG)E_2 and leukotriene (LT)C_4 production was observed in LPS-stimulated J774 and in A23187-stimulated PM, respectively. Similarly, the extract inhibited cytokine and NO production, affecting iNOS expression and ERK-1/2 phosphorylation in J774 macrophages. The commercial AM extract showed also anti-inflammatory effects in *in vivo* models. Indeed, the pre-treatment of animals with AM extract (100 mg/kg) 30 min before zymosan injection significantly reduced the peritoneal cellular infiltration and the production of pro-inflammatory mediators such as NOx, PGE_2, LTB_4, LTC_4, IL-1β and TNF-α. Similarly, in the ovalbumin (OVA) model of asthma, the pre-treatment with AM extract reversed OVA-induced bronchial hyperreactivity and partially restored the adrenergic bronchial relaxation. The protective effects on airway reactivity well correlated with positive effects of extract on airway remodelling and fibrosis (evaluated as α-SMA expression and Masson's Trichrome staining, respectively), and lung injury (evaluated with hematoxylin-eosin staining). The effects of AM extract were coupled to inhibition of the sensitization process, as indicated by a reduction of IgE plasma levels and pulmonary Th2 cytokine (IL-13 and IL-4) production induced by OVA sensitization.

In conclusion, our data demonstrated that this commercial extract of AM exerts *in vitro* and *in vivo* anti-inflammatory effects, suggesting its use as nutraceutic compound in inflammatory diseases.

Beneficial effects of a topical gel containing xyloglucan and pea protein to treat phenol-induced oral ulcers *in vivo*

Casili G, Cucinotta L, Campolo M, Paterniti I, Lanza M, Filippone A, Basilotta R, Cuzzocrea S, Esposito E

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres, 98166 Messina, Italy

Aphthous stomatitis, also known as canker sores, is characterized by recurrent painful aphthous ulcers and affects approximately 5 to 25% of the general population. The cause of aphthous stomatitis is idiopathic and multifactorial but is frequently triggered by an acute inflammatory response. Lifestyle modifications and topical treatments are often used to manage symptoms, but no definitive cure exists. Several studies have shown that a dysfunction of the oral epithelial tissue might be one of the initiating factors for aphthous stomatitis development. This study aims to evaluate the ability of a topical gel/ containing xyloglucan and pea protein (TXP/MD), two mucoprotective substances, to restore the integrity of the oral epithelial cells and manage inflammation associated with oral ulcers.
Oral ulcers were induced in Wistar rats by placing a cotton ball previously dipped into a solution of phenol, onto the left cheeks. After 24 h, all rats developed ulcers of 3 mm in diameter on the oral mucosa. TXP/MD was applied on the oral mucosa once a day for 2, 4 or 5 days from the day of ulcer induction. The oral mucosa from each group was processed to assess the degree of inflammation, hyperemia, and oedema. The left cheek tissue in rats treated with phenol were significantly infiltrated by neutrophils after 24 hours. TXP was able to effectively treat oral ulcer by reducing the degree of hyperemia and oedema in cheek tissue (figure 1) already at day 4. Moreover, TXP/MD significantly decreased the levels of inflammatory cytokines such as IL-2 and TNF-alpha after 4 days of gel application. A disrupted epithelial barrier can expose the immune cells of the lamina propria to oral antigens, which triggers the inflammatory response, a typical characteristic of canker sores. This study demonstrates the significant role of MD/TXP to counteract phenol-induced oral ulcer *in vivo*. MD/TXP contains mucomimetic substances that can effectively manage oral ulcers by restoring the oral mucosal tissues integrity, helping to reduce inflammation. TXP/MD represents an alternative safe and effective treatment that targets aphthous stomatitis at the source.

Immunomodulatory properties of *Ulva pertusa* extract in a mouse model of DNBS-induced colitis

Filippone A, Mannino D, Ardizzone A, Casili G, Lanza M, Cucinotta L, Campolo M, Esposito E.
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres, 98166 Messina, Italy

Inflammatory bowel diseases (IBDs), including Crohn's Disease (CD) and Ulcerative Colitis (UC), represent gastrointestinal (GI) disorders in which abdominal pain, discomfort, and diarrhea are the major symptoms. The immune system has been considered to play an important role in the pathogenesis of IBD and, in particular, the innate immune response has the faculty to induce gut inflammation in IBD patients. Indeed, an inappropriate mucosal immune response to normal intestinal constituents is a main feature of IBDs, thus leading to an imbalance in local pro- and anti-inflammatory species. In this perspective, natural compounds have been suggested as a valid approach to the management of various GI diseases. The green alga *Ulva pertusa*, belonging to the Ulvaceae family, showed powerful antioxidant and anti-inflammatory properties in the context of IBDs. We aimed to evaluate the effect of *Ulva pertusa* extract on the immunoinflammatory pathways as well as in the relief of abdominal pain through a murine model of Dinitrobenzene sulfonic acid (DNBS)-induced colitis. The *in vivo* model was induced by DNBS intrarectal installation (4 mg in 100 µl of 50% ethanol) while *Ulva pertusa* treatments were administered orally every day up to day 5. *Ulva pertusa* treatments significantly reduced abdominal pain and modulated the immune system and related-inflammatory pathways that were altered after colitis induction. We demonstrated the immunomodulator ability of *Ulva pertusa* for inflammatory pathologies, suggesting its potential therapeutic use in IBDs.

A geranium for the health of lungs: a multi-target herbal product against viral infections

Carloni D.

Italian Society of Phytotherapy, Department of Physical Sciences, Earth and Environment, University of Siena, Siena, Italy.

The recent pandemic has severely tested the health organizations of all countries all over the world, both for the difficulty in establishing the correct therapeutic intervention but also for the serious social and health consequences determined by the infection. Viral infections have a high impact on the health of the affected populations, frequently causing situations of difficulty in the recovery of general health conditions and in particular in the correct restoration of the physiological immune response. It would be interesting to identify solutions that can allow healtcare professionals (HCPs) to effectively deal with some infections such as those caused by the influenza virus or more generally caused by respiratory viruses, without producing significant adverse reactions. Phytotherapy can be a useful tool, in particular if preparations with a certified and reproducible action such as Eps®7630 are taken into consideration, which is the standardized extract obtained from the roots of *Pelargonium sidoides* DC. It is a Traditional Herbal Medicinal Product that is indicated against numerous viral infections, in particular those caused by respiratory viruses; these properties, which are supported by several pre-clinical and clinical studies, confirmed by EMA Monographs and reviews such as those of the Cochrane Collaboration. The active constituents responsible for the activities of this geranium are mainly phenolic compounds such as gallic acid alongside more complex structures such as oligomeric and polymeric proanthocyanidins and gallotannins; the phytocomplex also highlights as markers of various species and rare oxygenated coumarins, including umkalin and artelin. The mechanism of action that justifies the immunomodulatory properties of Eps®7630 is essentially related to the support of the innate immunity response by modulating the activation and production of inflammatory citokines by macrophages. The response to aggression by various respiratory viruses is due to multiple antiviral factors that Eps®7630 up-regulates such as interferon involvement, activation of Natural Killer cells, release of defensins, cooperation with vitamin D activity and the important counteracting effect against oxidative stress caused by infection. The multi-target anti-infective action of *P. sidoides* is also effective against bacterial complications, showing *in vitro* activity; it should also be outlined that this natural product is able to control various clinical signs related to respiratory tract infections complications, thanks to secretolytic and secretomotor effects. The activities of this herbal products have been widely evaluated in adults, but there are studies with positive results also in the paediatric population and the elderly. The modulation of the immune response to virus aggression will be maximal by administering *P. sidoides* extract at the first signs of infection, i.e. when the immune system is activated. It is important to note that the activity of Eps®7630 has been confirmed against both acute and chronic problems and its safety is demonstrated after millions of doses administered.

***Valeriana officinalis*, *Ziziphus juhuba* and *Humuls lupulus* with melatonin: studies about neuromodulatory and protective effects for counteracting sleep disorders**

Libero ML, Chiavaroli A, Di Simone SC, Acquaviva A, Nilofar, Brunetti L, Recinella L, Leone S, Orlando G, Menghini L, Ferrante C

Department of Pharmacy, Botanic Garden "Giardino dei Semplici", "G. d'Annunzio" University, Via dei Vestini 31, 66100 Chieti, Italy

Before the onset of a specific therapy with first-line drugs for treating sleeping disorders, herbal extracts could represent an advantageous approach. In this study extracts from *Valeriana officinalis*, *Ziziphus jujuba*, *Humulus lupulus*, melatonin, and their pharmacological association were studied for potential application in the treatment of insomnia. [1,2] The neuromodulatory and protective effects induced by the extracts and melatonin was tested on hypothalamic neurons and tissue for evaluating biocompatibility. By orexin A gene expression and serotonin steady state level, in the hypothalamus, were evaluated neuromodulatory effects. In the present study, *V. officinalis* extract and melatonin were more ineffective than *H. lupulus* and *Z. jujuba* in modifying hypothalamic serotonin level. Thanks these studies, the extracts and melatonin showed antioxidant and anti-inflammatory properties and they were, furthermore, able to decrease the hypothalamic gene expression of orexin A and the steady state level of serotonin. In conclusion, the present study demonstrated the efficacy of the combination of extracts with melatonin, in particular the intrinsic scavenging/reducing activity, for the treatment of insomnia. Additionally, this study showed the protective and neuromodulatory effects in the hypothalamus, with a significant reduction of both orexin A gene expression and serotonin steady state level. However, further studies prove necessary for confirming the pharma-toxicological profiles, in terms of both efficacy and safety.

Evaluation of the efficacy of honokiol, neolignan from *Magnolia Officinalis* Rehder & E.H Wilson, in an *in vitro* model of microglial cellular senescence

Sasia C, Borgonetti V, Galeotti N

Università degli studi di Firenze, Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino (NEUROFARBA)

Honokiol (HNK), a neolignan extracted from the bark of *Magnolia Officinalis Rehder & E.H Wilson*, has been known since ancient times for its anti-diabetic, anti-cancer, antioxidant and anti-inflammatory activities (Rauf et al., 2021). Inflammation and cellular senescence are closely related to tumors, but also to neurological disorders, such as chronic pain. Inflammation and aging are significant modifier of microglial functions (Angelova & Brown, 2019). Microglia are considered resident macrophages of the central nervous system (CNS), where represent 10-15% of immune cells. They are involved in the maintenance of homeostasis, protection against pathogens and disorders of the CNS (Ginhoux & Prinz, 2015).Senescent-microglia profile is characterized by an hyperactivation leading to morphological changes, with less dendritic branching and a "dystrophic" morphology, an altered surveillance phenotype, a major activity and expression of β-galactosidase and a more sustained inflammatory response in reaction to damage, with an increased release of SASP (secretory phenotype associated with senescence) factors, including pro- inflammatory cytokines and ROS (Greenwood & Brown, 2021). In this work we investigated the effect of HNK in reducing microglial senescence and inflammation using an *in vitro* model of senescence and neuroinflammation, using microglial cells, in order to evaluate their therapeutic potential in the treatment of neurological disturbances.

In this work we investigated the effect of honokiol (HNK) and its active concentration in an *in vitro* model of microglial senescence, treating BV2 cells with LPS 500 ng/mL for 10 days (4h/day) every 72h, and in an *in vitro* model of neuroinflammation, treating BV2 cells with LPS 250 ng/ml. HNK was tested at four different concentrations (0.1, 1, 3 and 10 µM) in order to find the active concentration. At the end, specific tests were performed to evaluate the development of senescence and neuroinflammation and the effectiveness of the treatments. In the microglial senescence model, treatment with HNK (0.1, 1, 3 and 10 µM) was able to modulate cellular senescence parameters by reducing the expression and activity of β-galactosidase, a marker of cellular senescence. An increase in cell viability and a reduction in cell branching typical of dystrophic microglial cells were also observed. Evaluation of SASP (senescent associated secretory phenotype) showed that the treatments are also effective in reducing these markers. In the neuroinflammation model, treatment with HNK (0.1, 1, 3 and 10 µM), was able to reduce the number of activated cells and cell branching through morphological analysis and increase in the expression of the anti-inflammatory cytokine IL-10.

In conclusion, we can say that HNK possesses senolytic and senomorphic activity and is able to attenuate neuroinflammation. Therefore, we hypothesise that this treatment may be promising candidate in the management of neurological disorders associate to neuroinflammation and cellular senescence.

Gamma-oryzanol: a new drug repositioning as a neuroprotective compound

Abate G[1,2], Pucci M[1], Tirelli E[1], Bonini SA[1,2], Mastinu A[1,2], Maccarinelli G[1], Zizioli D[1], Pezzotta A[3], Grilli M[4], Memo M[1,2], Uberti D[1,2]

[1]Università degli Studi di Brescia, Dipartimento di Medicina Molecolare e Traslazionale (DMMT), Sezione di Farmacologia, Brescia.

[2]NutriFun4Health, Center for Research and Services on Nutraceuticals and Functional Food for Health, Università degli Studi di Brescia, Brescia.

[3]Università degli Studi di Milano, Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Milano.

[4]Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze del Farmaco, Laboratorio di Neuroplasticità, Novara.

γ-Oryzanol (ORY) is a mixture of ferulic acid esters and triterpene alcohols, found mainly in rice (Oryza sativa L) bran. ORY is known for its antioxidant and anti-inflammatory action, and it is also registered as a prescription drug in Japan for its anti-lipidemic and anti-glycemic properties. Our research group has demonstrated the action of ORY as an activator of the transcription factor NRF2. Our work shows that chronic administration of ORY in adult mice improves cognitive performance and is able to protect against LPS-induced neuroinflammatory damage. In addition, we showed ORY is able to modulate at the hippocampal level the expression of proteins involved in synaptic plasticity, neuroprotection, and mitochondrial metabolism. Our data also demonstrated how ORY is able to modulate neurogenesis by directing addressing progenitor cells toward a neuronal phenotype. The neurogenic effect was also demonstrated in a zebrafish Tg*(-3.1neurog1:GFP*) model. This effect is nullified in morphants in which the *nrf2a* and *nrf2b* genes have been silenced, suggesting that the neurogenic and neurotrophic action of ORY is mediated by NRF2.
Therefore, our results suggest that ORY is a potential molecule of pharmacological interest with neuroprotective action, being the Nrf2-ARE signaling system a candidate element in the design of novel therapeutic agents for brain disorders.

Anti-hyperalgesic properties of *Echinacea purpurea* in a mouse model of chemotherapy-induced neuropathy: alkamides versus polyphenols efficacy

Lucarini E[1], Micheli L[1], Maggini V[2], Ciampi C[1], Gallo E[2], Bogani P[3], Fani R[3], Pistelli L[4,5], Di Cesare Mannelli L[1], De Leo M[4,5], Firenzuoli F[2], Ghelardini C[1]

[1]*Department of Neurosciences, Psychology, Drug Research and Child Health—Neurofarba—Section of Pharmacology and Toxicology, University of Florence, Florence, Italy*

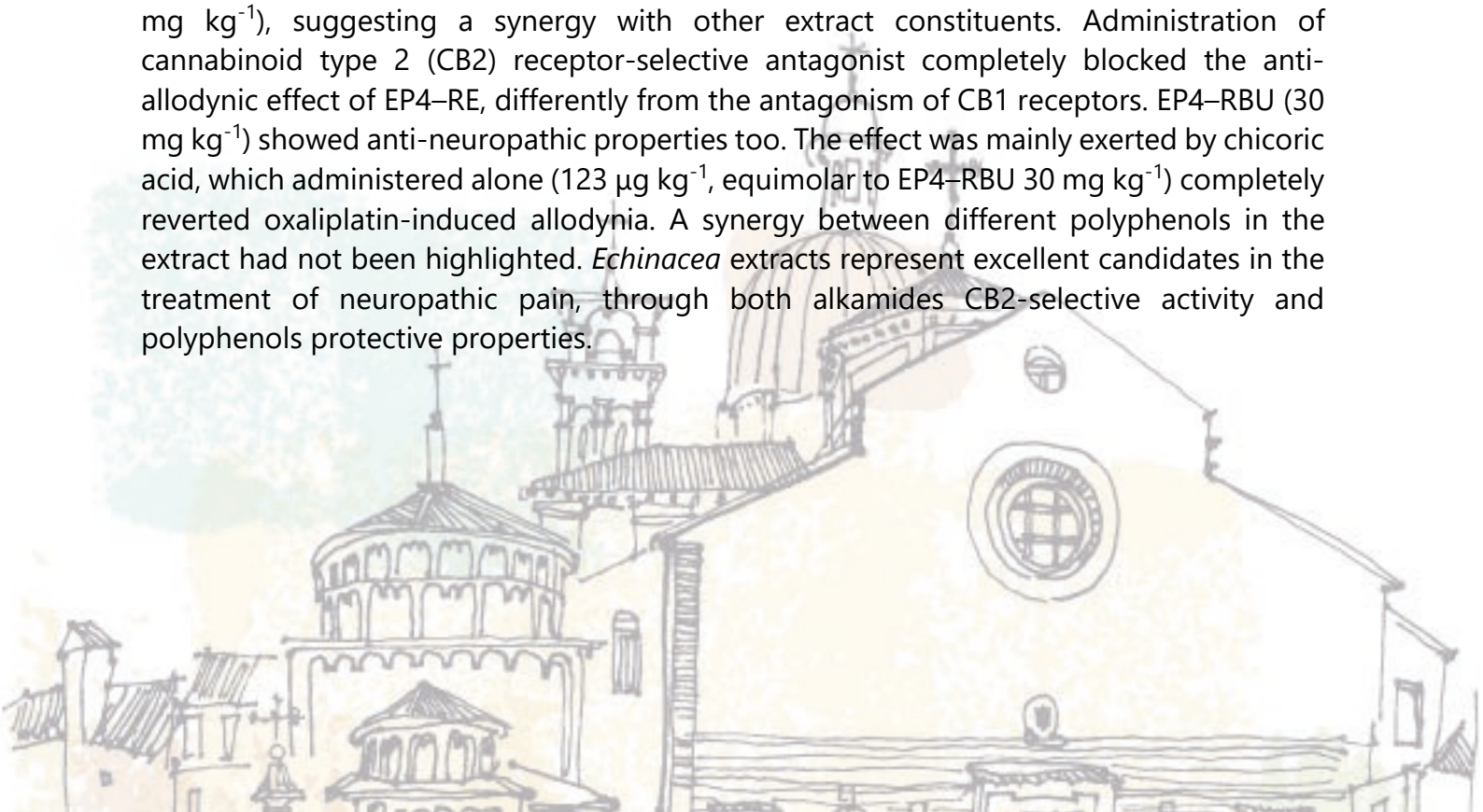
[2]*Research and Innovation Center in Phytotherapy and Integrated Medicine (CERFIT), Referring Center for Phytotherapy, Tuscany Region, Careggi University Hospital, Florence, Italy*

[3]*Department of Biology, University of Florence, Sesto Fiorentino, Italy*

[4]*Department of Pharmacy, University of Pisa, Pisa, Italy*

[5]*CISUP, Centre for Instrumentation Sharing Pisa University, Pisa, Italy*

Chemotherapy-induced neuropathy represents the main dose-limiting toxicity of several anticancer drugs, such as oxaliplatin, leading to chronic pain and an impairment of the quality of life. The management of chemotherapy-induced neuropathic pain is still difficult because of the limited efficacy and tolerability of available drugs. For this reason, identifying new effective and safe therapeutic strategies remains a strong medical need. *Echinacea purpurea* (L.) Moench (Compositae), is a plant known to relieve different types of inflammatory and neuropathic pain. While the action of the alkylamide component is well known, little is known about the analgesic efficacy of the other components. The purpose of this work was to assess the anti-hyperalgesic efficacy of two *Echinacea purpurea* extracts, a *n*-hexane extract (EP4–RE, rich in alkamides) and a butanolic extract (EP4–RBU, rich in polyphenols such as chicoric acid, caftaric acid and chlorogenic acid) obtained from plants grown *in vitro*. Both extracts have been characterized and tested in a mouse model of oxaliplatin-induced neuropathic pain, addressing the endocannabinoid system with alkamides and counteracting the redox imbalance with polyphenols. Thermal hypersensitivity was evaluated by the Cold Plate test. EP4–RE showed a dose-dependent anti-hyperalgesic profile. The extract was more effective than its main constituent, dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide (18 mg kg^{-1}, twofold to equimolar EP4–RE 30 mg kg^{-1}), suggesting a synergy with other extract constituents. Administration of cannabinoid type 2 (CB2) receptor-selective antagonist completely blocked the anti-allodynic effect of EP4–RE, differently from the antagonism of CB1 receptors. EP4–RBU (30 mg kg^{-1}) showed anti-neuropathic properties too. The effect was mainly exerted by chicoric acid, which administered alone (123 µg kg^{-1}, equimolar to EP4–RBU 30 mg kg^{-1}) completely reverted oxaliplatin-induced allodynia. A synergy between different polyphenols in the extract had not been highlighted. *Echinacea* extracts represent excellent candidates in the treatment of neuropathic pain, through both alkamides CB2-selective activity and polyphenols protective properties.



The transcription factor Nrf2 mediates the effects of *Antrodia camphorata* extract on neuropathological changes in a mouse model of Parkinson's disease

Capra AP, Lanza M, Cucinotta L, Casili G, Filippone A, Basilotta R, Campolo M, Paterniti I, Cuzzocrea S, Esposito E

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres, 98166 Messina, Italy

Parkinson's disease (PD) is a disorder that is characterized by progressive and selective neuronal injury and cell death. Recent studies provided accumulating evidence for a significant role of the immune system and neuroinflammation in PD pathogenesis. On this basis, many scientific articles have highlighted the anti-inflammatory and neuroprotective properties of Antrodia Camphorata (AC), an edible fungus containing various bioactive compounds. This study aimed to evaluate the inhibitory effect of AC administration on neuroinflammation and on oxidative stress, in a murine model of MPTP-induced dopaminergic degeneration. AC (10, 30, 100 mg/kg) was administered daily by oral gavage starting from 24 h after the first administration of MPTP and mice were sacrificed 7 days after MPTP induction. In this study, treatment with AC significantly reduced the alteration of PD hallmarks, increasing tyrosine hydroxylase expression and reducing the number of alpha-synuclein positive neurons. In addition, AC treatment restored the myelination process of neurons associated with PD and attenuated the neuroinflammatory state. Furthermore, our study demonstrated that AC was able to reduce oxidative stress induced by MPTP injection. In conclusion, this study highlighted that AC could be a potential therapeutic agent for the treatment of neurodegenerative disorder, as PD.

Cannabis sativa L. essential oils: anti-inflammatory and neuroprotective potential in a microglia cell model

Pieracci Y[1], Ascrizzi R[1,2], Russo L[1], Giacomelli C[1], Martini C[1], Trincavelli ML[1], Montanari M[3], Fulvio F[3], Bassolino L[3], Paris R[3], Pistelli L[1,2], Flamini G[1,2]

[1]*Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italia*

[2]*Centro Interdipartimentale 'NUTRAFOOD', Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italia*

[3]*CREA — Centro di Ricerca per Cerealicoltura e Colture Industriali, Via di Corticella 133, 40128 Bologna, Italia*

Cannabis sativa L. essential oil (EO) represents a high-value by-product obtained from the plant inflorescences, usually considered crop waste. In recent decades, hemp EO has gained great interest due to its several applications in various sectors. Since biological activities are strictly related to the content of secondary metabolites, a deeper knowledge of EO production and chemical composition is desirable to promote its use as a valuable by-product. Our previous studies (Pieracci et al., 2021, 2023) highlighted the influence of the hemp genotype and year of cultivation, as well as its phenological stage, on both the EO chemical composition and hydrodistillation yield. Despite its variability, hemp EO is consistently characterized by terpenes and phytocannabinoids as the main chemical classes; furthermore, among terpenes, sesquiterpenes tend to be the most variable components, both in their hydrocarbon and oxygenated forms (Pieracci et al., 2021, 2023).

Taking this into account, the present study aims to evaluate the ability of the EOs obtained from two industrial genotypes of *C. sativa* characterized by different chemical compositions to counteract the microglia-mediated neuroinflammation using a human microglia cell model (HMC3).

The EOs were obtained by hydrodistillation in a Clevenger apparatus from the air-dried apical parts of the selection S435 and the cv. Eletta Campana, cultivated in an open field in Bologna (Italy), and harvested at seed maturity. The EOs were analysed by Gas Chromatography coupled with Mass Spectrometry, and then evaluated for their cytotoxicity, ROS production, and influence on pro- and anti-inflammatory cytokines in a microglia cell model.

The analysed EOs showed a comparable relative amount of phytocannabinoids, almost totally represented by cannabidiol (CBD), but different percentages of the hydrocarbons and oxygenated forms of sesquiterpenes: the former class was more abundant in Eletta Campana, while the latter in S435. Among these classes, β-caryophyllene, α-humulene, and caryophyllene oxide represented the most important components, according to literature data, as well as to our previous studies. Concerning the cytotoxicity assay, S435 EO decreased the viability of HMC3 cells at concentrations of 50 µg/ml, while lower concentrations did not show a significative difference from the control. Conversely, the EO of Eletta Campana revealed a decrease of viability at 10 µg/ml. Since one of the mechanisms responsible for cell death is the accumulation of ROS, the ability of the EOs to influence their production was also assessed, although neither reduction nor increase in the ROS levels were shown. Finally, the investigation of the influence of the EOs on the genic expression of the pro-inflammatory cytokines IL-6 and IL-8, the anti-inflammatory cytokines IL-10, and the inflammatory transcription factor NFkB was performed by real-time RT-PCR. Eletta Campana, which is richer in sesquiterpene hydrocarbons, showed a greater anti-inflammatory activity, as it simultaneously decreased the expression of IL-6 and IL-8 and increased that of IL-10,

while S435, characterized by a prevalence of oxygenated sesquiterpenes, did not show the same outcomes.

Effects of a chronic treatment with a microdose of the Psilocybe mushroom-derived alkaloid psilocybin on the behavior of C57BL/6J mice

Nasini S[1], Colognesi M[1], Tidei S[1], De Martin S[1], Banzato M[1], Mattarei A[1], Folli F, Pappagallo M, Manfredi PL, Comai S

[1]*Department of Pharmaceutical and Pharmacological Sciences, University of Padua, 35122 Padua, Italy*
[2]*Department of Health Sciences, University of Milan, 20122 Milan, Italy*
[3]*Relmada Therapeutics, Coral Gables, FL 33134, USA*
[4]*Department of Biomedical Sciences, University of Padua, 35122 Padua, Italy*
[5]*Department of Psychiatry, McGill University, Montreal, QC H3A1A1, Canada*
[6]*IRCSS San Raffaele Scientific Institute, 20132 Milan, Italy. *These authors equally contributed.*

Traditional medical and religious uses of psilocybin containing mushrooms among American indigenous populations have been practiced for hundreds if not thousands of years, generally in the context of psychedelic experiences. Recently, there has been a surge in the phenomenon of psilocybin 'microdosing': regular ingestion of psylocybin containing mushrooms at quantities that cause minimal or no acute effects, with the expectation of a range of general health and psychological benefits. Because of the limited clinical and preclinical knowledge in the field of microdosing psilocybin paired with the lack of information about safety, in this pilot study we aimed at testing whether a non-psychedelic dose of psilocybin given repeatedly for 14 days to mice was tolerated and induced changes in anxiety- and depression-like behaviors and working memory.

The behavioral pharmacology experiments were conducted in adult (4 months old) C57BL/6J mice treated for 14 days with an intraperitoneal injection of vehicle (control group, n = 10 mice) or 0.05 mg/Kg psilocybin (treatment group; n=10 mice). The weight of the mice was monitored during the 14-day treatment, and on day 15, mice were tested in the T-Maze and Elevated Plus Maze (EPMT) tests while on day 16 in the Open Field (OFT) and Forced Swim (FST) tests.

We did not observe any effect of psilocybin on the weight of the mice over the two weeks (Two-way ANOVA for repeated measures; interaction time x treatment: $F(6, 108) = 1.420$, $p= 0.214$; factor treatment: $F(1, 18) = 0.2676$, $p= 0.611$). No differences between mice treated with vehicle and psilocybin were noted in the EPMT concerning the time spent in the open ($p=0.31$) and close ($p=0.26$) arms. In the T-maze, we found that mice treated with 0.05 mg/Kg psilocybin had significantly higher percentage of spontaneous alternations than those receiving vehicle ($t=2.375$, $df=18$, $p=0.029$). During the 20 min of the OFT, we did not observe any effect of psilocybin on the total distance traveled ($p=0.37$), and on the number ($p = 0.58$) and duration ($p = 0.37$) of visits in the central area of the arena, as well as on stereotyped behaviors (number of rearings ($p = 0.73$) and time spent grooming ($p = 0.39$)). Finally, the treatment with psilocybin did not affect the time of immobility in the FST compared with vehicle ($p = 0.86$).

This is the first study reporting the behavioral effects in mice of a repeated treatment with a low non-psychedelic dose of psilocybin. A 14-day treatment in adult C57BL/6J mice was safe and did not seem to affect multiple tests related to emotional behavior. Interestingly, we found a statistically significant signal for enhancement of the functioning of the working memory in animals treated with psilocybin that needs confirmation in future studies.

Pharmacognostic study of *Artemisia arborescens* (Vaill.) L., essential oil composition and biological activity

Polito F[1], Papaianni M[2], Woo S[3], Malaspina P[4], Cornara L[4], De Feo V[1]

[1]*Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy*

[2]*Department of Agricultural Sciences, University of Naples Federico II, Via Università 133, 80055 Portici, Italy*

[3]*Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy*

[4]*Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy*

The genus *Artemisia* (Asteraceae) includes about 500 species mainly distributed in the temperate zones of the Northern hemisphere [1]. *Artemisia arborescens* (Vaill.) L., also known as silver sage, and tree wormwood, is a Mediterranean aromatic multi-branched shrub from 1 to 2 m tall, with silver grey-green, deeply divided leaves, covered by a dense tomentum. The plant has a strong scent, and it is widely used in the culinary and alcoholic beverages industries [2], beside than for various ethnopharmacological uses [3]. The aim of this study was to study its leaves and branches, collected before the flowering stage.

Micro-morphological and anatomical investigation were carried out, showing the secretory ducts and trichomes in which essential oil (EO) is produced. EO was obtained by steam-distillation with a yield of 0,12%. Its chemical composition was achieved by GC and GC-MS, showing the presence of *trans*-thujone (24,2%), camphor (18,9%), aromadendrene (6,5%), and camphene (6,0%) as the maior compounds. The composition largely agrees with literature data [4,5].

The biological activity of the EO was assayed *in vitro* conditions against the bacterial plant pathogens of agricultural interest [6] *Xanthomonas campestris* pv. *campestris* (Gram-) and *Pseudomonas syringae* pv. *tomato* (Gram+). The EO had a minimum inhibitory concentration (MIC) when used undiluted [100% v/v], and 90%, 85% and 76% of growth inhibition when diluted 1:10, 1:100 and 1:1000. This antimicrobial activity was also confirmed by cellular material release assay. The results were in line with the antimicrobial test. Samples treated displayed a release of nucleic acids similar to the control treated with ethanol. In addition to the antimicrobial activity, the biofilm formation with or without the EO was evaluated using crystal violet staining according to Papaianni et al. [7]. The results demonstrated that the EO was able to reduce the *Xanthomonas campestris* pv. *campestris* biofilm with a decrease of 80%. The complex of data shows that the *A. arborescens* EO can find application as a potential alternative biocontrol product against plant pathogens.

Anti-virulence activity of abietane diterpenoid from *S. rosmarinus* Spenn. against methicillin-resistant *Staphylococcus aureus*

Iobbi V[1], Parisi V[2], Bernabè G[3], De Tommasi N[2], Bisio A[1], Brun P[3]

[1]*Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148 Genova, Italy*

[2]*Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Salerno, Italy*

[3]*Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35121, Padova, Italy*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious public health concern because it can cause severe infections that are difficult to treat. Quorum sensing (QS) is a cell-cell communication process that allows bacteria to survive as multi-cellular organizations and it regulates several physiological activities include virulence and biofilm formation. Several diseases are caused by biofilm-associated MRSA infections in which the accessory gene regulator (*agr*) quorum-sensing system is thought to play an important role. AgrA is a protein member of the LytR family of response regulators that is produced by the *agr* locus in *S. aureus*. Agr is considered the prototype quorum-sensing regulator system in Gram-positive bacteria. Interfering with the quorum sensing signal in MRSA can have several beneficial effects. By disrupting the ability of MRSA to coordinate its behaviour, it may be possible to reduce the virulence of the bacteria and to make them more susceptible to antibiotics and other antimicrobial agents. This could potentially lead to the development of new treatments for MRSA infections. In this study, we aimed to evaluate the anti-virulence activities of abietane diterpenoids isolated from *Salvia rosmarinus* against MRSA. The aim of this work was also to evaluate the possibility of using plant biomass of *S. rosmarinus* after the harvest of stem apex for the sale in vegetable markets. LC-MS analysis of the hydroalcoholic extract followed by NMR-based quantification of the rosmarinus ecotype "Eretto Liguria" underlined the presence of diterpenoids, triterpenoids, polyphenolic acids, and flavonoids. The abietane diterpenoids (carnosic acid, carnosol, 7-*O*-methylrosmanol and 12-*O*-methylcarnosic acid) were isolated from the methanolic extract of the residual aerial parts of the plant after harvesting. The quantification of carnosic acid in rosemary extract was carried through qNMR using 1D-NOESY sequence. Results showed that the carnosic acid content was 93.2±4.4 mg/g of dry extract, consistent with data reported for rosemary samples collected in the Mediterranean area. Carnosic acid was the most abundant abietane diterpenoid in the "Eretto Liguria" rosemary ecotype. Among the tested compounds, carnosic acid showed a significant reduction in the expression of genes involved in toxin production (*agrA* and *rnaIII* genes), coding keys components of the QS in MRSA. In MRSA, carnosic acid at 0.05 mg/mL inhibited biofilm formation, thus confirming its efficacy as biofilm preventing agent. Carnosic acid was also investigated for its specificity of *Staphylococcus* species. Moreover, the absence of cell toxicity for the possible use in clinical disease was established.

Targeting AgrA in MRSA is considered a novel strategy to the use of compounds that can specifically inhibit or disrupt the function of AgrA in MRSA, by blocking the ability of AgrA to bind to DNA, or the AgrA phosphorylation cascade. Docking simulations showed the binding interaction pattern of carnosic acid on AgrA active site.

Isolation of diterpenes from *Salvia officinalis* L. and their antimicrobial activity and mechanism

Donadio G, Nocera R, Dal Piaz F, De Tommasi N

Department of Pharmacy, University of Salerno, Fisciano, Italy; [2]Department of Medicine, Surgery and Dentistry; University of Salerno, Baronissi

According to Cragg et al. 2020[1] there is an urgency to discover new molecules with antimicrobial activity and leads for the treatment of infectious disease. Presently, the effectiveness of a decisive and rapid global health response to infectious diseases is threatened by antibiotic resistance due to systematic abuse and excessive use of antibiotics in human medicine. Indeed, the massive or improper use of such drugs in humans, animals, or agriculture results in the development of drug-resistant microorganisms evolved under this strong selective pressure. In 2015, aware of the great problem of antibiotic resistance, the WHO decided to adopt the Global Action Plan on Antimicrobial Resistance, based on five strict objectives: To improve awareness and understanding of antimicrobial resistance; to strengthen knowledge and the amount of data; to reduce the incidence of infections through effective hygiene measures; to optimize the use of antimicrobial drugs in human and animal health; and to increase investment in new drugs, diagnostic tools, vaccines, and other interventions.[2] In recent years, our research group is involved in the study of antibacterial activity of plant small molecules against strains responsible for pathologies like human oral caries. Extracts from different *Salvia* species have been investigated for several biological activities and many reports showed the antimicrobial potential of these species [3]. Starting from this consideration, we screened the dichloromethane extract from the aerial parts of *Salvia officinalis L*., (Lamiaceae). Then, the components of the extract were separated by chromatographic approaches and their structures elucidated by NMR a MS analysis. Obtained results indicated that the manool, a labdane diterpene, was the most active component. Monool showed a significant efficacy against Streptococcus mutans (ATCC 25275) with MIC values of 6.5 µg/mL. The molecular mechanism of manool remains still unclear. Therefore, a proteomic-based approaches was carried out to identify the target(s) of manool in *Streptococcus mutans*, the main oral pathogen involved in dental caries and in other systemic diseases. Firstly, expression proteomic studies on microorganisms treated or not with a manool were carried out to define the pathways involved in the bioactivity of this diterpene. Drug Affinity Responsive Target Stability (DARTS) coupled with mass spectrometry was used as a quick and reliable approach to identify potential cellular targets of manool. The analysis of proteins of *S.Mutans*, incubated with sublethal concentration of manool, evidence the modulation of ATP-binding cassette (ABC) transporters. Members of this superfamily are implicated in the translocation of a diverse range of molecules across membranes and are involved in bacterial pathogenesis. This results candidates ABC proteins as target of manool. Finally, preliminary studies were also conducted aimed at evaluating the effect of manool treatment on *S. mutants* vesiculation, as this process is supposed to be partially responsible for bacteria ability to escape from antimicrobial drugs. Our results confirmed the ability of manool to interact with *S. mutants* proteins playing different roles in proliferation and survival, suggesting a possible use of this compound to develop new antibacterial drugs.

The phytobiome of the medicinal plant *Origanum vulgare*: linking the bacterial endophytic communities to the essential oil

Semenzato G[1], Del Duca S[2], Vassallo A[3], Ascrizzi R[4], De Leo M[4], Pistelli L[4], Mucci N[5], Greco C[5], Padula A[5], Palumbo Piccionello A[6], Puglia AM[6], Emiliani G[7], Maggini V[8], Firenzuoli F[8], Fani R[1]

[1]*Department of Biology, University of Florence, Sesto Fiorentino, Italy*
[2]*Council for Research in Agriculture and Economics (CREA-AA), Florence, Italy*
[3]*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*
[4]*Department of Pharmacy, University of Pisa, Pisa, Italy*
[5]*Unit for Conservation Genetics (BIO-CGE), Institute for Environmental Protection and Research, Ozzano dell'Emilia, Italy*
[6]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy*
[7]*Institute for Sustainable Plant Protection (IPSP), National Research Council (CNR), Sesto Fiorentino, Italy*
[8]*Research and Innovation Center in Phytotherapy and Integrated Medicine (CERFIT), Referring Center for Phytotherapy, Tuscany Region, Careggi University Hospital, Florence, Italy*

Antimicrobial resistance is a global concern associated with high morbidity and mortality. Multidrug-resistance bacteria (MDR) may be untreatable with conventional antibiotics; hence, it is important to prioritize the development of alternative therapies. In this context, medicinal and aromatic plants might play a key role since they represent a natural source of bioactive molecules. In particular, antibacterial and antifungal activities have been reported for plants belonging to the *Origanum* genus and their essential oils (EOs). One of the advances in addressing these issues is the discovery that microorganisms residing inside the plant may contribute to the production of metabolites of pharmaceutical interest.

The aims of this work are i) to characterize the bacterial endophytic community (i.e., the phytobiome) associated with *Origanum* species and subspecies to select a collection of bacteria able to synthesize antimicrobial molecules, and ii) to check whether the EO aroma profile might be influenced by the presence of the endophytes and/or if some EO compounds might be directly synthesized by the endophytes themselves.

To this purpose, endophytic bacterial communities were isolated from different *Origanum* plant compartments. EOs were hydrodistilled from the same plants and their chemical composition was determined by gas chromatography coupled with mass spectrometry (GC-MS). The structure and the composition of the bacterial communities were firstly investigated through a Random Amplified Polymorphic DNA (RAPD) analysis and 16S rDNA gene sequencing revealing a high degree of biodiversity and a low degree of strains sharing among *Origanum* species and subspecies and the different compartments of the same plant. Endophytes' ability to inhibit the growth of a panel of MDR human pathogens was also evaluated through the cross-streaking method. This analysis showed that the endophytes synthesize bioactive molecules with a strong antibiotic activity, including volatile organic compounds (VOCs). The GC-MS analysis of such compounds revealed that some of the VOCs synthesized by endophytes are also present in the EO chemical profile of the host plant.

Data obtained suggest the existence of selective forces able to determine the structure and composition of the microbial community associated with different medicinal plants but also with different compartments of the same plant. The ability of endophytes to produce volatile molecules able to inhibit the growth of MDR bacteria highlights the enormous

biotechnological and pharmacological potential of endophytes. It might also suggest their involvement in the determination of the EO aromatic profile of the plant with which they are associated.

New sesquiterpenes from *Zanthoxylum rhoifolium* Lam. barks

Di Stasi M[1], De Tommasi N[2], Parisi V[2], Hernandez V[3], Braca A[1]

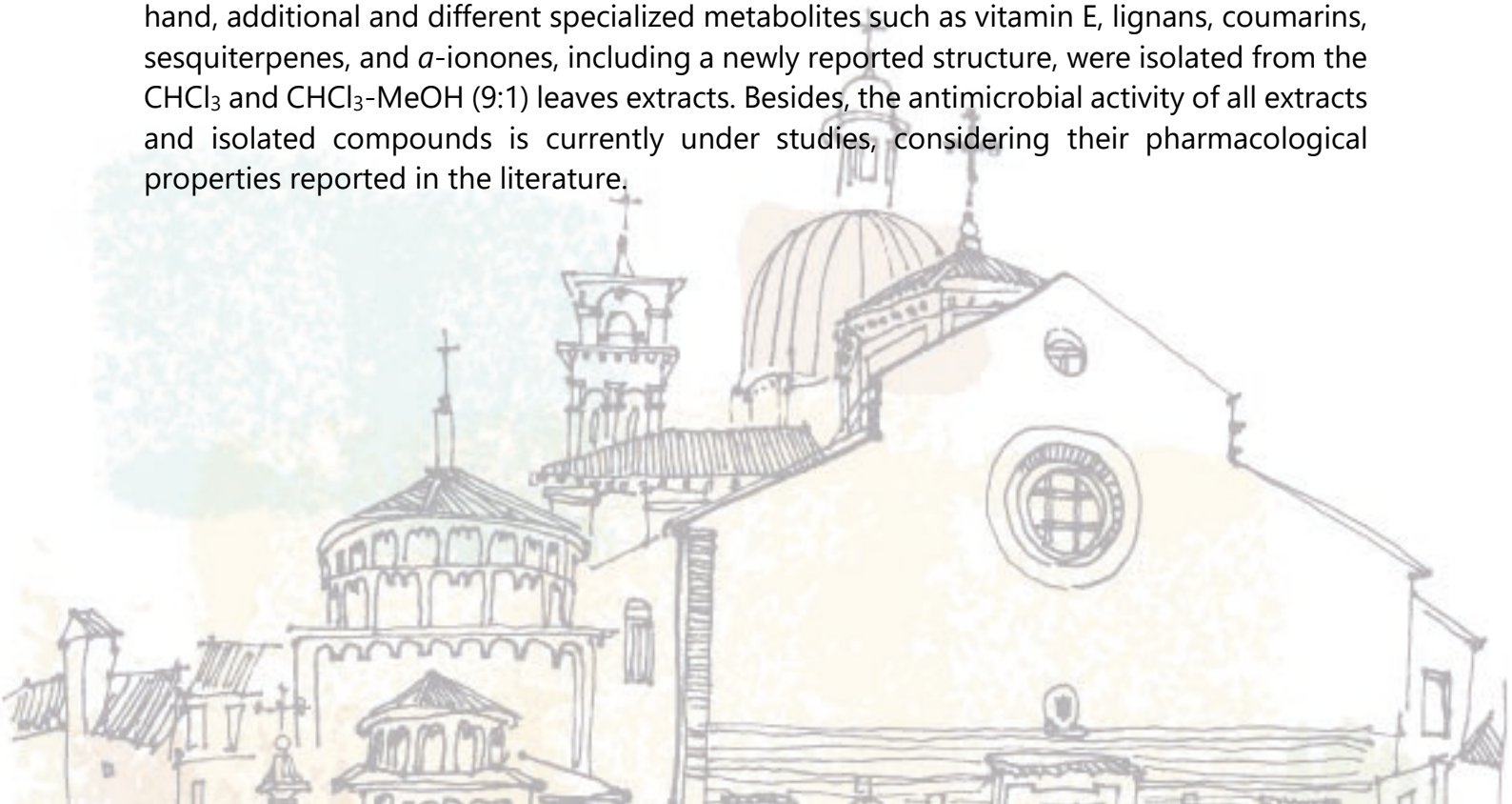
[1]*Dipartimento di Farmacia, Università di Pisa, Via Bonanno 33, 56126, Pisa, Italy.*

[2]*Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II, 84084 Fisciano (SA), Italy.*

[3]*Departamento de Farmacognosia y Medicamentos Organicos, Universidad de los Andes, Merida 5101, Venezuela.*

Zanthoxylum rhoifolium Lam. is a South American native tree belonging to the Rutaceae family, commonly known as Indian ash. It is mostly found in the eastern rainforest of the Atlantic coast, in the states of Minas Gerais and Rio de Janeiro, and in the North-East in the states of Piauí and Ceará [1]. The plant is traditionally employed in medicine for inflammatory, microbial and malaria processes and, to be specific, the roots are used as a febrifuge, gastroprotector and tonic, while the barks are used to treat flatulence, colic, dyspepsia and for their antinociceptive activity [2-3]. In previous phytochemical studies carried out on the bark, leaves, and fruits extracts of the plant, alkaloids, coumarins, lignans, terpenes, and flavonoids have been isolated [4-7]. Furthermore, the $CHCl_3$-MeOH extract, obtained from the plant bark showed in our previous preliminary work fungistatic effect on *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, and *Clonostachys rosea* [8].

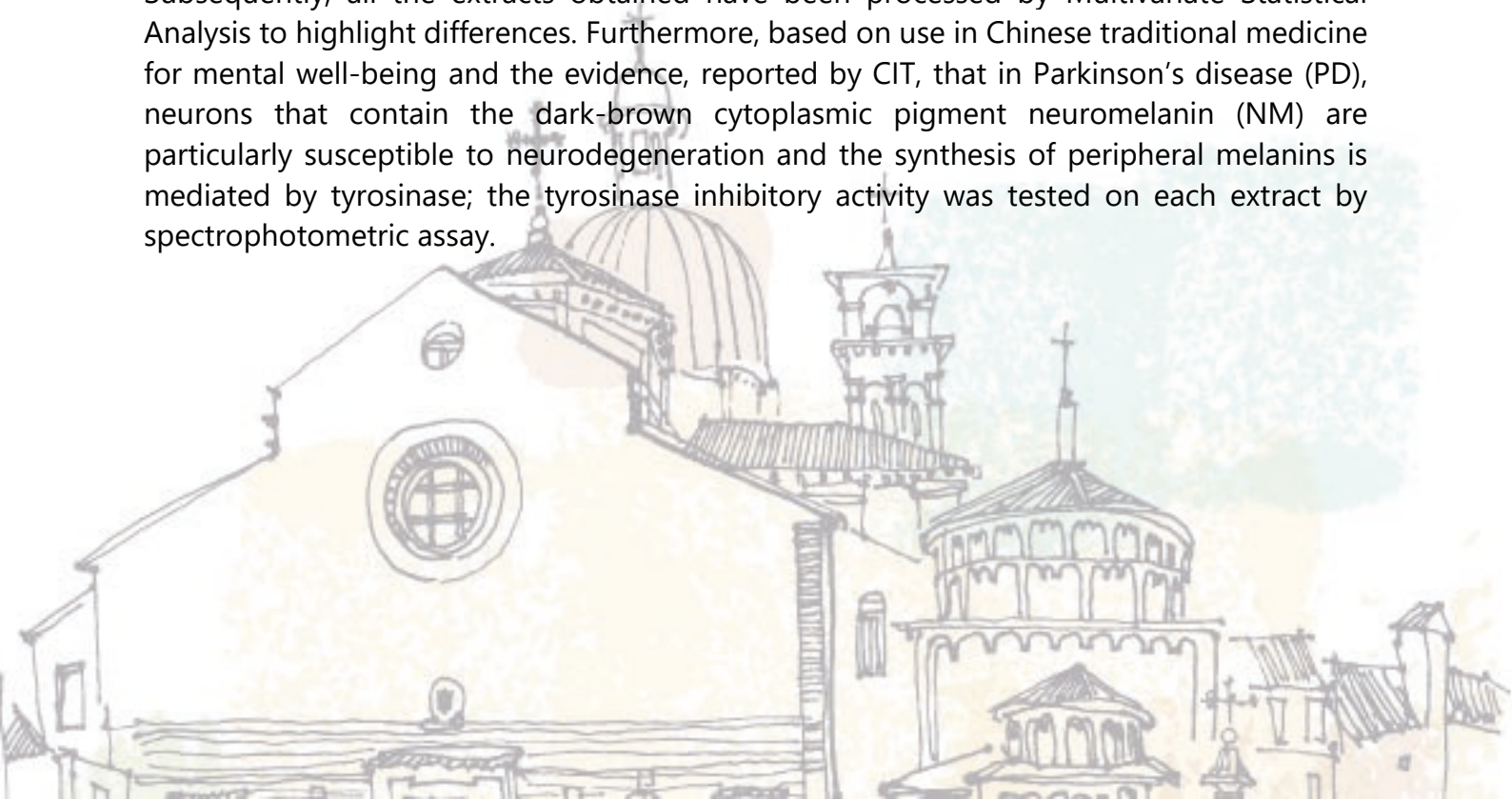
In the present study, leaves and barks separately were subjected to static maceration using solvents of increasing polarity, petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH, and after comparing the chromatographic profiles of the extracts with UHPLC-ESI-HR-Orbitrap/MS, the apolar and medium polarity extracts were considered for phytochemical investigation. The study of the extracts was conducted using different chromatographic techniques such as Sephadex LH-20, Biotage® flash chromatography, and RP-HPLC. The structural characterization of the obtained compounds was performed using 1D and 2D NMR and mass spectrometry. A variety of sesquiterpenes were isolated from the petroleum ether extract of the barks, four of which were never previously isolated, while several alkaloids and some flavonoids were isolated from the $CHCl_3$-MeOH (9:1) extract. On the other hand, additional and different specialized metabolites such as vitamin E, lignans, coumarins, sesquiterpenes, and *a*-ionones, including a newly reported structure, were isolated from the $CHCl_3$ and $CHCl_3$-MeOH (9:1) leaves extracts. Besides, the antimicrobial activity of all extracts and isolated compounds is currently under studies, considering their pharmacological properties reported in the literature.



Metabolite profile of green extracts of *Schisandra chinensis* fruits and evaluation of Tyrosinase inhibitory activity

Polcaro L, Piacente S, Masullo M
Dipartimento di Farmacia, Università degli Studi di Salerno

In recent years, there has been a growing interest in developing ecological and environmentally friendly methods for natural product extraction. These methods belong to "green extraction", which allows for obtaining a higher extraction yield using a lower amount of solvents and energy. It also helps to reduce the extraction time compared to conventional extraction methods like maceration. The current project is aimed at the application of green extraction techniques like ultrasound-assisted extraction (UAE), and dynamic solid-liquid extraction (SLDE-Naviglio) on *Schisandra chinensis* Turcz. Baill. fruits belonging to the Schisandraceae family [1]. Its fruits are called magnolia berries or five-flavor fruits because possess five basic flavors: salty, sweet, sour, pungent (spicy), and bitter. They have long been used as a sedative and tonic agent in case of physical exhaustion and to prevent fatigue [2]. *Schisandra chinensis*'s fruits were submitted to different extraction protocols like decoction, according to Pharmacopeia XII, macerations, UAE and SLDE-Naviglio prepared using EtOH and EtOH/H_2O (50:50, 75:25, 100:0) as bio-solvents. All the extracts obtained have been analyzed firstly by LC-MS, NMR, and HR-MAS, to obtain the metabolite profile. Based on these results, chromatographic processes have been applied to isolate bioactive compounds. The structural determination of isolated compounds has been performed by spectroscopic methods, including 1D-(^{1}H and ^{13}C) and 2D-NMR experiments as well as MS analysis. A total of 33 compounds corresponding to lignans belonging to different classes were identified. The major bioactive constituents of *Schisandra chinensis* belonging to the dibenzocyclooctane type such as schisandrin, schisandrin B, schisandrin C, schisantherin A, schisantherin B, deoxyschisandrin, gomisin A etc. Molecules like abscisic acid, gomisin J, henricin, galcatin, schisandrathera C ed intherioterin A have never been reported in *S. chinensis* specie, while the conocarpol hasn't been reported in *Schisandra* genus. Subsequently, all the extracts obtained have been processed by Multivariate Statistical Analysis to highlight differences. Furthermore, based on use in Chinese traditional medicine for mental well-being and the evidence, reported by CIT, that in Parkinson's disease (PD), neurons that contain the dark-brown cytoplasmic pigment neuromelanin (NM) are particularly susceptible to neurodegeneration and the synthesis of peripheral melanins is mediated by tyrosinase; the tyrosinase inhibitory activity was tested on each extract by spectrophotometric assay.



^{1}H NMR-based metabolomic analysis of the lichen *Cladonia foliacea*.

Nozella AH[1], Debiasi L[1], Gheza G[2], Trincia S[1], Vallese C[2], Nascimbene J[2], Ferruccio Poli F[1], Mandrone M[1]

[1]Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

[2]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

Lichens are known to produce a wide variety of special metabolites, most of which are exclusively produced by them (Shukla et al. 2010, Phytochemistry Review). Their antimicrobial, antioxidant, antiviral, anticancer, antigenotoxic, anti-inflammatory, analgesic and antipyretic activity have been reported (Ranković et al. 2015, Lichen Secondary Metabolites). The subject of this study, *Cladonia foliacea*, is a lichen commonly found in lowland dry habitats of Italy, capable of producing usnic acid, zeorin, fumarprotocetaric and protocetaric acids, which are responsible for the antimicrobial, antioxidant and antigenotoxic activity of its extracts (Yilmaz et al. 2004, Journal of Biosciences; Anar et al. 2016, Toxicology and Industrial Health)

Untargeted metabolomics applied to natural products is an interesting approach to obtain an overview of the metabolites present in an extract. This is relevant for quality control and to distinguish between different species, as well as to compare samples growing under different conditions in order to extract information on the biological significance of the produced metabolites.

The aim of this work is to follow the variation in the metabolome across the year of *C. foliacea* harvested in 5 different sites of the dry grasslands of the Valle del Ticino area, located at the boundary between Lombardia and Piemonte regions, with diverse environmental conditions. The monthly collected samples were air dried, pulverized with liquid nitrogen, extracted with deuterated methanol and water (1/1 v) analyzed by ^{1}H NMR.

The PCA based on the ^{1}H NMR profiles highlighted several variations in the metabolome both during time and depending on the harvesting location. In particular, samples collected in December were the only ones showing the presence of formic acid and having a highest concentration of protocetaric and usnic acid, alanine, and GABA.

January samples from three locations were more similar between each other, characterized by a higher concentration of sucrose, trehalose and fumarprotocetaric acid. While another sample was more similar to that of December due to its higher concentration of fumaric acid and lower concentration of fumarprotocetaric acid. Another sample showed a higher amount of usnic acid, GABA, and alanine, positioning it further away from the samples from the same season.

Lastly, the samples from February were characterized by a higher concentration of fumaric acid and glucose, except for the sample from two samples that had a more important presence of inositol positioning them away from the others.

Despite the study being on its first steps, the results found up to this stage are relevant since they lead to a clear change of the metabolism of this species along the progression of the months. The developed PCA model was validated, and it resulted capable of detecting the subtle differences between the samples from each location and each month leading to a possible capability for the identification of a *C. foliacea* sample based on the chemical composition of its extract.

Venerdì 16 giugno

Lettura Magistrale

Plant biodiversity to promote health and nutrition: re-discovering ancient varieties inside the *Prunus* genus

De Leo M

Dipartimento di Farmacia, Università di Pisa, via Bonanno 33, 56126 Pisa; Centro Interdipartimentale di Ricerca "Nutraceutica e Alimentazione per la Salute", Università di Pisa, via del Borghetto 80, 56124 Pisa, Italy; Centro per l'Integrazione della Strumentazione dell'Università di Pisa (CISUP), Università di Pisa, Lungarno Pacinotti 43/44, 56126 Pisa

Biological diversity and human health are strictly connected and their preservation, by strengthening the awareness of this linkage, is nowadays a global priority and central to sustainable development [1,2]. However, the biodiversity, including plant diversity, is declining alarmingly due to climate change, anthropogenic activities, and other natural factors, thus its conservation, at local and global level, is vital for the planet's future [3]. Plants have historically been recognized as a source of active ingredients that remain unsurpassed for their great chemical diversity due to the richness in biodiversity [4]. As a part of intervention strategies, the promotion of production and consumption of indigenous and underutilized plant species with great potential benefits to human health is hopeful to generate co-benefits for the ecosystem's health. In this context, Italian *Prunus* varieties locally consumed or at risk of extinction were investigated for their content in bioactive constituents and their potential as health-promoting agents. The *Prunus* genus, belonging the Rosaceae family, comprises more than 200 species distributed worldwide, including ornamental plants appreciated for their flowers and species widely cultivated for their delicious fruits. The richness in nutritional compounds as well as in polyphenols makes *Prunus* fruits promising health-promoting foods and source of potential therapeutic agents [5]. Despite the widespread use, many ancient and local *Prunus* varieties remain poorly studied and under threat of extinction. Through a bioprospection plan supported by an expert botanist different ancient Italian *Prunus avium* L., *Prunus domestica* L., and *Prunus salicina* Lindl. varieties were collected in the Casentino area, the National Park of the Tuscan Archipelago (Elba Island), and the inland areas of Sannio territory. All varieties were deeply investigated through a multidisciplinary approach, including metabolomics and biological assays, the results of which were finally linked by bioinformatic tools. Metabolomic fingerprints highlighted the high content of hydroxycinnamic acids, flavonoid glycosides, procyanidins, and anthocyanins, all expressing strong radical scavenging activity and good potential antiangiogenic activity in chick chorioallantoic membrane and in zebrafish embryos. A series of bioinformatic approaches (principal component analysis, hierarchical clustering, Pearson correlation matrices and correlation networks) were applied to simultaneously compare all fruits varieties based on chemical and biological data, highlighting the individual and total role of the identified compounds in the observed bioactivity. The overall integrated data strongly highlighted the high value of *Prunus* varieties as valuable resource to be promoted to preserve agrobiodiversity and to sustain the human health and well-being.

Application of wood distillate boosts antioxidant properties of *Solanum lycopersicum* L. (red tomato)

Fedeli R[1], Marotta L[2], Panti A[2], Carullo G[2], Fusi F[2], Gemma G[2], Butini B[2], Saponara S[1], Vannini A[1], Campiani G[2], Loppi S[1,3]

[1]*Department of Life Sciences, University of Siena, 53100 Siena, Italy;*

[2]*Department of Biotechnology, Chemistry and Pharmacy, University of Siena, 53100 Siena, Italy;*

[3]*BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples 'Federico II', 80138 Napoli, Italy*

Bio-stimulants may be useful substitutes of chemical fertilizers to boost the productivity and quality of crops. Several bio-based products are currently on the market; one of the newest and most promising is wood distillate (WD), which is a byproduct of the pyrolysis of plant biomass from waste forest timber used for bio-energy production. Wood distillate is rich of biologically active compounds such as polyphenols, improving plant fitness and yield performance. Its foliar application has been extensively studied, and results have shown to highlight the antioxidant profile of cultivated crops. In this context, WD has been used as foliar additive for *Solanum lycopersicum L.* Its application improved fresh weight (+16.0%), content of soluble sugars (*i.e.*, glucose (+32.9%), fructose (+24.4%), and total (+27.8%) antioxidant pool content (*i.e.*, polyphenols (+17.9%), flavonoids (+58.1%)), and lycopene content (+51.9%). No substantial difference in the mineral component has been observed between controls and the WD-treated tomato fruits, except for the phosphorus, which showed a significant reduction in WD-treated tomatoes by 24.1% The two tomatoes have been subjected to extraction by means of acetone/water mixture for further chemical characterization. The ^{1}H-NMR and ESI-MS analyses of the extracts revealed the presence of different fatty acids, but also amino acids and sugars. In particular, the WD-treated tomato showed the presence of pyroglutamic acid and phloridzin derivatives, but also dihydrokaempferol, naringenin glucoside, cinnamic acid and kaempferol-3-O-glucoside. These results clearly show the efficacy of using WD in improving the yield and nutritional qualities of edible parts of crops. Further analyses will be able to evaluate in cellular studies the antioxidant profile emerged *in vitro*.

Wild roses of the Apennines: metabolomic analysis and antioxidant activity

Trincia S[1], Tarozzi C[1], Nozella HA[1], Mandrone M[1], Chiocchio I[1], Mossetti U[2], Poli F[1]

[1]Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

[2]Botanic Garden and Herbarium, University Museum Network, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

The genus *Rosa* includes about 150 European species used in traditional medicine, in cosmetics and food industry.

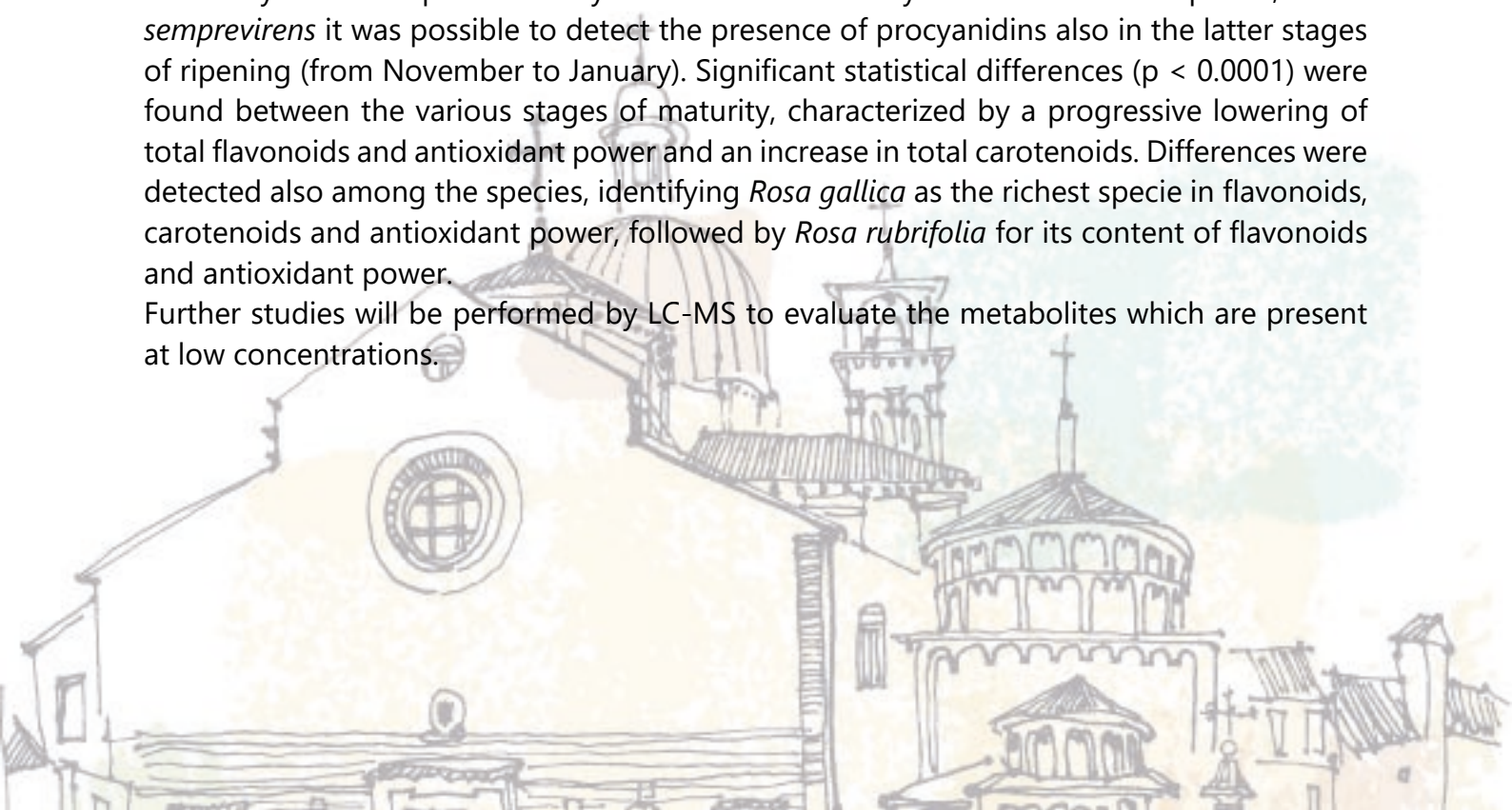
This work was based on eight species of wild *Rosa* from the Apennines (*Rosa arvensis* Hudson, *Rosa canina* L., *Rosa cinnamomea* L., *Rosa dumalis* Bechst., *Rosa gallica* L., *Rosa rubrifolia* Vill., *Rosa sempervirens* L., *Rosa tomentosa* Sm.) grown *ex situ* in the botanical garden of Bologna to minimize the morphological and phytochemical differences due to environmental influences.

The aim was to compare the rosehips of these species during the ripening process (from June 2022 to January 2023). In particular, it was performed a ^{1}H NMR-based metabolomic analysis (Mandrone et al. 2021, Food Control, 127, 108141) together with total flavonoid and carotenoid assay and *in vitro* antioxidant activity test (DPPH).

The PCA (Principal Component Analysis) developed on ^{1}H NMR spectra allowed us to follow the trend of maturation of the rosehip, highlighting how during the ripening process there was a decrease in procyanidins, amino acids and quinic acid and a consequent increase in glucose, citric and formic acid. An intermediate stage was also identified (during September), in which there was a high production of glycine, potentially due to hydric stress. Moreover, the PCA showed different trends of metabolomic variation over time for some species. In particular, *R. gallica* had the highest content of procyanidins also during September when other species were already lowering these compounds.

Compared to the others, *R. rubrifolia* and *R. tomentosa* had a premature ripening, and *R. rubrifolia* showed a high content of proline during diverse stages of its maturation. Considering the role of this amino acid as an osmolyte, its high content could reflect a higher sensitivity of this species to hydric stress. Differently from the other species, in *R. semprevirens* it was possible to detect the presence of procyanidins also in the latter stages of ripening (from November to January). Significant statistical differences ($p < 0.0001$) were found between the various stages of maturity, characterized by a progressive lowering of total flavonoids and antioxidant power and an increase in total carotenoids. Differences were detected also among the species, identifying *Rosa gallica* as the richest specie in flavonoids, carotenoids and antioxidant power, followed by *Rosa rubrifolia* for its content of flavonoids and antioxidant power.

Further studies will be performed by LC-MS to evaluate the metabolites which are present at low concentrations.



Chemical composition and biological activity of essential oils and crude extract from apical shoots and resin of *Pinus nigra* subsp. *laricio* Poiret

Fucile M[1], Perri M[1], Marrelli M[1], Zicarelli L[1], Lupia C[2], Statti G[1], Conforti F[1]

[1]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende, Italy*

[2]*National Etnobotanical Conservatory, Castelluccio Superiore, 85040 PZ, Italy*

Pine extracts are known for their beneficial properties, such as anti-inflammatory, anti-neurodegenerative, antitumoral, antimicrobial[1] and cardioprotective[2]. *Pinus nigra* subsp. *laricio* Poiret grows wild in Corsica and, in the Italian territory, in Sicily and Calabria. In Calabria, it grows on siliceous soils in forested areas at a height of 800-1500 a.s.l. This study investigated the phytochemical content and the biological activities of resin essential oil and ethanol extract and essential oil from apical shoots. The total ethanolic extract was portioned between *n*-hexane, dichloromethane and ethyl acetate, successively. The essential oils from apical shoots and resin were extracted by steam distillation and hydrodistillation, respectively. The chemical composition of essential oils and apolar fractions (*n*-hexane and dichloromethane) of the ethanolic extract were characterized by GC-MS. The main constituents of the essential oils were α-pinene (23,2 %), β-pinene (6,52 %) and limonene (5,74 %) for apical shoots and α-terpinolene (14,29 %) for resin. DPPH free radical scavenging activity (DPPH) and β-carotene bleaching test were used to evaluate the potential antioxidant capacity of all the samples. The best antioxidant activity for both tests was observed for the ethyl acetate fraction. Total phenolic content and total flavonoid content were also evaluated for apical shoots ethanolic extract. All the samples were tested for their inhibitory effect on NO production on the cellular line RAW 264.7. The best result, in this case, was obtained from the resin essential oil with an IC_{50} value of a 43.50 ± 1.18 μg/mL. Moreover, α-amylase and lipase inhibitory activities were evaluated.

Pharmacognostic evaluation of *Monarda didyma* L. "summitas cum floribus" growing in Trentino, Northern Italy

Smeriglio A[1], Trombetta M[1], Ingegneri M[1], Germanò MP[1], Miori L[2], Battistini G[2], Malaspina P[3], Cornara L[3]

[1]Department of Chemical, Biological, Pharmaceutical and Enviromental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy

[2] Areaderma S.r.l., Via per Trento 16, 38042 Baselga di Pinè (TN), Italy

[3]Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy

Monarda didyma L. is an aromatic herb of Lamiaceae, native to eastern North America, and known with different common names including Bee Balm, Bergamot, and Oswengo tea. This last common name is related to the traditional uses of this plant to prepare a digestive and expectorant tea by the North American Oswego Indians. In the eighteenth century the plant was introduced in Europe, where it began to be cultivated in Austria, Switzerland, and Germany, and then also in Northern Italy. Many different therapeutic indications have been reported for this species, including colic, dyspepsia, dysmenorrhea, cardiopathy, cold, fever, insomnia, and others. Starting from 2018, *M. didyma* L. 'summitas cum floribus' appears in Annex 1 of the Belfrit List.

Despite the innumerable studies available in literature on the essential oil obtained by distillation of the flowering tops, to date few data are available on the micro-morphological and phytochemical features, nor on biological properties of the crude extract. Considering this, after a preliminary investigation of the macro and micro-morphology of the flowering tops by light and scanning electron microscopy, we investigated the phytochemical profile by LC-DAD-ESI-MS analysis, and the antioxidant and anti-inflammatory properties of ethanolic and hydroglyceric extract (EE and HE, respectively) obtained by the Extractor Naviglio®.

The inflorescence consists of a terminal head of bright scarlet-red flowers, subtended by a whorl of red-tinged leafy bracts. The red colour of these aerial portions is due to the abundance of anthocyanins. Their presence was highlighted by light microscopy analysis in epidermal cells of the corolla, calyx, and leafy bracts, and even within the long uniseriate non-glandular trichomes, and in the stalk cells of capitate trichomes spread on these portions.

Preliminary phytochemical studies have already identified the HE as the richest in total phenols (1.45 ± 0.081 vs. 0.35 ± 0.019 g gallic acid equivalents /100 g of fresh weight) and especially in flavonoids (0.98 ± 0.070 vs. 0.26 ± 0.007 g rutin equivalents/100 g of fresh weight). These data were confirmed by LC-DAD-ESI-MS analysis which allowed to characterize the phytochemical profile of the flowering tops. Sixty-nine polyphenols were detected. From the semi-quantitative analysis, in agreement with what was observed by the light microscopy analysis, anthocyanins result the predominant compounds with Cyanidin 3-*O*-sambubioside which represents the most abundant compound in both extracts, followed by Delphinidin 3-*O*-glucoside and Malvidin 3-*O*-glucoside, in EE and HE, respectively.

The antioxidant (DPPH, TEAC, FRAP and ORAC) and anti-inflammatory activity (albumin denaturation assay and protease inhibitory activity) of both extracts were investigated by

several *in vitro* cell-free assays based on different environment and reaction mechanisms, comparing the results with the reference standards trolox and diclofenac sodium. According to the phytochemical results, HE showed the best biological activity in all the assays carried out with half-inhibitory concentrations (IC_{50}), expressed as fresh weight, ranging from 0.007-1.31 mg/mL and 0.47-10.31 mg/mL for antioxidant and anti-inflammatory activity, respectively.

Given the promising results obtained, *in vivo* anti-angiogenic activity studies aimed at evaluating a possible cosmeceutical use of one or both extracts of *M. didyma* flowering tops are currently ongoing on chicken chorioallantoic membranes by using retinoic acid as reference compound.

A microemulsion approach to address the solubility and cutaneous bioavailability issues of *Stachys parolinii* extract

Vanti G[1], Milonaki E[2], Anagnostou M[2], Tomou E.-M[2], Grifoni L[1], Bergonzi MC[1], Karioti A[3], Skaltsa H[2], Bilia AR[1]

[1]Department of Chemistry "Ugo Schiff" (DICUS), University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

[2]Section of Pharmacognosy & Chemistry of Natural Products, School of Health Sciences, Department of Pharmacy, National & Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771 Athens, Greece

[3]Laboratory of Pharmacognosy, School of Pharmacy, Aristotle University of Thessaloniki, University Campus, 54124, Thessaloniki, Greece

Several *Stachys* L. (Lamiaceae) species were extensively used in the traditional medicine of many countries to treat a great variety of disorders and diseases, including stress, inflammations and healings of the skin, stomach disorders, tumors, etc. In addition, they are considered a great source of phytochemicals with potential therapeutic applications. Accordingly, they are gaining much attention for the screening of their bioactive metabolites [1].

In this study, a methanolic extract of *Stachys parolinii* Vis., collected in central Greece, was obtained from the aerial plant parts. The phytochemical profile was analyzed by HPLC-PDA-MS and NMR techniques. Furthermore, 12 constituents were so far isolated by different chromatographic techniques. Despite the promising therapeutic activity of this extract, its low aqueous solubility requires a suitable dosage form for achieving optimal cutaneous administration and bioavailability.

This work aimed to develop an oil-in-water microemulsion for cutaneous use [2]. After an accurate screening of excipients (oils, surfactants, co-surfactants) suitable for microemulsion development and compatible with skin and damaged skin, we explore the solubility of the extract in the selected excipients by the flask shake method for 3 hours and visual inspection. Solutol HS 15, Transcutol P, and Capryol 90 were chosen for the high solubility of the extract. According to previous studies, we fixed the surfactant/co-solvent ratio at 1:1, 1:2, 2:1, and we constructed different pseudo-ternary phase diagrams as a pre-formulation study by the titration method to investigate the microemulsion existence regions. The dynamic light scattering technique was applied by the Zetasizer Pro to measure the size and polydispersity index (PdI) of the dispersed phase of formed microemulsions. All the results gave sizes around 25 nm and a PdI around 0.15. A preliminary evaluation of the extract solubility showed that the selected microemulsions could easily dissolve 5 mg/mL of extract in 15 minutes. The next studies will be focused on improving the solubility, and expectably the bioavailability, of the extract by optimizing the microemulsion composition and fully characterizing the obtained formulation to provide a proper dosage form for the cutaneous application of the *S. parolinii* extract. *In vitro* studies to test the antibacterial activity of the extract are still ongoing.

Effects of an oral solution containing xyloglucan and pea proteins on a murine model of gastroesophageal reflux disease

Ardizzone A, Mannino D, Casili G, Campolo M, Paterniti I, Lanza M, Filippone A, Repici A, Bova V, Cuzzocrea S, Esposito E

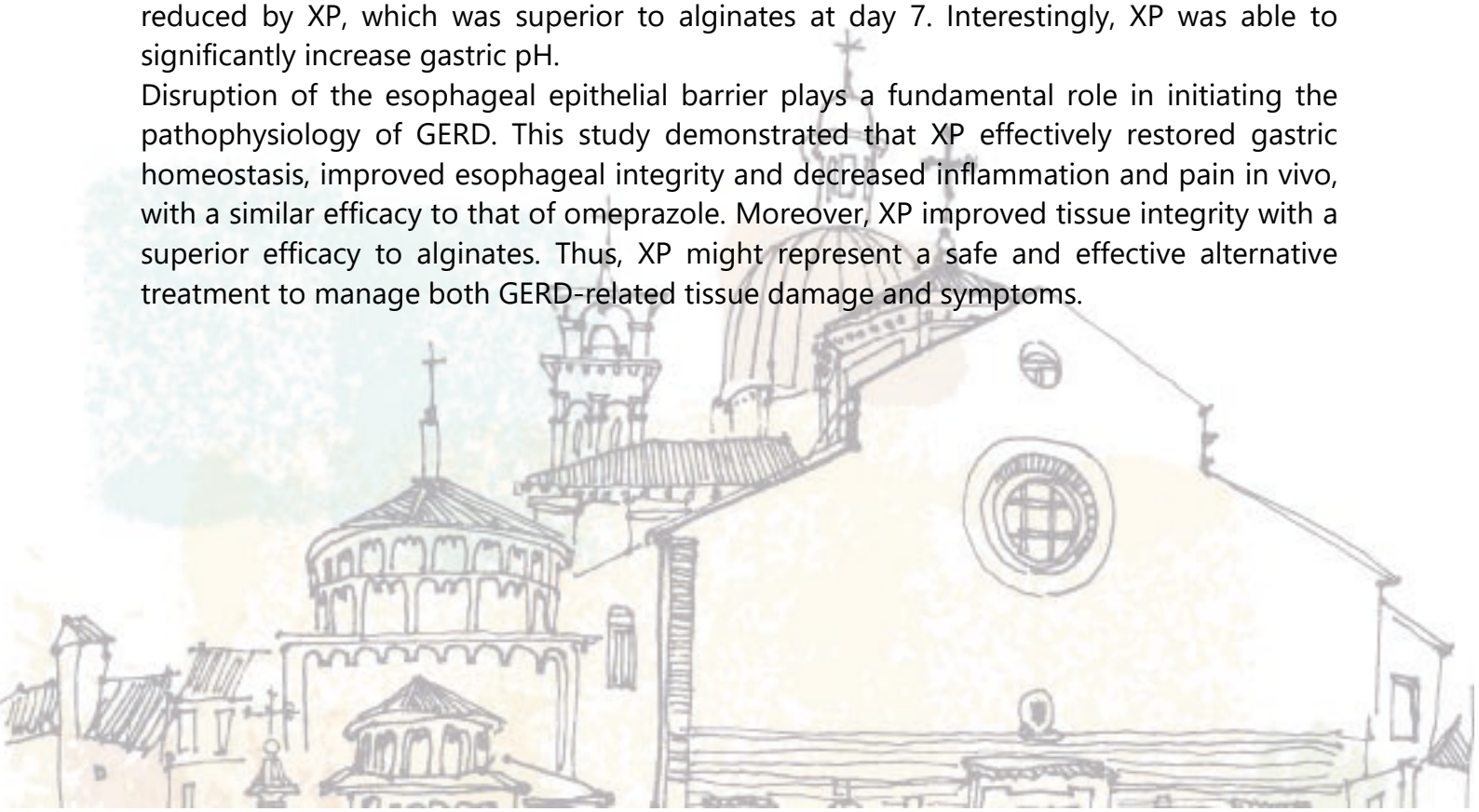
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres, 98166 Messina, Italy

Gastroesophageal reflux disease (GERD) is the most common foregut disease, affecting about 20% of the adult population, and is characterized by a constant exposure of the mucosal cells to gastric acid and enzymes, which results in modification of the mucosal tissue integrity and persistent symptoms including heartburn and regurgitation. Although GERD is associated with an esophageal epithelial barrier dysfunction, pharmacological therapies mainly aim to reduce the acidity of the gastroesophageal environment rather than protect the integrity of the esophageal tissue. Therefore, restoring the esophageal epithelium integrity as well as controlling gastric acidity, may represent an efficient approach to improve symptoms and prolong remission periods. This study aims to evaluate the efficacy of an oral solution containing xyloglucan and pea proteins (XP) in reestablishing the integrity of gastroesophageal tissues and decreasing gastric pain in a GERD murine model.

To induce GERD, C57BL/6 mice were alternatively overfed and fasted for 56 days. Mice were then treated with XP, sodium alginate, omeprazole or omeprazole+XP twice daily for 7 days. Gastric pain and inflammatory markers were evaluated after 3 and 7 days of treatment. After sacrifice, esophagi and stomachs were surgically removed for macroscopic and histological examination.

Gastric pain was significantly reduced at day 3 and 7 by XP, omeprazole and omeprazole+XP, while alginates were ineffective at day 3. XP significantly reduced gastric macroscopic damage and demonstrated the same efficacy as omeprazole in reducing esophageal damage. XP significantly reduced histological damage, with an efficacy comparable to that of omeprazole, but superior to that of alginates. Inflammatory markers were significantly reduced by XP, which was superior to alginates at day 7. Interestingly, XP was able to significantly increase gastric pH.

Disruption of the esophageal epithelial barrier plays a fundamental role in initiating the pathophysiology of GERD. This study demonstrated that XP effectively restored gastric homeostasis, improved esophageal integrity and decreased inflammation and pain in vivo, with a similar efficacy to that of omeprazole. Moreover, XP improved tissue integrity with a superior efficacy to alginates. Thus, XP might represent a safe and effective alternative treatment to manage both GERD-related tissue damage and symptoms.



Glucoerucin, a glucosinolate contained in *Eruca sativa* Mill., prevents the metabolic syndrome in mice fed a high-fat diet: modulation of the browning process and the irisin pathway

Flori L[1], Testai L[1], Miragliotta V[2], Lazzarini G[2], Piragine E[1], Citi V[1], Pagnotta E[3], Matteo R[3], Righetti L[3], Di Cesare Mannelli L[4], Ghelardini C[4], Martelli A[1], Calderone V[1]

[1]*Department of Pharmacy, University of Pisa, Italy*

[2]*Department of Veterinary Sciences, University of Pisa, Italy.*

[3]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca Cerealicoltura e Colture Industriali (CREA-CI), Bologna, Italy*

[4]*Department of Neuroscience, Psychology, Drug Research and Child Health-Neurofarba-Pharmacology and Toxicology Section, University of Florence, Italy*

A condition of generalized metabolic impairment shows changes in the subject's phenotype only in the advanced stage, but silently begins with a complex of molecular alterations. Metabolic syndrome does not refer to a single disease, but to a cluster of risk factors for cardiovascular diseases and diabetes. Adipose tissue (AT) is one of the most affected areas, starting from the early stages of cellular and tissue alteration. AT can be classified into two different phenotypes, white adipose tissue (WAT) and brown adipose tissue (BAT), and both play a pivotal role in regulating energy storage and thermogenesis while contributing to the regulation of many physiological processes. Timely intervention in the early stages is essential and natural products have effective pharmacological properties in a preventive/therapeutic perspective. From this point of view, plants belonging to the *Brassicaceae* family can positively act in metabolic alterations. The aim of this experimental study was to investigate the beneficial effect of the glucosinolate glucoerucin (GER) from *Eruca sativa* Mill. in a condition of metabolic syndrome in high-fat diet (HFD) fed mice. Treatment of 48 male Balb/c mice for 10 weeks with GER 10 mg/kg/die per os showed a containment of body weight and BMI compared to untreated animals fed with HFD. GER also positively influenced the lipid and glycemic profile. A significant improvement in the metabolic profile was observed in the HFD+GER group. In fact, the circulating levels of leptin, glucagon, and insulin were significantly reduced and the levels of adiponectin showed an increasing trend. GER was able to contain visceral WAT (vWAT) deposition, white adipocytes size increase and the inflammatory processes, in favor of an increased metabolic activity and the BAT deposition. Under specific endogenous or exogenous stimuli, WAT can partially modify its phenotype or reprogram the differentiation process of preadipocyte precursors, acquiring a brown-like adipose tissue phenotype, through a process called browning, promoting the maintenance of correct lipid and glycemic homeostasis. Irisin, a myokine mainly released by skeletal muscle, is an endogenous stimulus able to trigger browning. On the other hand, if a reduction in FNDC5 level in skeletal muscle from HFD group was associated with reduced circulating irisin levels, an increase for both FNDC5 and irisin was clear in GER supplemented animals. This scenario seemed to reflect on the thermogenic activity of the WAT, highlighting a positive trend of UCP1 levels. The results obtained in this experimental study showed beneficial effects of GER in the prevention of metabolic disorders associated with HFD, improving the lipid and glycemic profile and allowing the containment of body weight, BMI and the reduction of vWAT. GER was also able to mitigate the meta-inflammation process affecting WAT and to positively modulate the metabolic profile.

Finally, it is worth noting that most of these positive effects in the management of metabolic alterations may be related to its ability to stimulate the browning process and the thermogenic activity of vWAT by modulating the irisin pathway.

A *Cynara cardunculus* L. leaves standardized extract promotes *in vitro* intestinal epithelial differentiation and barrier function through activation of AMPK/SIRT1 pathway.

Muscarà C, Salamone FL, Molonia MS, Saija A, Cimino F, Speciale A

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy

Perturbations of the intestinal epithelial barrier function lead to an increase in intestinal permeability with the consequent entry of harmful substances from the external environment. This condition of intestinal hyperpermeability, also known as leaky gut syndrome, has been correlated to some intestinal pathologies such as inflammatory bowel diseases, therefore interest in plant substances capable of improving the function of the epithelial barrier is growing recently. It has been shown that polyphenols, secondary metabolites contained in different types of fruits and vegetables, are able to improve intestinal health and that this effect is at least partly due to their ability to modulate the AMPK/SIRT1 pathway. Therefore, the aim of this work was to evaluate the *in vitro* effect on intestinal epithelial barrier function of a standardized and commercially available extract obtained from the leaves of *Cynara cardunculus* L. (CCLE), rich in polyphenols such as chlorogenic acid, luteolin and their derivatives, which represent a waste product of the food industry.

Caco-2 cells were seeded and treated with CCLE (5, 10 or 15 µg/mL) from seeding to day 18. For the *in vitro* evaluation of epithelial monolayer formation, trans-epithelial electrical resistance (TEER) was monitored throughout the differentiation period. At day 18, fluorescein permeability and alkaline phosphatase activity were measured, and calcium switch assay was carried out. Western blot analysis was used to evaluate tight junctions (TJs) expression (claudin-1, occludin, and ZO-1). In addition, p-AMPK protein expression was evaluated with western blot analysis, whereas Real-Time PCR technique was employed to investigate SIRT1 mRNA expression. A series of experiments were conducted exposing Caco-2 cells to Compound C (Comp. C), a selective AMPK inhibitor.

CCLE treatment stimulated epithelial barrier function, significantly increasing TEER values at day 18 and reducing paracellular permeability to fluorescein. These functional improvements were related to the ability of CCLE to stimulate the differentiation of intestinal epithelial monolayers as demonstrated by the increase in alkaline phosphatase activity and TJs proteins expression. Furthermore, the Ca^{2+} switch assay confirmed the important role played by CCLE in the assembly and stabilization of TJs leading to an improvement of the intestinal epithelial barrier function. Finally, thanks also to the use of Comp. C, it was confirmed that the beneficial effects of CCLE on the stimulation of epithelial cell differentiation and on the intestinal barrier function were related to the ability of the extract to activate the AMPK/SIRT1 pathway.

In conclusion, these data demonstrate the power of CCLE to improve the intestinal barrier function and support the hypothesis that this beneficial effect is due to the ability of the polyphenols contained in the extract to activate the AMPK/SIRT1 pathway. Finally, these data promote the use of agricultural waste products, such as the leaves of *Cynara cardunculus* L., to obtain extracts rich in bioactive polyphenols potentially useful for the health of the intestinal epithelial barrier.

Glycyrrhiza glabra L. extracts: phytochemical characterization and inhibition of pro-inflammatory cytokines and JAK/STAT signalling pathway in LPS-stimulated RAW 264.7 cells

Perri M.R, Marrelli M, Zicarelli L, Conforti F, Statti G
Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036, Rende, CS, Italy

The hypothesis that chronic inflammation supports cancer promotion was confirmed and elucidated over the past years, as well as the evidence that among these phenomena, a lot of modulated signalling pathways play a key role. In particular, it was recognized that the aberrant activation of the JAK/STAT cascade is associated to proliferation, invasion and metastasis signals (1). Currently, there is a growing interest in the discovery of products deriving from plants able to perform biological activities. *Glycyrrhiza* genus (Fabaceae) is widespread all over the world and includes about 30 species, among which the *Glycyrrhiza glabra* L. is the most important. According to the ancient Greek etymology, the term *Glycyrrhiza* means "sweet radix" and traditionally identify liquorice, a perennial herb native to the Mediterranean basin but also cultivated in Russia, India and China. It has a large use tradition in folk medicine: it was prescribed in the times of Roman empire and, in traditional Chinese medicine, it is considered an essential herbal medication (2,3). The aim of this research was to perform a phytochemical characterization and the assessment of the potential anti-inflammatory activity of different *Glycyrrhiza glabra* extracts. Four *Glycyrrhiza glabra* root samples belonging to different geographical areas from Southern Italy and Morocco were subjected to extraction and hydrolysis process, in order to remove the glycoside fraction within the phytocomplex. Both raw and hydrolyzed obtained extracts were characterized by means of HPLC, where three main liquorice standards (isoliquiritigenin, glycyrrhizin and 18β-glycyrrhetinic acid) were identified and quantified. Then, in order to evaluate the ability of the extracts to inhibit the release of pro-inflammatory (TNF-α, IL-6) anti-inflammatory (IL-10) cytokines and NO mediator and to downregulate the JAK/STAT signalling pathway, ELISA tests and Western blot analyses were carried out in RAW 264.7 cell lines previously stimulated with lipopolysaccharides (LPS). *Glycyrrhiza glabra* hydrolyzed samples (100 µg/mL) significantly inhibited release and production of TNF-α and IL-6 pro-inflammatory cytokines better than raw extracts. Samples were also investigated for their ability to inhibit the JAK/STAT signalling pathway: also in this case, the hydrolyzed extracts downregulated both phosphorylated JAK2 and STAT3 proteins.

Investigation of olive leaf extracts from pruning wastes

Vaccaro F, Baini G, Cappellucci G, Miraldi E, Biagi M

Università degli Studi di Siena, Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente. Strada Laterina, 8 Siena

Stress is a natural mechanism occurring in the body in response to extremely varied perturbations, including physical, psychological, metabolic, environmental, biological insults or changes, 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, namely adaptogens, has provided interesting indications about herbal products with the peculiar ability to regulate stress response, but this is one of the topic where clinical outcomes are far to be fully elucidated by mechanistic investigation.

This work aims to deepen these types of investigations and therefore different models of cellular stress have been developed; for the first time, herbal products derived from by-products, such as olive leaves was taken into account. Olive leaf extracts (OLEs) were analyzed 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 various areas of southern Italy, we produced two types of OLEs: 70% v/v ethanol extracts and decoctions. Their content in total polyphenols, total triterpenes and oleuropein was analyzed, through colorimetric assays and HPLC-DAD analysis: we found that OLEs have an important percentage of oleuropein, polyphenols and triterpenes, and, compared with standardized dry extract of US Pharmacopoeia, the phytocomplex is qualitatively equivalent to it. Subsequently, OLEs were tested on SH-SY5Y cells to evaluate their cytotoxicity at 4h and 24h and they showed to be well tolerated even at high concentrations. To set up different models of cellular stress, cells were firstly treated with various types of stressors: oxidative stress with H_2O_2, glutamate, bacterial lipopolysaccharide (LPS), serum deprivation. Then the SH-SY5Ys underwent to the same stress conditions, but with a 24h pre-treatment with OLEs. Up to now, OLEs provided interesting results, as hypothesized, in the protection from acute oxidative stress, and against serum deprivation that, again, upregulates intracellular ROS level. The antioxidant power of OLEs were quantified through DPPH and ORAC assay: in both cases a very high antioxidant capacity of OLEs emerged, with ethanolic extracts being slightly superior compared to decoctions, reflecting the respective content in polyphenols and oleuropein. Furthermore, HPLC-DAD analyses pre- and post-reaction with DPPH were carried out: although the main anti-radical constituent consumed in the reaction was oleuropein, actually the whole phytocomplex participated in the radical scavenging reaction. Finally, from preliminary pharmacokinetic investigations, carried out both through ADME prediction software and through INFOGEST simulated digestion and dynamic microfluidics coupled with biomimetic epithelial absorption, hydroxytyrosol (the main product of oleuropein degradation, as well as the most antioxidant molecule contained in olive leaves) showed to be more absorbed at the intestinal level and through the blood-brain barrier compared to oleuropein. This first investigation further paves the way for a neuroprotective application of olive leaf extracts from pruning wastes.

Sulforaphane promotes apoptotic and ferroptotic cell death in leukemia cells

Greco G[1], Schnekenburger M[2], Catanzaro E[3], Turrini E[4], Ferrini F[5], Sestili P[5], Diederich M[6], Fimognari C[4]

[1]Dipartimento di Chimica "Giacomo Ciamician", Alma Mater Studiorum-Università di Bologna, 40126 Bologna, Italia.

[2]Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, 9 rue Edward Steichen, L-2540 Luxembourg, Luxembourg.

[3]Cell Death Investigation and Therapy (CDIT) Laboratory, Department of Human Structure and Repair, Ghent University, 9000 Ghent, Belgium.

[4]Dipartimento di Scienze per la Qualità della Vita, Alma Mater Studiorum-Università di Bologna, Corso d'Augusto 237, 47921 Rimini, Italy.

[5]Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino Carlo Bo, 61029 Urbino, Italy.

[6]Department of Pharmacy, College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea.

Cell death is classified as either accidental (necrosis) or programmed (apoptosis and autophagy). Other mechanisms of non-apoptotic programmed cell death (PCD), such as ferroptosis, have recently been discovered and characterized. Several plant-derived compounds, including artesunate, piperlongumine, and whitaferin A, were identified as inducers of ferroptosis. Sulforaphane is an isothiocyanate formed by the activity of the enzyme myrosinase on the glucosinolate glucoraphanin, a bioactive compound found in cruciferous vegetables. Sulforaphane (SFN) exhibits a marked antitumor activity. Although its ability to induce apoptosis is well established, its ability to activate non-apoptotic PCD mechanisms is still poorly defined. The aim of this study was to determine whether SFN could cause ferroptosis in U-937 acute myeloid leukemia cells.

Cell death mechanisms were investigated analyzing the nuclear morphology by fluorescence microscopy after the staining of cells with Hoechst 33342 and propidium iodide. The protein expression of glutathione peroxidase 4 (GPX4) was analyzed by flow cytometry, while the intracellular levels of glutathione (GSH) and malondialdehyde (MDA) were measured spectrophotometrically.

SFN lowered U-937 cell viability in a dose-dependent manner. SFN increased the percentage of apoptotic cells after 24 hours of treatment. The fraction of necrotic cells, instead, was never greater than 10%, except at the highest tested concentration where it increased to around 70%. The pan-caspase inhibitor Z-VAD-fmk (Z-VAD) nearly entirely suppressed apoptosis caused by SFN at low concentration, demonstrating its pro-apoptotic activity. Pre-treatment with Z-VAD, on the other hand, had no effect on cells treated with the highest concentration of SFN, implying the engagement of non-apoptotic PCD mechanisms. To investigate the role of ferroptosis, U-937 cells were pre-treated with the lipid peroxidation inhibitor ferrostatin-1 (ferr-1) with or without Z-VAD before being exposed to SFN at high concentrations for 24 hours. Ferr-1 partially recovered cell viability, but the most prominent effect was a shift from necrosis to apoptosis. We next explored the main processes involved in ferroptosis, since our findings showed that SFN at high concentrations may trigger this type of cell death. SFN dramatically lowered intracellular GSH levels and GPX4 protein expression in a time-dependent manner. Furthermore, SFN enhanced lipid peroxidation, as demonstrated by a rise in MDA intracellular levels.

Our results indicate that the ability of SFN to trigger different PCD in a dose-dependent manner underlies its cytotoxic effect. At low concentrations, SFN induced apoptosis, while at higher concentrations it activated ferroptosis. Furthermore, SFN switched one mode of cell death to another. Overall, our findings corroborate the pleiotropic nature of SFN, making its use as an anticancer drug very attractive and paving the way to future studies.

Cannabidiol loaded in liposomial formulation enanches antigenotoxic effect against camptothecin

Grifoni L[1], Brunha C[2], Sendão T[2], Dias A[2], Oliveira R[2], Bilia AR[1]

[1]*Department of Chemistry, University of Florence, Florence*

[2]*Centre of molecular and environmental biology (CBMA), University of Minho, Portugal*

Cannabidiol (CBD) is a pleiotropic non-psychoactive constituent of *Cannabis* being pursued as a therapeutic treatment for multiple conditions [1]. Studies reporting pharmacokinetic data of CBD in humans have evidenced a scarce bioavailability after oral administration. Currently, CBD conventional formulations are challenging because of unpredictable release and absorption. Rational design of nanodelivery systems can provide a practical alternative [2]. In the present study CBD was nanoencapsulated in liposomes to enhance stability, solubility, and bioavailability. Developed deformable nanoliposomes were made of Phospholipoid 90G, cholesterol and Tween20, exhibited ideal physical characteristics in terms of size (147.6±1.2 nm), polydispersity index (0.193±0.070) and Z-potential (-45.81±1.43 mV). Moreover, high percentage recovery and encapsulation efficiency, 99.01±1.41% and 90.55±1.22% respectively, were found using HPLC technique. The antigenotoxic potential of CBD both unformulated and loaded in liposomes (LP-CBD) was evaluated in *Schizosaccharomyces pombe* yeast model, using camptothecin (CPT) as a toxicity-inducing agent. First a spot assay was performed. By observing fluorescence microscopy, cells treated with CPT revealed some alterations, in particular an abnormal elongation when compared to negative control. CPT co-treated cells using both, individually, CBD and LP-CBD evidenced a decrease cell size, suggesting their potential in mitigating the damage caused by CPT. Even if the difference between CBD and CBD-LP was noticeable, evidence of different results came from cell cycle analysis. After ninety minutes, only the treatment containing CBD-LP showed the same results of negative control. According to this study CBD possesses antigenotoxic properties. In addition, the higher activity of CBD was found in CBD-LP, probably due to the highest permeation across *Schizosaccharomyces* membrane.

Zosterabisphenone C, a new diarylheptanoid heterodimer from the seagrass *Zostera marina,* shows cytotoxic effects in colon cancer

De Cicco P[1], Cacciola NA[2], Amico R[1], Grauso L[3], Scarpato S[1], Mangoni A[1], Borrelli F[1]

[1]*Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy.*

[2]*Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy.*

[3]*Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy.*

The marine environment is a rich source of biologically active molecules for the treatment of human diseases, including cancer. To date, several marine active ingredients have been approved by the Food and Drug Administration for the treatment of various cancers (1). Seaweeds are a valuable resource for bioactive natural compounds (2,3). *Zostera marina* L. (Fam. Zosteraceae) is a common and easily accessible seagrass that is widely distributed in the North Atlantic and North Pacific. To date, several phenolic compounds in *Z. marina* have been well studied, particularly as antimicrobial or antifouling agents (4), including zosteraphenols, new tetracyclic diarylheptanoids (5). Here we report the isolation of unique diarylheptanoid dimers named zosterabisphenone (ZBP) A, B and C and the elucidation of their potential anti-cancer activity (6,7). Samples of *Z. marina* (unrooted plants, freshly washed ashore and air-dried) were extracted with acetone, and the extract was successively subjected to SiO2 column chromatography, Sephadex LH -20 chromatography and reversed-phase HPLC to obtain pure ZBPs. To investigate the antitumor activity of the ZBPs, we performed cytotoxic assays on two cancer cell lines [the colorectal human cancer cell line HCT116 and the hepatic cancer cell line Hep G2 (0.1-10 µM, 24 h and 48 h exposure)] and on a healthy human colonic epithelial cell line (HCEC). Finally, we investigated the anti-cancer activity of the more active ZBP in vivo using a human colon cancer xenograft model. ZBP-A showed weak cytotoxic effects on HCT116 at the highest concentration and time period tested (23% inhibition) and no effects on Hep G2. In contrast, ZBP-B significantly reduced HCT116 cell viability (97.4% inhibition at 10 µM, 48h) and impaired Hep G2 cell viability only at the highest concentration tested. ZBP-C significantly reduced HCT116 cell viability (73% inhibition at 10 µM, 48h), while it did not reduce Hep G2 cell viability at any of the concentrations tested. To gain insight into the anticancer effects of ZBP, we selected compound ZBP-B and performed further studies on HCT116 cells. To evaluate selectivity on cancer cells versus healthy cells, cytotoxic studies were also performed on HCEC. ZBP-B reduced the viability of HCEC by only 7%. Annexin V/ PI assay showed that ZBP-B (10 µM) induced apoptosis in about 60% of cells after 24 h of treatment. The pro-apoptotic activity was also confirmed by the activation of caspase-3, by the cleavage of its substrate poly(adenosine diphosphate ribose) polymerase (PARP) and the activation of caspase-8 and -9. In addition, our results showed that ZBP-B decreased the protein expression of the anti-apoptotic proteins Bcl-2 and c-myc. These results suggest that ZBP-B exerts a selective cytotoxic effect on colorectal cells (HCT116 cells) due to activation of both the intrinsic and extrinsic apoptotic pathways. In mice bearing a tumor, treatment with ZBP-B (20 mg-kg-1, daily peritumorally) significantly reduced tumor growth compared to the vehicle group. Our results suggest that ZBP-B has the potential to inhibit the progression of colon cancer and could therefore be proposed as a lead compound for the development of new antitumor drugs in colorectal cancer.

Perillaldehyde from *Ammodaucus leucotrichus* as a new ferroptosis inducer with relevant clinical potential

Turrini E[1], Catanzaro E[2], Maffei F[1], Guerrini A[3], Krysko DV[2], Fimognari C[1]

[1]*Department for Life Quality Studies, University of Bologna, Rimini, Italy*

[2]*Cell Death Investigation and Therapy (CDIT) Laboratory, Department of Human Structure and Repair, Ghent University, Ghent, Belgium*

[3]*Pharmaceutical Biology Lab., Research Unit 7 of Terra&Acqua Tech Technopole Lab., Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy*

Many anticancer drugs derive from natural sources and have been explored for their ability to induce non-apoptotic cell death mechanisms, such as necroptosis, ferroptosis or pyroptosis, as an important strategy to fight cancer (Greco et al. Cancers 2021;13:304). In particular, ferroptosis induction is an emerging strategy to treat cancer and contrast the tricky issue of chemoresistance due to apoptosis resistance (Wu et al. Front Oncol 2020;10, 571127). Ferroptosis is a regulated form of cell death, which is carried out by blocking the system Xc- cystine/glutamate antiporter or glutathione peroxidase 4 (GPX4), resulting in a defective glutathione (GSH)-redox system. The consequent inability to quench the reactive oxygen species (ROS) leads to the accumulation of lipid ROS, crucial for the execution of ferroptotic cell death (Dixon et al. Cell 2012;149:1060–72). This work aimed to elucidate the anticancer mechanisms evoked by perillaldehyde (P), a monoterpenoid isolated from *Ammodaucus leucotrichus* Coss. & Dur, a medicinal annual plant belonging to the family *Apiaceae* that grows in the Saharan and Sub-Saharan countries, commonly known as "hairy cumin".

We investigated the antileukemic potential of P in HL-60 promyelocytic leukemia cells through the analysis of its cytotoxic potential. The analysis of cell death was performed using specific inhibitors and the cell-impermeant fluorescent probe Sytox Green, followed by cytofluorimetric analysis. We characterized the P mechanism of action through the analysis of lipid peroxidation, GPX4 expression and GSH intracellular level using flow cytometry and spectrophotometry. Furthermore, we analyzed the release of ATP triggered by P via a multimode multiplate reader measuring luminescence. To preliminarily assess the clinical relevance of P, we tested its ability to induce cell death on patient-derived acute myeloid leukemia (AML) biopsies, using Sytox Blue as viability dye. To round the study off, we tested its selectivity towards tumor cells using peripheral blood mononuclear cells from healthy donors and Sytox Green as viability dye.

P-induced cell death was not antagonized by neither necroptosis nor apoptosis's inhibitors. However, all tested ferroptosis inhibitors significantly counteracted P's cytotoxic effects, envisaging this compound as a promising ferroptosis inducer. Specifically, P induced ferroptosis through increase in lipid peroxidation, decrease in GPX4 protein expression, and depletion of intracellular GSH. Besides, it stimulated the active secretion of ATP, one of the necessary events in the induction of adaptative immune response (Garg et al. EMBO J 2012;31:1062–79), prompting further studies to disclose its possible nature as an immunogenic cell death inducer (Fucikova et al. Cell Death Dis 2020;11:1–13). We recorded a similar mechanism of action and potency on patient-derived AML biopsies compared to HL-60 cells. Moreover, P showed lower toxicity on normal cells compared to both HL-60 cells and AML biopsies, thus partial selectivity.

Altogether, these data depict a favorable risk-benefit profile for P and reveal its peculiar antileukemic activity, which qualifies this natural product to proceed further through the drug development pipeline.

Acmella oleracea extracts from _in vitro_ seedlings: chemical characterization and a novel preclinical application on the acute treatment of chemotherapy-induced neuropathy

Zonfrillo B[1], Bellumori M[1], Firenzuoli F[3], Maggini V[2], Di Cesare Mannelli L[1], Ghelardini C[1], Mulinacci N[1], Innocenti M[1]

[1]*Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence*

[2]*CERFIT, Research and Innovation Center in Phytotherapy and Integrated Medicine, Careggi University Hospital, Florence*

[3]*Department of Biology, University of Florence*

Acmella oleracea (L.) R.K. Jansen (Asteraceae), popularly known as jambu, is commonly used as food ingredients in typical Amazon cuisine, and as a traditional medicinal herb. It is a rich source of several bioactive compounds, notably alkylamides, flavonoids and other phenolic compounds. The anti-inflammatory, antioxidant, and pain-relieving properties of this plant have been extensively studied, whereas less is known about its properties in the treatment of persistent neuropathic pain.

This research involved the study of aerial parts (AP) and roots (R) of *Acmella* seedlings grown *in vitro* and developed starting from regenerating lines derived by organogenesis[1]. The study aimed to characterize the phytochemical profile of the extracts from aerial parts and roots of the plant, focusing on the evaluation of its characteristic secondary metabolites, i.e. alkylamides and phenolic compounds. The compounds were recovered in two extracts: one hydroalcoholic (HAE) and one lipophilic (HE). These extracts were tested on a mouse model of chemotherapy-induced neuropathy (CIN), one of the most debilitating side effects of anti-cancer drugs. A total of 24 compounds (12 phenols and 12 alkylamides) were detected by HPLC-DAD-MS[2]. The chromatographic profiles of the extracts of the aerial parts and roots showed a similar pattern of secondary metabolites, in particular a pool of cinnamoyl derivatives and several alkylamides, whose main component was spilanthol. The extracts were also analyzed by ^{1}H-NMR spectroscopy to have a fingerprinting of the main organic compounds present in the sample. The two tissues presented quantitative differences in secondary metabolites: the aerial parts were rich in phenols and alkylamides (respectively 8.68 ± 0.31% mg/g DE and 2.77 ± 1.50% mg/g DE in HAE extracts), while the roots were almost twice as rich in phenols (14.15 ± 1.50% mg/g DE in HAE extracts) but alkylamides were in a negligible amount. The extraction and fractionation procedure allowed the separation and concentration of the two classes of secondary metabolites in different extracts for evaluating the biological effect of spilanthol and alkylamides alone or in combination with phenols.

Preliminary results on CIN model highlight the acute therapeutic effect of phenolic components of *Acmella oleracea* aerial parts and roots extracts against oxaliplatin-induced neuropathy, increased by the presence of spilanthol. The results candidate *Acmella* hydro-alcoholic extracts as a novel approach against neuropathic pain.

Evaluation and characterization of the cytotoxic profile of two compounds extracted from the Caribbean sponge *Smenospongia aurea* in a panel of human cancer cell lines

Pellicioni V[1], Greco G[2], Costantino V[3], Teta R[3], Esposito G[3], Fimognari C[1]

[1]Dipartimento di Scienze per la Qualità della Vita, Alma Mater Studiorum – Università di Bologna, 47921 Rimini, Italia

[2]Dipartimento di Chimica "Giacomo Ciamician", Alma Mater Studiorum-Università di Bologna, 40126 Bologna, Italia

[3]Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, 80131 Napoli, Italia

The marine environment accounts for more than 70% of the surface of the earth, and marine organisms represent valuable sources of bioactive molecules. Among them, sponges are responsible for the production of a wide variety of secondary metabolites essential for their survival, characterized by great chemical diversity and multiple biological effects. Such secondary metabolites yielded great interest due to their potential pharmacological activities, including anti-cancer activity.

The aim of the study was to assess the cytotoxic profile and characterize the cell death mechanism induced by the compounds smenamide-A and smenolactone-D, a chlorinated peptide/polyketide hybrid and a chlorinated polyketide, respectively, extracted from the Caribbean sponge *Smenospongia aurea*. In particular, their activity was tested on a panel of human cancer cell lines: breast cancer (MCF-7), epidermoid carcinoma (A431), and leukemia (Jurkat). Smenamide-A (1 nM-20 µM) did not reduce cell viability in any of the tested cell lines. In contrast, smenolactone-D (1-10 µM) was cytotoxic in a concentration-dependent manner in all the tested cell lines. As Jurkat cells resulted to be the most sensitive, the subsequent experiments were conducted on this cell line.

In order to understand the mechanism of cell death induced by smenolactone-D, we used specific inhibitors of different cell death mechanisms. In particular, inhibitors of apoptosis (zVAD-fmk), ferroptosis (deferoxamine [DFO], vitamin E, ferrostatin-1), and necroptosis (necrostatin-1s) were used. Moreover, N-acetylcysteine (NAC) was employed to investigate the role of reactive oxygen species (ROS) in smenolactone-D-induced cell death. The results show a significant increase in cell viability following pretreatment with NAC and zVAD-fmk, while an almost complete recovery of cell viability was obtained following pretreatment with vitamin E. These data suggest that the cytotoxicity triggered by smenolactone-D relies on a combined mechanism of action involving both the induction of apoptosis and ferroptosis.

Ferroptosis is a form of non-canonical cell death activated by an iron-dependent accumulation of lipid ROS, resulting from direct or indirect inhibition of the GPX4 enzyme. To confirm the involvement of ferroptosis in the mechanism of action of smenolactone-D, we analyzed 1) the intracellular levels of malondialdehyde, one of the final products of lipid peroxidation, and 2) GPX4 protein expression. Treatment with the compound doubled the cellular malondialdehyde amount compared to control cultures and caused a time-dependent decrease in GPX4 expression.

Altogether, the results obtained in the present study outline an interesting *in vitro* cytotoxic potential of smenolactone-D, whose multifaceted mechanism makes it an interesting lead compound for the management of diseases such as cancer, paving the way for the synthesis of structural analogs.

Antiproliferative effects of Cannabis with low content of tetrahydrocannabinol: potential in the treatment of psoriasis

Rizzi L, Pellati F, Bresciani E, Molteni L, Meanti R, Corsi L, Torsello A
Università degli Studi di Milano- Bicocca
Università degli Studi di Modena e Reggio Emilia

Psoriasis is a multiform systemic disease characterized by chronic inflammation and uncontrolled proliferation of keratinocytes. The pathogenesis is complex and not fully understood. Classic therapeutic strategies, and most recent biological drugs have major undesirable effects. New drugs are needed to improve tolerability and effectiveness on the disease. In this contest, scientific evidence has proposed the use of cannabinoids as a potential treatment of psoriasis.

It is known that Cannabis sativa (CS) contains in its phytocomplex several compounds that have been suggested for the therapy of skin and immune-mediated pathologies. These compounds appear to exert their immunomodulatory properties by induction of apoptosis, suppression of cell proliferation, inhibition of synthesis and liberation of inflammatory cytokines, and production of chemokines. Based on this evidence, the purpose of this research was to evaluate in vitro the antiproliferative activity of different phytocomplexes extracted from 3 CS varieties characterized by a low content of tetrahydrocannabinol (THC). Extracts of CS Carmagnola and CS Santhica are characterized by high concentrations of cannabidiol (CBD), while extracts obtained from CS Bernabeo have high levels of cannabigerol (CBG).

Immortalized human keratinocytes (HaCaT) were incubated with increasing concentrations of the 3 CS extracts to study their antiproliferative activity. Cell viability was measured by MTT cell viability assays, while apoptosis and necrosis were evaluated by the Annexin V tests. CS Carmagnola extracts, CBD and CBG, caused a reduction in the proliferation of keratinocytes. CS Santhica extract activated the apoptotic process, increased Bax and decreased Bcl-2 and induced a tendency to increase the synthesis and activation of Caspase 3 and Caspase 7. Treatment with CBD, instead, did not induce changes in apoptotic markers, but caused a significant decrease in the levels of interleukins IL-23A, IL-1β and IL-6.

Overall, these results indicate that CS extracts exert their antiproliferative effects by reducing cell proliferation and/or inhibiting the synthesis and release of inflammatory cytokines.

Through confocal microscopy we have also shown that dying HaCaT keratinocytes have a reduced cell volume, with thickening of the cytoplasm, and the formation of vacuoles in the nucleus probably related to an autophagic process.

In conclusion, our results suggest that CS extracts can regulate keratinocyte proliferation, promote apoptosis and the inhibit inflammatory cytokines. Further studies will help to better clarify the molecular mechanisms involved.

Phytochemical and pharmacological investigation of industrial hemp inflorescences by-products

Acquaviva A[1,2,3], Di Simone SC[1], Libero ML[1], Nilofar[1], Chiavaroli A[1], Recinella L[1], Leone S[1], Brunetti L[1], Orlando G[1], Menghini L[1], Ferrante C[1]

[1]*Dipartimento di Farmacia, Università degli Studi "G. d'Annunzio" Chieti, Italia*

[2]*Facultad de Ciencias, Universidad de Granada, Spagna*

[3]*Veridia Italia S.R.L., Pescara, Italia*

Industrial hemp is characterized by a huge number of by-products, such as inflorescences, that have been recently recognized as high-quality sources of biomolecules and bioactive extracts with pharmaceutical interest. The present research is a comparative investigation on phytochemical and pharmacological properties of extracts obtained from female and male inflorescences of different industrial hemp varieties, namely *Futura 75, Carmagnola selezionata, Eletta campana, Kompolti,* and *Tisza*.

The water extract obtained from female inflorescences of variety *Futura 75* showed the presence of cannabidiol, cannabidiolic acid and rutin as the prominent phytocompounds. In the in vitro system, the hemp extract was able to protect human keratinocytes and fibroblasts from cytotoxicity and apoptosis induced by oxidative stress. Moreover, modulatory effects on IL-6 were found. In isolated rat skin, the extract reduced hydrogen peroxide-induced l-dopa turnover, prostaglandin-E2 production and the ratio kynurenine/tryptophan, thus corroborating anti-inflammatory and antioxidant effects. The present findings showed the efficacy of hemp water extract from *Futura 75* as skin protective agent.

The essential oils (EOs) of *Futura 75*, *Carmagnola selezionata* and *Eletta campana* showed E-caryophyllene and α-pinene as the prominent terpenes, whereas the cannabidiolic acid was the terpenophenol present at higher concentration. The EOs inhibited the growth of all tested dermatophytes species. In isolated skin specimens, EOs prevented the hydrogen-peroxide-induced synthesis of prostaglandin E_2, consistent with the intrinsic anti-inflammatory activity. Finally, in H1299 cells, all tested EOs reduced the gene expression of ACE-2 and TMPRSS2, as well.

The prominent terpenes in the EOs of *Kompolti* and *Tisza* were iso-caryophyllene, α-humulene, and β-caryophyllene oxide, whereas cannabidiol and cannabigerolic acid were the main terpenophenols, respectively. Both EOs inhibited the viability of different cancer cells. The EOs also produced antimycotic effects towards different dermatophyte species. Therefore, the present findings highlight the pharmacological properties of the *Futura 75*, *Carmagnola selezionata*, *Eletta campana*, *Kompolti* and *Tisza* essential oils, which deserve further investigations.

The phytochemical and biological properties of pollen extracts from male inflorescences of industrial hemp is almost completely unexplored in the literature. Male flowers in flowering crops represent a waste product that is selected manually. To date, hemp pollen represents an innovative product that could be advantageous for an implementation of the supply chain with a view to optimizing processes and limiting waste. The water and hydroalcoholic extracts from hemp pollen were found particularly rich in phenolic compounds, such as hydroxytyrosol, coumaric acid, and hesperetin. The brine shrimp (*Artemia salina*) lethality test revealed a higher toxicity of the hydroalcoholic extracts. In analogy, in the *daphnia*

magna test, no toxicity attributable to the extracts was highlighted. Additionally, in prostate PC3 and myocyte C2C12 cells no relevant changes in vitality induced by the extracts were detected. The extracts were found effective as inhibitors of different bacterial and fungal strains. This study confirms the novelty of a product obtained directly from bees, suggesting the hemp pollen extracts as innovative sources of antimicrobial agents.

Overall, the obtained experimental data validate the innovativeness of industrial hemp by-products extracts that can reserve promising applications in food, pharmaceutical, and cosmetic sectors.

Gynostemma pentaphyllum (var. Ginpent) protects against acute peripheral inflammation and motor alteration

Mac Sweeney E[1], Bonini SA[1], Premoli M[1], Maccarinelli G[1], Zhang L[2], Lucini L[2], Memo M[1,*], Mastinu A[1,*]

[1]Department of Molecular and Translational Medicine, Division of Pharmacology, University of Brescia, 25123 Brescia, Italy

[2]Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy

**NutriFun4Health, Center for Research and Services on Nutraceuticals and Functional Food for Health, Brescia, Italy*

Gynostemma pentaphyllum (var. Ginpent) (GP) is a perennial plant that belongs to the Cucurbitaceae family. It grows spontaneously in South and East Asia and it was widely used in traditional medicine. According to many in vitro and in vivo studies, GP extracts have demonstrated antioxidant, anticancer, anti-inflammatory, antidiabetic and antilipidemic activity, among others. This study aims to investigate the effect of the treatment with GP dry extract on animal models with acute peripheral inflammation and motor alteration induced by lipopolysaccharide (LPS 7.5 mg/kg). Initially, the GP dry extract secondary metabolite content was determined by means of UHPLC-ESI/QTOF-MS. Mice were then pre-treated with the GP extract (for three days) and on the third day with LPS. The GP effect on motor coordination was investigated by performing two motility tests: the pole and rotarod tests. To evaluate the GP extract anti-inflammatory activity, an enzyme-linked immunosorbent assay (ELISA) was also performed using mice's serum. The UHPLC-ESI/QTOF-MS analysis identified many secondary metabolites with potential anti-inflammatory activity, such as saponins, polyphenols and phytosterols. The results of the motility tests showed that the pre-treatment with GP had a protective effect against motor alteration induced by LPS. In addition, the GP treatment reduced the expression of inflammatory cytokines, such as IL-1β and IL-6, as shown by the ELISA data. In conclusion, this study highlights the therapeutic value of GP as an anti-inflammatory agent for inflammatory-based disorders. Further studies are needed to determine if the GP protective effect against inflammation is due to single molecules or to the phytocomplex.

Artocarpus tonkinensis protects mice against collagen-induced arthritis: decreases Th17 cell function and suppresses osteoclastogenesis

Adorisio S[1], Ayroldi E[2], Delfino DV[1,2]

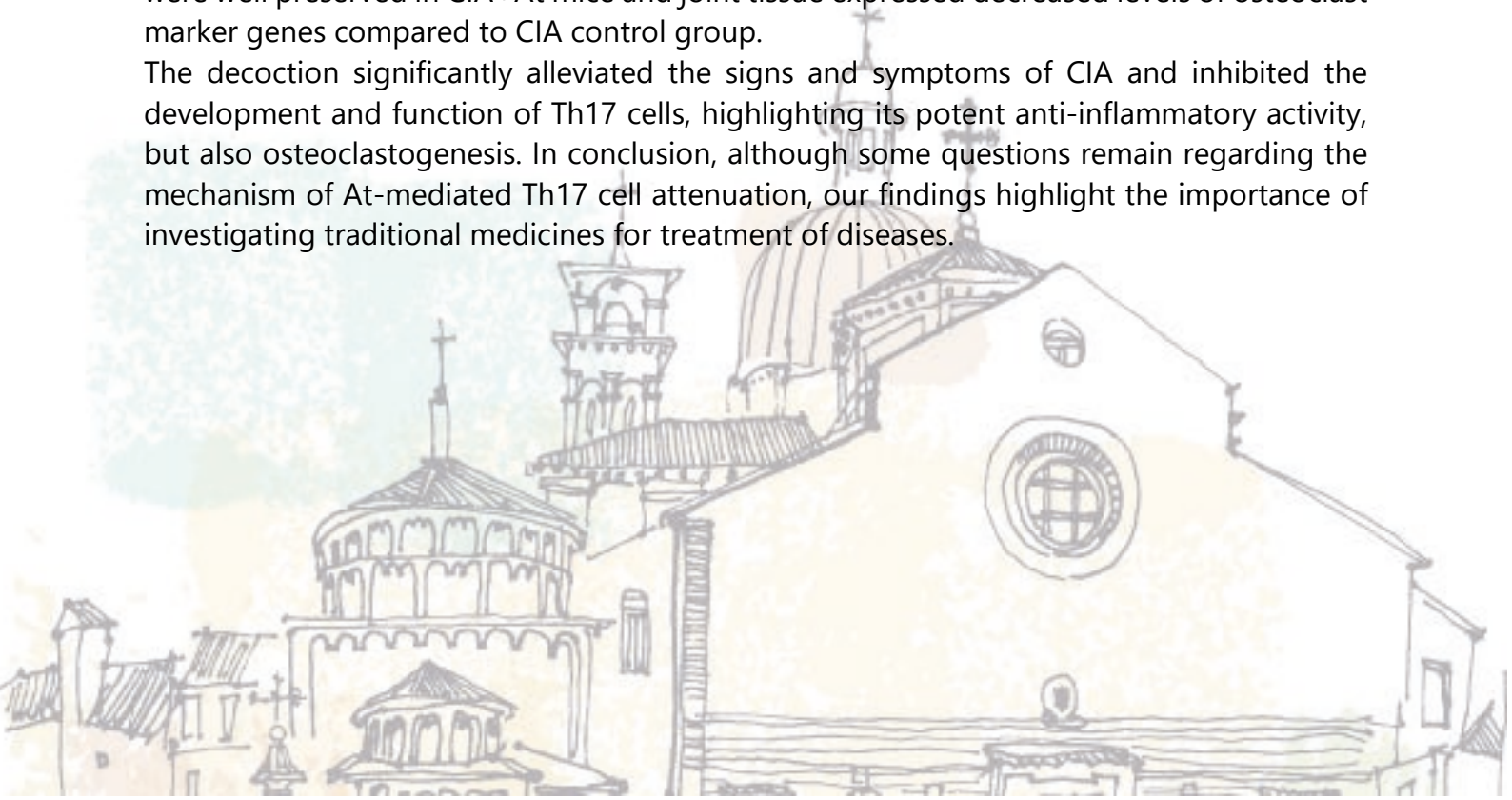
[1] *Foligno Nursing School, Department of Medicine and Surgery, University of Perugia, Perugia, Italy.*

[2] *Department of Medicine and Surgery, University of Perugia, Perugia, Italy.*

Artocarpus tonkinensis (Moraceae) is a tree that grows in north Vietnam whose leaf decoction is used as a traditional remedy by the Hmong ethnic group to treat arthritis and backache. Our study evaluated the decoction's efficacy and mechanism of action in DBA/1J mice with collagen-induced arthritis (CIA). CIA are also characterized by chronic joint inflammation mediated by IL-22 produced by Th17 cells, which stimulates synovial fibroblasts to induce cell proliferation and secretion of other inflammatory cytokines, and the initiation of osteoclastogenesis. Moreover, IL-6 produced by fibroblasts responding to Th17 cell-derived IL-17 amplifies inflammation and stimulates synovial tissue in an autocrine manner. Thus, IL-17 maintains the inflammatory cycle via activity of downstream cytokines. We investigated whether also macrophage cell population might be targeted by A. tonkinensis decoction assessing if it could interfere in macrophage acquisition of a pro-inflammatory phenotype, and decrease formation of osteoclasts, key cells in the rheumatoid arthritis joint damage.

Mice treated with the decoction (At) either from the first collagen immunization or after CIA development experienced significantly less joint edema and inflammatory infiltration, whereas CIA-induced cartilage damage could only be prevented by early At treatment. Autoimmune gene expression profiles showed that Th17 cell-associated chemokine CCL20 and cytokines IL-6, IL-17, and IL-22 were strongly downregulated by At. Reduced expression of IL-2, IL-17, IL-22, and FasL in lymph node cells from At-treated mice was further confirmed by real-time PCR. The decoction also inhibited polarization of Th17 cells from CD4+ splenic T cells according to levels of IL-17 and RORC, a Th17 cell-specific transcription factor. Protective effect of At against bone loss was also enlightened in vivo, bone and cartilage were well preserved in CIA+At mice and joint tissue expressed decreased levels of osteoclast marker genes compared to CIA control group.

The decoction significantly alleviated the signs and symptoms of CIA and inhibited the development and function of Th17 cells, highlighting its potent anti-inflammatory activity, but also osteoclastogenesis. In conclusion, although some questions remain regarding the mechanism of At-mediated Th17 cell attenuation, our findings highlight the importance of investigating traditional medicines for treatment of diseases.



Anti-oxidant and anti-inflammatory effects of ellagic and punicic acid in an *in vitro* model of cardiac fibrosis

Pallio G, Minutoli L, Irrera N, Squadrito F
Department of Clinical and Experimental Medicine, University of Messina, Via C. Valeria, 98125 Messina, Italy

Cardiac fibrosis is a pathological process characterized by an excessive deposition of extracellular matrix (ECM) and an increased production of fibrillar collagen in the cardiac interstitium, mainly caused by the activation of cardiac fibroblasts and their transition into myofibroblasts. Oxidative stress is deeply implicated in the patho- genesis of cardiac fibrosis both directly and via its involvement in the tumor growth factor β1 (TGF-β1) signaling. Ellagic acid (EA) and punicic acid (PA) are the main components of the Punica granatum L (pomegranate) fruit and seed oil respectively, whose antioxidant, anti-inflammatory and anti-fibrotic effects have been previously described. Therefore, the aim of this study was to investigate the effects of EA or PA or EA+PA in an in vitro model of cardiac fibrosis. Immortalized Human Cardiac Fibroblasts (IM-HCF) were stimulated with 10 ng/ml of TGF-β1 for 24 h to induce a fibrotic damage. Cells were then treated with EA (1 μM), PA (1 μM) or EA+PA for additional 24 h. Both EA and PA reduced the pro-fibrotic proteins expressions and the intracellular reactive oxygen species (ROS) accumulation. The anti-oxidant activity was also observed by Nrf2 activation with the consequent TGF-β1-Smad2/3-MMP2/9 and Wnt/β-catenin signaling inhibition, thus reducing collagen production. EA and PA significantly inhibit NF-κB pathway and, consequently, TNF-α, IL-1β and IL-6 levels: the greater effect was observed when EA and PA were used in combination. These results suggest that EA, PA and in particular EA+PA might be effective in reducing fibrosis through their antioxidant and anti-inflammatory properties by the modulation of different molecular pathways.

Essential oil composition, antioxidant activity and leaf micromorphology of five Tunisian *Eucalyptus* species

Romano B[1], Maresca DC[1], Polito F[2], Cornara L[3], De Feo V[2], Ercolano G[1], Ianaro A[1]

[1]Department of Pharmacy, School of Medicine and Surgery, University of Napoli Federico II, Via D. Montesano, 49, 80131 Napoli, Italy

[2]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy

[3]Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy

Eucalyptus species have been widely employed in the projects of reforestation in Tunisia. Although their ecological functions are controversial, these plants are indeed important to counteract soil erosion, and represent a fast-growing source of fuelwood and charcoal wood. In the present study, we considered five Eucalyptus species, namely Eucalyptus alba, E. eugenioides, E. fasciculosa, E. robusta, and E. stoatei cultivated in the Tunisian Arboreta. The aim was to carry out the micromorphological and anatomical characterization of the leaves, the extraction and phytochemical profile of the essential oils (EOs), and the evaluation of their biological properties.

These EOs showed in vitro antioxidant activity, and reduced the oxidative cellular stress as shown by their activity on reactive oxygen species (ROS) production, and modulation of the expression of antioxidant enzymes, such as glutamate-cysteine ligase (GCL) and heme oxygenase-1 (Hmox-1). Moreover, the EOs inhibited the production of nitric oxide (NO), showing anti-inflammatory activity. The data collected suggest that these EOs may be considered a promising therapeutic strategy for inflammation-based diseases and may represent an additional value for the economy of Tunisia.

Bergamot polyphenols improve hepatic inflammation, and reverse altered thalamus metabolism and brain structure in a mouse model of non-alcoholic fatty liver disease

Musolino V[1], Cardamone A[2], Coppoletta AR[2], Nucera S[2], Ruga S[2], Bosco F[2], Guarnieri L[2], Macrì R[2], Scarano F[2], Bava I[2], Caminiti R[2], Lorenzo F[2], Tucci L[3], Lupia C[2], Serra M[2], Mollace R[2], Maiuolo J[1], Carresi C[2], Muscoli C[2], Palma E[2], Gliozzi M[2], Mollace V[2]

[1]Laboratory of Pharmaceutical Biology, Department of Health Sciences, Institute of Research for Food Safety & Health IRC-FSH, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy.

[2]Department of Health Sciences, Institute of Research for Food Safety & Health IRC-FSH, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy.

[3]H&AD Srl, 89032, Bianco, Italy.

Citrus bergamia Risso et Poiteau, known as bergamot, is a small tree belonging to the Rutaceae family, with persistent, simple and alternate leaves. The blade is ovate-oblong or lanceolate with a dark-green upper surface and light-green lower surface. The actinomorphic flowers are pentamerous, bisexual, and fragrant. Fruit is a slightly flattened subglobose to pyriform berry (hesperidium). Although the geographical origin of bergamot is still uncertain, it grows almost exclusively in the coastal area of Calabria from Villa S. Giovanni to Gioiosa Jonica, where the climate and environmental conditions are favorable to its cultivation. Bergamot is mainly known for its essential oils, obtained by the fruit peel, and widely used in cosmetic industry, whereas the other parts of the plant are considered as waste products. Among them, the "pastazzo", obtained after fruit peeling and squeezing, have been studied for its nutraceutical potential due to the typical polyphenol composition. To extract polyphenols, "pastazzo" was minced with water and the obtained solution was passed through a polystyrene absorbing resin column. Following the elution and evaporation step, the concentrated polyphenolic solution was dried in a spray dryer system to achieve the powder of bergamot polyphenolic fraction (BPF). Phytochemical characterization showed that BPF is mainly composed by flavanones, flavones, and their 3-hydroxy-3-methyl-glutaryl derivatives which lend hypolipemic, hypoglycemic, anti-oxidative and anti-inflammatory properties in the treatment of metabolic syndrome disorders, such as Non-alcoholic fatty liver disease (NAFLD). NAFLD represents a continuum of events characterized by hepatic fat accumulation which can progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and in some cases hepatocellular carcinoma. NAFLD have wider implications than NASH; indeed, it can involve several organs and systems, the brain included. The aim of this investigation was to perform a preclinical study to evaluate BPF effects on NASH implications in the diet-induced animal model of NAFLD (DIAMOND). Groups of 8 weeks old mice were randomly assigned to fed chow diet and tap water (NCNW) or high fat diet with sugar water (WDSW). WDSW animals were treated with vehicle or 50 mg/kg/day BPF, via gavage once daily over 11 weeks, starting from week 16. NCNW mice did not receive any treatment. Animals were weighed before starting diet regimen and once a week till the end of the study. At the baseline and weeks 4, 8, 20, 27, the thalamic neurochemical profile and total cerebral brain volume (TCBV) were evaluated using ^{1}H-MRS. The results showed that BPF reduced ALT, AST, triglycerides, LDL-C, total cholesterol, and fasting glucose. It significantly improved NASH resolution ($p<0.001$) and the SAF scores ($p<0.05$). BPF reduced markers of oxidative stress, circulant pro-inflammatory cytokines, JNK and p38 MAP kinase activity. BPF did not reduce the number of mice with

fibrosis but improved collagen proportional area ($p < 0.05$) and procollagen I and III expression. BPF prevented TCBV reduction and the increase in thalamic levels of total N-acetylaspartate, total creatine, total choline, and taurine. Collectively our results showed that BPF resolves NASH and ameliorates pathophysiological features of NASH along with restoring the cerebral energy metabolism and preventing brain inflammation.

Identification of an induced-neurodegeneration preclinical model for the study of plant extracts with anti-glycantion activity

Pucci M[1], Abate G[1,2], Tirelli E[1], Mastinu A[1,2], Memo M[1,2], Uberti D[1,2]

[1]Università degli Studi di Brescia, Dipartimento di Medicina Molecolare e Traslazionale (DMMT), Sezione di Farmacologia

[2]NutriFun4Health, Center for Research and Services on Nutraceuticals and Functional Food for Health, Università degli Studi di Brescia.

Advanced-glycation-end-products (AGEs) are nonenzymatic glycation products that are formed in the presence of elevated glucose levels and are implicated in the development of many chronic and degenerative diseases, including diabetes, cardiovascular disease, and Alzheimer's disease. AGEs induce oxidative and inflammatory damage through structural and functional alterations in proteins, and through activation of the AGEs receptor (RAGE) pathway. A limitation for testing the central-level effects of novel antiglycating compounds is the lack of suitable preclinical models. Our research group characterized a mouse model treated with Methylglyoxal (MG), a highly reactive AGEs precursor that is formed both endogenously and in food. In our study, chronic administration of high dosages of MG induced cognitive impairment accompanied by increased gene and protein expression of RAGE, pro-inflammatory cytokines, and an imbalance in redox homeostasis. In addition, MG-treated animals show hippocampal alterations in the amyloidogenic pathway of Amyloid Precursor Protein (APP), which is involved in Alzheimer's disease. The aim of the study is to screen through the "high-throughput" model for natural compounds and plant extracts with potential anti-glycating activity that will be selected for preclinical study.

Lettura Magistrale

EU Regulatory framework on food supplements

Di Giorgi V (Ministero della Salute, Roma)

Phytotherapy in the treatment of chronic low-grade inflammation

Santagà D.
A.V.D. Reform

Phytotherapy represents a valid intervention aid in different phases of low grade chronic inflammation. It has been seen that one of the major culprits of inflammaging and immunosenescence is the transcription factor NFkB.
The main plants that inhibit the release of NFkB are Curcuma Longa, Boswellia Serrata, Zingiber Officinalis, Silymarin from Sylibum marianum.
These plants are particularly useful as they simultaneously manage to activate another transcription factor fundamental in the response to inflammaging, i.e. NRf2.

Tested to reach the highest quality: Labomar's approach for ensuring quality, safety and efficacy of nutraceutical products

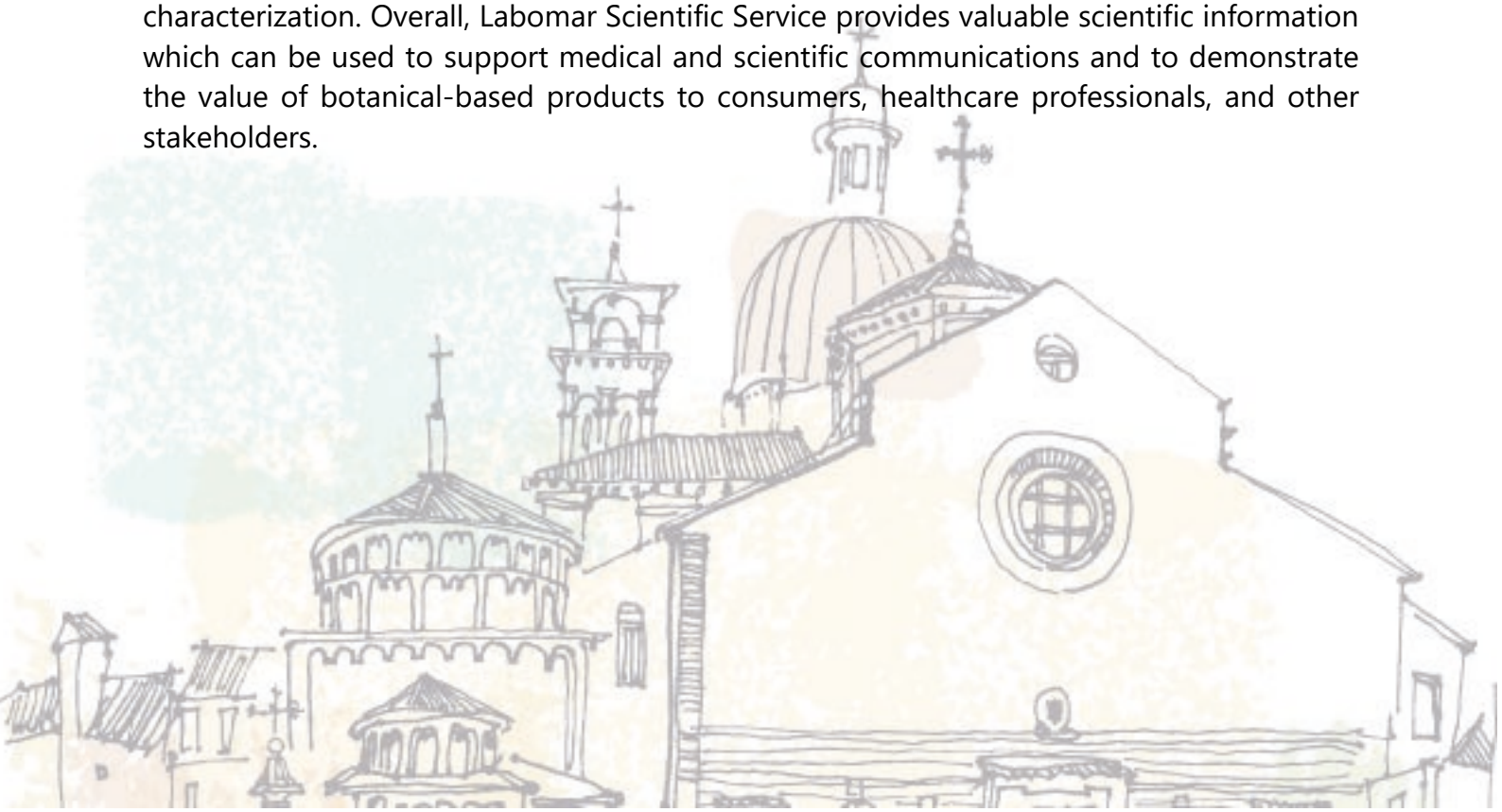
Amadio E, Bassetto R, Perin S, Zanatta S
Labomar SPA

Labomar is a highly innovative Contract Development and Manufacturing Organization (CDMO) specialized in the production of nutraceuticals, medical devices, and food supplements. Labomar offers a full-service support to its clients, from the selection of raw materials to the delivery of the finished product.

The production facility is equipped with state-of-the-art equipment and technologies that allow to manufacture products that meet the highest quality standards. The company is committed to sustainability and environmental responsibility. The company also places a strong emphasis on ethical and social responsibility, and they work closely with their suppliers and partners to ensure that their products are produced in a socially responsible and sustainable manner.

The company's R&D team consists of researchers and formulators who work together to develop innovative products and services that meet the highest quality standards and comply with relevant regulations. In addition to developing products, Labomar's R&D team also provides a wide range of services and solutions to their customers.

In this context, Labomar Scientific Service is a new R&D department that has been created to provide a wide range of services that help to drive the development of innovative, high-quality products that meet the evolving needs of the industrial sector. The department's services include pre-clinical studies and analytical services all of which are designed to ensure products safety, effectiveness, and compliance with relevant regulations. In more details Labomar Scientific Service provides studies to demonstrate: i) efficacy and properties of raw materials, herbal extracts, and nutraceutical products; ii) bioaccessibility and release of phyto-active ingredients; iii) the mechanism of action and the non-pharmacological activity of “Substance-Based Medical Devices” and the iv) phyto-active components characterization. Overall, Labomar Scientific Service provides valuable scientific information which can be used to support medical and scientific communications and to demonstrate the value of botanical-based products to consumers, healthcare professionals, and other stakeholders.



Valorization of by-products from agro-foods for pharmaceutical, cosmetic, nutraceutical applications

Terenzi C[1], Medri F[1], Davani L[1], Montanari S[1], De Simone A[2], Andrisano V[1]
[1]*Department for Life Quality Studies, University of Bologna, Corso D'Augusto 237, 47921 Rimini, Italy*
[2]*Department of Drug Science and Technology University of Torino, via P. Giuria 9, 10125 Torino, Italy*

The agro-food by-products, whose annual volumes are estimated to be approximately 5 billion tons of biomass residues globally, when not further treated, are discarded as waste, generating several environmental problems[1]. These by-products (such as hulls, shells, peels, squeezing residues, seed residues etc.) have proved to be valorized in pharmaceutical, nutraceutical and cosmetic fields thanks to their content in bioactive molecules, such as polyphenols and proteins[2]. Therefore, in the present work some waste products of the Romagna agro-food industries, legumes (soy, peas, green beans and beans) and fruits (peaches, apricots, apples and tomato) were selected for further valorization. Mainly, the aim of the research was the chemical characterization of the by-products in terms of bioactive molecules. Then, in view of valorizing the waste products, normally discarded, we compared their content profile with the one resulted from their related final products commercially available (fruit juices and tomato sauce, best-selected legumes, squeezed okara, etc.). This work concerned, primarily, the development and optimization of a high yield extraction, ultrasound-based method to obtain polyphenols. Later, the extracted fractions were characterized applying two different UV-VIS spectrophotometric assays. First, the Total Phenolic Content (TPC)[3] was carried out in order to evaluate total polyphenolic content and, secondly, the Total Antioxidant Status (TAS)[4] was performed to determine their antioxidant activity. Then, a chromatographic HPLC-DAD method was optimized and validated with the purpose of defining the polyphenolic profile for each by-products[5]. Afterwards, a comparison of the polyphenolic profile in legume and fruit extracts was carried out, between that of the final products and the correspondent by-products. Moreover, a proteins extraction methodology was optimized and the extracted proteins were quantified by the Kjeldahl method. Fruit by-products resulted to have a greater antioxidant activity in the range of 3,41 ± 0,39 to 7,26 ± 0,82 mmol Trolox/100 g of dry weight (DW) sample) justified by an higher polyphenolic content (4,25 ± 0,50 to 7,87 ± 0,82 mmol GAE/ 100 g DW), in comparison with legumes by-products (in the range of 1,79 ± 0,30 to 3,32 ± 1,17 mmol Trolox/100 g DW). The latter, on the contrary, demonstrated to have an higher content of proteins (from 13,5 to 34,5 g/100 g of DW). Furthermore, the comparison between final products and correspondent by-products highlighted how all these residues still represent an important source of bioactive compounds, which has to be valorized, in order to be used in cosmetic, pharmaceutical and nutraceutical fields, in a green circular bioeconomy prospect.

Nutraceutical and nutritional composition of an Italian wild pears (*Pyrus communis* var. Zingaro) fruits at different maturation stages

Alderotti F[1,2], Dos Santos Nascimento LB[1], Centritto M[2], Sobolev AP[3], Gori A[1,2], Brunetti C[2]

[1]*University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI) (Florence), Italy.*

[2]*National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Sesto Fiorentino (Florence), Italy.*

[3]*National Research Council of Italy, Institute for Biological Systems, "Annalaura Serge" Magnetic Resonance Laboratory, Via Salaria, Km 29.300, 00015 Monterotondo, Italy.*

Wild fruits are receiving much attention because of their low agronomical input and high nutritional value, which can vary according to the ripening stage. Pear (*Pyrus communis* L.) is one of the most important fruit crops in Europe, and Italian ancient wild varieties may provide an opportunity for their sustainable exploitation for their nutraceutical properties. This study aimed to characterize and compare the nutritional (amino acids, organic acids, and sugars) and nutraceutical (phenolic compounds) composition of fruit peels and pulps of an Italian wild variety of pear (P. *communis* var. Zingaro) during different stages of maturation (S1, unripe; S2, beginning of maturation; S3, partially mature; S4, almost mature; S5, mature; S6, very mature). The visual aspect of pulp and peel coloring were evaluated by measuring the browning index. NMR analysis of the pulp extracts was conducted to identify different classes of primary and secondary metabolites. Phenolic compounds identification and quantification in pulp and peel extracts were performed using HPLC-DAD and mass spectrometry (MS). Further, FRAP and DPPH assays were conducted to evaluate the antioxidant capacity of both peel and pulp extracts.

During maturation, peels changed from yellow (S1) to orange at S3 and finally brown at S6; while the pulp remained yellowish from S1 to S4 and suddenly darkened at the mature stage (S5), as revealed by the browning index. Such browning may be due to the oxidation of phenolic compounds coupled with the lower malic acid content. NMR analysis of the pulp extracts allowed the identification of 10 organic acids, 11 amino acids, 5 soluble sugars, and other important nutraceutical compounds, including vitamin B8 (choline). Overall, all the compounds identified in the pulp decreased after S4, reaching the lowest content in S6. The total phenolics content (TPC; sum of flavonoids, hydroxybenzoic acid derivatives, catechin derivatives, and hydroxycinnamic acid derivatives) decreased in both pulp and peel extracts throughout the ripening. Pulp extracts had the highest TPC at S1-S2, while peels at S1-S3. Furthermore, peel showed to be richer in TPC than pulp, due to the protective role of phenolics against biotic and abiotic stresses. In line with the TPC results, FRAP and DPPH assays revealed a higher antioxidant capacity at S1-S2 for pulp extracts and at S1-S3 for peel extracts compared to the other maturation stages. In conclusion, we observed that the first three maturation stages (S1–S3) were the most abundant in phenolics, indicating that the less mature stages of the fruits might be the most suitable for nutraceutical purposes due to the highest phenolic content. These results confirm that the wild ancient pear "Zingaro" is a rich source of nutritional compounds and phytochemicals, showing to be a promising neglected variety suitable to be further valorized in the nutraceutical and food sectors.

Pharmacovigilance of cannabidiol for medicinal use and as a food supplement

Calapai F[1,2], Ammendolia I[1,2], Cardia L[3], Currò M[2], Cacciola A[2], Esposito E[1], Calapai G[4], Mannucci C[4]

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.*
[2]*Department of Clinical and Experimental Medicine, University of Messina, Italy.*
[3]*Department of Human and Pediatric Pathology "Gaetano Barresi", University of Messina, Italy.*
[4]*Department of Biomedical and Dental Sciences and Morphological and Functional Imaging, University of Messina, Italy.*

Cannabidiol (CBD) is a substance contained in the *Cannabis sativa* plant. It is present in two different licensed drugs, one also containing delta-9-tetrahydrocannabinol (THC), indicated for pain from muscle spasms in multiple sclerosis and another drug authorized as an anticonvulsant against severe epileptic infantile syndromes. CBD has also gained favor with consumers of natural products for general health and well-being, to obtain relief from inflammatory processes, pain, anxiety, mood and sleep disorders. Despite its growing diffusion, the safety profile of CBD is not yet sufficiently known. For this reason, data on CBD-related suspected adverse reactions (SARs) in EudraVigilance, a system controlled by the European Medicines Agency (EMA), was collected and analyzed. In EudraVigilance, CBD-related SARs are traceable in individual cases safety reports (ICSRs) either for CBD as medicinal product licensed by national authorities or CBD contained in not-medicinal (not licensed as drugs) CBD products. ICSRs for both the two kinds of products, including serious SARs related to CBD consumption were analyzed by age and gender, suspected adverse reactions, indication, concomitant medications.

Risk profile emerging from real world data obtained through the analysis of SARs contained in EudraVigilance shows some differences between the use of CBD as medicinal product as antiepileptic agent and consumption of CBD as not-medicinal product. Precautions to be adopted for CBD medical use as antiepileptic are: awareness of use with other drugs, appropriate surveillance of potential adverse effects, more attention towards possible aggravation of epilepsy and drug effectiveness. Consumption of CBD not-licensed as medicinal product shows the raising of a different risk profile characterized more frequently, on descending order, by mental disorders, hepatic disorders, aggravation of epilepsy, neurological disorders, hematologic disorders. Potential risk related to CBD not medicinal products requires attention to the use of high doses, adequate surveillance of potential adverse effects, greater attention to possible liver toxicity and drug interactions of CBD and, in the case of antiepileptic therapy, surveillance of possible aggravations of the epilepsy.

Potential efficacy of Alliaceae and Brassicaceae edible plants in patients with type 2 diabetes: a systematic review and meta-analysis of clinical trials

Piragine E[1], Petri D[2], Martelli A[1], Lucenteforte E[3], Calderone V[1]

[1]*Department of Pharmacy, University of Pisa*

[2]*Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa*

[3]*Department of Clinical and Experimental Medicine, University of Pisa*

Type 2 diabetes (T2D) is one of the most common metabolic disorders in the elderly and is associated with high morbidity, mortality, and healthcare costs. Recent projections indicate that the global prevalence of T2D will increase dramatically in the coming years, pointing out the need to develop novel strategies to prevent the onset and progression of this "silent killer". Many pre-clinical studies have demonstrated the potential role of natural organosulfur compounds in reducing blood glucose levels in experimental models of T2D. The main dietary source of these compounds is represented by edible plants belonging to the *Alliaceae* (i.e., garlic and onion) and *Brassicaceae* (i.e., broccoli, rocket, and cabbage) botanical families. They contain alliin (the precursor of polysulfides) and glucosinolates (the precursors of isothiocyanates), respectively. Both polysulfides and isothiocyanates share the ability to slowly release hydrogen sulfide (H_2S), an emerging endogenous regulator of glucose homeostasis. This peculiar feature makes *Alliaceae* and *Brassicaceae* plants potential candidates in the prevention and treatment of metabolic disorders associated with unbalanced glucose metabolism, including T2D. However, results from clinical studies investigating the potential glucose-lowering properties of edible plants rich in organosulfur compounds are poor and conflicting, and previous meta-analyses have not provided conclusive evidence on this intriguing topic.

The aim of this work was to evaluate the efficacy of edible plants from *Alliaceae* and *Brassicaceae* in reducing blood glucose levels in patients with T2D.

We performed a systematic review with meta-analysis of clinical trials by searching four databases (i.e., Medline via PubMed, CENTRAL via the Cochrane Register of Clinical Trials, Scopus and EMBASE). The protocol has been published in the PROSPERO database (CRD42022320428). Inclusion criteria were defined following the PICOD scheme (P: patients with T2D; I: use of edible plants from *Alliaceae* or *Brassicaceae*, their extracts or isolated compounds; C: patients receiving placebo, standard antidiabetic therapy, or no treatment; O: measurement of blood glucose levels at the end of treatment; D: controlled clinical trials, both randomized and non-randomized). The methodological quality (risk of bias) of the included studies was assessed with the Cochrane tool for clinical trials. A random-effects meta-analysis was performed with the RevMan software (version 5.4).

16 clinical trials were reviewed, and 12 studies were included in the meta-analysis. Results showed that consumption of *Alliaceae* and *Brassicaceae* significantly reduced fasting blood glucose (FBG) levels in patients with T2D compared to the placebo group (mean reduction: -12.67 mg/dl [95% confidence interval (CI): -19.66; -5.68]). Noteworthy, the "combination therapy" between *Alliaceae/ Brassicaceae* and traditional antidiabetic drugs (e.g., metformin and sulfonylureas) led to a further decrease in FBG levels compared to the conventional therapy (mean reduction: -6.75 mg/dl [95% CI: -12.62; - 0.88]). Regular consumption of these

edible plants was well-tolerated, with mild gastrointestinal adverse events occurring in a few patients.

In conclusion, organosulfur-containing edible plants share a promising nutraceutical potential in the treatment of T2D. Furthermore, our preliminary results support a possible "combination therapy" between *Alliaceae/Brassicaceae*, or supplements thereof, and standard antidiabetic drugs, to provide better control of blood glucose levels in T2D patients.

Development nutraceutical ingredients to slow biological aging

Campisi M[1†], Cannella L[1†], Baumann J[2], Pavanello S[1]

[1]*Occupational Medicine, Department of Cardiac, Thoracic, and Vascular Sciences and Public Health, University Hospital of Padova, Padova, Italy.*

[2]*Mibelle Group Biochemistry, Bolimattstrasse 1, 5033 Buchs, Switzerland.*

[†]*Contributed equally*

Manipulations to decelerate biological aging and extend health span are major challenges for health given the social and healthcare costs of aging population. Excessive oxidative stress and inflammation are primary drivers in biological aging and age-related diseases. Didymin, a natural bioactive flavonoid and the major polyphenol of *Monarda didyma L.*, has showed a crucial anti-inflammatory role. Our clinical study aims to investigate the potential benefits of a 12-weeks supplementation of *Monarda didyma L.* extract on reducing / delaying the biological aging of a susceptible population of aging workers.

This is a randomized, placebo-controlled, double-blind, parallel design and monocentric pilot study. Study population comprises 80 participants, aged 45-65 years, randomly allocated to both Intervention group (supplementation of *Monarda didyma L.* extract) and placebo control group (placebo supplementation) for a 12-weeks period of treatment. All study participants, who have been enrolled at the Occupational Medicine Unit – Azienda Ospedale Università of Padua providing a written informed consent, underwent clinical examination, an interview with structured questionnaires (demographic data, lifestyle, information quality of life and sleeping patterns) and collection of fasting blood for evaluation of indicators of biological aging as primary outcomes (DNA methylation age, telomere length, mitochondrial DNA copy number), as well as secondary outcomes including basic biochemistry and inflammation markers (emocrome, glycemia, insulin, total cholesterol, low and high density lipoproteins, triglycerides, creatinine, telomerase expression, C-reactive protein, interleukin-6, aspartate transaminase, alanine transaminase, gamma-glutamyl transferase) and salivary sample for cortisol analysis. Clinical examination and sample collection, to assess primary and secondary outcomes, will be also performed at the follow up after 12-weeks of this treatment. Tracking of additional parameters (heart rate, sleep, steps number) are recorded along the study period by using a wearable MiBand 7 watch (Xiaomi), as well as a daily diary to determine compliance and record effects on stress, well-being and health status.

The study was registered in the database for Protocol Registration and Results System.ClinicalTrials.gov PRS (NCT05399966), registration date: May 17, 2022. The local Ethics Committee Azienda Ospedaliera Padova approved the study protocol (5518/AO/22; Study Code: EMODISU) on December 6, 2022.

80 participants, aged 45-65 years, female (N=40) and male (N=40) have been recruited between February 28 and March 15, 2023 and the follow up after 12-weeks of this treatment is planned between May 24 and June 9, 2023.

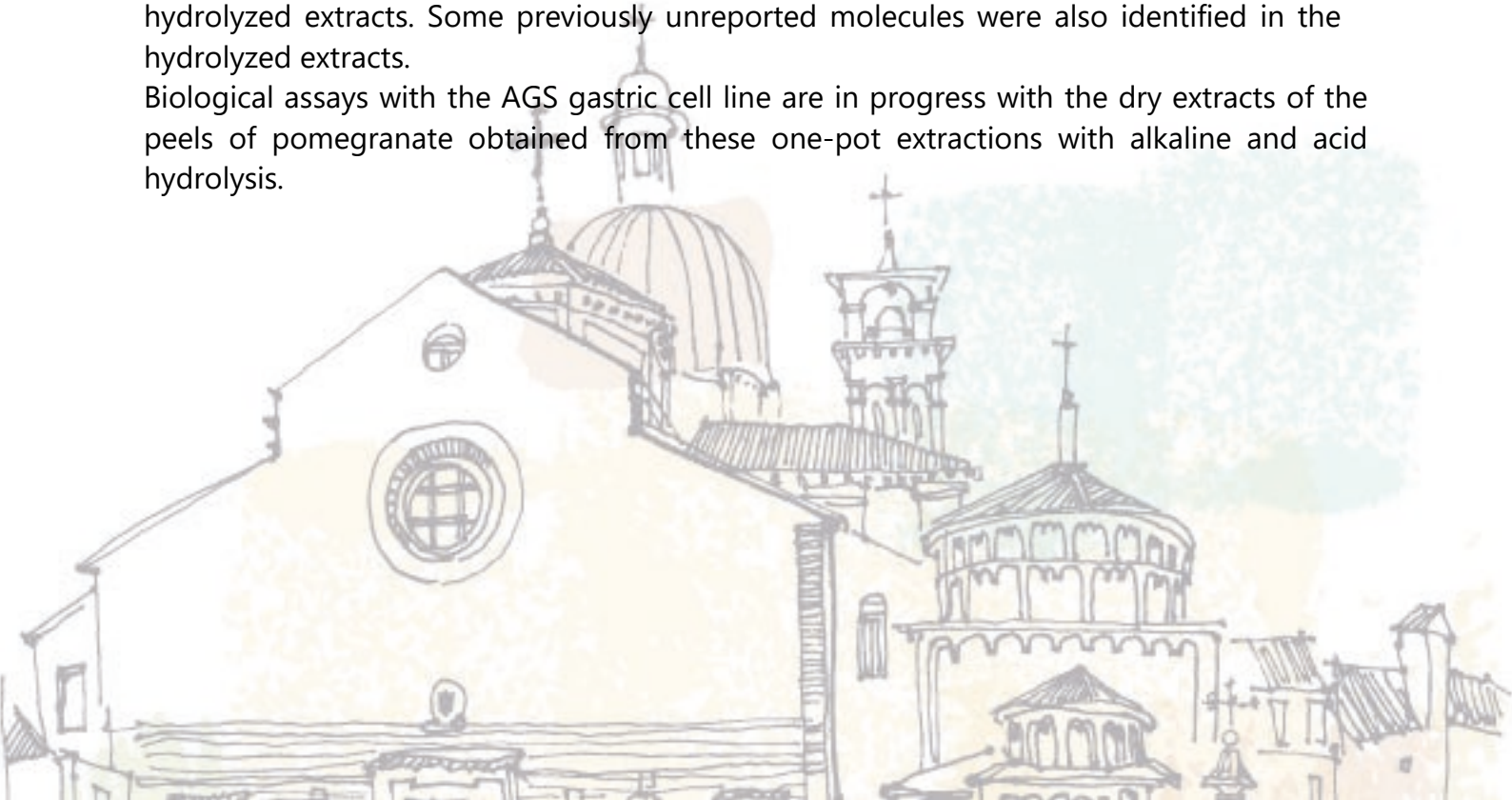
The success of this research could represent a turning point in the healthy aging research, leading to rejuvenation by means of a sustainable and safe intervention, accessible to everybody in order to extend health span.

Sabato 17 giugno

Enhancement of pomegranate peel by recovering the phenolic compounds: one-pot extraction with hydrolysis

Ugolini T, D'Agostino S, Cecchi L, Khatib M, Innocenti M, Mulinacci N
Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence

The peel of the pomegranate (*Punica granatum* L.) is produced in large quantities as a by-product of the juice industry. Because it contains polysaccharides, anthocyanins and polyphenols, all with a broad spectrum of bioactivity and possible applications, it is a high value biomass. The transformation of such by-product from waste to resource is nowadays of great importance, from both an economic and an ecologic perspective.
In our work we have developed three one-pot extraction methods with chemical hydrolysis applied to pomegranate peel. The aim was to obtain extracts with lower molecular weight phenolic derivatives to improve their bioavailability and bioactivity compared to their non-hydrolyzed analogues. As far as we know, data on the extraction of pomegranate peel under alkaline conditions are not available in the literature so far.
For alkaline hydrolysis we used food-grade sodium bicarbonate at two different concentrations (0.3 % and 0.6 %), while for acid hydrolysis it was applied a method previously proposed (1). Our extracts were characterized using HPLC-DAD-MS detecting ca. 40 compounds. The concentration in total hydrolysable tannins was quantified by HPLC-DAD. The optimized methods were applied to four different pomegranate peel samples of the Wonderful and G1 varieties. Samples were collected in three different harvesting seasons from two Italian regions (Marche and Emilia-Romagna).
Our methods have been optimized with regards to extraction ratio, reaction time and amount of base or acid. Our results demonstrated that both alkaline and acid hydrolysis methods are viable solutions for producing extracts with a modified phenolic profile with lower amount of punicalagins and higher gallic and ellagic acid concentrations than non-hydrolyzed extracts. Some previously unreported molecules were also identified in the hydrolyzed extracts.
Biological assays with the AGS gastric cell line are in progress with the dry extracts of the peels of pomegranate obtained from these one-pot extractions with alkaline and acid hydrolysis.



Green biotechnology for human feeding

Dalla Costa V, Piovan A, Filippini R
Department of Pharmaceutical and Pharmacological Sciences – University of Padova

The global population is expected to grow in the next years and so the food demand. This increased demand for food cannot be sustained due to limited amount of arable land; furthermore, the sustainability in food production and the threat of crop losses due to climate change and plant diseases are playing an increasingly important role and need to be taken into account.

Some of these issues could be countered using new and ethically more justifiable technologies such as plant cellular agriculture, which can fill the gap between food demand and supply in a sustainable way [1,2].

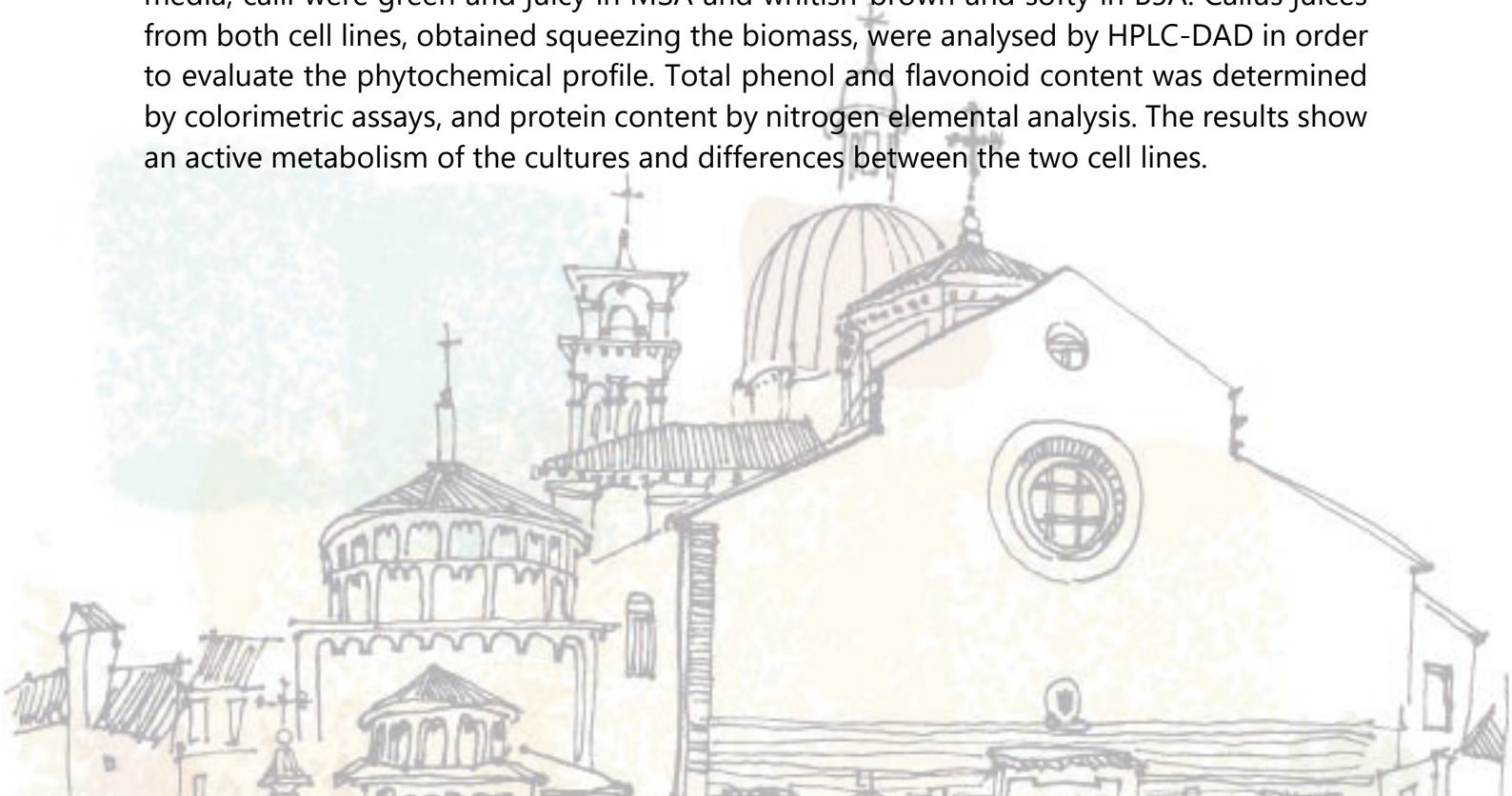
In vitro plant cell cultures can be established of any plant species, whether they are food plants or endangered species with a particular metabolic profile, and could produce nutritious, safe and healthy food together with chemicals and innovative materials, while minimizing resource input such as energy, land and water, in addition to gaining seasonal and geographical independence and reducing waste [3].

As grapes are one of the richest sources of phytochemicals, with potential beneficial effects on human health [4], the aim of this work was to establish *Vitis labrusca* L. var. Isabella *in vitro* cell cultures to study their potential use for nutritional and healthy food.

For this purpose, *Vitis labrusca* leaves, a cultivation by-product, were used as starting material for undifferentiated cell culture establishment.

The first step to establish *in vitro* cell culture was to test the best harvesting period and sterilization conditions. To obtain callus induction, leaf explants were cultured on two different basal media, supplemented with sucrose (30 mg/L) and 2,4-D (1.3 mg/L), NAA (0.25 mg/L) and K (0.25 mg/L), namely MSA and B5A. Calli were obtained with high frequency from leaves harvested in full spring, on both media.

In the successive subcultures significant differences were revealed between the two culture media, calli were green and juicy in MSA and whitish-brown and softy in B5A. Callus juices from both cell lines, obtained squeezing the biomass, were analysed by HPLC-DAD in order to evaluate the phytochemical profile. Total phenol and flavonoid content was determined by colorimetric assays, and protein content by nitrogen elemental analysis. The results show an active metabolism of the cultures and differences between the two cell lines.



The chemical assessment of the glucosinolate-myrosinase system: analytical challenges and technical pitfalls

De Nicola GR

CREA Centro di ricerca Orticoltura e Florovivaismo, Via dei Fiori, 8, 51017 Pescia (PT), Italia

Glucosinolates (GLs) are bio-relevant thiosaccaridic secondary metabolites present in a large number of plants, mainly of the order Brassicales, including economically important leaf and root vegetables, edible flowers, oilseed, and ornamental crops cultivated worldwide. GLs display a remarkable structural homogeneity based on a hydrophilic β-D-glucopyrano unit, a O-sulfated anomeric (*Z*)-thiohydroximate function connected to a rather hydrophobic amino acid-derived side chain whose constitution, depending on plant species, is the sole structural variant. To date around 140 GLs have been identified which are categorized into three classes depending on the precursor amino acid: aliphatic GLs (methionine, isoleucine, leucine, or valine), aromatic GLs (phenylalanine or tyrosine), and indole GLs (tryptophan). The occurrence of GLs coincides with the presence of specific thioglucosidases, the myrosinases, which can hydrolyze these compounds into a variety of reactive transient and stable products, some of which are recognized bioactive compounds responsible for various physiological actions as evident from many epidemiological and clinical studies. In particular, isothiocyanates (ITCs) are low molecular weight electrophiles with proven health-promoting effects such as anticarcinogenic, anti-inflammatory, neuroprotective, anti-obesity, and antiviral activity. Notwithstanding the increasing body of evidence around the biological activities, the correct determination of GLs in plant materials and derived products, such as botanicals and nutraceuticals, still represents an analytical challenge requiring highly specialized expertise and know-how. Indeed, depending on the plant species, tissue, GL content, complexity of the GL profile, and experimental aim, different choices need to be made regarding the methods of extraction, isolation and analysis. A series of analytical methods have been developed to identify diverse structures and quantify GL content directly, or indirectly by detecting the products obtained upon specific enzymatic hydrolysis. The most commonly used methods are based on the HPLC-PDA analysis of desulfo GLs according to the EU ISO 9167-1 official method originally designed for analysis of *Brassica* oilseeds and later variously adapted to suit the needs of researchers examining GL of other plant species, tissue types and different plant preparations. We describe here the main approaches for GLs analysis highlighting some advantages and critical points. Moreover, examples of technical pitfalls are given, such as artifacts of desulfation of radish extracts containing glucoraphenin, as well as artifacts originated in the GC-MS injection port when analyzing the renown dietary ITC *R*-sulforaphane and its homologues. Importantly, our experience underlines that relying on libraries does not always lead to a correct identification of GLs and GL hydrolysis products without knowing the behavior of standard samples in the applied analytical conditions.

Environmental impact of phytoextracts: the case of *Melia azedarach* L.

Popescu VS[1], Mac Sweeney E[1], Abate G[1,4], Pucci M[1,4], Tirelli E[1,4], Zhang L[2], Peron G[1], Gianoncelli A[1,4], Ribaudo G[1,4], Bulgari D[3,4], Gobbi E[1], Lucini L[2], Uberti D[1,4], Memo M[1,4], Mastinu A[1,4]

[1]*University of Brescia, Molecular and Translational Medicine Department.*
[2]*Catholic University of Sacred Heart, Piacenza and Cremona Campus, Piacenza, Italy.*
[3]*University of Milan, Department of Food, Environmental and Nutritional Sciences.*
[4]*NutriFun4Health, Center for Research and Services on Nutraceuticals and Functional Food for Health, Brescia.*
Corresponding Author: Vlad Sebastian Popescu (v.popescu@studenti.unibs.it).

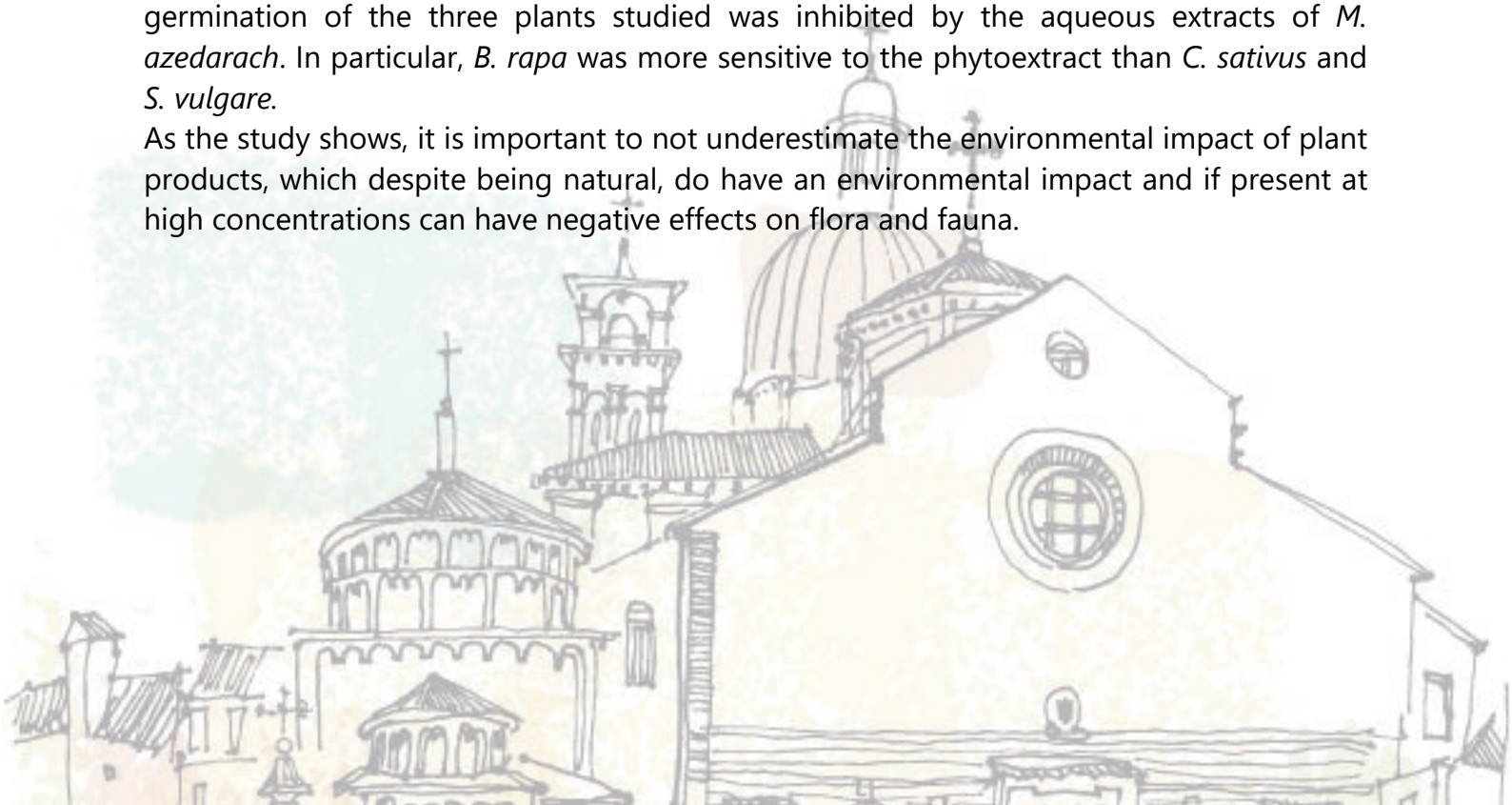
Melia azedarach L., also known as chinaberry tree, is a deciduous tree native to southeast Asia and northern Australia but has become an invasive species in almost all continents. In traditional Chinese medicine, *Melia azedarach* is used orally and topically as an antiparasitic and antifungal medicine. Clinical studies have shown numerous properties of the plant, including antidiabetic, cytotoxic, antibacterial and antioxidant activities.

The main objective of the study was the phytochemical and biological characterization of *M. azedarach* phytoextract, with the specific aim of evaluating its toxicological effects on in-vitro and in-vivo models.

The chemical analysis of the phytoextract was performed by UHPLC/Q-TOF-MS and HPLC-DAD. MTT assay was performed to investigate the phytoextract toxicity on a human neuroblastoma cell line (SH-SY5Y) and a primary fibroblast cell line (FB-21). Moreover, a toxicity assay on zebrafish was executed. Finally, to assess the environmental impact of the phytoextract, a germination assay on *Brassica rapa* L., *Cucumis sativus* L. and *Sorghum vulgare* L. was performed.

Chemical analysis of the phytoextract revealed the presence of numerous terpenes and polyphenols. In particular, the extract was rich in LMW phenolics, flavonoids and limonoids. The results showed that the phytoextract was toxic from 0.8 µg/mL in FB-21 cells and 3.12 µg/mL in SH-SY5Y cells. As for the toxicity assay on zebrafish, this showed very significant toxicity and morphological abnormalities at the two highest concentrations. Finally, the germination of the three plants studied was inhibited by the aqueous extracts of *M. azedarach*. In particular, *B. rapa* was more sensitive to the phytoextract than *C. sativus* and *S. vulgare*.

As the study shows, it is important to not underestimate the environmental impact of plant products, which despite being natural, do have an environmental impact and if present at high concentrations can have negative effects on flora and fauna.



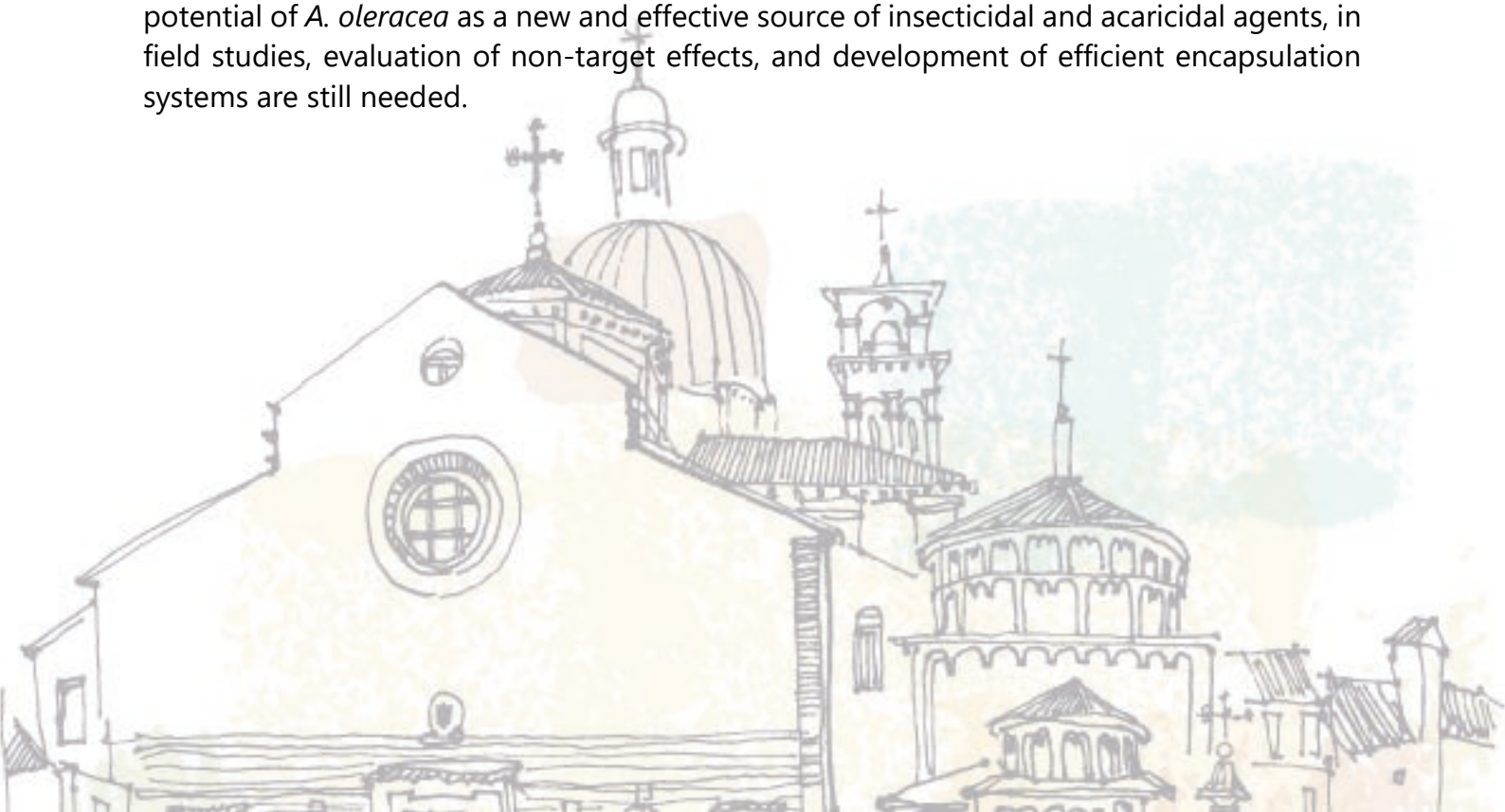
Acmella oleracea ("jambù", Asteraceae): a spilanthol-rich source for the development of effective green insecticides and acaricides

Ferrati M[1], Spinozzi E[1], Baldassarri C[1], Cappellaci L[1], Benelli G[2], Petrelli R[1], Maggi F[1]

[1]*School of Pharmacy, Chemistry Interdisciplinary Project (ChIP), University of Camerino, Via Madonna delle Carceri, 62032 Camerino, Italy*

[2]*Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 856124 Pisa, Italy*

Acmella oleracea (L.) R.K. Jansen, traditionally called Jambù, is a plant belonging to the Asteraceae family encompassed with a wide range of applications and ethnobotanical uses. Especially, this plant is commonly known for its anesthetic activity and consequently used as a remedy for toothache. It is native to South America and cultivated all over the world for medicinal, food, and cosmetic purposes. The interest in this plant is inherent to its essential oil and extracts, which contain spilanthol, the main *N*-alkylamide of the phytocomplex. This compound has shown, among others, a high pesticidal activity thanks to its good ability to penetrate and affect the central nervous system (CNS) of insects. In recent years, the insecticidal and acaricidal potential of *A. oleracea* products has been investigated against several target species of both medical (e.g., ticks, mosquitoes, houseflies) and agricultural (e.g., moths, aphids) importance. The effectiveness of this plant drew the attention of agricultural industries depending on the recent interests in sustainable plant-based insecticides. As a matter of fact, traditional chemical pesticides are responsible for extensive pollution, human pathologies caused by the presence of residues in food, and the development of resistance phenomena in target organisms. Moving plant-based insecticides from laboratory to field is still difficult, mainly due to regulatory issues. In this regard, *A. oleracea* is worthy of considerable attention. Indeed, its application in foodstuffs should reduce the risk to human health once used as a biopesticide and facilitate the registration process. Furthermore, jambù is already widely cultivated for nutraceutical and cosmetic purposes, and consequently, there is a huge availability of plant biomass to be exploited on an industrial level to develop botanical insecticide formulations. Notwithstanding the potential of *A. oleracea* as a new and effective source of insecticidal and acaricidal agents, in field studies, evaluation of non-target effects, and development of efficient encapsulation systems are still needed.



C*arlina acaulis*, a traditional medicinal plant for insect vectors management

Spinozzi E[1], Ferrati M[1], Baldassarri C[1], Maggi F[1], Pavela R[2], Benelli G[3], Aguzzi C[4], Zeppa L[4], Cappellacci L[1], Palmieri A[5], Petrelli R[1]

[1]*Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Via Madonna delle Carceri, 62032 Camerino, Italy*

[2]*Crop Research Institute, Drnovska 507, 161 06 Prague 6, Czech Republic*

[3]*Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 856124 Pisa, Italy*

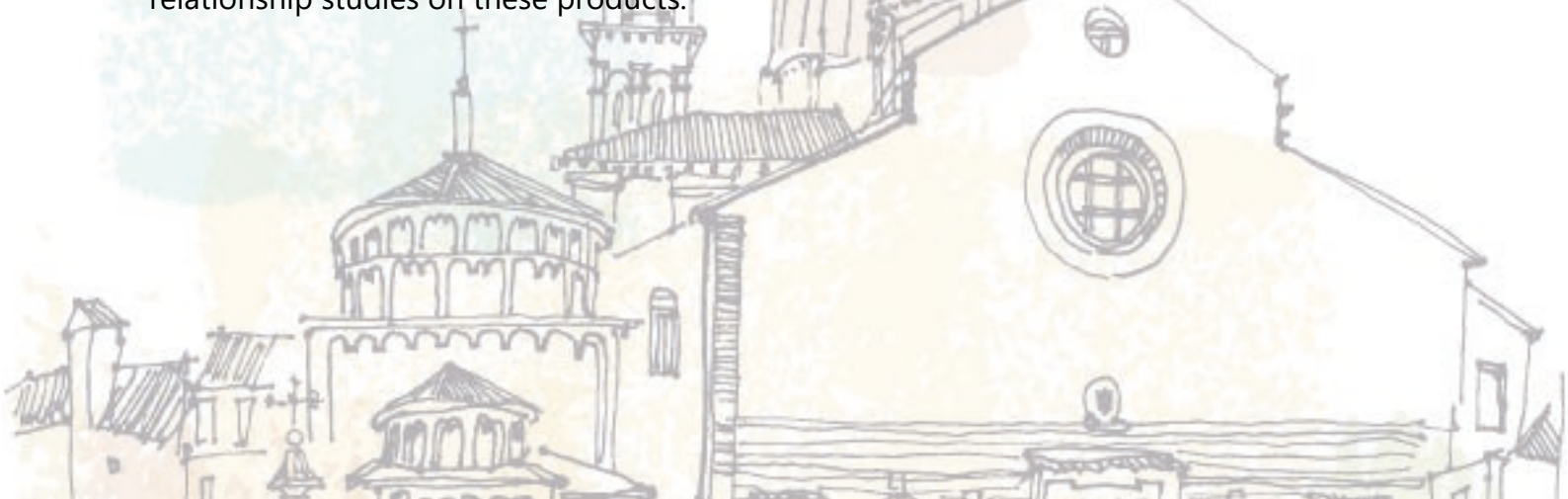
[4]*School of Pharmacy, University of Camerino, Via Madonna delle Carceri 9/C, 62032 Camerino, Italy*

[5]*School of Science and Technology, Chemistry Division, Università di Camerino, via Madonna delle Carceri, 62032 Camerino, Italy.*

Vector-borne diseases represent a concerning threat to global public health. Furthermore, the overuse of conventional insecticides led to the onset of vectors' resistance with an increase in the prevalence of illnesses as Japanese encephalitis, leishmaniasis, dengue, malaria, filariasis, onchocerciasis, and schistosomiasis. In this scenario, the implementation of vectors management is crucial and botanical insecticides represent promising alternative sources of insecticidal molecules.

Carlina acaulis (Asteraceae) is a perennial plant native to the European Alpine regions, with a great history as traditional remedy, being considered one of the most known medicinal plants in Europe. Its root essential oil (EO) is mainly characterized by the predominance of an aromatic C_{13} polyacetylene, i.e. carlina oxide, which showed various biological activities. Our research group focused on the scarcely investigated insecticidal potential of the EO and carlina oxide against several targets of medical and agricultural importance. Between the numerous targets tested, the EO and carlina oxide have been revealed as highly effective against *Culex quinquefasciatus* Say (LC_{50} of 1.31 and 1.39 µg mL^{-1} on 3rd instar larvae, respectively), which is considered a major lymphatic filariasis and arbovirus vector, transmitting St. Louis encephalitis, Western equine encephalitis and West Nile. The EO and carlina oxide were also effective on *Musca domestica* L. (EO LD_{50} = 2.74 and 5.96 µg fly^{-1}, on male and female adults), which is an important carrier of pathogens that are responsible for more than one hundred human and animal diseases of public concern.

Given the promising results obtained with the natural counterparts, different functionalized carlina oxide analogues were synthetized and tested on *Cx. quinquefasciatus* and *M. domestica* to assess their insecticidal potential. The results on the two vectors were highly promising for some of the compounds tested. Moreover, their toxicity on human keratinocytes was significantly reduced if compared to carlina oxide. The presented work could pave the way to the production of carlina oxide analogues and to structure-activity relationship studies on these products.



Cynara cardunculus L. suppresses adipogenesis in 3T3-L1 adipocytes via AMPK signaling pathway activation

Molonia MS[1,2], Salamone FL[1], Speciale A[1], Muscarà C[1], Saija A[1], Cimino F[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy.

[2]Foundation Antonio Imbesi, Messina, Italy.

Adipose tissue is an important energetic storage organ where adipocytes play a central role in maintaining lipid homeostasis and energy balance, by storing triacylglycerols or releasing free fatty acids in response to changes in energy demands. In presence of obesity, an imbalance between energy requirement and expenditure occurs, with consequent accumulation of energy in the form of lipids in white adipose tissue (WAT) and expansion of total body mass.

Recently has been detected the ability of white adipocytes to transform, in response to several stimuli, into a phenotype tending to brown adipose tissue (BAT), through a process called "*browning*". In this regard some research has shown, in fact, that the activation of the thermogenesis process, via upregulation of the key sensor of energy metabolism AMP-activated protein kinase (AMPK), represents an important target to counteracting obesity and related disorders.

Therefore, the objective of this study was to investigate the *in vitro* beneficial effects of a standardized extract obtained from the leaves of *Cynara cardunculus* L. (CCLE), rich in chlorogenic acid and luteolin derivates, on adipogenesis inhibition in 3T3-L1 cells through the involvement of the thermogenic process.

CCLE at two different concentrations (5 and 10 µg/mL) was added to 3T3-L1 cells during the 10 days of the adipocyte differentiation process. CCLE effects on lipid accumulation were evaluated through Oil Red O staining, whereas the main markers involved in adipogenesis and thermogenesis were assessed via Western blot and real-time PCR techniques.

Data showed that CCLE treatment during 3T3-L1 differentiation markedly inhibited lipid accumulation and suppressed the expression of the main markers involved in the adipogenic process (C/EBPβ, PPARγ, FABP4, and FASN). Furthermore, CCLE significantly increased the thermogenic process via the upregulation of pAMPK protein levels, and UCP1 and PGC1α mRNA expression. However, pretreatment with compound C, a specific AMPK inhibitor, prevented the mitotic clonal expansion (MCE) of preadipocytes and abolished the inhibitory effects of CCLE on C/EBPβ and PPARγ expression, thus suggesting that the antiadipogenic effect of the extract is closely related to AMPK signaling pathway activation.

Therefore, these results clearly indicate that CCLE extract inhibits adipogenic differentiation in 3T3-L1 cells via activation of the thermogenic process, opening thereby new perspectives for the use of this natural extract for the prevention and management of obesity.

Walnut (*Juglans regia* L.) and chestnut (*Castanea sativa* Mill.) leaves as precious by-products for the treatment of skin aging induced by environmental pollution

Baini G, Cappellucci G, Biagi M, Miraldi E

Department of Physical, Earth and Environmental Sciences, University of Siena Via Laterina 8, 53100 Siena, Italy

The highly anthropized and industrialized environments typical of our time have caused strong repercussions on human health. Being the organ most exposed to air pollution, the skin undergoes tissue and cellular alterations due to an excess of pro-inflammatory and pro-oxidant elements.

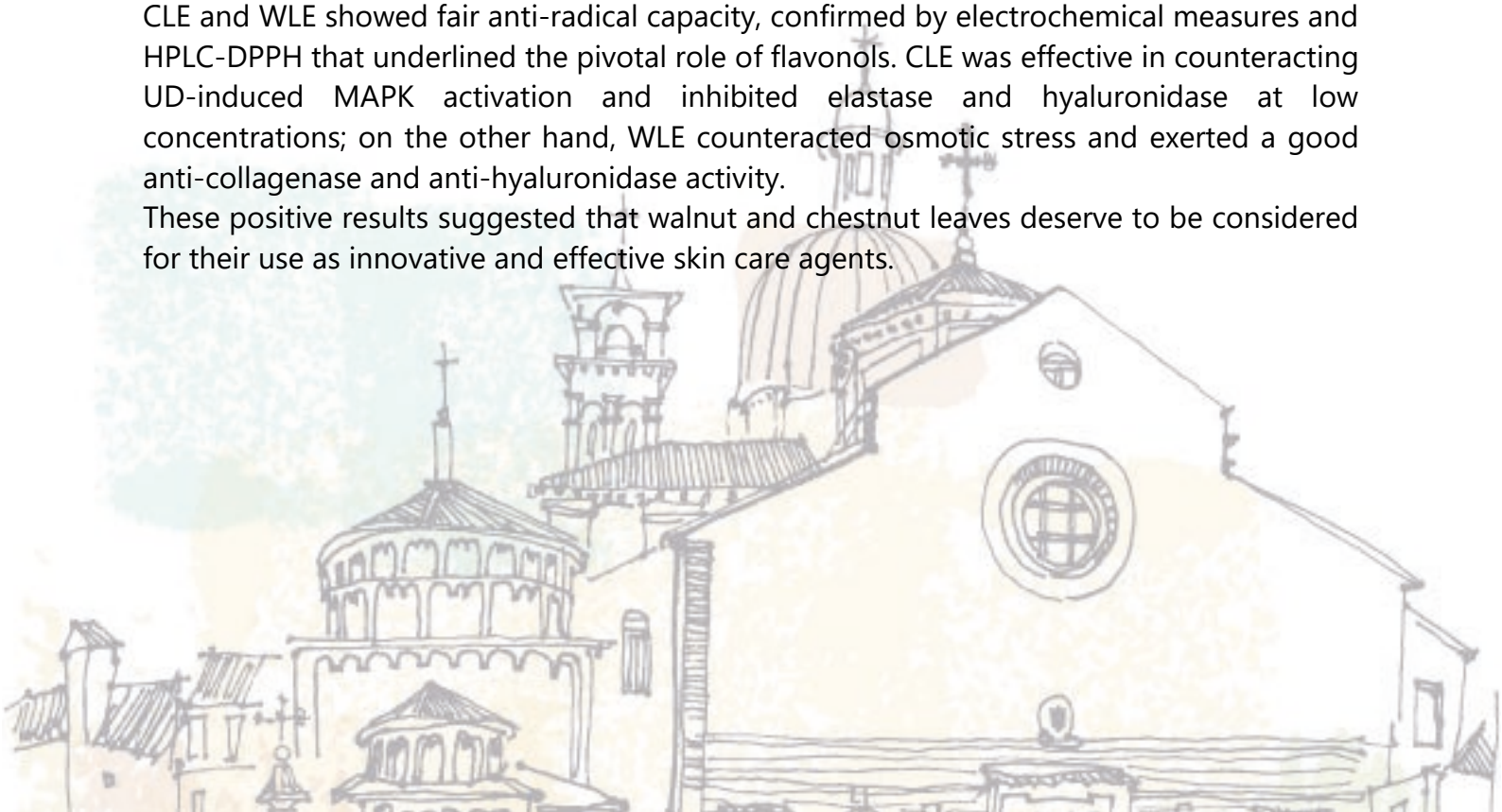
In order to investigate and develop new natural products derived from Italian and in particular Tuscan species, walnut (*Juglans regia* L., Juglandaceae) and chestnut (*Castanea sativa* Mill., Fagaceae), two species cultivated mainly for their edible part, respectively the seeds and fruits, have been taken into consideration. Both species are also known for their ethnobotanical use: walnut leaves are used to flavor and protect cheese from dust and pests; chestnut leaves tea is registered to treat dandruff and skin diseases.

With the aim of better studying the phytochemical profile and biological characteristics of walnut and chestnut leaves, two economic by-products, abundant but little exploited, a large *multistep* experimental protocol was developed and validated, consisting of extracts optimization, cell-free and cell-based tests, to evaluate the biological activities of selected extracts in stress models from air pollution stimuli in human keratinocytes (HaCaT) also including urban dust (NIST®SRM®1648a) (UD) stimulation: cell viability, osmotic toxicity, intracellular ROS level, cytokines release, MAPK activation and extra-cellular matrix degrading enzymes inhibition.

Both extracts were characterized by a high polyphenolic content (>4% m/m dry weight), walnut leaves were also rich in triterpenes (>2.5% m/m d.w.). For both species the best time for harvesting the leaves turned out to be September/October, before the harvesting of the fruit. Ethanol 60% v/v was the best extraction solvent for both species. The optimized extract CLE (chestnut leaves) and WLE (walnut leaves) were tested.

CLE and WLE showed fair anti-radical capacity, confirmed by electrochemical measures and HPLC-DPPH that underlined the pivotal role of flavonols. CLE was effective in counteracting UD-induced MAPK activation and inhibited elastase and hyaluronidase at low concentrations; on the other hand, WLE counteracted osmotic stress and exerted a good anti-collagenase and anti-hyaluronidase activity.

These positive results suggested that walnut and chestnut leaves deserve to be considered for their use as innovative and effective skin care agents.



Assessment of antidiabetic, anti-obesity, and neuroprotective potential of two *Cannabis sativa* L. extracts

Mazzara E, Petrelli R, Mustafa AM, Caprioli G, Dall'Acqua S, Sut S, Nuñez S, López V, Cásedas G, Moliner C, Bonacucina G, Maggi F, Cespi M

[1]*School of Pharmacy, University of Camerino, Camerino, Italy*

[2]*Department of Pharmaceutical and Pharmacological Sciences, Natural Product Laboratory, University of Padua, Padua, Italy*

[3]*Department of Pharmacy, Faculty of Health Sciences, Universidad San Jorge, Zaragoza, Spain*

Industrial hemp (*Cannabis sativa* L.) is a plant containing numerous bioactive compounds such as cannabinoids, terpenes, and polyphenols. During the microwave-assisted extraction (MAE) of volatile terpenes, two by-products are produced, namely the aqueous residue rich in bioactive phenolics, and the residual deterpenated plant material, which can be employed for further extraction and purification of phytocannabinoids. It is noteworthy that these can be potentially exploited in the food, cosmeceutical, nutraceutical, and pharmaceutical sectors. The two by-products were obtained during the MAE optimized run; specifically, the aqueous residue was freeze-dried, while the residual biomass was extracted with *n*-hexane, to be then analysed. HPLC-DAD-MS^n analysis led to the quantification of several bioactive compounds. In particular, the aqueous residue presented luteolin-7-O-glucuronide and apigenin glucuronide as the main detected phenolic derivatives, while the hexane extract showed an interesting cannabinoids profile, especially in the decarboxylated form, with CBD as the predominant one. The two extracts were evaluated in terms of antidiabetic and anti-obesity potential, through both enzymatic and non-enzymatic assays. As a result, the aqueous residue showed a powerful α-glucosidase inhibition, and a modest efficacy in terms of inhibition of advanced glycation end (AGE) products. On the other hand, the residual deterpenated biomass did not display significant biological activity. The two extracts were then studied for their antioxidant profile, and their neuroprotective potential. The assays on *in vitro* antioxidant capacity (DPPH radicals neutralization, and superoxide radical scavenging activity), as well as inhibition of physiological enzymes such as acetylcholinesterase (AChE), and monoamine oxidase A (MAO-A) were performed. In addition, Neuro-2a cell line was selected to test the cytotoxic and neuroprotective potential of these extracts. Both extracts demonstrated remarkable antioxidant capacity, and especially the aqueous extract stood out in the DPPH, and superoxide radical uptake tests. In enzyme inhibition assays, the aqueous extract exerted noteworthy AChE and MAO-A inhibitory activity. Neuro-2a assays evidenced that the aqueous extract was not cytotoxic, and exhibited cytoprotective properties against hydrogen peroxide, and antioxidant response reducing reactive oxygen species (ROS) production. This work demonstrated that these extracts could be a source of compounds, namely cannabinoids and glycosidic flavones, with potential beneficial effects on human health, especially against metabolic and neurodegenerative disorders. Particularly, the aqueous extract, whose potential as an antioxidant and pharmacological agent appears to be understudied with respect to the cannabinoid extract, resulted to be promising for possible exploitation.

Humulus lupulus L. not only for beer! investigation of the bitter taste receptors involved in its mechanism of action

Lela L[1], Ponticelli M[1], Russo D[1,2], Faraone I[1,3], Kioussi C[4], Stevens JF[4,5], Milella L[1]

[1] *Department of Science, University of Basilicata, via dell'Ateneo Lucano 10, 85100 Potenza, Italy*

[2] *Spinoff Bioactiplant, via dell'Ateneo Lucano 10, 85100 Potenza, Italy*

[3] *Innovative Startup Farmis s.r.l., Via Nicola Vaccaro 40, 85100, Potenza, Italy*

[4] *Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR, 97331, USA*

[5] *Department of Chemistry, Oregon State University, Corvallis, OR, 97331, USA*

Bitter taste, one of five basic taste qualities, is considered a key defense mechanism against poisoning by potentially toxic substances. The variety of bitter compounds originating from plants is innumerable. Whereas some bitter chemicals are toxic (strychnine) and should not be ingested, other compounds exhibit health beneficial effects. *Humulus lupulus* L., commonly known as hop, is a perennial climbing plant belonging to the Cannabaceae family particularly rich in bitter principles. Its main use is in the brewing industry (approximately 97%) [1]. In fact, it is an essential ingredient in imparting the bitter taste to beer and protecting against microbes through its specialized metabolites. About hundreds of specialized metabolites belonging to different chemical classes have been identified in hop female inflorescences, stems, rhizomes, and leaves. The inflorescences are the most studied because of their use in the brewing process: hop resins consist of bitter acids, including α-acids (humulone, adhumulone, cohumulone) which are responsible for the foam stability and bitterness of the beer and can isomerize into iso-α-acids and β-acids (lupulone, adlupulone, colupulone) which are less stable and can be destroyed during beer brewing processes [1;2]. These compounds are involved in maintaining glucose homeostasis and reducing body weight and fat mass [3-5]. Their appetite-suppressing effect could be useful as a diet complementary agent by stimulating the secretion of gastrointestinal hormones, lowering postprandial blood glucose, modulating gut motility, and reducing food intake. The mechanisms of action of bitter molecules are not yet fully understood. Therefore, this work aims to identify intestinal-derived hormones (such as GLP-1) or bitter taste receptors (Tas2rs) in the intestinal tract as possible targets of hop bitter substances. In particular, hop inflorescences were extracted by maceration technique using a hydroalcoholic mixture (EtOH/H_2O 55%) and the main hop bitter compounds as α-acids (cohumulone 6.14±0.19 mg/g DW; adhumulone+humulone 2.04±0.11 mg/g DW) and β-acids (colupulone 7.04±0.54 mg/g DW; adlupulone+lupulone 2.10±0.02 mg/g DW) were quantified by HPLC-DAD [6]. The mechanism of action related to satiating hormone secretion was determined in STC-1 cells, an intestinal neuroendocrine cell line commonly used for this purpose. The bitter hop extract treatment (100 μg/mL) doubled the secretion of GLP-1, compared with the untreated cells by using ELISA assay. Furthermore, the expression of some genes involved in the bitter taste transduction mechanism was detected by qRT-PCR. Hop extract up-regulated Tas2rs gene expression, in particular, *Tas2r138* receptor, which was increased by 3-fold after stimulation with the extract, as well as the expression of *Plcβ2* and *Trpm5* genes which have been shown to act as key effectors in taste transduction. Hop effects in secreting gut hormones were mediated by calcium channels such as *Casr* or L-type voltage-sensitive Ca^{2+} channels underlying the involvement of calcium signals in the mechanism. In conclusion, hop extract could represent a promising source of bitter compounds with satiating effects.

Addition of polyphenolic extracts of *Myrtus communis* and *Arbutus unedo* fruits to whey: valorization of a common dairy waste product as a functional food

Detti C[1], Nascimento LB[1], Gori A[1,2], Vanti G[3], Amato G[4], Nazzaro F[4], Bilia AR[3], Ferrini F[1,2], Centritto M[2], Brunetti C[2]

[1]*Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, 50144, Florence, Italy*

[2]*National Research Council of Italy (CNR), Institute for Sustainable Plant Protection (IPSP), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Firenze, Italy*

[3]*Department of Chemistry, Ugo Schiff", University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy*

[4]*National Research Council of Italy (CNR), Institute of Food Sciences (ISA), Via Roma 64, 83100 Avellino, Italy*

Myrtus communis L. and *Arbutus unedo* L. are two typical species of the Mediterranean basin. Their fruits, rich in polyphenols, are important sources of biomolecules that may be used as nutraceuticals or food additives. Whey milk constitutes one of the most polluting by-products of cheese manufacturing. However, this waste product can be utilized as an ingredient to functionalize foodstuffs thanks to its large content of proteins and mineral nutrients. In this context, adding polyphenolic-rich extracts in whey could be attractive for the further valorization of this byproduct. To this end, the aim of this study was to obtain a polyphenolic enriched extract from *M. communis* and *A. unedo* fruits and investigate the chemical, microbiological, and physical stability of the additions performed in whey.

To do that, fresh, dried, and lyophilized fruits were extracted using two different methods: decoction and ethanolic ultrasound-assisted extraction. Then, the most polyphenolic-rich extracts were added to whey in two forms: powder (powder addition, PA) and liquid (liquid addition, LA) and stored at 4°C for 60 days. During the storage period, their chemical, microbiological, and physical properties were evaluated every 15 days (T_0, T_{15}, T_{30}, T_{45}, T_{60}). In particular, the polyphenolic stability and the presence of lactic acid bacteria in samples, and the aggregation structure inside the whey added with extracts were performed.

The results obtained demonstrated that the decoction of fresh fruits of *M. communis* and *A. unedo* can be used to maximize the amount of polyphenols and be further added in two different forms, liquid (LA) and powder (PA), to the whey to impart extra healthy properties. Furthermore, the addition using *M. communis* extracts, in both types of forms (LA and PA), to whey produced a chemically stable product for 60 days, with high polyphenolic content. Concerning, the *A. unedo* extracts, the LA showed to be a better choice compared to the powder one (PA) in terms of stability. Furthermore, in all samples tested, the microbiological analysis showed the presence of lactic acid bacteria suggesting the potential use of lactose and polyphenols as prebiotics. Finally, the physical analysis indicated a more homogeneous distribution after the addition of the extracts in whey. The additions performed did not change the macroscopic aspects of whey. Despite this, *M. communis* extracts slightly affect the architecture of casein micelles, while *A. unedo* extract modifies casein micelles to a greater extent due to the presence of tannin compounds.

In conclusion, whey is a nutritionally valuable by-product with numerous potentially beneficial effects on human health, and the developed novel functional product obtained by the addition of decoctions of *M. communis* and *A. unedo* fruits showed to have good stability

in terms of chemical, microbiological, and physical properties suggesting its potential use to create innovative products that may be employed in the nutraceutical sector.

Central sardinia's (Italy) native food plant, *Lactuca longidentata*: an exploration of its health benefits

Nilofar, Di Simone SC, Flores GA, Acquaviva A, Libero ML, Orlando G, Menghini L, Ferrante C
Botanic Garden "Giardino dei Semplici", Department of Pharmacy, "Gabriele d'Annunzio" University, Via dei Vestini 31, 66100 Chieti, Italy

The objective of this study was to investigate the biological properties and phytochemical composition of *Lactuca longidentata*, an endemic species found in Sardinia, Italy. Water extracts from the leaves and roots were analyzed for phenolic composition, scavenging and reducing properties, and enzyme inhibition. In addition, in vitro studies were conducted to assess the biocompatibility of the extracts, including an allelopathy assay, brine shrimp and *Daphnia magna* toxicity tests, and viability assay on C2C12 myocytes. The antimicrobial properties of the extracts were also evaluated against various Gram positive and Gram negative bacteria, yeasts, dermatophytes, and fungi commonly found in public swimming pools.

Colorimetric analyses revealed that the leaves of *L. longidentata* were richer in total phenols and flavonoids than the roots, while chromatographic analyses confirmed that the leaves contained higher levels of catechins and chicoric acid. The leaf extract was also found to be the most effective antiradical agent. Within biocompatibility limits (concentration <200 µg/mL), the leaf extract demonstrated notable efficacy in reducing the growth of *Escherichia coli* and *Trichophyton tonsurans* (MIC < 10 µg/mL), while the root extract exhibited similar potency against the swimming pool fungal species *Rhodotorula*, *Auxarthron ostraviense*, and *Trichothecium roseum*.

Overall, the results of this study shed light on new potential uses for Lactuca longidentata as a health-promoting agent, in addition to its traditional use as a food.

Safety and efficacy of red yeast rice phytocomplex and lovastatin: a comparative analysis

Rigillo G[1], Baini G[2], Miraldi E[2], Pani L[1,3], Tascedda F[4], Biagi M[2]

[1]*Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia*

[2]*Department of Physical Sciences, Earth and Environment, University of Siena, Siena*

[3]*Department of Psychiatry and Behavioral Sciences, University of Miami School of Medicine*

[4]*Department of Life Science, University of Modena and Reggio Emilia*

In the last 20 years, the demand for red yeast rice-based food supplements has significantly increased as consumers have been looked for natural alternatives to manage blood cholesterol levels without the adverse effects associated with synthetic statins. Actually, monacolin K, derived from red yeast rice (RYR) and chemically identical to lovastatin, inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, offering a natural alternative to synthetic drugs. In 2018, despite the health claim approved in 2011, the European Food Safety Authority (EFSA) raised concerns about the safety of monacolin K consumption, especially for vulnerable populations, and highlighted the lack of knowledge on RYR phytocomplex. Consequently, in 2021, the European Commission established a new maximum intake limit < 2.99 mg/day for monacolins.

This study aimed to investigate the safety and efficacy of RYR phytocomplex compared to the sole monacolin K (=lovastatin) by analyzing eight different RYR samples with different monacolin K and secondary monacolins content. Efficacy was evaluated through a validated cell-free enzymatic assay, while an integrated *in vitro* simulated digestion and *in silico* ADME prediction were employed to compare the pharmacokinetics of different samples and lovastatin. The safety of RYR was assessed by monitoring cytotoxicity in intestinal, hepatic, kidney, and skeletal muscle cells using cell viability assays. Furthermore, muscle damage-related targets and myokines were measured by qRT-PCR in myoblasts exposed to prolonged non-toxic stimuli.

Results demonstrated that RYR samples have a large chemical variability not only related to monacolin K content, in lactone and hydroxy-acid form, but also as regards pigments, secondary monacolins, polyphenols, and triterpenes. The enzymatic test revealed that all samples were more effective in inhibiting HMG-CoA activity than lovastatin at equivalent monacolin K content. Although secondary monacolins had a weaker effect than monacolin K, a synergistic effect was observed within the phytocomplex. Additionally, the phytocomplex facilitated higher bioaccessibility of monacolin K in RYR compared to lovastatin. Regarding cell viability, lovastatin and RYR samples exhibited no toxic effects up to 150 µg/mL on intestinal, hepatic, and kidney cells; however, variable effects were observed on skeletal muscle cells at medium-high concentrations. Nevertheless, at physiological non-toxic concentrations, RYR samples did not impact the expression levels of myokine IL-6, autophagy factors, and catabolic markers of protein balance, unlike synthetic statin.

These results suggest that RYR and lovastatin share similar biological activities, but the phytocomplex's contribution from secondary monacolins, pigments, polyphenols, and triterpenes ensure a superior safety profile for this botanical food supplement. However, it is important to note that the composition of RYR phytocomplex can vary significantly between different products on the market. This variability highlights the need for standardization and quality control measures to ensure the safety and efficacy of this

botanical food supplement. In conclusion, our study provides evidence supporting the enhanced efficacy, bioaccessibility, and safety profile of RYR phytocomplex compared to lovastatin demonstrating its potential as a natural and effective option for hypercholesterolemia management.

The flavonoid luteolin attenuates colitis and M1 macrophage activation via TRPM8

Cicia D[1,2], Biscu F[2], Ferrante C[3], Iannotti FA[4], Nanì MF[1], Lucariello G[1], Amico R[1], Orlando G[3], Matteoli G[2], Capasso R[5], Izzo AA[1], Pagano E[1]

[1]*Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples (Italy)*

[2]*Translational Research Center for Gastrointestinal Disorders (TARGID) Department of Chronic Diseases, Metabolism and Ageing (CHROMETA), KU Leuven, Leuven (Belgium)*

[3]*Department of Pharmacy, University 'G. D'Annunzio' Chieti-Pescara, Chieti (Italy)*

[4]*Institute of Biomolecular Chemistry ICB, CNR, Pozzuoli, Naples (Italy)*

[5]*Department of Agricultural Sciences, University of Naples Federico II, Naples (Italy)*

Diet significantly influences the development and progression of inflammatory bowel disease (IBD) [1]. As a matter of fact, dietary therapy is the first line treatment for patients with mild-to-low Crohn's disease [2]. Here, we show that the dietary flavonoid luteolin attenuates intestinal inflammation *via* the temperature-sensitive cation channel TRPM8 (transient receptor potential melastatin type-8).

Firstly, we evaluated the affinity of a number of dietary compounds for the TRPM8 ion channel. A subset of natural compounds with reported anti-inflammatory activity was screened by using the molecular docking analysis with Autodock Vina. The ligands with higher affinity to TRPM8 (based on their Ki values, i.e., rutin and luteolin) were also tested in the intracellular calcium assay on transfected HEK293 stably overexpressing recombinant TRP channels.

Luteolin, but not rutin, was able to block TRPM8 (IC_{50} 3,091±0,326 μM), without affecting other TRP channels, namely TRPA1 and TRPV1.

The *in vitro* anti-inflammatory effect of luteolin was evaluated in wild type (WT) bone marrow derived macrophages (BMDMs). 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 BMDMs supernatant. Metabolic reprogramming of proinflammatory M1macrophages was measured by the Seahorse analysis. To verify if the anti-inflammatory effect of luteolin was mediated by TRPM8, *in vitro* experiments were also carried out on *Trpm8*$^{-/-}$ BMDMs. Finally, luteolin was evaluated in the dextran sodium sulphate (DSS) model of colitis. FACS analysis was used to characterize the myeloid population in colonic lamina propria in DSS- and DSS+luteolin-treated mice.

In vitro, luteolin-treated macrophages showed a significant reduction of the main pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) and an increase in IL-10 production. Moreover, luteolin treatment altered the metabolic signature of proinflammatory WT M1 BMDMs restoring a naïve like metabolism. Of note, luteolin had no effect on *Trpm8*$^{-/-}$ BMDMs, suggesting a TRPM8-mediated anti-inflammatory effect. *In vivo*, luteolin oral administration reduced colitis severity in mice both in terms of disease activity index and histological damage. This effect was associated to a significant reduction in the recruitment of pro-inflammatory myeloid cells.

In conclusion, our experiments have identified luteolin, a flavonoid widely found in the plant kingdom, as a new TRPM8 blocker. Luteolin, *via* TRPM8, exerts an anti-inflammatory effect on macrophages, reducing their pro-inflammatory capacity and metabolic switch. *In vivo*, luteolin oral administration mediates an impairment in the innate immune response during intestinal inflammation and attenuates colitis severity in mice.

Naringenin promotes lysosomal regeneration: a potential therapeutic strategy for Hereditary Spastic Paraplegia

Guarato G[1], Feltrin S[1], Dianin F[1], Vantaggiato C[2], Napoli B[1], Gumeni S[3], Orso G[1]

[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Largo E. Meneghetti 2, Padova, Italy

[2]Scientific Institute IRCCS E. Medea, Laboratory of Molecular Biology, Via D. L. Monza 20, 23842 Bosisio Parini, Lecco, Italy

[3]Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece

Hereditary spastic paraplegia (HSPs) is a group of neurodegenerative disorders characterized by progressive spasticity and weakness in the lower limbs, due to retrograde axonal degeneration of the corticospinal tracts [1]. Despite the increasing understanding of disease mechanisms, to date, HSPs remain uncurable conditions [2]. We recently performed the first combined *in vitro/in vivo* pharmacological screening in models of the HSP form SPG15 demonstrating that lysosomes are a key pharmacological target to rescue SPG15 phenotype and identifying potential therapeutic compounds [3]**.** All the compounds selected from the screen act on lysosomal regeneration, through *ex novo* biogenesis induced by TFEB (Transcription factor EB) nuclear translocation or the activation of autophagic lysosomal reformation (ALR), or on TPC2 (two-pore channel 2) lysosomal calcium channels improving lysosomal function and fusion [4,5]. Unfortunately, the most effective compound identified, SMER28, has not been registered for any clinical trials and no information is available on its tolerability and pharmacokinetics, and further investigations are necessary for the transition from preclinical to clinical studies. Thus, we selected another promising compound, Naringenin (NAR), as a potential positive modulator of lysosomal function to test in our preclinical HSP models. NAR is a natural compound found in citrus and tomato species that has already been tested in clinical trials. NAR is known to cross the blood-brain barrier and exhibits potent neuroprotective activity, as demonstrated by several works using preclinical models of Parkinson's and Alzheimer's diseases [6]. In this work, we tested the therapeutic potential of NAR in SPG4 and SPG15 *Drosophila* and patient-derived cell models and evaluated its mechanism of action.

Drosophila RNAi *D-Spastin* and *D-Spastizin* strains, ubiquitous and neuron driver lines were used for loss of function studies. Eclosion rate and climbing activity assays were performed to highlight developmental and locomotor differences. ALR analysis was performed as previously described [3]. qPCR was used to evaluate TFEB activation [7]. Nar complexed with hydroxypropyl-β-cyclodextrin was chronically administrated at 0.5 mM in the food.

Dspastin (SPG4) and Dspastizin (SPG15) *Drosophila* and cellular models show autophagy alterations, accumulation of enlarged lysosomes and ALR defects. ALR is a process of lysosome biogenesis from autolysosomes after cargo degradation. During ALR, tubular structures extrude from the autolysosomes to generate not acidic proto-lysosomes that mature into functional lysosomes [8]. Chronic treatment with NAR was able to restore the lysosomal size and number and to induce lysosome tubulation in the HSP models, both *in vitro* and *in vivo*. NAR promotes the formation of tubular structures extruding from autolysosomes during ALR similarly to SMER28 [3]. Moreover, NAR activates TFEB, thus promoting *ex novo* lysosomal biogenesis. Finally, NAR treatment rescued the lethality

phenotypes associated with the loss of spastin and rescues the locomotor deficit we observed in both HSP fly models.
Our overall findings identified NAR as a potential therapeutic compound in the treatment of these HSP forms by restoring the lysosomal homeostasis, acting via lysosomal regeneration, through ALR activation or promoting *ex novo* lysosome biogenesis.

In vivo effect of sumac fruit (_Rhus coriaria_ L.) on skin inflammation

Sangiovanni E[1], Fumagalli M[1], Pelin M[2], Piazza S[1], Maranta N[1], Pozzoli C[1], Martinelli G[1], Sosa S[2], Dell'Agli M[1]

[1]Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", Università Degli Studi di Milano, Via Balzaretti 9, 20133 Milan, Italy;

[2]Department of Life Sciences, University of Trieste, Via Fleming 22, 34127 Trieste, Italy.

Rhus coriaria L., commonly known as sumac, is a plant that belongs to the Anacardiaceae family, whose fruit is widely used in the Mediterranean area as a spice or condiment. From a health point of view, sumac finds traditional uses as a remedy for various pathological conditions, including wound healing and inflammation. In our laboratory, we have recently demonstrated that an ethanolic extract of sumac fruit decreased some inflammatory markers (IL-8, MMP-9, VEGF, and ICAM-1) in human keratinocytes (HaCaT) by acting on the transcription factor NF-κB[1].

In this work we investigated the anti-inflammatory properties of sumac fruits in an *in vivo* model of skin inflammation.

For this purpose, an acetone (ARC) and an ethanol macerated extract (mERC) were prepared (5 g of dried pulverized fruits in 50 mL acetone or ethanol, respectively). Both the extracts had a very similar total phenol content (TPC), with values of 160 and 170 mg EAG/g, respectively for ARC and mERC, and a similar profile of inhibition for IL-8 secretion in HaCaT cells (measured by ELISA assay) stimulated with TNFα or phorbol myristate acetate (PMA).

The *in vivo* anti-inflammatory activity of sumac fruit extracts was evaluated as inhibition of Croton oil-induced dermatitis in CD-1 mouse ear. Six hours after dermatitis induction, the extracts showed a dose-dependent reduction of:

- edema (-31% and -46% for mERC and ARC at 1 mg/cm^2, respectively; -57% for indomethacin at 0.1 mg/cm^2);
- leukocyte infiltration (-29% and -36% for mERC and ARC at 1 mg/cm^2, respectively; -36% for indomethacin at 0.1 mg/cm^2).

Gene expression was then evaluated using a 384-well PCR array of mouse genes involved in the inflammatory process (RT2 ProfilerTM PCR array: PAMM-011ZE Mouse Inflammatory Cytokines and Receptors, QIAGEN Sciences, USA). After 6 h from its cutaneous application, croton oil induced a statistically significant expression of 19 genes in auricle tissues, which encode for chemokines, chemokine receptors (e.g., CCR2 and CXCR2), interleukins (e.g., IL-13 and IL-6), and interleukin receptors (e.g., IL-2RG). Both the extracts significantly reduced the expression of IL-13, TNFRSF11B, TNFSF11, and VEGFA, but only ARC inhibited CCL8, CXCL10, IL-3, and IL-6.

In conclusion, the anti-inflammatory activity of sumac fruits has been confirmed *in vivo*. ARC and mERC reduced edema, leukocyte infiltration, and the expression of some genes involved in the inflammatory process. In general, *in vivo* the topical anti-inflammatory activity of ARC was higher than that of mERC. Future studies will be aimed at the phytochemical characterization of the extracts, to identify the molecule or class of molecules responsible for the observed activity.

A novel integrated *in vitro/in silico* approach to investigate species of ethnobotanical interest with wound healing and immunomodulatory activities

Cappellucci G, Baini G, Vaccaro F, Miraldi E*, Biagi M**
Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente, Strada Laterina 8, Siena.

The ethnobotanical study of medicinal plants has always been a source of inspiration for the development of new pharmaceutical products; however, today there is a need to find new methods of preclinical analysis that are able to properly assess and predict pharmacodynamic and pharmacokinetic processes of the complexity of herbal products. In this perspective, one modern screening tool for assessing the molecular targets that may be involved by the molecules characterizing a given phytocomplex is network pharmacology (NP), a computational bioinformatic approach that examines the effect of molecules both at the level of the interactome and at the level of the pathology/disease.
Thus, starting from two species widely used in traditional medicine, but scarcely investigated such as Calluna vulgaris L. Hull (CVE) and Sedum telephium L. (SED), for immune system and wound healing, respectively, in this study we wanted to assess the involvement of certain molecular targets using NP as a first screening tool to be coupled to ethnobotanical surveys, based both on the most important international literature and on interviews conducted in Italy and through inter-university collaborations, it emerged that SED is still one of the species most commonly used in the traditional way for wound healing, by applying the fresh leaf juice directly on the wound. On the other hand, for CVE, ethnobotanical tradition suggests that this species has always been used as water decoction or maceration, mainly for urinary tract disorders, but also to strengthen the immune system.
The phytochemical analyses of traditional preparations showed, for both species a polysaccharide prevalence accompanied by a high polyphenols content, hydroxycinnamic derivatives, arbutin, flavanones and flavonols in CVE and flavonols glysosides in SED.
Following the chemical analyses, research was directed towards the evaluation and study of the mechanisms of action towards immunomodulatory activity for CVE and tissue repair processes for SED.
NP analysis, carried out by cross-referencing data from different softwares (Swiss Target, SEA, GeneRecommender), showed the involvement of factors involved in the modulation of the immune system by many CVE molecules (TNFα, IL-2, PI3K, IKBKB, VDR, ERK2) and in tissue repair processes for SED molecules such as VEGF, FGF and TGF.
PBMC isolated from the peripheral blood of healthy volunteers and THP-1 were used to perform biological assays to evaluate the molecular mechanisms underlying the immune modulation operated by CVE, and HaCaT and HFF were used to test the ability of SED to heal skin lesions. In all tests quantitative ELISA dosages were used.
In vitro experiments showed a good correspondence with the findings of NP studies, thus demonstrating that the integrations of ethnobotany-NP-validated in vitro dosages can be a feasible approach for a preliminary assessment of the biological activity of medicinal plants, especially in the case of poorly investigated species.

Modulation of neuroinflammation by essential oils obtained from different hemp varieties

Barbalace MC[1], Freschi M[1], Rinaldi I[1], Mazzarra E[2], Petrelli R[2], Maraldi T[3], Malaguti M[1], Maggi F[2], Hrelia S[1], Angeloni C[1]

[1]Department for Life Quality Studies, Alma Mater Studiorum, University of Bologna, Italy.

[2]School of Pharmacy, University of Camerino, Italy.

[3]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy.

Nowadays, the progressive aging of the population due to the increase in longevity is a tangible event for the worldwide population. It represents a success for the scientific community thanks to the progress of science, but at the same time, it will mean a great challenge for the immediate future. Indeed, with the increase in life expectancy, we have witnessed a parallel increase in chronic-degenerative diseases, especially neurodegenerative diseases which mainly affect the elderly population. The etiology of neurodegenerative diseases is multifactorial, but they share key pathological features like oxidative stress and neuroinflammation. During neuroinflammation, a crucial role is played by microglia which constitute resident immune cells in the central nervous system. Nevertheless, pathological conditions like Alzheimer's disease, Parkinson's disease, multiple sclerosis, stroke, and other neurological disorders, are associated with the hyperactivation of microglial cells. This phenomenon leads to the release of several pro-inflammatory mediators like cytokines and reactive oxygen species (ROS) leading to neuronal damage. Recently, the regulation of microglial immune-related functions through the endocannabinoid system has emerged as a potential neuroprotective strategy. Essential oils (EOs) obtained from hemp inflorescences are rich in interesting bioactive molecules like terpenes and terpenoids able to prevent the progression and help in the management of several chronic-degenerative diseases including neurodegenerative diseases. In this regard, improving the knowledge of different hemp varieties or cultivars to obtain EOs enriched in specific bioactive phytochemicals may be advantageous. This study aimed to investigate the anti-inflammatory properties of EOs obtained from inflorescences of different hemp varieties in BV-2 microglial cells. Cells were treated with increasing concentrations of EOs for 2 h and then activated with LPS 100 ng/mL for 24 h. One of the tested EOs, named Gorilla Glue (GG), demonstrated promising anti-inflammatory potential. Nitric oxide production, measured by Griess assay, was significantly reduced by $5x10^{-3}$ µL/mL EO treatment with respect to LPS. The treatment with EO decreased the gene expression of inflammatory mediators such as IL-1β, IL-6, TNF-α, COX-2, iNOS, and NLRP3 and increased the gene expression of anti-inflammatory mediators such as IL-4 and Arg1 compared to LPS. The anti-inflammatory effect could be ascribed to the significant reduction of the NFkB-nuclear translocation, probably mediated by the inhibition of the phosphorylation of p38 MAPK, as evidenced by immunofluorescence and Western Blot analyses, respectively. GG EO was able to counteract LPS-induced oxidative stress by significantly reducing ROS levels and increasing GSH levels. The main compounds found in GG EO were (E)-caryophyllene (17.6%), selina-3,7(11)-diene (12.5%), selina-4(15),7(11)-diene (7.1%) and α-humulene (4.7%). We tested (E)-caryophyllene and α-humulene for their anti-inflammatory activity. α-humulene was able to counteract the increase of all the pro-inflammatory mediators tested, while (E)-caryophyllene only upregulated the anti-

inflammatory cytokine IL-4. These results suggest a specific contribution of different bioactive compounds to the positive effects shown, thus suggesting this EO is a promising protective agent in neurodegenerative diseases due to its anti-inflammatory properties.

Bio-pharmacological properties of *Pelargonium quercetorum* Agnew extracts: focus on potential application as agents against inflammatory bowel diseases

Chiavaroli A, Libero ML, Di Simone SC, Acquaviva A, Nilofar, Recinella L, Leone S, Brunetti L, Cicia D, Izzo AA, Orlando G, Zengin G., Menghini L, Ferrante C

[1]*Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy*

[2]*Department of Pharmacy, School of Medicine, University of Naples Federico II, 80131 Naples, Italy*

[3]*Physiology and Biochemistry Laboratory, Department of Biology, Science Faculty, Selcuk University, 42130 Konya, Turkey*

Pelargonium quercetorum Agnew, belonging to the Gerianaceae family, is a medicinal plant traditionally used by the local population in the Hakkari region of Turkey and in the Kurdistan region of Iraq for treating intestinal worms [1,2].

In the present study, the chemical composition and biological properties of different polarity extracts of *P. quercetorum* were investigated. Specifically, phenolic compounds were assayed through colorimetric and chromatographic analysis. Intrinsic enzyme inhibition (against α-glucosidase, α-amylase, cholinesterases, and tyrosinase) and scavenging/reducing properties were assayed as well. The extracts were also studied in an ex vivo experimental model of colon inflammation, constituted by isolated colon specimens exposed to LPS, in order to reproduce the burden of inflammation and oxidative stress occurring during colitis, in vivo [3]. In this context, the gene expression of pro-inflammatory biomarkers, including cyclooxygenase-2 (COX-2) and tumor necrosis factor α (TNFα) were assayed. Additionally, the gene expression of vascular endothelial growth factor A, hypoxia-inducible factor 1α (HIF-1α), deeply involved in the so-called inflammatory-to-cancer transition, was also measured in human colon cancer HCT116 cells. In the same cell model, the gene expression of transient receptor potential cation channel subfamily M (melastatin) member 8 (TRPM8), possibly involved in colon carcinogenesis, was conducted as well.

The extracts showed a different qualitative and quantitative content of phytochemicals, with water and methanol extracts being richer in total phenols and flavonoids, among which are flavonol glycosides and hydroxycinnamic acids. This could explain, at least in part, the higher antioxidant effects shown by methanol and water extracts, compared with ethyl acetate extract. By contrast, the ethyl acetate was more effective as cytotoxic agent against colon cancer cells, and this could be related, albeit partially, to the content of thymol and to its putative ability to downregulate TRPM8 gene expression. Additionally, the ethyl acetate extract was effective in inhibiting the gene expression of COX-2 and TNFα in isolated colon tissue exposed to LPS.

Overall, the present results support future studies for investigating protective effects against gut inflammatory diseases.

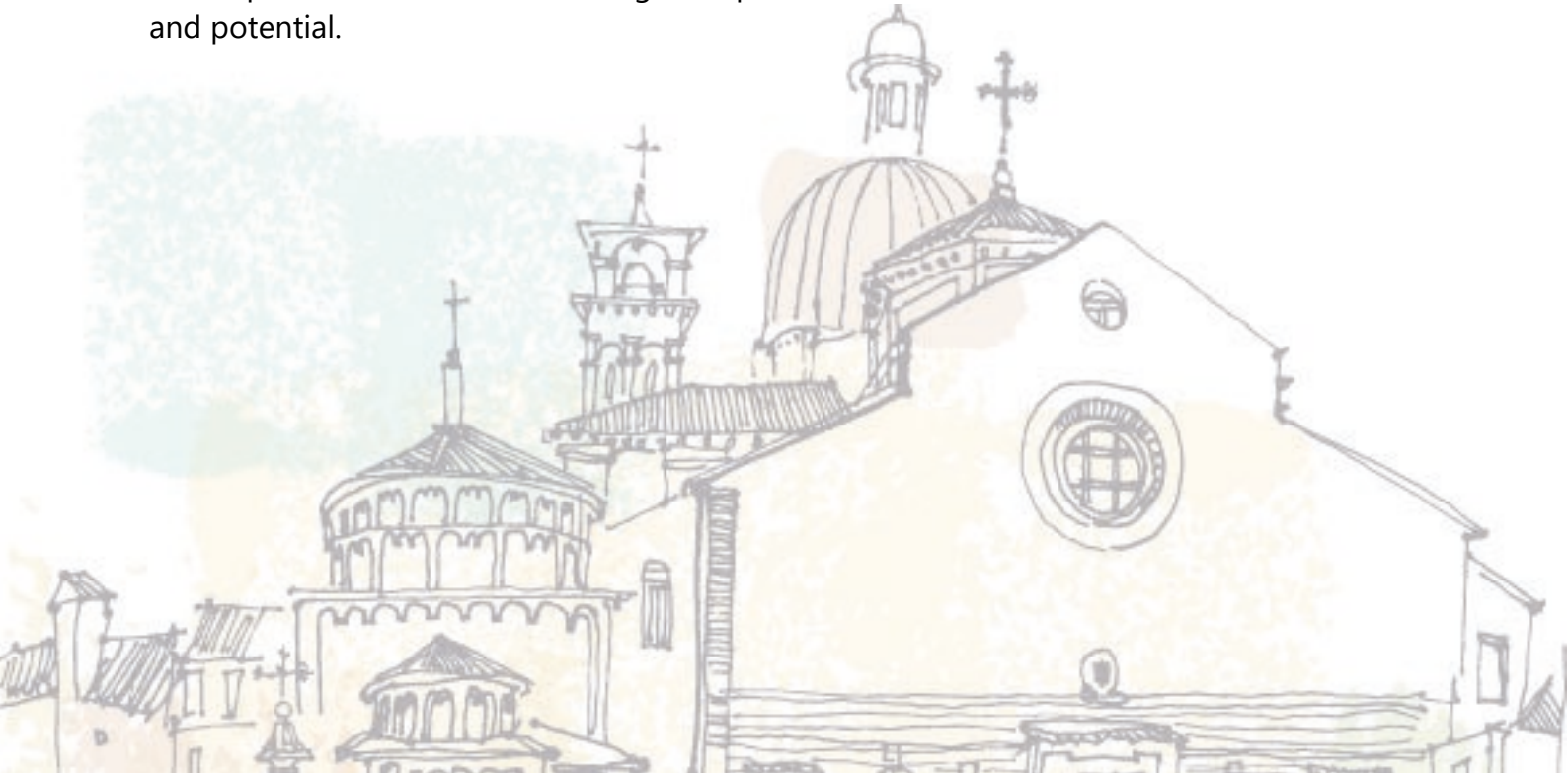
Elenco abstract poster

Venerdì 16 giugno

P1. Characterization of polyphenolic composition in *Leccinum Scabrum* and *Leccinum Versipelle* mushrooms

Medri F[1], Terenzi C[1], Davani L[1], Montanari S[1], De Simone A[2], Andrisano V[1]
[1]*Department for Life Quality Studies, University of Bologna, Corso D'Augusto 237, 47921 Rimini, Italy*
[2]*Department of Drug Science and Technology University of Torino, via P. Giuria 9, 10125 Torino, Italy*

Wild edible mushrooms are widely appreciated in gastronomy, medicine and pharmacology. In spite of the substantial amount of studies on mushrooms, which have recently received considerable attention, many species are not investigated yet. The purpose of the study was to examine Leccinum Scabrum and Leccinum Versipelle wild edible mushrooms as potential sources of antioxidants. This work concerned, primarily, the application of an ultra-sound based extraction (UAE) method and the development and optimization of a microwave assisted extraction method (MAE) to obtain a higher yield of polyphenols with a lower use of solvents and sample. Later, the mushrooms extracted fractions were characterized applying the UV-VIS spectrophotometric assay Total Phenolic Content (TPC) in order to evaluate the total content of phenolic compounds. Furthermore, the identification and the quantification of individual polyphenolic compounds in these extracts was conducted by a high-performance liquid chromatography coupled with diode array detection (HPLC-DAD) method. TPC results show that both species of Leccinum have a similar polyphenols content, with TPC values ranging from an average of 919.3 (Leccinum Scabrum) to 956.6 mg GAE/100g DW (Leccinum Versipelle). The HPLC-DAD analysis was carried out with 24 phenolic compounds as standards, among which the structures of 2 polyphenols were identified in the sample injection: t-cinnamic acid and p-coumaric acid. Other 5 compounds were attributed to the hydroxy-benzoic acid class. The obtained phenolic profiles revealed the richness of phenolic acids and hydrophilic phenolic compounds. These results place these species of mushrooms among food products with a considerable antioxidant content and potential.



P2. Chemical characterization of the volatile and non-volatile profiles of *Achillea nana* L. aerial parts by SPME-GC/MS, GC/MS and HPLC/MS-MS, techniques

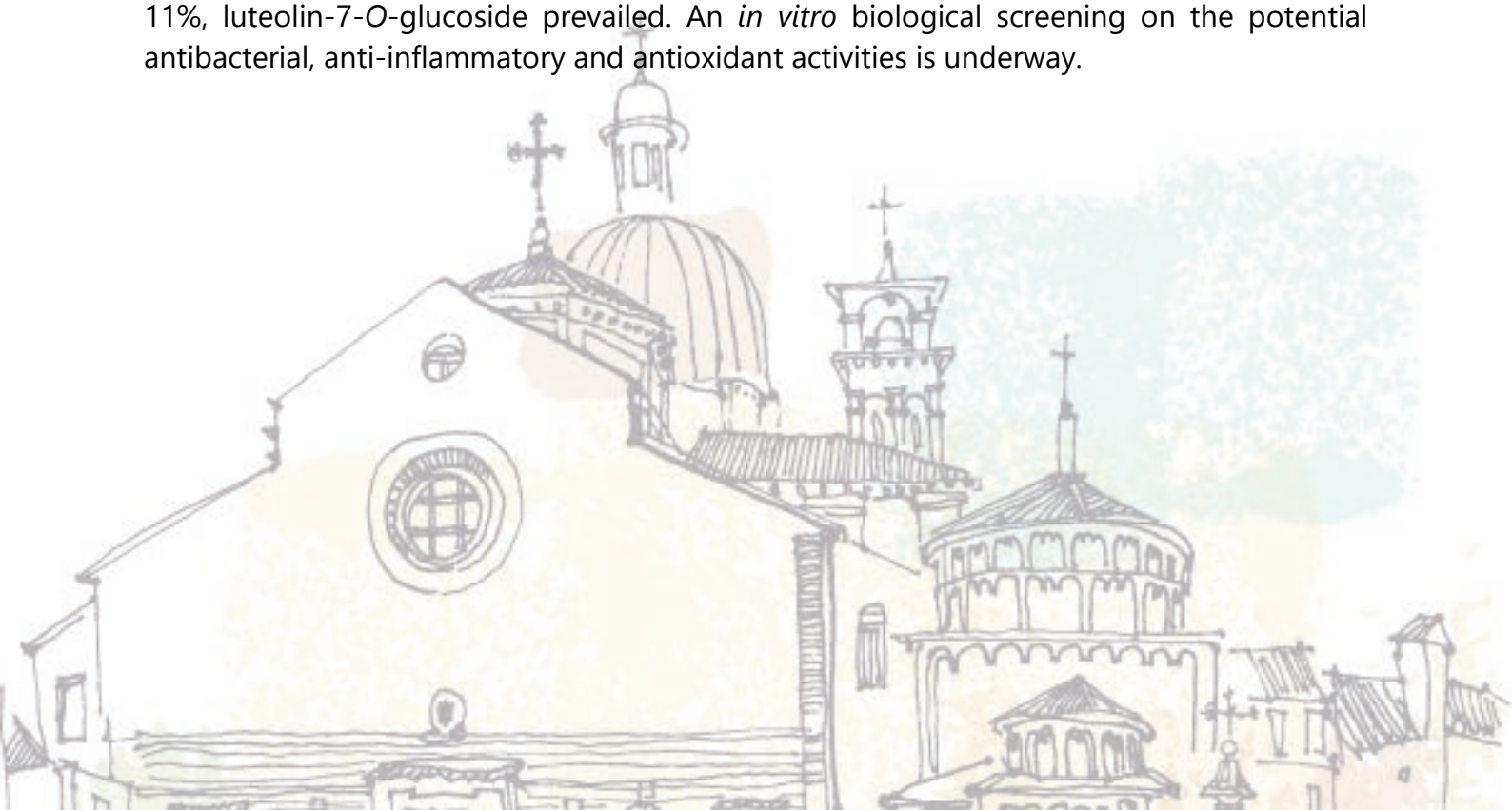
Argentieri MP[1], Garzoli S[2], Meroni G[3], Martino PAM[3], Soggiu A[3], Bonizzi L[3], Zecconi AA[3], Iriti M[3], Vitalini S[3]

[1]Department of Pharmacy – Pharmaceutical Sciences, Universita' degli Studi di Bari "Aldo Moro", via Orabona 4, 70125, Bari, Italy

[2]Department of Drug Chemistry and Technology, Sapienza University, P. le Aldo Moro 5, 00185, Rome, Italy

[3]Department of Biomedical, Surgical and Dental Sciences, Universita' degli Studi di Milano, via G. Pascal 36, 20133, Milan, Italy

Achillea nana L. (Asteraceae) is a perennial, calcifuge, herbaceous plant that grows on mountain rocks and scree, from 1800 to 3000 metres in the Alpine area. It is a strongly aromatic species, with flowers gathered in dense corymbs formed by 4-8 capitula and stems up to 15 cm, simple, erect and tomentose (Pignatti, 1982). Its traditional use is poorly documented. In Piedmont it was known for the diuretic, diaphoretic and febrifugal properties (Chiovenda-Bensi, 1955; Lomagno and Lomagno Caramiello, 1970, 1977) while in Lombardy the preparation of infusions and digestive liqueurs is reported (Vitalini et al., 2015). The aerial parts of *A. nana* were collected at flowering stage in July 2022 in Valtellina (Valle dei Forni, Sondrio, Italy) and identified according to Flora d'Italia (Pignatti, 1982). SPME-GC/MS technique was performed to describe the volatile chemical profile of the sample as it is after drying. Monoterpenes accounted for the major fraction with terpinolene (35.4%), *α*-pinene (35.2%) and β-terpinene (12.2%) as the most abundant compounds. The sample, subsequently powdered, was then extracted with solvents of increasing polarity (petroleum ether, PET; dichloromethane, DCM; methanol, MeOH). The components of the PET and DCM extracts were characterized using GC/MS. The obtained results highlighted the presence of hydrocarbon and terpene compounds. The MeOH extract was subjected to HPLC/MS-MS analysis which allowed to highlight a series of flavonoids and phenolic acids. The latter were the main compounds representing 89% of the total. In particular, 5-*O*-chlorogenic acid and 3.5-dicaffeoylquinic acid were the most abundant. Among the flavonoids, amounting for 11%, luteolin-7-*O*-glucoside prevailed. An *in vitro* biological screening on the potential antibacterial, anti-inflammatory and antioxidant activities is underway.



P.3 Chemical characterization of the volatile compounds of 12 species within the genus *Salvia* L. cultivated at the Botanic Garden of Pisa and study of the relationship with their geographical origin.

Bozzini MF[1], Pieracci Y[1], Peruzzi L[2,3], D'Antraccoli M[3], Ciampi L[3], Pistelli L[1,4], Flamini G[1,4]

[1]*Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy;*

[2]*Dipartimento di Biologia, Università di Pisa, Via Derna 1, 56126 Pisa, Italy*

[3]*Orto e Museo Botanico dell'Università di Pisa, Via Luca Ghini 13, 56126 Pisa, Italy*

[4]*Centro Interdipartimentale 'NUTRAFOOD', Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy*

The genus *Salvia* L., with more than 1000 classified species and a cosmopolitan geographical distribution, represents the largest group within the Lamiaceae family[1]. An important collection dedicated to this genus is located at the Botanic Garden of the University of Pisa since 2013 and has been enriched to currently host 77 taxa and 8 hybrids. The particular structure of the stamens has represented the feature for which all the sages have been grouped into a single genus, but the first phylogenetic molecular studies have shown that the genus *Salvia* L., as traditionally circumscribed, is not monophyletic: the species have been distributed in four clades (deriving from distinct evolutionary lines), which are closely related with their geographical distribution[2].

In this study, the volatile organic compounds (VOCs), of the essential oils (EOs) and spontaneously emitted by flowers and leaves of 12 species of *Salvia*, belonging to the collection of the Botanic Garden, were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). In particular, essential oils were obtained from the dried plant material by hydrodistillation with a Clevenger apparatus, while the volatile compounds spontaneously emitted by flowers and leaves were individually analyzed *in vivo* through the Headspace Solid-Phase Micro-Extraction (HS-SPME) technique, using a polydimethylsiloxane (PDMS) fiber, kept in contact with the headspace (HS), containing the VOCs, for a time ranging from few minutes to half an hour. Both the EOs, previously diluted with *HPLC-grade n*-hexane, and the HSs were then injected into GC-MS apparatus. The identification of the chemical compounds was based on the comparison of their retention times with those of pure samples, as well as on the comparison between their Linear Retention Indices (l.r.i.) and on computer matching against commercial and home-made mass spectra libraries. The major chemical classes detected were monoterpenes and sesquiterpenes, both in their hydrocarbon and oxygenated forms. The complete chemical compositions of EOs and HSs, were subsequently subjected to Multivariate Statistical Analysis using the Hierarchical Cluster Analysis (HCA) method, obtaining a grouping of the different species according to their chemical similarities and differences. The clustering allowed to identify the presence of a good correspondence between the EOs chemical profiles of the 12 species and both their geographical origin and partition into clades, with only two exceptions. The same matching was not found in the analysis of flowers and leaves HSs. Given that all the 12 species have been cultivated in the same environmental conditions, similarities and differences shown in the volatile profiles can be considered genetically determined.

P4. *Calamintha nepeta* Savi: ethnobotany, phytochemistry and pharmacological properties

Lupia C[1], Marrelli M[2], Perri MR[2], Zicarelli L[2], Conforti F[2], Duca A[3], De Biasio F[3], Canora G[1], Salamone E[1], Statti G[1,2]

[1]*National Etnobotanical Conservatory, Castelluccio Superiore, 85040 PZ, Italy*

[2]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende, CS, Italy*

[3]*EVRA Srl Soc. Benefit, Loc. Galdo, 85044 Lauria, PZ, Italy*

Calamintha nepeta Savi is a perennial plant, 30 to 80 cm high, with white to lilac and purple flowers, which belongs to the Lamiaceae family (1).

Much information is known about the ethnobotanical uses of this species, which was utilized to flavor salads and other dishes, but also to perfume rooms and against wood moths. In the popular tradition, small brooms and bunches used to clean wine vessels were produced with this plant. *C. nepeta* was also used together with walnut leaves to slow down the undesired potatoes budding during the storage.

Moreover, in the folk culture, it was utilized to make rudimentary puppets used in the old Italian godparenthood rituals of "comparaggio". In the traditional medicine, this plant, previously crushed with stones, was used for wound healing and as repellent and it was considered an excellent remedy against insect and viper bites, both in humans and animals. The flowering tops infusion was useful in case of hemoptysis and as a vermifuge, while the leaves were inserted into the carious tooth to relieve toothache. Moreover, the plant, macerated with milk overnight, was used to treat intestinal diseases (2).

The pharmacological properties of *C. nepeta* have been investigated and it is currently known that the medicinal parts are leaves and aerial parts (1,3). The drug is a diaphoretic and expectorant and it can be used in nutraceuticals to support fluidity of bronchial secretion and regulate the sweating process (1,3). Moreover, it could be useful for helping the digestive system and improving sleep quality (1,3,4).

Calamintha nepeta aerial parts were collected in Calabria and extracted with methanol through maceration (48 h x 3, plant to solvent ratio 1:10 g/mL). The raw extract was then sequentially partitioned using solvents with increasing polarity: petrol ether, dichloromethane and ethyl acetate. The phytochemical composition was verified with gas-chromatography-mass spectrometry (GC-MS). The total phenolic and flavonoid contents were also assessed (5). The *in vitro* antioxidant potential was measured using the DPPH and the β-carotene bleaching assays (6). Moreover, the inhibitory activity on the production of the pro-inflammatory mediator nitric oxide (NO) was verified in LPS-stimulated RAW 264.7 macrophages (7). The ethyl acetate fraction and the raw extract showed radical scavenging activity, while the petrol ether fraction demonstrated the best inhibitory effects on NO production. Obtained results confirm that *C. nepeta* represents a promising source of active principles.

P5. The synergistic inhibitory effect of fennel, lavender and oregano essential oils on nitric oxide production: an optimization by a mixture design methodology

Marrelli M, De Luca M, Perri MR, Zicarelli L, Statti G, Conforti F

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, via Pietro Bucci, 87036 Rende (CS), Italy

The synergistic effects of phytochemicals are attracting much attention in an effort to boost their biological activity (1). A number of these studies focus on the possible synergism of essential oils (2,3). The rationale for using a combination of botanicals is to produce a dynamic product, whose effects are greater than the sum of known and unknown chemical components, as synergistic responses need to be distinguished from simple additive responses (4). Different mechanisms could be responsible for the potential increase of the bioactivity, such as the improvement of the bioavailability of natural compounds or the modulation of multiple signaling pathways (1).

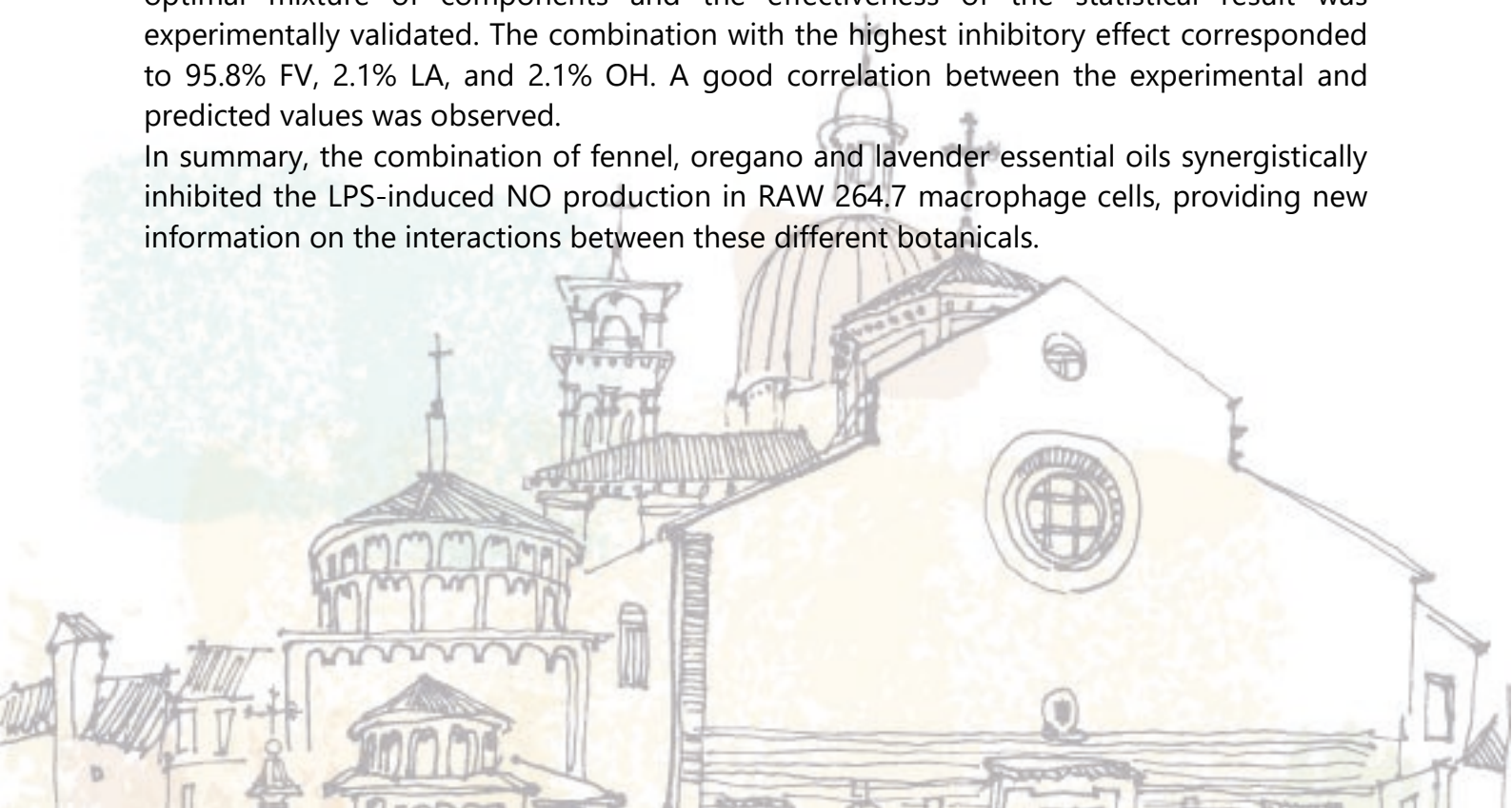
The present study was designed to optimize the combination of the essential oils (EOs) from three different aromatic plants*:* *Foeniculum vulgare* subsp. *piperitum* (C.Presl) Bég. (FV), *Lavandula austroapennina* N.G. Passal., Tundis & Upson (LA) and *Origanum heracleoticum* L. (OH). The mixtures were evaluated for their inhibitory effects on the production of nitric oxide (NO) in LPS-stimulated RAW 264.7 macrophages. The concentration of nitrite in cell culture media was determined by the Griess reaction (5).

The *in vitro* inhibitory activity of the single essential oils was firstly assessed. All the samples were effective in inhibiting the NO production, with IC_{50} values equal to 104.9 ± 4.2, 182.0 ± 9.9 and 347.8 ± 5.6 μg/mL for LA, OH and FV, respectively. The chemical composition of investigated EOs was also verified with gas chromatography-mass spectrometry (GC-MS).

A formulation based on the combination of the three EOs was then developed using a mixture design approach. To this goal, a statistical Simplex Lattice Mixture design was applied.

Using a Design of Experiments (DoE) approach, a multivariate model was able to predict the optimal mixture of components and the effectiveness of the statistical result was experimentally validated. The combination with the highest inhibitory effect corresponded to 95.8% FV, 2.1% LA, and 2.1% OH. A good correlation between the experimental and predicted values was observed.

In summary, the combination of fennel, oregano and lavender essential oils synergistically inhibited the LPS-induced NO production in RAW 264.7 macrophage cells, providing new information on the interactions between these different botanicals.



P6. Chemical composition and bioactivities of *Rosmarinus officinalis* L. cultivated and wild extracts in Campania region

Francolino R[1], Martino M[1], Caputo L[1], Amato G[1], Chianese G[2], Formisano C[2], De Feo V[1], Ercolano G[2], Ianaro A[2], De Martino L[1]

[1]*Department of Pharmacy, University of Salerno, Fisciano, Italy,*

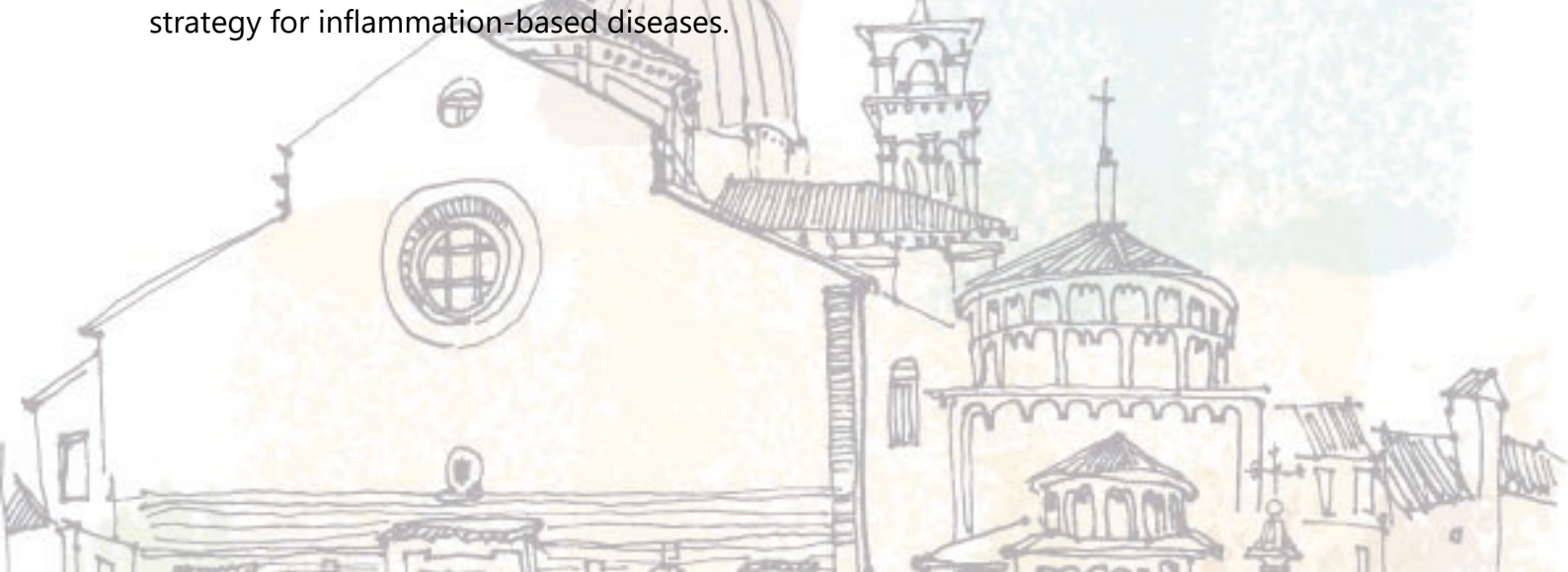
[2]*Department of Pharmacy, University of Napoli, Napoli, Italy*

Rosmarinus officinalis L. is an aromatic, evergreen plant from the Lamiaceae family. Several studies have shown the multiple activities of rosemary extracts, such as antioxidant, antibacterial, hypoglycaemic, anticancer, hepatoprotective, anti-inflammatory, and antithrombotic. The environmental conditions (soil, altitude, weather) could change the chemical composition of rosemary extracts and their biological activities. The purposes of this study are to compare the chemical profile and bioactivities of hydroalcoholic extracts derived from cultivated (CRO) and wild (WRO) *Rosmarinus officinalis* L. The plants were collected in Campania region. The chemical composition of the extracts is performed by LC-MS. LC–MS analysis revealed the presence of a wide range of phenolic compounds in the extracts, including flavonoids, phenolic and terpenes. The extracts were further submitted to NMR analysis to highlight the occurrence of primary metabolites.

The total polyphenolic content (TPC) by Folin- Ciocalteau method and total flavonoid content (TFC) by aluminium chloride colorimetric method are determined by spectrophotometric assays. The antioxidant activity is evaluated by two different tests: DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power). The extracts of cultivated and wild *R. officinalis* shown a similar content of polyphenols and flavonoids. The antioxidant activity of rosemary extracts is associated with the amount of TPC and TFC. The extracts shown an interesting antioxidant activity in both antioxidant assays.

The enzymatic activities studied include anti-acetylcholinesterase (AChE) and anti-butyrylcholinesterase (BChE) by the Ellman's assay and α-amylase by Benfeld's assay. The extracts showed similar enzyme activities for the α-amylase and BChE assays, while in AChE assay WRO shown a better activity than CRO. Finally, to better characterize the antioxidant effects of the R. extracts, in vitro studies were performed with the J774A.1 murine macrophage cell line. As expected, pretreatment with *Rosmarinus officinalis* extracts, significantly reduced the production of ROS and impaired mitochondrial mass as assed by using the DCF-DHA and the Mitotracker green fluorescent probes. All these activities exerted by the extracts are closely related to their similar phytochemical profile.

The data collected suggest that these extracts may be considered a promising therapeutic strategy for inflammation-based diseases.



P7. Re-evaluation of Tuscan *Crocus sativus* L. wastes by metabolomic fingerprint and *in vitro* antiviral test against SARS-CoV-2

Cioni E[1], Di Stasi M[1], Iacono E[2,3], Luminare AG[4], Gambineri F[4], Bazzichi A[4], Lai M[2,5,6], De Leo M[1,6], Braca A[1,6], Pistello M[2,5,6]

[1]*Department of Pharmacy, University of Pisa, Pisa, Italy*

[2]*Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy*

[3]*Department of Medical Biotechnologies, University of Siena, Siena, Italy*

[4]*ARCHA srl, Pisa, Italy*

[5]*Virology Unit, Pisa University Hospital, Pisa, Italy*

[6]*CISUP, Center for Instrument Sharing of the University of Pisa, Pisa, Italy.*

Crocus sativus L. (saffron), belonging to the Iridaceae family, is a perennial plant reaching up to 25 cm in height that requires for growing a continental Mediterranean climate [1]. Italy is among the major producers of saffron in Europe, after Greece and Spain [2]. The plant is cultivated only to recover the stigmas from its flowers to obtain the spice, which is widely used in the food sector both for particular taste and intense colour. The remaining part of the flower (almost all) is waste. Previous chemical characterization studies of saffron bioresidues revealed the presence of flavonoids such as quercetin and kaempferol glucosides [3,4] known in the literature as bioactive agents, useful for their antioxidant and antiviral properties [5].

The aim of this work was to re-evaluate Tuscan saffron by-products by preparation of a hydroalcoholic extract, followed by its chemical quali-quantitative UHPLC-HR-Orbitrap/ESI-MS analysis and *in vitro* test of the antiviral effect against SARS-CoV-2. The raw material was provided by the Tuscan "Montegrappa Farm by Anastasia Vecchiarelli" (Grosseto, Italy) and extracted by static maceration with EtOH-H_2O 60% v/v immediately after harvesting. Extracts were injected in triplicate solutions into the LC-MS system and the chemical fingerprint was obtained by tentative identification of the components based on HR-MS data. The metabolomic study of saffron by-products extract confirmed the presence of quercetin, kaempferol, isorhamnetin derivatives (as mono- or diglucoside and in acetylated form) and showed the occurrence of crocins. Kaempferol-*O*-sophoroside resulted the most abundant kaempferol derivative contained in the saffron extract (316.5 ± 5.3 mg/100 g of fresh flower (FF)), followed by kaempferol (14.8 ± 1.4 mg/100 g FF). For the antiviral assay, VERO E6 cells were infected with the SARS-CoV-2 clinical strain VR PV10734 obtained from the Unit of Virology, AOUP, Pisa. The SARS CoV-2 antiviral activity of the saffron extract was evaluated by counting the number of infected cells in the presence and absence of extract (High Content Confocal Microscopy) and by quantifying SARS CoV-2 genomes by Real-Time PCR. Saffron by-products extract reduced SARS-CoV-2 viral genomes and the frequency of cell infection by 2-fold respect to the untreated control.

In conclusion, these encouraging results, which fit into a perspective of enhancing the small local agri-food chain, showed the high chemical and biological value of the Tuscan saffron wastes phytocomplex as a good source of health-promoting specialized metabolites.

P8. Coumarins from ethyl acetate extract of gum ammoniacum

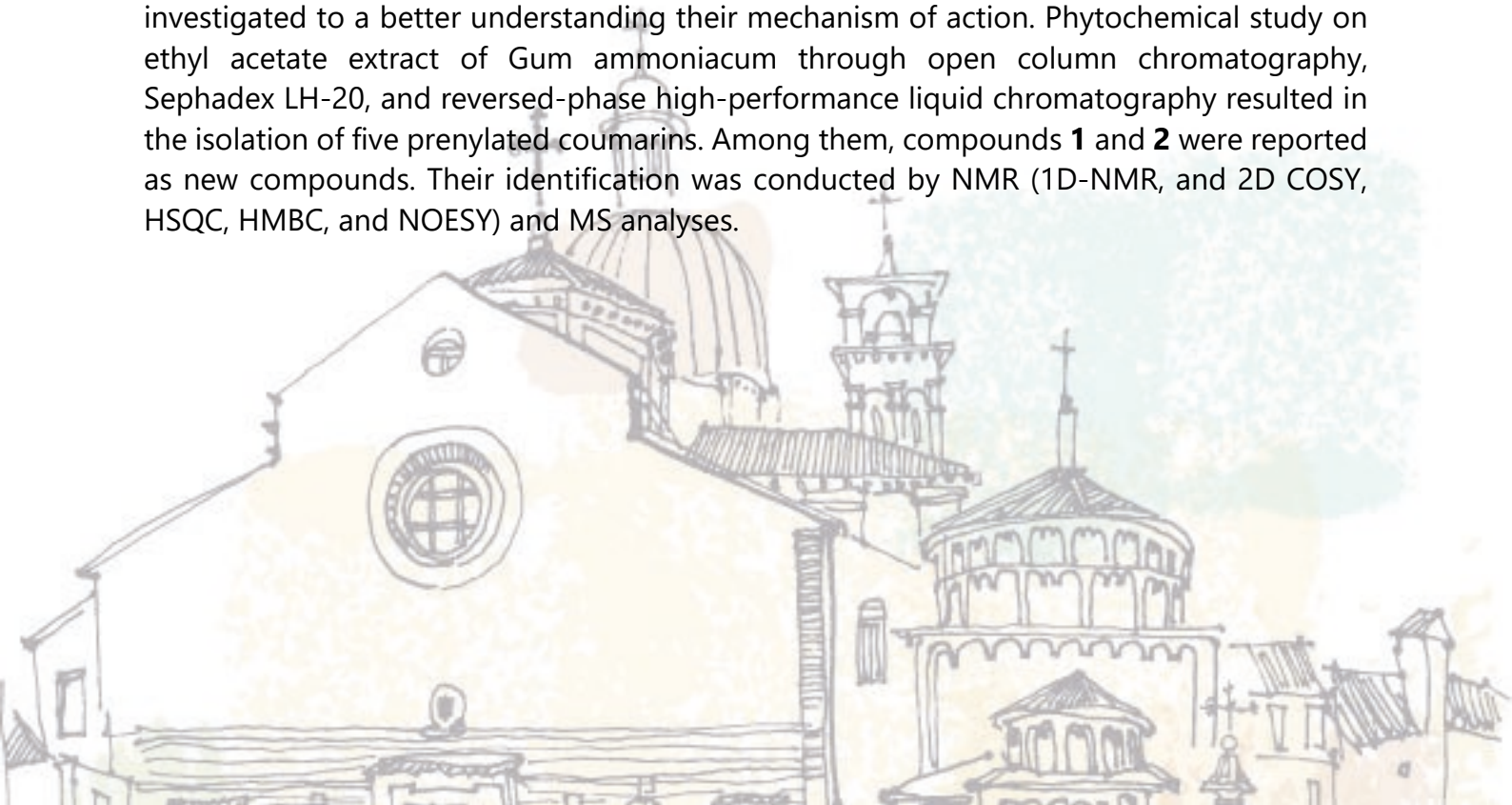
Kharatha M[1,3], Alilou M[2], Donadio G[3], De Tommasi N[3], Moridi Farimani M[1]

[1]Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Evin, Tehran, Iran.

[2]Institute of Pharmacognosy, University of Inssbruck, Innrain 80-82, 6020 Innsbruck, Austria.

[3]Department of Pharmacy, University of Salerno, 84084 Fisciano (SA), Italy.

Dorema genus (Apiaceae) comprises 12 accepted species of which seven species in flora of Iran, among them two are endemic *D. ammoniacum* D. Don and *D. aucheri* Boiss [1]. *D. ammoniacum* is a perennial plant growing in arid and semi-arid regions of central Iran [1]. This species is well known for its exudate, a medicinal gum resin, known as "Ushaq" or "Persian ammoniacum" [2]. In Iranian traditional medicine, ammoniacum gum is used as an expectorant, stimulant and antispasmodic, and to treat catarrh, asthma, chronic bronchitis and enlargement of liver and spleen [3]. *Dorema* gum resin showed some bioactivities including antibacterial, antifungal, cytotoxic, and acetylcholinesterase (AChE) inhibitory effects. Free salicylic acid, ammoresinol, dshamirone, sesquiterpene chromandiones and coumarins have been isolated and chracterized from *D. ammoniacum* [3]. Coumarins are found in over 150 different species of plants belonging to almost 30 different families, including *Rutaceae*, *Clusiaceae*, *Guttiferae*, *Caprifoliaceae*, *Oleaceae*, *Nyctaginaceae*, and *Apiaceae*. These compounds accumulate in fruits, vegetables, roots, flowers, leaves, bark and stems. Coumarins display a wide range of biological activities consist of antimicrobial, antifungal, antiviral, antioxidant, anticancer. [4] The aim of the present study was to investigate *D. ammoniacum* gum resin constituents and to evaluate their antibacterial activities. Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) was determined by the reference protocol of Clinical and Laboratory Standards Institute: CLSI (2009) against different species of the Gram-negative such as *Escherichia coli, Citrobacter, Salmonella, Pseudomonas aeruginosa* and Gram-positive such as *Staphylococcus aureus, mutans and epidermidis, Klebsiella pneumoniae, Bacillus subtilis and clausii, and Enterococcus fecalis*. Compounds showing a promising antimicrobial activity will be further investigated to a better understanding their mechanism of action. Phytochemical study on ethyl acetate extract of Gum ammoniacum through open column chromatography, Sephadex LH-20, and reversed-phase high-performance liquid chromatography resulted in the isolation of five prenylated coumarins. Among them, compounds **1** and **2** were reported as new compounds. Their identification was conducted by NMR (1D-NMR, and 2D COSY, HSQC, HMBC, and NOESY) and MS analyses.



P.9 Moderate deficit irrigation can modulate the secondary metabolites of *Pistacia lentiscus* L. fruits during ripening

Muti L[1], Vanacore D[2], Brunetti C[1,2], Dos Santos Nascimento L[1], Goracci G[1], Alderotti F[1], Detti C[2], Bilia AR[3], Ferrini F[1,2], Gori A[1,2]

[1]University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Sesto Fiorentino, Florence 50019, Italy

[2]National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Sesto Fiorentino, Florence and Turin 50019 and 10135, Italy

[3]Department of Chemistry, University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino, Italy

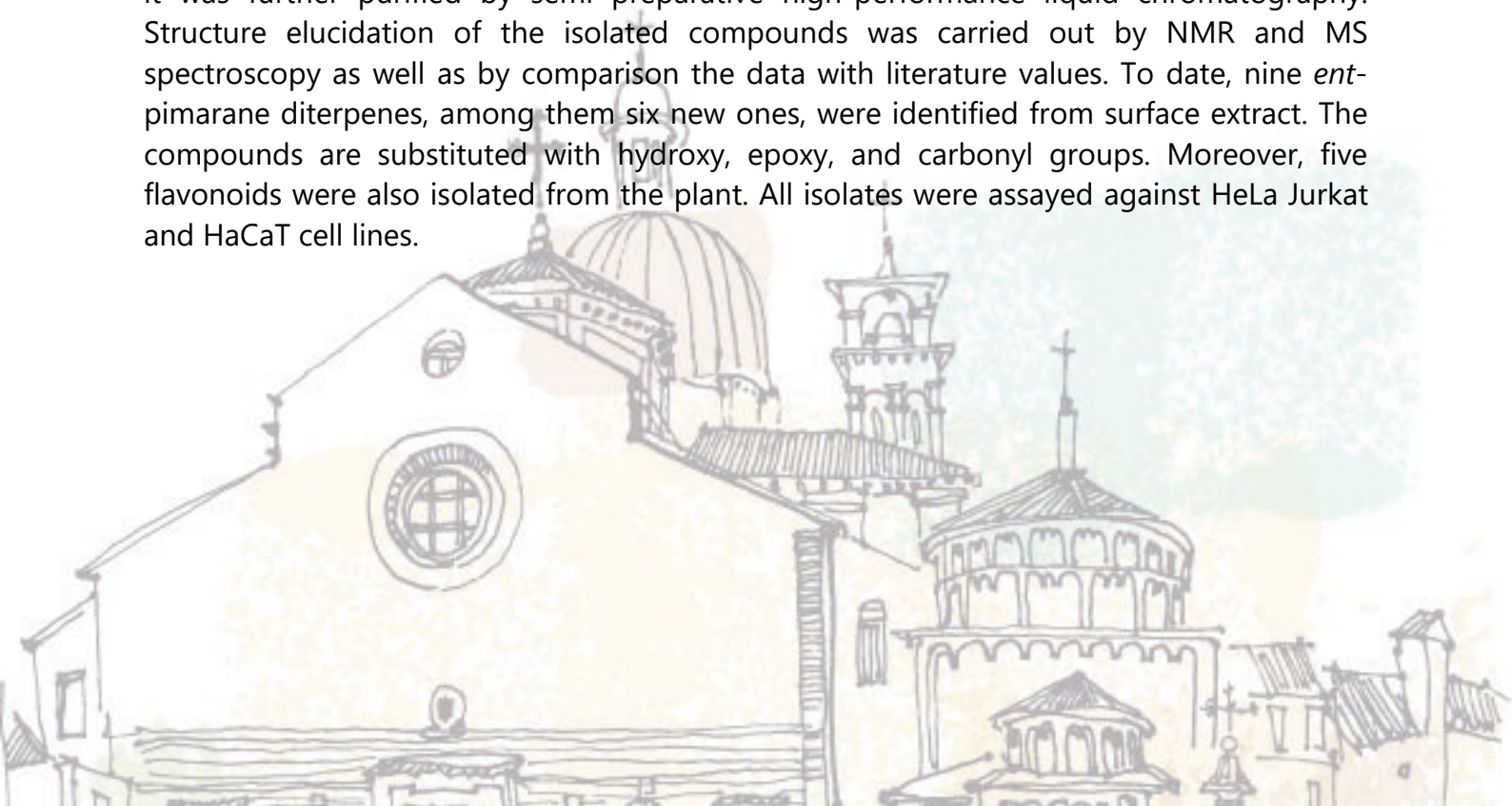
Pistacia lentiscus L. (PL) is a shrub of the Anacardaceae family whose fruits are used to produce an edible oil. This plant is one of the most common species in the Mediterranean basin and is highly adaptable to different abiotic stresses, particularly drought. PL fruits are also rich in health-promoting phytochemicals owing to their polyphenol and terpene abundance. There is evidence that controlled water deficit may increase the concentration of secondary metabolites, thereby potentially improving plant antioxidant defenses and fruit quality. The aim of the present study was to assess the changes in secondary metabolites of PL fruits in response to moderate water deficit applied in the last two ripening stages (December-January). Water deficit was applied to six five-year-old PL potted plants providing 70% of the fraction of transpirable soil water, while six well-watered plants were irrigated daily to pot capacity. Measurements of soil moisture, plant water relations, gas exchange, and chlorophyll fluorescence were performed to monitor the plant's physiological responses to stress. HPLC-DAD analyses of polyphenols and GC-MS analysis of terpenes were also conducted. The results show that the ripening of PL fruits was affected by the irrigation treatment, both for their polyphenolic and terpene content. In addition, water stress had a different effect on the phytochemical composition depending on the fruit ripening stage. These results are important for understanding the effects of water stress on PL physiological performances and how can be used to modulate PL fruit quality, especially regarding the production of antioxidant compounds beneficial for human health.

P10. Isolation and biological evaluation of *ent*-pimarane diterpenoids from *Lavandula pubescens* Decne

Pouramin S[1], Parisi V[1], Bader A[2], Rosa E[1], De Tommasi N[1]
[1]*Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II, Fisciano, SA, Italy.*
[2]*Department of Pharmacognosy, Umm Al-Qura University, 21955 Makkah, Saudi Arabia.*

The flora of Saudi Arabia is very rich due to its environmental diversity. Recently the flora of this region has attracted the attention of several groups of scientists to screen and detect the most promising plants as a source of anticancer agents[1,2,3]. Thus, the objective of our project is the screening of some Saudi Arabian medicinal plants for their cytotoxic effect and to isolate the active constituents to determine the probable mechanism of antiproliferative activity. Several plants that belonging to the Lamiaceae family collected in Saudi Arabia were screened on HeLA (human cervical carcinoma), JURKAT (human T-lymphocyte), and HaCaT (human epidermal keratinocyte) for this purpose. From the screening, the *L. pubescens* surface mixture extract exhibited and interesting antiproliferative activity towards HeLa (61±3.1 µg/ml) and Jurkat (73±2.5 µg/ml) cell lines, while it did not show cytotoxic activity on the nontumorigenic HaCaT cells.

Lavandula ssp are indigenous to the regions bordering the Mediterranean Sea, from southern Europe through north and east Africa, in the Middle East, and southwest Asia. Saudi Arabia is known as one of the main geographical areas of lavender species diversity and endemism and it has been suggested as a centre of origin for the genus[3], five naturally growing lavender species: *L. atriplicifolia* Benth*, L. citriodora* A. G. Mill*, L. coronopifolia* Poir., *L. dentata* L., *and L. pubescens* Decne are endemic in this country[4]. Uses of *L. pubescens*, as medicinal plants in Saudi Arabia have been reported particularly as antimicrobial, and decoction of leaf is given in headache and cold[4]. The extract of the surface mixture of *L. pubescens* (4.1 g) was obtained by dipping 200 g of fresh leaves into 2100 ml of dichloromethane for less than 30 sec and then the extract was dried under reduced pressure at 40 °C. The obtained extract was separated by Silica gel column chromatography and then it was further purified by semi preparative high-performance liquid chromatography. Structure elucidation of the isolated compounds was carried out by NMR and MS spectroscopy as well as by comparison the data with literature values. To date, nine *ent*-pimarane diterpenes, among them six new ones, were identified from surface extract. The compounds are substituted with hydroxy, epoxy, and carbonyl groups. Moreover, five flavonoids were also isolated from the plant. All isolates were assayed against HeLa Jurkat and HaCaT cell lines.



P11. Phytochemical characterization and biological activities of *Stenomesson miniatum* bulb extract, a medicinal plant of the Andes

Lianza M[1], Fimognari C[1], Nuzillard JM[2], Renault JH[2], Antognoni F[1], Bonvicini F[3], Poli F[4], Greco G[5], Turrini E[1]

[1]*Department for Life Quality Studies, University of Bologna, Corso D'Augusto 237, 47921 Rimini, Italy*
[2]*University of Reims Champagne-Ardenne, CNRS, ICMR UMR 7312, 51097 Reims, France*
[3]*Department of Pharmacy and Biotechnology, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy*
[4]*Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy*
[5]*Department of Chemistry, University of Bologna, Via Selmi 2, 40126 Bologna, Italy*

The fresh bulbs of *Stenomesson miniatum* (Herb.) Ravenna, a species belonging to Amaryllidaceae family, were traditionally employed by the Andean healers to treat some types of tumors and abscesses [1]. The aims of this study were to characterize the alkaloid-enriched extract from the bulbs of this plant with a poorly investigated phytochemical profile, and to provide support through chemical analysis to the therapeutic properties mentioned in the ethnobotanical studies. The topical use of the ointment prepared from the fresh bulbs suggested a possible cytotoxic action against skin cancer and antibacterial activity against bacteria triggering skin infections. Hence, the cytotoxic effect against A431 human epidermoid carcinoma cells was determined, and the antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were carried out. The alkaloid-enriched extract was obtained by acid-base extraction, and a centrifugal partition chromatography (CPC) in pH-zone refining mode was then carried out, allowing to obtain thirteen chemically simplified fractions, six of which consisting of almost pure alkaloids, and the remaining in mixtures of alkaloids. By means of ^{13}C NMR-based dereplication, eleven known Amaryllidaceae alkaloids were identified without resorting to compound purification. Another compound, never reported in literature, was found to be an extraction artefact. The bulb extract showed activity against A431 tumor cells with an IC_{50} of 3.3 µg/mL after 72h of treatment. The analysis of the fractions allowed the identification of pretazettine and haemathamine as the alkaloids mainly responsible of the cytotoxic effect. The extract and the fractions were also tested against an in vitro model of hematological tumor, *i.e.* Jurkat leukemia cells. The extract inhibited the Jurkat cells growth with an IC_{50} of 10.9 µg/mL after 72h of treatment. The latter cytotoxic effect was attributed, at least in part, to the presence of haemanthamine. Conversely, no antibacterial activity was recorded against the targeted bacterial strains for any of the investigated samples. However, Amaryllidaceae alkaloids are not the only active compounds produced by this plant, the antibacterial use can be due to polyphenols, several of which exhibit antibacterial activity [2]. In conclusion, results obtained demonstrate that *S miniatum* turned out to be a new source of Amaryllidaceae alkaloids endowed with cytotoxic activity.

P12. Phytochemical profile, cytotoxicity and redox state modulatory activities of olive leaf (*Olea europaea* L.) enriched extract in prostate cancer cell lines

Malfa GA[1,2], Luca T[3], Siracusa L[4], Di Giacomo C[1,2], Bianchi S[1,2], Sergi A[1], Castorina S[3], Acquaviva R[1,2]

[1]*Dept. Drug and Health Sciences, University of Catania, Viale A. Doria 6, 95125 Catania, Italy*

[2]*Research Center on Nutraceuticals and Health Products (CERNUT), University of Catania, Viale A. Doria 6, 95125 Catania, Italy*

[3]*Dept. Medical, Surgical Sciences and Advanced Technology, University of Catania, Via Santa Sofia 95123 Catania, Italy*

[4]*Inst. of Biomolecular Chemistry, Italian National Research Council ICB-CNR, 95126 Catania, Via Paolo Gaifami 18, 95126 Catania, Italy*

Olea europaea L., belonging to the Oleaceae family, is an evergreen slow-growing fruit tree well adapted to drought and poor soils and also resistant to salinity. This plant shows a life expectancy of over 1000 years, as attested by the different monumental trees across the Mediterranean area, including Italy, with ancient olive trees that are approximately several thousand years old. It is native to Asia Minor and Syria and cultivated in all Mediterranean climate areas of the world, representing today one of the most important crops in the Mediterranean basin.

Olea europaea L. derivatives have been widely used in traditional remedies in European and Mediterranean countries such as Greece, Spain, Italy, France, Turkey, Israel, Morocco, and Tunisia for the antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties (1). These effects are related to biologically active secondary metabolites such as phenolic acids, secoiridoids, flavonoids, and triterpenes which constitute the phythocomplex of olive leaves and fruits.

The Mediterranean diet, which is rich in olive products, is a testament to *Olea europaea* bioactive compounds as having positive effects on health and is strictly associated with reduced incidences of cancer and cardiovascular disease.

Experience from ethnomedicine, together with extensive laboratory findings on the anti-cancer effects of a variety of bioactive food components, have indicated that flavonoids and triterpenic compounds could play an important role in the prevention and treatment of cancer (2,3). Secoiridoids and triterpenes possesses antiangiogenic action and are able to inhibit tumor cell growth and invasiveness.

In view of these considerations, we analyzed the phytochemical profile, the antioxidant activity, and the cytotoxic effect of an aqueous enriched extract obtained from olive leaves, collected in the flowering time in Syracuse (Sicily, Italy), on two different prostate cancer cell lines LNCaP and PC3, and in normal fibroblasts HFF-1. Prostate cancer is the sixth most common cancer worldwide, the third most common cancer in men, and the second leading cause of cancer-related deaths in men in the West.

The quantitative and qualitative analysis confirmed the high content of phenolic compounds in *O. europaea* enriched extract (HPLC-DAD analysis, Folin-Ciocalteu and aluminium chloride method) and the related antioxidant activity (DPPH test, ROS and RSH quantification), but also highlighted a significant cytotoxic effect, confirmed by MTT test, on two prostate cancer cell lines. Remarkably, the extract resulted non-toxic for HFF-1 at the concentrations and exposure times tested, so showing a good selectivity towards tumor cells. The underlying

cytotoxicicity was associated with evident morphological changes and further investigated by LDH release assay, which allowed to establish necrosis as the main cell death mechanism. Our study requires further investigation but can be useful for the research community in developing new therapeutic preventive strategies and novel drug targets for the treatment of cancer, which may be of interest to health specialists and affect public health.

P13. Cytoprotective properties of the ethyl acetate extracts from *Anacyclus maroccanus* Ball. e *Anacyclus radiatus* Loisel aerial parts

Percaccio E[1], Sissi S[2], Vitalone A[1], Mazzanti G[1], Markouk M[2], Di Giacomo S[1,3], Di Sotto A[1]

[1]*Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy.*

[2]*Laboratory of Agri-Food, Biotechnology and Valorization of Plant Resources, Phytochemistry and Pharmacology of Medicinal Plants Unit, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco.*

[3]*Department of Food Safety, Nutrition and Veterinary Public Health, National Institute of Health, Rome, Italy*

Anacyclus species (Fam. Asteraceae) are endemic plants from Morocco greatly exploited by the local population for primary health care, as inexpensive and available sources of drugs. They are traditionally used by Moroccan people to treat several ailments, among which digestive disorders, likely due to their antioxidant, anti-inflammatory, analgesic, and antimicrobial properties [1]. In our previous study we highlighted promising antioxidant, hypoglycemic, and antiglycative properties of the ethyl acetate extracts from the aerial parts of two *Anacyclus* ecotypes, namely *A. maroccanus* Ball. and *A. radiatus* Loisel, likely associated to their polyphenolic composition [1]. In line with this evidence, present study was aimed at evaluating the cytoprotective properties of the ethyl acetate extracts from *A. maroccanus* and *A. radiatus* towards the oxidative damage induced by the pro-oxidant agent tert-butyl hydroperoxide (tBOOH, 500 µM) and hyperglycemia (6 mg/ml glucose) in human cell lines [2]. To this aim, pancreatic adenocarcinoma (Bx-PC3) and cholangiocarcinoma (Mz-ChA-1) cells, and noncancerous intrahepatic cholangiocytes (H69) were exploited. The cells were exposed to the treatments for 24 h under both pre-treatment and co-treatment protocols, then the the cytotoxicity was evaluated by the MTT assay and the intracellular ROS levels by the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) test, according to Di Giacomo et al. [3]. Preliminary cytotoxicity assays highlighted the extracts were nontoxic up to 100 µg/ml in all the tested cells. Under our experimental conditions, tBOOH induced about a 50-60% reduction of cell viability in Mz-ChA-1, Bx-PC3, and H69 cells. As expected, the extracts of both ecotypes were able to counteract the tBOOH cytotoxicity up to 70-80% in Mz-ChA-1. The extracts also decreased the intracellular ROS levels, almost doubled by tBOOH, with a greater efficacy of *A. radiatus* in Bx-PC3 with respect to *A. maroccanus* (60% vs 40% increase of cell viability respectively); both samples reduced the ROS levels by about 52% and 65%, respectively. At last, *A. maroccanus* completely restored cell viability and lowered the ROS basal levels, despite a weak effect of *A. radiatus* in H69 cells. Regarding the hyperglycemia model, glucose (6 mg/ml) induced a 45% and 60% lowering of cell viability in Bx-PC3 and H69 cells. The *A. maroccanus* and *A. radiatus* extracts induced only a slight cytoprotection, in terms of cell viability and ROS levels in H69 cells. By contrast, they showed protective properties against hyperglycemia damage in H69 cells, by inducing a 24% increase of cell viability and a 32% lowering ROS. Altogether, present results highlight a possible interest in these species as natural sources of bioactive compounds and/or phytocomplexes and suggest further studies in order to characterize their possible application for nutraceutical purposes, with possible benefits for Moroccan economy.

P14. Use of phytotherapics and nutraceuticals in colorectal cancer patients undergoing chemotherapy.

Di Grazia D[1], Giacchi D[2], Gaspari M[1], Allegra S[1], Armando T[2], Storto S[2], Chiara F[1], De Francia S[1], Mussa MV[2]

[1]Department of Clinical and Biological Sciences, University of Turin, Italy

[2]Department of Public Health, University of Turin, Turin, Italy

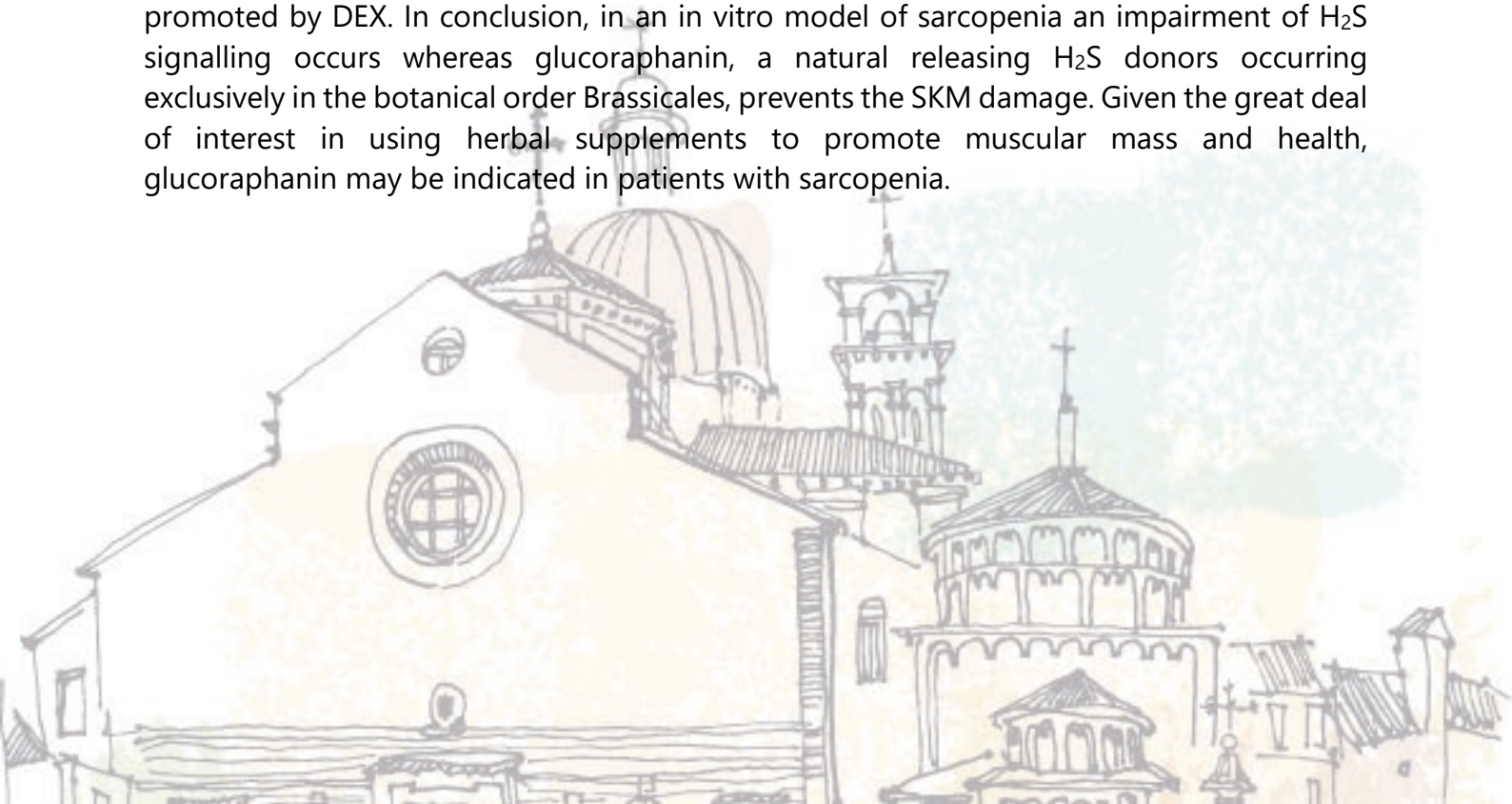
Phytotherapy is increasingly used to treat different diseases, including colorectal cancer.
Colorectal cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related death in the world. Patients with colorectal cancer use phytotherapeutic substances to reduce the side effects of oral chemotherapy, but in some cases drug-drug and/or drug-food interactions occur. For this reason, the aim of the study was to describe phytotherapeutic substances and food that colorectal cancer patients use most commonly, and to determine whether their use can increase side effects.
A prospective observational descriptive study has been conducted between February and August 2020. Data were collected through the administration of a questionnaire when the patients were admitted at the ''Sub-Alpine Oncology and Hematology Center'' (C.O.E.S.) in the Molinette University Hospital of the City of Health and Science of Turin. Data were collected from 29 patients (16 men and 13 women) taking oral chemotherapy, for a total of 54 accesses (27 entrances for men and 27 entrances for women).
In this study it was recorded that 76% of patients use phytotherapeutic substances. In the results was emerged that the most frequently recorded side effects have been headache and alopecia in 98%, vomiting in 96%, nausea in 91%, skin toxicity in 89% and epigastric pain in 85% of patients.
Multiple comparisons were made to identify any differences related to sex, side effects, phytotherapeutic substances and substances taken. Considering the variable sex and side effects, it was found a statistically significant presence of epigastric pain: 29.8% of men and none among women patients. Subsequently, comparisons were analysed between the various independent variables of phytotherapeutic substances and side effects. Green tea was related with nausea ($p<0,001$), epigastric pain ($p<0,001$) and in some cases may cause hepatotoxicity. Evaluating turmeric use, it has been seen that a wide use was related to hepatotoxicity, an increase of circulating chemotherapy drug, nausea ($p<0,001$) and vomiting ($p<0,001$). Chamomile was associated with nausea ($p=0,001$), skin toxicity ($p<0,01$) and alopecia ($p<0,001$) and, eventually, aloe use has been related to mucositis ($p<0,01$). Considering food, fennel and grapefruit taking were related with headache ($p<0,001$) and skin toxicity ($p<0,01$).
Products with a phytotherapeutic basis are consumed on the advice of acquaintances or doctors, and, for this reason, it is extremely important to deepen the studies, to ensure the best possible patient care. This study seems to suggest that the simultaneous intake of phytotherapy and oral chemotherapy may increase the toxicity of the therapy drug.
Research is needed to expand the knowledge of the interactions between phytotherapics, foods and drugs used for cancer patients.

P15. The beneficial effect of glucoraphanin in an *in vitro* model of sarcopenia

Esposito E[1], Mitidieri E[1], Micheli L[2], Ciampi C[2], Cirino G[1], Ghelardini C[2], Di Cesare Mannelli L[2], Sorrentino R[3], d'Emmanuele di Villa Bianca R[1]

[1]Department of Pharmacy, School of Medicine and Surgery, University of Naples, Federico II, Naples, Italy
[2]NEUROFARBA Department, University of Florence, Florence, Italy
[3]Department of Molecular Medicine and Medical Biotechnology, School of Medicine and Surgery, University of Naples, Federico II, Naples, Italy

Glucoraphanin is one of the prominent glucosinolates in cruciferous vegetables, largely indicated for its health benefits [1]. It is a sulfur-containing compound with the peculiarity to be a slow-release hydrogen sulfide (H_2S) donor [2]. H_2S is an endogenous gaseous mediator synthesized from L-cysteine mainly by the action of cystathionine-γ-lyase (CSE) and cystathionine-ß-synthase (CBS). It is increasingly recognized as an important signalling molecule found in several districts and tissues, including the skeletal muscle (SKM) [3]. Sarcopenia is a muscle-wasting syndrome characterized by loss of SKM mass and function associated with an increased likelihood of adverse outcomes including falls, fractures, physical disability, and mortality [4]. To date, it remains an under-recognized syndrome and therefore poorly managed in routine clinical practice. Here, in an in vitro model of sarcopenia based on damage induced by dexamethasone (DEX) in C2C12-derived myotubes, we evaluated the involvement of H_2S signaling and the protective potential of endogenous and exogenous sources of H_2S, i.e., glucoraphanin, L-cysteine (endogenous source of H_2S) and 3-mercaptopyruvate (endogenous and exogenous source of H_2S). C2C12-derived myotubes were treated with DEX (1µM, 48h) to induce muscle atrophy [5]. DEX-induced sarcopenia caused a reduction in H_2S signaling in terms of both the expression of CBS and CSE and the production of H_2S. DEX induced the apoptotic processes promoting caspase-3 activity that was prevented by glucoraphanin and mercaptopyruvate but not by L-cysteine treatment. In parallel, all compounds tested reduced O_2^- levels and protein carbonylation and in turn, the oxidative unbalance evoked by DEX. Glucoraphanin, L-cysteine and 3-mercaptopyruvate compounds were also able to prevent the morphological and morphometrical alterations promoted by DEX. In conclusion, in an in vitro model of sarcopenia an impairment of H_2S signalling occurs whereas glucoraphanin, a natural releasing H_2S donors occurring exclusively in the botanical order Brassicales, prevents the SKM damage. Given the great deal of interest in using herbal supplements to promote muscular mass and health, glucoraphanin may be indicated in patients with sarcopenia.



P16. Phytotherapy use to treat chemotherapy-related side effects in lung cancer patients

Gaspari M[1], Bettonte G[2], Di Grazia D[1], Allegra S[1], Armando T[2], Storto S[2], Chiara F[1], De Francia S[1], Mussa MV[2]

[1]Department of Clinical and Biological Sciences, University of Turin, Italy

[2]Department of Public Health, University of Turin, Turin, Italy

Phytotherapy has been used since ancient times for the treatment of many diseases, one of the fields of greatest use is oncology. In fact, patients use herbal medicines to counteract the side effects of chemotherapy; however, these remedies, although natural, can lead to interactions if taken simultaneously with other medicines. Lung cancer is the second type of tumour with higher incidence and the most common cause of cancer death globally. The aim of this work was to investigate the use of phytotherapeutic substances and foods in a group of patients with lung cancer undergoing oral chemotherapy, and to identify the most frequent adverse effects. Eventually, all the collected data were evaluated considering males and females patients in two different subgroups.

A prospective observational descriptive study has been conducted between January and August 2020. Data were collected through the administration of a questionnaire when the patients were admitted at the ''Sub-Alpine Oncology and Hematology Center'' (C.O.E.S.) in the Molinette University Hospital of the City of Health and Science of Turin. Data were collected from 35 patients (15 women and 20 men) for a total of 66 accesses (36 women and 30 men).

We showed that 69% of people who entered in the facility have assumed at least one phytotherapeutic product. The adverse effects most frequently experienced were asthenia (64%), constipation (32%) and skin toxicity (27%). Some statistically significant comparisons have been observed between the variable "sex" and the variable "side effects" with regard to the presence of diarrhea ($p<0,01$), headache ($p<0,05$) and epigastric pain ($p<0,05$). Other statistically significant comparisons resulted between the variable "phytotherapics" and the variable "side effects". Chamomile was related with mucositis ($p<0,05$) and alopecia ($p<0,05$). Green tea was associated with higher levels of alopecia ($p<0,01$). The last statistically significant comparison was between the variable "foods" and the variable "side effects". The fennel was one of the most assumed food (53% of accesses in the facility) and was correlated with dysgeusia ($p<0,05$). Cabbage use was related to dysgeusia ($p<0,01$), epigastric pain ($p<0,05$) and alopecia ($p<0,01$). Milk was related with dysgeusia ($p<0,01$). Almost all the statistically significant results obtained from our work showed higher toxicity given by chemotherapy when patients assumed phytotherapics and foods. Ginger ($p<0,05$) and garlic ($p=0,001$) were the only phytotherapeutic substances that were correlated with lower levels of asthenia.

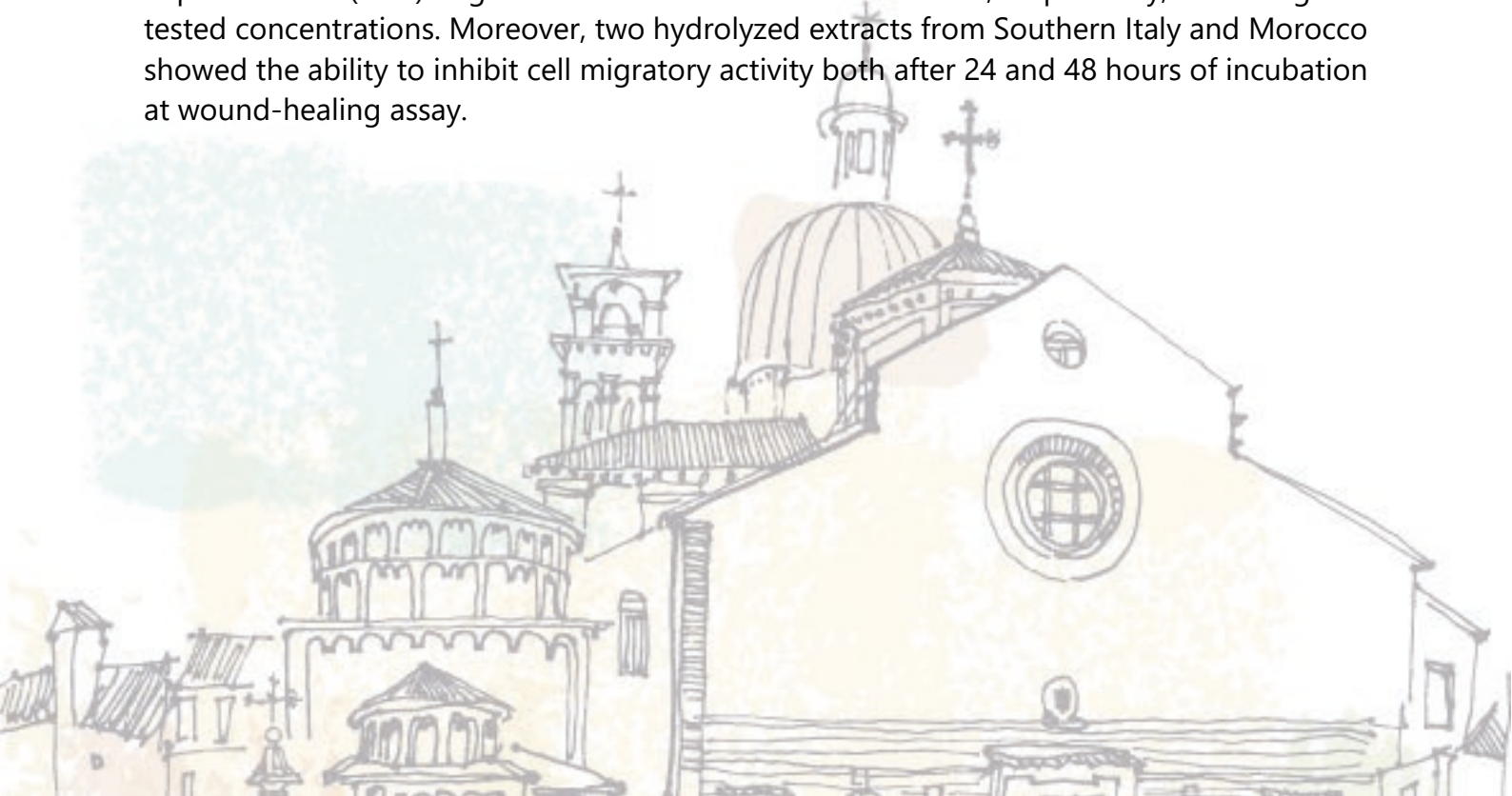
For some phytotherapics there is evidence that they are effective in the field of oncology. However, this study seems to suggest that their use concomitant with oral chemotherapy may increase the toxicity of chemotherapeutic drugs. It is important that the nurse in collaboration with the doctor carefully evaluate the products that are taken by patients at home, not only focusing on drugs, but also going to investigate the intake of herbal products and foods. To date there are few studies that have certified a clinical significance of phytotherapeutic substances in oncology. Further research is needed to evaluate the

interactions between phytotherapeutic products, foods and the drugs used for the treatment of cancer patients.

P17. Anti-proliferative and anti-migratory potential of *Glycyrrhiza glabra* L. extracts on breast and gynecological cancer cell lines

Perri MR, Marrelli M, Zicarelli L, Conforti F, Statti G
Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036, Rende, CS, Italy

Cancer is one of the leading causes of death worldwide and the last World Health Organization (WHO) projection (2016-2060) shows how cancer related deaths could exceed those from ischemic heart disease in the next years (1). Breast cancer represents the second leading cause of cancer death among women worldwide, even if, due to screening programmes and advances in medicines, the mortality rate is declining (2). Another cluster of diseases accounting for 15-20% of all cancer diagnosed in women is represented by gynecological malignancies, among which uterine, ovarian and cervical cancers are the most widespread forms (3). In the recent years, an even increasing attention to products derived from the vegetable kingdom, as a source of bioactive compounds able to exert a wide range of biological activities, is gaining much attention. *Glycyrrhiza glabra* L., commonly known as liquorice, is a species belonging to Fabaceae family, largely diffused and cultivated all over the world. It was traditionally used as adjuvant in gastric and pulmonary disorders, as well as, according to Chinese Traditional Medicine, for the treatment of tuberculosis and peptic ulcer. Currently, it is industrially used in confectionery, liqueurs and soft drinks (4).
Here, different *Glycyrrhiza glabra* raw and hydrolyzed extracts belonging to different geographical areas (Southern Italy, Morocco and Hungary) were investigated for their anti-proliferative and anti-migratory potential on different breast and gynecological cancer cell lines. Among breast cancer cell lines (MCF-7 and MDA-MB-231), only the *Glycyrrhiza glabra* hydrolyzed extracts showed a mild anti-proliferative activity on MCF-7 cells at the highest tested concentration (60 µg/mL), while no relevant activity was assessed for MDA-MB-231 cell line. As regards the anti-proliferative activity on different gynecological cancer cell lines (SiHa, HeLa, C33A and A2780 cells), the best results were assessed for *Glycyrrhiza glabra* hydrolyzed extracts on both C33A and A2780 cell lines, a cervical cancer Human Papillomavirus (HPV) negative and an ovarian cancer cell lines, respectively, at the highest tested concentrations. Moreover, two hydrolyzed extracts from Southern Italy and Morocco showed the ability to inhibit cell migratory activity both after 24 and 48 hours of incubation at wound-healing assay.



P18. Pigmented potatoes and their preventive effect against LPS-induced inflammation in THP-1 macrophages

Toccaceli M[1], Marinelli A[1], Bassolino L[2], Pacifico D[2], Parisi B[2], Nicoletti F[2], Lo Scalzo R[3], Mandolino G[2], Petroni K[1]

[1]Dipartimento di Bioscienze, Università degli Studi di Milano, Milano

[2]CREA – Centro di Ricerca per la Cerealicoltura e Colture Industriali, Bologna

[3]CREA – Centro di Ricerca per l'Ingegneria e le Trasformazioni Agroalimentari, Milano

Pigmented potatoes represent a source of several phytonutrients known for their health-promoting potential. While the most common tubers are rich in phenolic acids like chlorogenic acid (CGA) which confers the typical white/yellow color, the pigmented varieties originate from the accumulation of pigments like carotenoids and/or anthocyanins in the flesh and/or in the skin conferring different shades of orange, red or purple. As naturally-occurring antioxidant and antinflammatory compounds, these bioactives could serve as a strategy to counteract chronic inflammation. This prolonged inflammatory condition represents a risk factor of various noncommunicable diseases among the leading causes of death, including cancer, obesity, cardiovascular and neurodegenerative disorders. Considering that carotenoid consumption inversely correlates with the incidence of many chronic diseases such as obesity, diabetes and cancer [1], whereas anthocyanins were found to counteract many inflammation-related diseases including neuroinflammation, cardiovascular disorders and obesity [2, 3], we aimed to study the potential antinflammatory effect of three potato varieties differently enriched in these phytonutrients. We compared the effect of extracts derived from CGA-rich, carotenoid-rich and anthocyanin-rich upland potato varieties named Kennebec (yellow skin and white flesh), Desirée (red skin and yellow flesh) and Bleuet (purple skin and flesh) respectively.

After the characterization of the phytonutrients composition of tubers extracts by HPLC-DAD and spectrophotometric analysis, their antinflammatory potential has been tested on THP-1 derived macrophages insulted with LPS. Human THP-1 monocytes were first differentiated in macrophages via PMA-treatment, pre-treated with extracts and then co-treated with extracts and LPS. The dose-dependent effects on gene expression and/or protein levels of pro-inflammatory mediators were evaluated.

Our results showed that at higher doses all the three extracts exerted a nearly comparable antinflammatory activity *in vitro*, whereas, when provided at lower concentrations close to those detected in human plasma after potato consumption, only Desirée- and Bleuet-derived extracts were able to counteract LPS-induced inflammation. Thus, these upland potato varieties may represent an economical and resilient source of bioactive compounds able to prevent chronic inflammation.

P19. *In vitro* evaluation of the neuroprotective effect of *Achillea millefolium* L. flowering part extract

Freschi M, Barbalace MC, Angeloni C, Malaguti M, Hrelia S
[1]*Department for Life Quality Studies, Alma Mater Studiorum University of Bologna, Bologna, Italy*

Neurodegenerative diseases are a chronic phenomenon characterized by an alteration of the cell function and structure in the central nervous system (CNS), that lead to an increased neuronal death. Neurodegenerative diseases, including Alzheimer's disease, are multifactorial disorders that exhibit neuroinflammation and oxidative stress as major causative factors. In particular, neuroinflammation promotes the development and the progression of neurodegeneration through the activation of microglial cells in the brain. During neuroinflammatory processes, activated microglia release proinflammatory mediators that contribute to the progression of the neuronal damage. Nowadays, although there are different therapeutic strategies clinically used to counteract neurodegeneration, all of them are unable to stop the progression of these pathological conditions and, moreover, cause significant side effects in the patients. Identifying natural molecules that can counteract microglia activation through the reduction of the release of pro-inflammatory mediators could be a promising strategy for the prevention and possible treatment of these disorders.

Therefore, increasing interest is being focused on the use of herbal preparations for the treatment and the prevention of these disorders. The Asteraceae family, including Achillea millefolium L, has historically been associated with extensive use in medical fields for the treatment of numerous disorders and diseases and for its anti-inflammatory action. In this study, the potential anti-inflammatory effect of the extract of the flowering part of Achillea millefolium L. was evaluated in an *in vitro* model of neuroinflammation, the BV-2 microglial cell line activated with lipopolysaccharide (LPS). The extract was characterized by HPLC/DAD and was particularly rich in bioactive compounds among which the most abundant were caffeic acid, rutin, and rosmarinic acid. Cell viability and intracellular levels of reactive oxygen species (ROS) were determined by MTT spectrophotometric assay and fluorescent 2',7'-dichlorodihydrofluorescein diacetate (H2DCF-DA) assay respectively. The fuorimetric assay with fluorescent monochlorobimane (MCB) probe was used to assess the intracellular levels of reduced glutathione (GSH). The levels of the proinflammatory cytokines and enzymes, NO-induced synthase (iNOS), interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), were measured through retrotranscription and amplification by RT-PCR. The results showed that *Achillea millefolium L*. extract causes a significant decrease in intracellular ROS levels compared with LPS-treated cells and an increase in intracellular GSH levels compared with controls, with no reduction in cell viability. In addition, the extract is able to modulate the expression of inflammatory mediators at the gene level through significantly reducing the expression of all genes tested (iNOS, IL-1β, TNF-α).

Considering the data obtained in this study, it is possible to conclude that the extract tested shows anti-inflammatory effects. Thus, extracts of Achillea millefolium L. could be considered as promising sources of nutraceutical compounds. Its use could lead, in the future, to the development of new formulations that are useful in counteracting pro-inflammatory processes by helping to slow down the progression of neurodegenerative diseases.

P20. *In vitro* protective effects of a standardized extract of *Opuntia ficus indica* L. and *Olea europaea* L. against indomethacin-induced intestinal epithelial cells injury

Salamone FL[1], Muscarà C[1], Molonia MS[1,2], Saija A[1], Cimino F[1], Speciale A[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy

[2]Foundation Antonio Imbesi, Messina, Italy.

The pathogenesis of enteropathy induced by non-steroidal anti-inflammatory drugs (NSAIDs) is still unclear, and there are no established treatments. NSAIDs can cause increased intestinal permeability and the reduction of intestinal mucus granting luminal substances, such as bile acid and intestinal bacteria, to access to the mucosa. These in turn lead to inflammation through neutrophils infiltration, upregulation of proinflammatory mediators, and the production of reactive oxygen species. Strategies of therapy are often represented by physical protection of mucosa with mucoadhesive materials and by treatment of correlated inflammatory and cytotoxic events. Nowadays, research is focused on natural substances as protective agents for treatment and prevention of NSAID-induced small intestinal damage. In this study, we used a model of intestinal epithelial cells injury induced by NSAID indomethacin, to investigate the *in vitro* protective effects of a standardized extract (OFI+OE) containing polysaccharides obtained from cladodes of *Opuntia ficus indica* L. with mucoadhesive properties, and polyphenols from leaves of *Olea europaea* L. known for multiple biological activities.

Caco-2 human intestinal epithelial cells were cultured for 18 days post confluence to obtain fully differentiated cells. Then, cells were pre-treated with OFI+OE (200 and 400 µg/mL) for 24 hours and subsequently exposed to indomethacin 1 mM for 24 hours to simulate *in vitro* pro-inflammatory and cytotoxic events correlated to mucosal diseases. Monolayer integrity and function were evaluated by analysis of fluorescein permeability. Western blot analysis and qPCR techniques were used to determinate proteins and genes expression involved in oxidative stress, in inflammatory NF-κB pathway and in apoptotic process. In addition, reactive oxygen species (ROS) and Total Antioxidant Activity (TAA) levels evaluation allowed to clarify OFI+OE protective effects on redox imbalance involved in mucosal diseases.

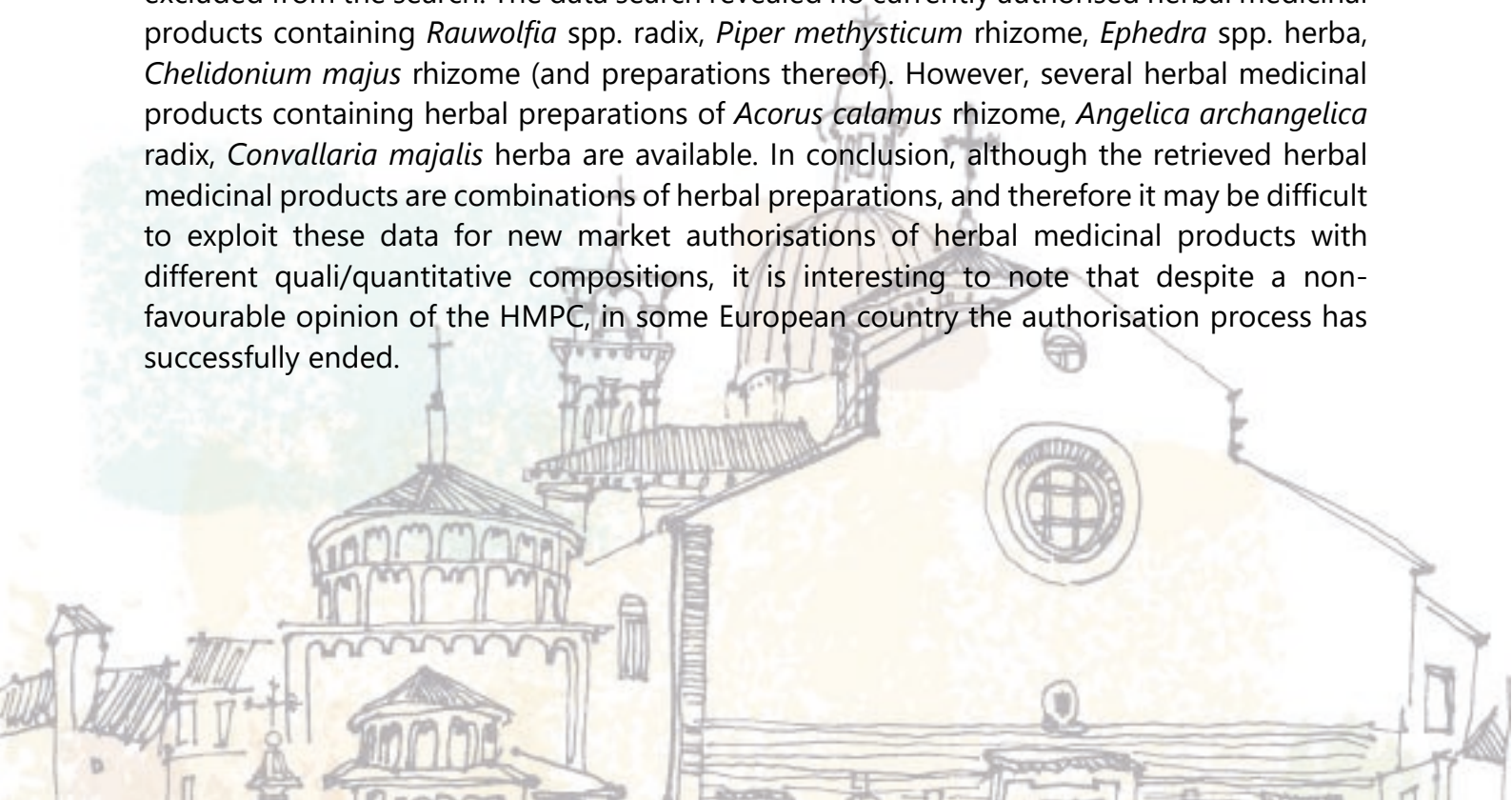
Pre-treatment with OFI+OE was able to prevent intestinal epithelial barrier damage induced by indomethacin, as demonstrated by the decrease in fluorescein permeability. Furthermore, OFI+OE enhanced the redox status, reducing ROS intracellular production and increasing TAA values, and inhibited NF-κB pathway and pro-inflammatory cascade. High ROS levels, stimulated by indomethacin, were able to induce endoplasmic reticulum (ER) stress and apoptotic cell death, but OFI+OE prevented indomethacin-induced apoptosis as demonstrated by its effects on Bcl-2/Bax ratio and Caspase-3 protein levels.

Finally, herein we demonstrate the protective effects of *Opuntia ficus indica* L. and *Olea europaea* L. combined extract against indomethacin-induced intestinal epithelial cells injury, via modulation of oxidative, inflammatory, and apoptotic signaling pathways. In conclusion, this study provides a basis to the possibility of using this natural combined extract as a strategy for treatment and prevention of gastrointestinal mucosal damage.

P21. Focus on some herbal substances associated with safety concerns

Carlucci V, Rodda M, Brunasso Cattarello L, Gillio Tos E, Baldone G, Conto A
Pharma Business Unit, ChemSafe Srl, Colleretto Giacosa (TO), Italy

The requirements and procedures for marketing authorization/registration of a medicinal product for human use in the European Community primarily laid down in Directive 2001/83/EC. Traditional use registration is a simplified registration procedure allows the registration of traditional herbal medicinal products without requiring tests and trials on safety and efficacy. To support European Union (EU) Member States, the Committee on Herbal Medicinal Products (HMPC) focuses on two main tasks: establishing EU monographs; drafting an EU list of herbal substances, preparations and combinations thereof.
In September 2015, the HMPC published the *Public statement on prioritisation for assessment of herbal substances associated with safety concerns* in which decided that priority should not be given to assessment of some herbal substances (i.e., *Acorus calamus*, rhizome; *Angelica archangelica*, radix; *Chelidonium majus*, rhizome; *Convallaria majalis*, herba; *Ephedra* spp., herba; *Piper methysticum*, rhizome; *Rauwolfia* spp., radix) because it is likely that an EU herbal monograph would not be established due to the probability of an unfavourable benefit-risk assessment. The HMPC pointed out that it is possible that these herbal substances and preparations thereof can be the active substance(s) in registered or authorised medicinal products, provided that an adequate dossier meeting all the requirements and providing sufficient information to result in a positive benefit-risk assessment is presented by the applicant.
Registered or authorised medicinal products containing, as active substance(s), the listed herbal substances associated with safety concerns may be used to support new marketing authorizations. Hence, a data search was carried out in March 2023 on twenty-six European national medicine registers aimed at investigating whether one or more of the previously listed herbal substances and preparations thereof are the active substance(s) in currently registered/authorized medicinal products. Homeopathic or anthroposophic specialties were excluded from the search. The data search revealed no currently authorised herbal medicinal products containing *Rauwolfia* spp. radix, *Piper methysticum* rhizome, *Ephedra* spp. herba, *Chelidonium majus* rhizome (and preparations thereof). However, several herbal medicinal products containing herbal preparations of *Acorus calamus* rhizome, *Angelica archangelica* radix, *Convallaria majalis* herba are available. In conclusion, although the retrieved herbal medicinal products are combinations of herbal preparations, and therefore it may be difficult to exploit these data for new market authorisations of herbal medicinal products with different quali/quantitative compositions, it is interesting to note that despite a non-favourable opinion of the HMPC, in some European country the authorisation process has successfully ended.



P22. Fatty Acid composition, Antioxidant, and *in vitro* Anti-inflammatory activity of five cold-pressed *Prunus* seed oils, and their Anti-biofilm effect against pathogenic bacteria

Amato G[2], Fratianni F[1], d'Acierno A[1], Ombra MN[1], De Feo V[2], Ayala-Zavala J[3], Coppola R[4], Nazzaro F[1]

[1]*Institute of Food Science, National Research Council of Italy (CNR), Avellino, Italy*
[2]*Department of Pharmacy, University of Salerno, Fisciano, Italy*
[3]*Centro de Investigacion en Alimentacion y Desarrollo, Hermosillo, Mexico*
[4]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*

The genus *Prunus* originating from Asia belongs to the Rosaceae family, Amygdaloideae subfamily. Several *Prunus* species are highly appreciated by consumers; they are studied for their nutritional and bioactive properties positive to human health. Such peculiarities are linked to the prevention of different diseases and disorders, including age-related declines We focused our attention on the cold-pressed seeds oils of some species of *Prunus*: apricot (*P. armeniaca* L.), peach (*P. persica L.) Batsch,* cherry (*P. avium* L.), plum (*P. domestica* L.), and black cherry (*P. cerasus* L.), evaluating the fatty acid (FA) composition, the antioxidant and the *in vitro* anti-inflammatory properties, as well as the biofilm-inhibitory activity on some pathogenic bacterial strains. GC-MS was performed for the analysis of FAs. The most abundant FAs were oleic and linoleic acids; other FAs identified were linolenic, palmitic, palmitoleic and stearic acids. The antioxidant property was carried using different tests: the DPPH, the FRAP, and the ABTS • + assays. The denaturation assay performed on bovine serum albumin (BSA) was used to evaluate the *in vitro* anti-inflammatory activity. Black cherry seed oil exhibited the best antioxidant activity (IC_{50}: 7.28 µg/mL) compared to the cherry (7.68 µg/mL), peach (9.04 µg/mL), plum (9.49 µg/mL) and apricot (10.01 µg/mL) The oils showed low IC_{50} values for the *in vitro* anti-inflammatory activity, ranging from 3.29 µg (apricot) to 4.34 µg (cherry). Linoleic and stearic acids impacted the antioxidant activity of the oils, while oleic, linolenic and palmitoleic acids showed beneficial effects as anti-inflammatory agents, as shown by the correlation data. The anti-biofilm activity was evaluated using five pathogenic strains: *Acinetobacter baumannii, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus*, through the crystal violet test, while the MTT test was used to evaluate the inhibition of mature biofilm after 24 h and the metabolic activity of the bacterial cells. The oils were able to inhibit both the pathogen biofilm formation (up to 71.40% inhibition) and to act against the mature biofilm and their metabolism, with different potency depending on the strain, with values up to 61.54%. The oils analyzed could provide interesting insights and potential applicability, opening new opportunities for their use. In fact, they represent a valuable source of some healthy FAs and they showed potential antioxidant and anti-inflammatory *in vitro* activity. The inhibitory action exerted by these oils both against the formation of the biofilm and against the mature biofilm, opens up new application perspectives in the health and food fields: these oils could represent potential natural agents to counteract the onset of infections caused by pathogens difficult to eradicate.

P23. Pomegranate by-products valorization: cell-based antioxidant activity of the hydroalcoholic leaf extract

Marengo A[1], Sanna C[2], Foglietta F[1], Serpe L[1], Rubiolo P[1]

[1]Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Pietro Giuria 9, 10125 Torino, Italia

[2]Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari,
Via S. Ignazio da Laconi 13, 09123 Cagliari, Italia

Punica granatum L. (Lythraceae family), commonly known as pomegranate, is a very popular plant native to Central Asia, but thanks to its adaptability it has now spread to different parts of the world. Besides the food consumption of the edible fruit, various parts of the pomegranate have been used since ancient times for their healthy properties. Indeed, non-edible parts such as peels, seeds, bark and leaves are a source of a plethora of specialized metabolites (tannins, alkaloids, flavonoids, terpenes) of interest to the pharmaceutical and cosmetic fields[1]. This work focuses on the valorization of leaf extract of pomegranate originated from Sardinia. Pomegranate leaves are usually considered as a by-product of the pomegranate cultivation. However, they are rich in active compounds and have therefore traditionally been used to treat various ailments, such as sore throat, fever, and urinary tract infections. There are already data in the literature confirming the beneficial properties of extracts derived from pomegranate leaves and exploring new potential health benefits for pharmaceutical and cosmetic applications[2,3,4]. This work aims to investigate the antioxidant potential of the ethanolic extract of pomegranate leaves on human umbilical endothelial (HUVEC) and human dermal fibroblast (HDF) cell lines, focusing on some pure compounds characteristic of the total extract (TE). In particular, the antioxidant activity was evaluated as a primary antioxidant by simultaneous incubation of the cells for 5 hours with the TE/compound and the prooxidant stimulus (H_2O_2, 500 µM) or as a secondary antioxidant by a preincubation of 24 hours with the TE/compound followed by incubation with H_2O_2 for 5 hours. The data obtained from the cell viability analysis were also confirmed by the analysis of reactive oxygen species production (ROS). Interesting results were obtained for TE on HUVEC cells, especially as a secondary antioxidant. In contrast, a slight activity was observed on HDF cells. The chemical composition of the extract, analyzed by high-performance liquid chromatography coupled to a photodiode array and tandem mass spectrometry (HPLC-PDA-MS/MS), revealed the presence of polyphenols (luteolin, apigenin, and ellagic acid derivatives), with ellagic acid (EA) and luteolin 4'-*O*-glucoside (LG) selected as the main analytes for further analysis. Briefly, EA showed a significant activity as a primary and secondary antioxidant on HUVEC cells, whereas only a statistically significant activity as a primary antioxidant was observed on HDF cells. Since LG showed a toxicity towards the tested cells, further experiments were performed with its aglycone, luteolin (LU). LU was able to demonstrate its role as a good primary antioxidant only in HDF cells.

Although further studies are needed, these preliminary cell-based results confirm the potential use of pomegranate leaf extracts and some of their constituents as food supplements and cosmetic preparations with antioxidant effects. This work also highlights the differential cellular response to different antioxidant stimuli extracted from *P. granatum* leaves.

P24. Study of an herbal preparation composed by a combination of *Ruscus aculeatus* l. and *Vitis vinifera* L. extracts, magnolol and diosmetin to address chronic venous diseases through an anti-inflammatory effect and AP-1 modulation

Nocera R, Eletto D, Santoro V, Parisi V, Bellone ML, Izzo M, Tosco A, Dal Piaz F, Donadio G, De Tommasi N

[1]*Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy*

[2]*MathTechMed - Research Center Mathematics for Technology, Medicine and Biosciences University of Ferrara, 44121 Ferrara, Italy*

[3]*Department of Medicine, Surgery and Dentistry, University of Salerno, 84081 Baronissi, Italy*

Chronic venous disease (CVD) is an often-underestimated inflammatory pathological condition that can have a serious impact on quality of life. Previous studies have shown that the common inflammatory transcription factor AP-1[1] and Nf-kB play key roles in the initiation and progression of this vascular dysfunction. Functionally, AP-1 regulates the expression of many genes involved in pathological and physiological processes; one of the most relevant in the inflammatory process is that encoding for MCP-1[2], whose expression is strongly enhanced by the activation of AP-1. Given the ability of some plant metabolites to affect several inflammatory pathways, also inhibiting AP-1, the aim of this research was to set up an herbal preparation composed of a mixture of magnolol, diosmetin, *R. aculeatus* L. (Asparagaceae) root and *V. vinifera* L. (Vitaceae) seeds extracts, and to evaluate its efficacy in reducing CVD-related inflammation. Preliminarily, the composition of *R. aculeatus* L. (Asparagaceae) root and *V. vinifera* L. (Vitaceae) seeds extracts was investigated by HR-ESI-MS analysis and a multiple-reaction monitoring (MRM) method was used to quantify ruscogenin and procyanidin B1. Moreover, the cytotoxicity of magnolol, diosmetin, *V. vinifera* and *R. aculeatus* extracts was evaluated on human endothelial cells (HUVECs) using MTT assay. Based on the results obtained in these experiments, two herbal preparations were established, differing in the relative amount of the four components: DMRV-1 (diosmetin 6% (w/w), magnolol 10% (w/w), *V. vinifera* seeds extract 48% (w/w), and *R. aculeatus* extract 36% (w/w), and DMRV-2 (diosmetin 11% (w/w), magnolol 9% (w/w), *V. vinifera* seeds extract 45% (w/w), and *R. aculeatus* 35% (w/w)). Further cytotoxic investigation of these preparations led to the selection of DMRV-2 as the safest extract for the subsequent biological studies. Therefore, the efficacy of DMRV-2 as a potential anti-CVD agent was evaluated using ELISA assays, western-blot analyses and real-time PCR. The obtained results demonstrated that DMRV-2 induced a significant reduction of the cytokines IL-6 and IL-8 on LPS-stimulated HUVECs. Furthermore, it appeared to negatively modulate mRNA level expression of AP-1 subunits, thus preventing the expression of its target gene *mcp-1*. DMRV-2 was also shown to partially inhibit Nf-kB activation by reducing cytosol-nucleus translocation of that protein. All together, these results suggested that this herbal preparation could interact with multiple pathways involved in CVD onset and progression.

Sabato 17 giugno

P25. Use of acupuncture in cancer patients undergoing chemotherapy, immunotherapy, and radiation therapy

Allegra S[1], Di Nino A[2], Armando T[2], Storto S[2], Chiara F[1], De Francia S[1], Mussa MV[2]
[1]*Department of Clinical and Biological Sciences, University of Turin, Italy*
[2]*Department of Public Health, University of Turin, Turin, Italy*

Acupuncture is a technique that has been used since ancient times for the treatment of many diseases. It is also used for cancer-related conditions all over the world, and the evidence is growing year by year. Its action is carried out with the use of very fine needles applied to certain points connected to each other through so-called "meridians". This treatment complements but does not exclude traditional cancer therapy: potentially, it could reduce the possibility and intensity of side effects of therapy, enhancing therapeutic compliance. The aim of this work was to investigate the use of acupuncture in a group of patients undergoing chemotherapy (given intravenously or orally), immunotherapy, and radiation therapy. In addition, we described if patients undergoing acupuncture treatment have a side effects reduction with the same pharmacological treatment, compared to those who do not use it. Eventually, all the collected data were evaluating considering males and females patients in two different subgroup.
A prospective observational descriptive study has been conducted between July and December 2022. Data were collected through the administration of a questionnaire when the patients were admitted at the ''Sub-Alpine Oncology and Hematology Center'' (C.O.E.S.) in the Molinette University Hospital of the City of Health and Science of Turin. Data were collected from 323 patients (117 women and 206 men).
We showed that 55.1% of the patients had already heard about acupuncture technique. Only 11.5% of the total number of patients have practiced it at least once in their lifetime, but only 21.6% have practiced it to counteract the cancer therapy-related side effects. The complications most frequently experienced were asthenia (79.3%) and nausea (60.1%). Some statistically significant comparisons have been observed between the variable "gender" and the variable "side effects" with regard to the presence of alopecia (p=0.002), nausea (P=0.004), vomiting (p=0.003), constipation (p=0.005), diarrhoea (p=0.027), asthenia/tiredness (p=<0.001), mucositis (p=<0.001), and paresthesia/formicity in the fingers and toes (p=<0.001). Other statistically significant comparisons resulted between the variable "presence/absence of metastasis" and the variable "side effects" regarding the presence of asthenia/fatigue (p=<0.001), pain (p=0.008), skin rashes (p=0.021), and altered sensitivity (p=0.007). The last statistically significant comparison was between the variable "acupuncture use" and the variable "side effects" regarding nausea event (p=0.027).
Several evidences in the literature confirm the benefit of using acupuncture for side effects of cancer therapy, especially for nausea and vomiting, fatigue, pain, and gastrointestinal function. The number of the sample that has used acupuncture is really small, but with not inconsiderable benefits.
The field of alternative medicine and acupuncture is still unknown to many patients, but above all to many health professionals. In order to provide the patient with suitable

assistance in any healthcare situation, it is therefore necessary to spread acupuncture knowledge more widely. Moreover, more research is required to expand our understanding of the topic and to move this field toward higher standards of quality.

P26. Biotechnological strategies for enhanced production of antioxidant metabolites in *in vitro* cultures of *Isatis tinctoria* (Brassicaceae)

Davì F[1,2], Taviano MF[1], Nicosia N[3], Speranza J[4], Cavò E[1], Ragusa S[5], Arena P[1,2], Kwiecień I[6], Ekiert H[6], Miceli N[1]

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy*

[2]*Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy*

[3]*Division of Neuroscience, Vita Salute San Raffaele University, 20132, Milan, Italy*

[4]*Department of Genetics and Genome Biology, University of Leicester, United Kingdom*

[5]*PLANTA/Research, Documentation and Training Center, Via Serraglio Vecchio 28, 90123 Palermo, Italy*

[6]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna St., 30-688 Kraków, Poland*

The importance and growing commercial demand for plant secondary metabolites have led to a great increase in research focused on bioactive compound production using *in vitro* plant tissue culture systems. In this context, the agitated shoot culture of *Isatis tinctoria* L., a valuable medicinal plant, was selected to produce antioxidant compounds. The Murashige and Skoog (MS) medium variants containing different concentrations (0.1-2.0 mg/L) of plant growth and development regulators benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) were tested. Their influence on the growth of biomass, the accumulation of phenolic compounds as well as the antioxidant potential was evaluated. The MS variant with BAP/NAA 1.0/1.0 mg/L was found to be the best substrate for the *in vitro* production of phenolic compounds; moreover, the extract obtained from the biomass grown on this MS variant showed the best antioxidant efficacy both in the 1,1-diphenyl-2-picrylhydrazil (DPPH) test and the ferrous ion chelating activity assay (9.65±0.26 mg Trolox equivalents (TE)/g extract and IC_{50} 0.64±0.01 mg/mL, respectively). This variant was selected for further research aimed at improving the production *in vitro* of phenolics. Agitated shoot cultures were treated with different hormonal, abiotic, and biotic elicitors *i.e.,* Methyl Jasmonate (MeJa), Calcium Chloride ($CaCl_2$), Silver Nitrate ($AgNO_3$), and Yeast extract as well as with L-Phenylalanine (Phe) and L-Tyrosin (Tyr), precursors of phenolic metabolites. The quantitative determination of the total phenolic content (TPC) of hydroalcoholic extracts (MeOH 70%) obtained from the elicited/precursor feeding biomass was attained by a spectrophotometric method, the phenolic profile of the extracts was characterized by means of RP-HPLC and the antioxidant potential was evaluated.

The biomass extracts richest in TPC were those obtained after 72 h of supplementation with Tyr 2 g/L (49.37±0.93 mg GAE/g extract) as well as after 120 and 168 hours with Tyr 1 g/L (58.65±0.91 and 60.36±4.97 mg GAE/g extract); the HPLC analysis of the extracts led to the identification of six flavonoids namely vicenin-2, isovitexin, apigetrin, apigenin, quercitrin, quercetin, and nine phenolic acids, neochlorogenic, protocatechuic, chlorogenic, vanillic, caffeic, syringic, p-coumaric, ferulic, sinapic acids. Notably, the amount of flavonoids and phenolic acids detected in the elicited biomass was higher than that of the parental plant's leaves (cauline and basal). Regarding the elicitors, the highest TPC was achieved with $CaCl_2$ (20, 50 mM, 24 h). As far as the antioxidant properties, the best chelating activity was found for the extract obtained from biomass fed with Tyr 2 g/L, 72 h (IC_{50} 0.27±0.01 mg/mL), while the strongest radical scavenging properties (DPPH test) were highlighted for the extract obtained from biomass elicited with $CaCl_2$ 50 mM, after 24 h of incubation (25.14±0.35 mg

TE/g extract). In conclusion, the *in vitro* shoot culture of *I. tinctoria* supplemented with Tyrosin and/or $CaCl_2$ could represent a biotechnological source of compounds with antioxidant properties.

P27. Shoots formation by indirect organogenesis and VOCs production in *Acmella oleracea* L. ("jambù", Asteraceae)

Maggini V[1], Palagano E[2], Menicucci F[3], Cencetti G[2], Gallo E[1], Bettini PP[4], Michelozzi M[2], Bogani P[4], Firenzuoli F[1]

[1]Research and Innovation Center in Phytotherapy and Integrated Medicine (CERFIT), Referring Center for Phytotherapy, Tuscany Region, Careggi University Hospital, Florence, Italy

[2]National Research Council, Institute of Bioscience and Bioresources, (CNR-IBBR), Sesto Fiorentino, Italy

[3]National Research Council, Institute for the Chemistry of OrganoMetallic Compounds (CNR-ICCOM), Sesto Fiorentino, FI, Italy

[4]Department of Biology, University of Florence, Sesto Fiorentino, Italy

Acmella oleracea (L.) R.K. Jansen, also known as *Spilanthes acmella* Murr. or *Spilanthes oleracea* L., is a traditional medicinal plant (known as toothache plant) used in South America, Asia and Africa in the treatment of oral pain. Nowadays, *A. oleracea* extracts are attracting great interest as potent anti-ageing products with a botulinum toxin-like effect. For pharmaceutical, cosmetic and food applications, standardized cultivation methods are mandatory to increase plant material availability and to reduce phytochemical variability. We already demonstrated the effect of regeneration on the spilanthol (the most significant bioactive compound) accumulation in plant tissues. The present study evaluated for the first time the volatile profile of plant seedlings from regenerating lines derived by organogenesis and grown in *vitro*.

Comparison of morpho-physiological parameters, namely height, lateral branching, number of leaves and fresh weight of leaves and roots between *Acmella oleracea* plantlets germinated *in vitro* from seeds and shoots regenerants was conducted. No differences were highlighted in terms of stem and root fresh weights. In contrast, all regenerants showed a significant reduction of plant height and lateral branching capability compared to seed-derived plants, the latter being completely absent in seed-derived plants. These morphological traits are typical of plant somaclones. Significant variations were also observed in the number of leaves both between seed and *in vitro* regenerants and between regenerants only. Moreover, volatile organic compounds produced by the selected plantlets were detected and analysed. Regenerated shoots showed a higher concentration of monoterpenes with respect to the seed-derived plants. In particular, α-pinene, β-pinene, limonene, trans-β-ocymene resulted more abundant in regenerated shoots than in the seed-derived samples.

These results confirmed that i*n vitro* plant cultures could rapidly increase the availability of raw material through regeneration and multiplication systems. In addition, regenerating lines showed elevated quantities of bioactive molecules of pharmacological interest.

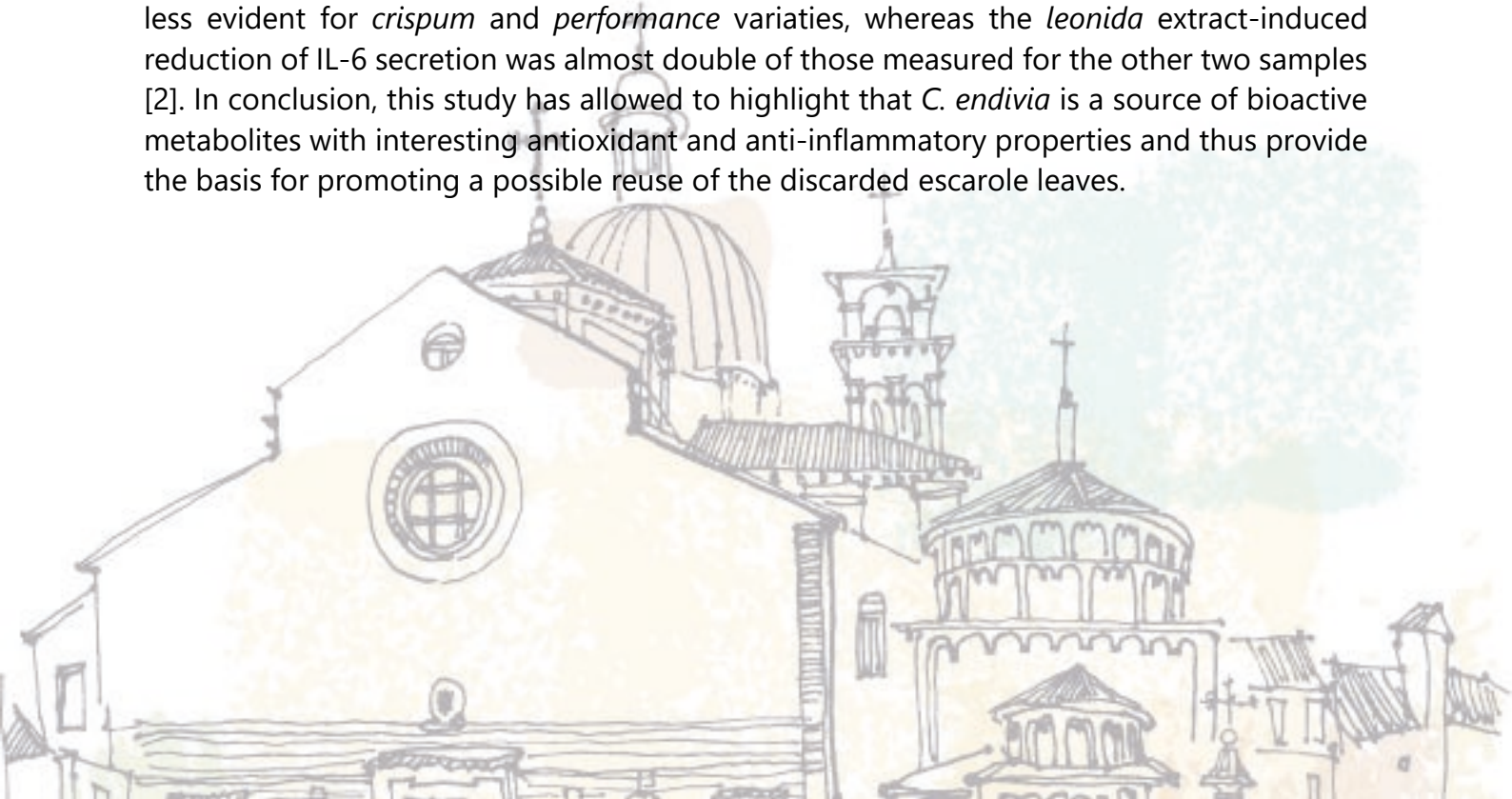
P28. The importance of (not) being a waste: the case study of *Cichorium endivia* L.

Parisi V[1], Bellone M[1], Santoro V[1], Vitiello M[1], Dal Piaz F[2], De Tommasi N[1], Donadio G[1]

[1]Dipartimento di Farmacia, Università di Salerno, 84084 Fisciano (SA), Italy;

[2]Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università di Salerno, 84084 Fisciano (SA), Italy;

Plants of genus *Cichorium* are largely cultivated as edible plants and, particularly in South Italy, play an important role in agricultural market [1]. The external leaves of *Cichorium endivia L.* (Asteraceae) (known as escarole) are a crop by product usually discarded. In this work the phytochemical, antioxidant, and anti-inflammatory activity of hydroalcoholic extract of discarded leaves from escarole: var. *performance*, var. *crispum* and var. *leonida,* grown in the Salerno hinterland, was carried out. The plant matrices were extracted using green methods, such as ultrasound-assisted extraction combined with hydroalcoholic solvents. Green extraction of naturals products represents a new frontier to meet the challenges of the 21st century, to preserve both the environment and population and in the meantime improve competition of industries to be more ecological, economic, and innovative. The obtained extracts were analysed by nuclear magnetic resonance (NMR) and liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS/MS) to obtain a phytochemical fingerprinting. 1D-NMR analyses highlighted the presence of amino acids, sugars, and organic acids. Moreover 35 specialized metabolites, phenolic acids, flavonoids and sesquiterpenes were identified by LC-HRMS/MS analysis. Quantitative analyses highlighted that var *leonida* was the richest in terms of sesquiterpenes, especially dihydrolactucin and its isomer, on the other hand the highest amount of flavonoids was detected in var. *performance*. Based on the chemical composition of extracts, their antioxidant and anti-inflammatory potential was investigated. Results in cell-free and cell-based assay showed an interesting antioxidant activity for tested varieties. Furthermore, the anti-inflammatory property of the escarole extracts was investigated on LPS- stimulated M0 macrophages. A 24-h treatment of cells with 100 µg/mL of each extract revealed that all the samples modulated IL-6 secretion from inflamed M0. However, this effect was significantly less evident for *crispum* and *performance* variaties, whereas the *leonida* extract-induced reduction of IL-6 secretion was almost double of those measured for the other two samples [2]. In conclusion, this study has allowed to highlight that *C. endivia* is a source of bioactive metabolites with interesting antioxidant and anti-inflammatory properties and thus provide the basis for promoting a possible reuse of the discarded escarole leaves.



P29. Essential oils as potential acetylcholinesterase and butyrylcholinesterase inhibitor. A case study: *Elettaria cardamomum* (L.) Maton essential oil

Pavarino M, Cagliero C, Marengo A, Bicchi C, Rubiolo P, Sgorbini B
Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italia

Alzheimer's disease (AD) is the most common dementia of old age worldwide, characterized by extensive neuronal death that occurs over time and in an irreversible manner. To the best of our knowledge, there is no treatment that can affect the progression of the disease. There are only symptomatic drugs that improve the patient's cognitive abilities and attempt to prevent the progression of the disease. These drugs are acetylcholinesterase (AChE) inhibitors such as donepezil, rivastigmine and galantamine, but in the late stages of AD, butyrylcholinesterase (BChE) levels increase significantly, while AChE levels decrease in the hippocampus and temporal cortex. Therefore, it is important to find effective BChE inhibitors that raise acetylcholine (ACh) levels in advanced AD to prevent the negative effects of AChE suppression. Inhibitors of both types of cholinesterase have recently gained popularity in AD drug discovery and are indeed one of the avenues being pursued for AD research. Essential oils (EOs), complex mixtures of hundreds of compounds, have traditionally been used to improve cognitive abilities and alleviate other symptoms associated with AD; in addition, their complexity in terms of bioactive molecules makes them an ideal target for research into inhibitors of these enzymes.

Sixty-nine essential oils from different plant species and botanical families (Annonaceae, Apiaceae, Asteraceae, Betulaceae, Burseraceae, Caryophyllaceae, Compositeae, Cupressaceae, Ericaceae, Geraniaceae, Lamiaceae, Lauraceae, Mirtaceae, Oleaceae, Pinacee, Piperaceae, Poaceae, Rosaceae, Rutaceae, Santalaceae, Stiracaceae, Verbenaceae, Zingiberaceae) were subjected toAChE and BChE *in vitro* colorimetric assay by the Ellman method and chemically characterized using gas chromatography coupled to mass spectrometry. Several essential oils were found to be potential AChE or BChE inhibitors, but the only EO that was found to be active in inhibiting both enzymes was *Elettaria cardamomum* (L.) Maton essential oil. Therefore, the study focused on finding specialized bioactive metabolites in this particular essential oil. *E. cardamomum* is a plant that belongs to the Zingiberaceae family and has been used for centuries in traditional Ayurvedic and Chinese medicine for a variety of different purposes in the form of infusions, decoctions, distillates and essential oils. The rapid spread of the use of the essential oil of this plant throughout the world and its use in food, medicine, and fragrance development makes it a very interesting matrix in the research of specialized bioactive metabolites. A bio-guided fractionation approach was used to isolate fractions/pure compounds that can be tested individually to evaluate their activity. *E. cardamomum* EO hydrocarbon fraction was found to be inactive towards both AChE and BChE; conversely, the oxygenated fraction was active towards both enzymes. These results demonstrate that the essential oil of *E. cardamomum* is a promising source of potential AChE and BChE inhibitors.

P30. *Foeniculum vulgare* Miller: revalorization of a local food waste

Santoro V, Rosa E, Donadio G, Polito F, Caputo L, De Feo V, De Tommasi N
Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy

Bio-wastes represents a significant issue for the agro-food industry and require strategic actions for their possible re-use. This research aims to the valorization of local fennel byproducts, that after harvest, constitute almost 60% of fennel biomass [1].
For this purpose, a qualitative study of the hydroalcoholic extract of edible and non-edible parts, leaves and stems, of *Foeniculum vulgare* Miller was carried out by liquid chromatography-high resolution mass spectrometry HRESIMS. The analysis led to the identification of secondary metabolites belonging to different chemical classes, as flavonoids, phenolic acids, amino acids and fatty acids. The results highlighted the presence a high amount essential amino acid in particular phenylalanine threonine and isoleucine. The potential antioxidant activity of the extracts was also investigated by DPPH e ABTS assays. Results show a moderate antioxidant activity for the hydroalcoholic extract. The chemical composition of the essential oils was also studied. The essential oils were obtained by steam-distillation with a yield of 0,001% for the edible parts and 0,002% for the waste. The chemical compositions were achieved by GC-MS and they turned out to be very similar, showing the presence of *trans*-anethole (57,1% in the edible parts and 51,5% in the waste, respectively) and limonene (23,3% in the edible parts and 26,7% in the waste, respectively) as the major components. The composition agrees with the literature where *trans*-anethole and limonene are present among the main components, even if not always the most abundant [2-5].
Finally, the antimicrobial activity of fennel essential oils was assessed by using the disk diffusion method against bacteria that are common food contaminants: *Staphylococcus aureus, Escherichia coli, Salmonella* sp., *Shigella* sp., *Enterococcus faecalis, Listeria monocytogenes* and *Acinetobacter baumannii*. The essential oils resulted active on almost all bacterial strains, especially on *Shigella* sp. with no significant difference between the antimicrobial activity of the two essential oils.

P31. Spilanthol-rich extracts from *Acmella oleracea* (L.) R.K. Jansen and their efficacy on stored product pests

Spinozzi E[1], Ferrati M[1], Piergentili A[1], Petrelli R[1], Cappellacci L[1], Maggi F[1], Angeloni S[1], Ricciutelli M[1], Drenaggi E[1], Boukouvala MC[2], Kavallieratos NG[2], Benelli G[3]

[1]Chemistry Interdisciplinary Project (ChIP) Research Center, School of Pharmacy, University of Camerino, Via Madonna delle Carceri 9/B, 62032 Camerino, Italy;

[2]Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos str., 11855, Athens, Attica, Greece;

[3]Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124, Pisa, Italy.

Since many years, the infestation of stored food products has represented a huge concern for human health being often responsible of many food allergies and other health issues. Given the increasing resistance to conventional pesticides, there is the urgent need of discovering alternative products. In this regard, botanicals represent promising candidates for the development of innovative, effective, and safe pesticidal agents. *Acmella oleracea* (L.) R.K. Jansen is a medicinal plant known as jambù that plays an important role in the pharmaceutical, nutraceutical, and cosmetic fields. Its different biological activities are mainly due to the presence of the *N*-alkylamide spilanthol, which has been recently shown as an insecticidal agent.

In this regard, the aims of this work were to optimize an extraction protocol to obtain spilanthol-rich extracts and test them against stored-product pests.

After the screening of extraction techniques and solvents, *n*-hexane and methanol extracts were characterized by HPLC-DAD-MS and selected for biological assays. In detail, *A. oleracea*-derived products, especially the *n*-hexane extract, were effective on most targets tested. The different susceptibility of the pests was probably linked to a higher spilanthol content in the *n*-hexane extract. This study confirms the possible exploitation of this plant as pesticidal agent by agrochemical industries.

P32. Development of a "foodomics" platform for monitoring the transformation process of aromatic plants for the food industry

Evangelista AR, Nitride C, Andolfo G

Department of Agricultural Sciences, University of Naples Federico II, Via Università, 100, 80055 Portici, NA, Italy

Recent advances in analytical methods in food science and technology have led to new modern approaches to assess food quality and safety. The aim at collecting molecular level evidence for building a multi-omics data model for aromatic plants. The multi-omics platform will inform food operators in the field of fresh, semi-processed and processed aromatic herbs about the impact of season, the time of harvesting and the impact of processing for enhancing the aroma. Basil (*Ocimum basilicum* L.), belonging to Lamiaceae is used as model herbal plant, it has a unique aroma due to essential oil which attracts the interest of consumers. A "fingerprint" for ingredient authentication and the monitoring of markers through during processing will be the key indicators used to feed the "Foodomic" model. Data will be collected using genomic, transcriptomic, proteomic, and metabolomic data. Foodomic platform applies high-resolving analytic technologies ranging from next-generation sequencing approaches to high-resolution mass spectrometry. The developed for basil will be then transferred to other herbal plants. All this will be done within the framework of a multidisciplinary and integrated exchange of scientific disciplines.

P33. Nutritional and health valorization of plants indigenous to Western Sahara

Chiozzini C[1], Maietti A[2], Tacchini M[1], Abdi Bellau ML[3], Maresca I[1], Sacchetti G[1], Guerrini A[1,4]

[1]Pharmaceutical Biology Lab., Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

[2]Department of Chemistry, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy

[3]Pharmacist of the Sahrawi refugee camps, Tindouf, Algeria (RASD)

[4]University Centre for International Development Cooperation, University of Ferrara, Ferrara, Italy

For 45 years Saharawi refugees (180000 people) have been waiting for a political agreement on independence that would allow their return to Western Sahara (Besenyö et al., *AARMS*, 2010, 9(1), 67–78), land they claim from Morocco. Their living conditions are drastic as the refugee camps are in the Hamada highland of the Algerian desert, one of the most inhospitable areas on the planet. Although the people are making great efforts to achieve a form of self-sufficiency (e.g., by growing fruit and vegetables and rearing goats) food is very dependent on humanitarian aid and the diet is not balanced, leading to malnutrition on the one hand and obesity on the other (Gozalbo et al., *Children*, 2020, 7(12), 264). The diet covers only 68% of energy needs, and malnutrition causes health problems (e.g., anaemia), mainly among women and children.

In addition, several diseases are closely linked to refugee status, such as dental illnesses and gastrointestinal or respiratory infections, a significant increase in cardiovascular and thyroid ailments, hypertension, asthma, and diabetes.

In this context, the traditional Sahrawi medicine, based on local medicinal plants, is the main tool to treat many of these disorders.

In this research project (Presidenza Consiglio dei Ministri, prat. 231/2019) it is outlined the nutritional and health profile of plant drugs collected in the wild in Western Sahara (Bir Lehlu), such as the leaves of *Atriplex halimus* L., and cultivated locally in experimental gardens, such as the leaves of *Moringa oleifera* Lam, *Ziziphus mauritania* Lam. and *Ammodaucus leucotrichus* Coss. The crude drug of *M. oleifera* evidenced a high protein content (23.12 ± 0.36 g/100g), an interesting nutritional value to compensate for the malnutrition of the Sahrawi population (Borhane Ziani et al., *J.Funct.Foods*, 2019, 53, 237-247). Experimental data also showed that the highest total fibre content is in *Z. mauritania* leaves (48.87 ± 0.91 g/100g) corresponding to 3.05 ± 0.13 g/100g of soluble fibre and 45.83 ± 1.04 g/100g of insoluble one. The highest mineral amount is attributable to the xero-halophytic species *A. halimus* (Hassine et al., *J.Exp.Bot.*, 2008, 59(6), 1315–1326), mainly due to a high iron and sodium content, followed by the high Fe values of *A. leucotrichus*. *M. oleifera* leaves have the highest lipid amount, with α-linolenic acid at around 71% and linoleic acid at 9%. Decoctions and mother tinctures of these drugs have been prepared. High content of total polyphenols was found mainly in the decoctions rather than in tinctures, while the antioxidant capacity (DPPH and FRAP tests) showed, quite unexpectedly, the highest activity in the hydroalcoholic extracts rather than in decoctions. The crude drugs with the highest polyphenol content and antioxidant activity are *M. oleifera* and *Z. mauritania*.

Antimicrobial activity was evaluated against four bacterial (*Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Staphylococcus aureus*) and two yeasts strains (*Candida glabrata* and *Candida albicans*). However, at the highest concentration tested (500 µg/mL), only weak

activity of *Z. mauritania* and *M. oleifera* extracts was detected against *Candida* strains. Experiments to formulate Fe-based food supplements are being carried out.

P34. Evaluating the effect of *in-vitro* digestion on the total polyphenol content of *Vitis vinifera* L. by-products

Mastellone G[1], Gennaro G[1], Marengo A[1], Sgorbini B[1], Larrea V[2], Cirrincione S[3], Lamberti C[3], Hernando I[2], Gai F[3], Rubiolo P[1], Cagliero C[1]

[1]*Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, I-10125 Torino, Italia*

[2]*Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camí de Vera, s/n, 46022, Valencia, España*

[3]*Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, I-10095 Grugliasco, Italia*

The agricultural industry is characterized by several areas of environmental concern, including large use of water, use of energy and chemicals, exploitation of soil and ecosystems, and production of solid wastes. In view of a circular economy model, agrifood by-products represent a sustainable source of bioactive compounds for feed, functional food, and food supplement industries. The production of wine is one of the most extensive agricultural activities in the world, generating a huge amount of organic waste material. Most of the waste is represented by grape pomace, followed by seeds, pulp, skin, stems, leaves and green pruning residues. These by-products have attracted attention in recent years for their valuable content of bioactive compounds, in particular polyphenols. The beneficial properties of these compounds are mainly correlated to their antioxidant activity which is well documented in *in-vitro* studies. However, the bioactivity and bioavailability of polyphenols *in-vivo* depend on the extent to which they are absorbed after oral ingestion.
This study aimed to investigate the effect of *in-vitro* static simulation of gastrointestinal food digestion on the total polyphenol content of different cultivars of *Vitis vinifera* L. green pruning residues. Grapevine pruning samples were subjected to sequential oral, gastric and intestinal digestion adopting the appropriate combination of electrolytes, enzymes, bile, dilution, pH and time of digestion. The untreated and digested samples were then subjected to a methanolic extraction, and the phytochemical profile was analyzed by ultra high performance liquid chromatography equipped with a photodiode array detector (UHPLC-PDA). The quali-quantitative comparison of the polyphenol content before and after the *in-vitro* digestion revealed that these compounds are highly stable under the tested conditions and the phenolic compounds are more abundant in the bio-accessible phase than in the excreted fraction.

P35. Antidysmenorroic effects of the non-psychotropic *Cannabis sativa* L. phytocomplex on isolated human myometrium

Occhiuto C[1], Giofrè S[2], Smeriglio A[2], Trozzi A[2], Tranchida PQ[2], Occhiuto F[2]

[1]Imbesi Foundation, University of Messina, Piazza Pugliatti, 1 -98122 Messina, Italy.

[2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina. Viale Ferdinando Stagno d'Alcontres, 31 - 98166 Messina, Italy.

Dysmenorrhea, menstrual cramps, is a menstrual disorder affects on average over 50 % of women. Specifically, the painful uterine cramping associated with menstruation is probably triggered by vasopressin and increased production of prostaglandins by the myometrium and endometrium which stimulate increased uterine contractions. The current method of treatment for dysmenorrhea, with non-steroidal anti-inflammatory drugs, blocks prostaglandins production and acts as a painkiller. Oral contraceptives agents or uterine relaxants are also used by some women in the treatment of dysmenorrhea. The endocannabinoid system (ECS) is a homeostatic system involved in many physiological and pathological conditions. The ECS regulates almost all levels of female reproduction, starting with oocyte production through to parturition. Dysregulation of the ECS is associated with the development of gynecological disorders. Cannabinoids that act at the ECS as specific agonists may potentially influence dysregulation and, therefore, represent new therapeutic options for the therapy of gynecological disorders such as menstrual pains (primary dysmenorrhea). Several research have evidenziated the important medicamentous potential of the phytocomplex obtained from the female inflorescences of non-psychotropic *Cannabis sativa* L. (THC < 0.2%), characterized by the presence (in addition to cannabinoids) of numerous and important ingredients such as terpenes.

To investigate the uterine relaxant effect of the phytocomplex of non-psychotropic *Cannabis sativa* L., isolated organ bath experiments have been conducted with the hexane extract from female inflorescences and dried leaves of the plant and with three of its pure active ingredients cannabidiol (CBD), cannabigerol (CBG), and myrcene (My), using human non-pregnant isolated uterus as experimental model.

Human uterus tissues were obtained from hysterectomy specimens coming from premenopausal women. Isolated uterus tissue strips were mounted in an organ-bath system, and the relaxation effect of plant extract, comparatively to CBD, CBG, My and nifedipine as positive control, was evaluated by cumulative addition to uterus strips. The responses of cumulative concentrations of extract, CBD, CBG and myrcene on spontaneous contractions and on prostaglandin E_2 (PGE_2) and vasopressin (V) induced submaximal contraction, were also investigated. Phytochemical analysis (GC × GC-QMS) was used to correlate the composition of extract with its uterolytic activity.

Cumulative dosing of plant extract induced concentration-dependent relaxation in uterus tissue. CBD, CBG and My demonstrated effects like to those highlighted by the extract but of less intensity. Simultaneous concentrations of CBD, CBG and My showed an additive relaxation effect. In addition, the plant extract was able to reduce the spasmogenic effects induced by PGE_2 and vasopressin of the precontracted human uterus.

P36. *Crepis vesicaria* L.: an underexploited herbal species with promising health-promoting effect.

Mangieri C[1], Carlucci V[1], Benedetto N[1], Cafarelli A[1], Covino MA[1], Lupia C[2], Milella L[1]
[1]*Department of Science, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy*
[2]*Conservatorio di Etnobotanica, Piazza Plebiscito 13, 85040 Castelluccio Superiore, Italy*

Crepis vesicaria L. (Cv), known as beaked hawk's beard, is an underexploited plant mainly used in Mediterranean cuisine and frequently employed in traditional medicine for its depurative, blood cleaning, and diuretic properties [1]. Despite the wide diffusion, knowledge of nutritional and bioactive compound profiles is scarce [2]. Therefore, we aimed to study the phytochemical composition and evaluate its health-promoting effects investigating its specialized metabolite content and antioxidant capacity in different plant organs. Flowers (CvF), roots (CvR), stems (CvS), pappus (CvP) and leaves (CvL), stored at the "*Conservatorio di Etnobotanica*", in Castelluccio Superiore, Basilicata, were air-dried and exhaustively extracted by dynamic maceration with 70% ethanol (1:30 *w/v*) [3]. The obtained extracts were analysed for their phytochemical constituents by evaluation of Total Polyphenols Content (TPC), Total Flavonoids Content (TFC), and Total Tannin Content (TTC) [4]. The highest content of polyphenols was found in CvR (74.62±1.19 mg of gallic acid equivalents/g DW), while the extract richest in flavonoids was CvF (10.20±1.20 mg of quercetin equivalents/g DW). Reported results show that roots are the organ richest also in condensed tannins (10.82±1.08 mg of catechin equivalents/g DW); whereas the other parts of the plant mainly contain hydrolysable tannins (CvS is the richest with 65.11±1.82 mg of tannic acid equivalents/g DW).

The antioxidant activity was evaluated by 4 complementary assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP), and *β*-Carotene Bleaching assay (BCB). Results were then compared by Relative Antioxidant Capacity Index (RACI) [5] and revealed that the extract with the strongest capacity to reduce the oxidative stress was CvL, which was consequently chosen for further studies.

The oxygen radical absorbance capacity (ORAC) of CvL was evaluated obtaining a value of 166.60±9.11 µmol Trolox equivalents/g DW. The biological activity of the CvL was evaluated on the human hepatocarcinoma cell line (HepG2), used as model cells. The extract showed no cytotoxic activity at the tested concentrations (10-400 µg/mL) and reduced the intracellular reactive oxygen species (ROS) level in stressed cells in a dose-dependent manner.

Considering the widespread use of *C. vesicaria* in the cuisine of the Mediterranean area, the study continued with the analysis of the cooking water (CvCW) and the cooked fresh leaves (CvCL), which were extracted by dynamic maceration with 70% ethanol, as above cited. CvCW showed the highest content of polyphenols (73.80±0.43 mg GAE/g DW), comparable to CvR. Antioxidant tests were performed on those extracts and RACI was calculated comparing CvCW and CvCL with the previously analysed extracts. CvCW turned out to be the extract with the best antioxidant properties, probably due to a greater affinity of the water with the specialized metabolites contained in the plant and therefore to their better extractability. The results obtained lead us to consider how the cooking water from the *C. vesicaria* leaves,

which is normally thrown away, could represent a source of bioactive compounds with beneficial properties for human health.

P37. Mosquitocidal and anti-inflammatory effects of hemp (*Cannabis sativa* L.) essential oils from monoecious, male, and female inflorescences and their encapsulation in nanoemulsions

Rossi P[1], Cappelli A[1], Marinelli O[2], Valzano M[1], Pavoni L[2], Bonacucina G[2], Petrelli R[2], Ianni M[2], Mazzara E[2], Ricci I[1], Maggi F[2], Nabissi M[2]

[1]School of Biosciences and Veterinary Medicine, University of Camerino, 62032 Camerino, Italy;
[2]School of Pharmacy, University of Camerino, Via Madonna delle Carceri, 62032 Camerino, Italy.

Among the numerous novel products obtainable from hemp (*Cannabis sativa* L.) waste plant material deriving from different industrial processes, the essential oil (EO) is gaining particular attention for its possible application in several fields, such as cosmetics, pharmaceuticals, and botanical insecticides. For the purpose, in the current work, we evaluated the chemical composition of EOs produced from different hemp varieties, namely Felina 32 and Carmagnola Selezionata (CS) employing monoecious, male, and female inflorescences, and we investigated their mosquitocidal properties on larvae and pupae of two main malaria vectors, *Anopheles gambiae* Giles and *An. stephensi* Liston. Then, in order to assess the safe use of hemp EOs for operators, the potential pro- or anti-inflammatory activity of hemp EOs along with their toxicological profile were evaluated on dermal fibroblasts and keratinocytes. Based on the interesting results obtained by insecticidal and anti-inflammatory studies, a preliminary investigation of EOs encapsulation into nanoemulsions (NEs) has been carried out to increase the poor physicochemical stability of the EOs. Felina 32 and CS inflorescences provided EOs with monoterpene and sesquiterpene hydrocarbons as the main constituents. This work evidenced the potential application of male inflorescences, which are usually discarded during hemp product processing. These EOs could be exploited as potential eco-friendly and sustainable biopesticides, due to their capability to neutralize mosquitoes, and their possible use to develop stable and safe formulations. The LC_{50} values detected in this study (<80 ppm) were lower, on average, than those found for several plant EOs, with the advantage of employing an industrial by-product. Regarding MTT assay and gene and protein expression analysis, EOs exhibited no cytotoxicity at the appropriate doses, and displayed an anti-inflammatory effect on the tested human cell lines. These outcomes encourage further applied research on hemp EOs to support their industrial exploitation.

P38. Potential beneficial effect of grape seed extract on LPS-induced gut permeability damage and oxidative stress

Nicolosi RM[1], Imperia E[2], Altomare AM[2,3], Guarino MPL[3,4], De Vita D[1], Pasqua G[1]

[1]Department of Environmental Biology, Sapienza university of Rome, P.le Aldo Moro 5,00185, Rome, Italy

[2]Food Sciences and Human Nutrition Unit, Campus Bio-Medico university of Rome, Via Alvaro del Portillo 21, 00128, Rome, Italy

[3]Research unit of Gastroenterology, Università Campus Bio-Medico di Roma, Via Alvaro del Portillo 21, 00128, Rome, Italy

[4]Unit of Gastroenterology, Fondazione Policlinico Campus Bio-Medico of Rome, Via Alvaro del Portillo 200, 00128, Rome, Italy.

Pomace is a by-product of the winemaking process and very frequently a cost burden for farms. It is possible to valorise those by transforming them into plant matrices which are used to extract bioactive metabolites. Specifically, the seeds that are isolated from the unfermented pomace (white winemaking) contain lipids, proteins, carbohydrates and 5-8% of polyphenols, depending on the cultivar. A large part of the polyphenols are procyanidins, in monomeric, dimeric, oligomeric (3 to 10 units) and polymeric (>10 units) forms. Procyanidins are thought to be the major responsible of the biological effects of grape seeds extract (GSE). Several authors reported the antimicrobial activity of grape seed extracts against specific strains of bacteria and fungi (1). It has been demonstrated a significant correlation between anti-*Candida* activity and the content of the flavan-3-ols in grape seed extracts, with a polymerization degree ≥4 (2). The aim of this work is to study the protective effects of grape seed extract on LPS-induced gut permeability damage and oxidative stress on Caco-2 cells. GSE was obtained by solid-liquid EtOH/H_2O (7:3 *v/v*) extraction at 40°C to preserve the stability of the active ingredients to allow their efficient recovery. The method used in this study and the extraction process conducted at Sapienza's innovative startup ViVita pharma allow the hydroalcoholic extraction mixture to be recovered and reused several times, reducing production costs, and carrying on a more sustainable process with a circular economy perspective. Chemical analysis of GSE was performed by high-performance liquid chromatography equipped with photodiode detector (HPLC-DAD) and nuclear magnetic resonance (NMR). HPLC-DAD analysis revealed a 70% content of total procyanidins in both the extract obtained from the virgin hydroalcoholic mixture and the extract obtained from the recovered hydroalcoholic mixture. From the ^{1}H NMR spectrum, it was possible to identify and quantify 24 metabolites classified as amino acids, organic acids, carbohydrates, and miscellaneous molecules. Among them, it is interesting to notice the presence of ascorbate, procyanidin B1 and polymeric procyanidins. The potential beneficial effect of GSE on LPS-induced gut permeability damage and oxidative stress was tested on an *in vitro* human intestinal epithelial cell line Caco-2, that after 21 days differentiates into normal mature enterocytes. Cells were challenged apically with LPS (bacterial lipopolysaccharide from a pathogenic strain of *Escherichia coli* 0111:B4) 10 µg/mL (3), GSE 6.25 µg/mL and LPS + GSE for 24 h. Epithelial barrier integrity was investigated measuring the paracellular flux of fluorescein isothiocyanate-dextran (FD-4) (3) from the apical to basolateral compartments, while reactive oxygen species (ROS) production was assessed by adding 2′,7′-dichlorodihydrofluorescein diacetate (H2-DCF-DA) (4). Our results demonstrate a protective role of GSE on LPS-induced epithelial barrier damage and ROS production by

LPS 10 μg/mL. Preliminary results suggest in an *in vitro* model, that GSE can prevent the intestinal epithelium permeability damage and ROS production induced by LPS, but further studies will be needed to better understand the mechanism underlying LPS-induced gut impairment and the antioxidant effect of GSE in its prevention and management.

P39. Preliminary investigation about morpho-anatomical and phytochemical features and antioxidant activity of leaves and flowers from Sicilian *Plumeria rubra* L. cv. "Classica palermitana"

Ingegneri M[1], Malaspina P[2], Betuzzi F[2], Di Gristina E[3], Cornara L[2], Trombetta D[1], Smeriglio A[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98166 Messina, Italy

[2]Department of Earth, Environment and Life Sciences (DISTAV), University of Genova, 16132 Genova, Italy

[3]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, 90128 Palermo, Italy

Plumeria rubra L. (Apocynaceae), also known as 'Frangipani', is an ornamental plant commonly grown in parks and home gardens for its beautiful fragrant flowers of various colour and size. It is native from Caribbean areas, but it has spread across the tropical and subtropical lands. The species is also present along Sicilian coastal areas, especially in Palermo, where it has been renamed 'Pomelia' and it has been widely cultivated as an attractive ornamental tree, becoming one of the official floral symbols of the city. The cultivar "Classica palermitana" or "Tonda palermitana" is characterized by a white corolla with a yellow centre, intensely scented.

Various ethnobotanical uses and pharmacological activities of *P. rubra* have been reported, such as antibacterial and antioxidant, anxiolytic, hypolipidemic effects and others. However, no studies are to date available about this old Sicilian cultivar.

Considering this, the aim of this study was to investigate the morpho-anatomical and phytochemical features of leaves and flowers of *P. rubra* cv. "Classica palermitana", also evaluating the potential antioxidant activity of their hydroalcoholic extracts.

The leaves, which measure up to 30 cm, were elliptic with entire margin and bright green coloured. They were amphistomatic with paracytic stomata, showing a dorsiventral mesophyll, and a single bicollateral vascular bundle in the midrib. Laticifers, irregularly dispersed in the ground tissues, were circular in transversal section, and reacted positively with Sudan III. Frangipani flowers were arranged in terminal or lateral cymes. Conical-papillate cells and non-glandular moniliform-like trichomes were observed on the petal's surface. Moreover, Sudan III highlighted the presence of nonarticulated laticifers also in the petals.

The phytochemical screening carried out on leaves and flowers extracts (LE and FE, respectively) revealed an interesting content of total phenols (2.70 ± 0.125 and 2.23 ± 0.066 g gallic acid equivalents/100 g of LE and FE, respectively). Flavonoids are the most abundant compounds (1.88 ± 0.082 and 1.46 ± 0.054 g rutin equivalents/100 g of LE and FE, respectively), with a flavan-3-ols content of 0.32 ± 0.02 and 0.23 ± 0.01 g catechin equivalents/100 g of LE and FE, respectively. Both LE and FE are also rich source of proanthocyanidins (1.69 ± 0.03 and 1.58 ± 0.02 cyanidin equivalents/100 g of LE and FE, respectively), showing a low polymerization index (0.19 and 0.15 for LE and FE, respectively), which indicates a greater presence of monomeric or oligomeric molecules. The antioxidant activity was investigated by four *in vitro* cell-free assays (DPPH, TEAC, FRAP, ORAC) based on different environment and reaction mechanisms. Furthermore, the iron-chelating capacity of both extracts was evaluated by ferrozine assay. According to the polyphenols content, LE showed, in all the assays carried out, the best antioxidant and iron-chelating activity (IC_{50} 4.85 - 529.28 µg/mL vs. 6.59 - 947.38 µg/mL of FE).

Further studies are ongoing to characterize the phytochemical profile and biological activity of the LE and FE, but these preliminary studies already allow to speculate promising nutraceutical and cosmeceutical applications for *P. rubra* cv. "Classica palermitana".

P40. The use of nanovesicles to enhance the antioxidant activity of an extract of *Onopordum illyricum* leaves

Sanna C[1], Caddeo C[2], Tuberoso CIG[2], Floris S[2], Masala V[2], Pintus F[2]

[1]*Department of Life and Environmental Sciences, University of Cagliari, Via S. Ignazio da Laconi 13, 09123 Cagliari, Italy*

[2]*Department of Life and Environmental Sciences, University of Cagliari, SS 554 - Bivio per Sestu, Monserrato, 09042 Cagliari, Italy*

In the last decades, there has been an increasing interest in the search for novel pharmacophores from plants because of their chemical diversity and versatility not matched by synthetic chemistry libraries. However, plant products, in the form of either isolated molecules or extracts, are often prone to degradation and possess poor aqueous solubility, limited permeability, and even toxicity, which limit their possible application in therapy. In this context, the use of delivery systems, especially nanocarriers, can be a valuable strategy to overcome these physicochemical and pharmacokinetic limitations[1]. Phospholipid vesicles are being successfully used to load plant extracts and deliver them to a number of targets via different administration routes, and several studies have demonstrated their greater therapeutic efficacy compared with conventional approaches[2].

In this study, we focused on *Onopordum illyricum* L. (Asteraceae), a wild thistle widespread in the Mediterranean basin, traditionally used for food and therapeutic purposes. Since it is used in Sardinia (Italy) to treat exanthematous skin[3], a potential topical application of vesicle formulations for the treatment of oxidative stress-related skin disorders is proposed. An 80% ethanol extract from *O. illyricum* leaves was qualitatively analyzed by (HR) LC-ESI-QTOF MS/MS in negative ion mode, and then formulated in two types of phospholipid vesicles (liposomes and Penetration Enhancer-containing Vesicles) with the aim of exploiting and enhancing its bioactivity for skin delivery.

The phytochemical analysis of the extract showed, in accordance with previous studies[4,5], the abundance of phenolic compounds, especially hydroxycinnamic acid and flavonol derivatives. Luteolin-glucuronide was the most abundant compound, followed by 1,5-di-*O*-caffeoylquinic acid and dicaffeoyl succinyl quinic acid.

The vesicles were characterized by size distribution, surface charge, stability, and entrapment efficiency. The latter was calculated based on the amount of 13 targeted phenolic compounds identified in the *O. illyricum* extract (quantified by LC-DAD analysis) and detected in the vesicle dispersions. In addition, their antioxidant power was evaluated through DPPH and FRAP assays and in cultured human keratinocytes, and their effects were compared to those exhibited by the *O. illyricum* extract solution.

The extract-loaded vesicles showed small size (<100 nm), high entrapment efficiency (even >90% for most phenolic compounds), and good long-term stability. Moreover, the extract-loaded vesicles exhibited remarkable antioxidant activity, as demonstrated by colorimetric assays and by enhanced reduction of intracellular reactive oxygen species (ROS) levels in stressed skin cells. Our results demonstrate that the antioxidant power of a phenolic-rich extract from *O. illyricum* leaves was enhanced by the incorporation in nanovesicles, supporting the role of nanotechnologies in allowing and even promoting the application of plant-derived products in therapy. Further investigation is certainly required to understand

the interactions between extract-loaded vesicles and cells and to assess the penetration of targeted phenolic compounds in the skin.

P41. The Effect of *Olea europea* L. extract and its compound oleuropein, on cardiotoxicity induced by doxorubicin

Maiuolo J, Musolino V, Gliozzi M, Mollace V

[1]Laboratoy of Pharmaceutical Biology,in IRC-FSH center, Department of Health Sciences, University "Magna Græcia" of Catanzaro, Italy

[2]IRC-FSH center, Department of Health Sciences, University "Magna Græcia" of Catanzaro, Italy

This research is interesting for two different reasons. The first one is the obtaining of oleuropein. Usually, oleuropein is extracted from olive oil or olives. In this research, we have obtained it from leaves, that are normally considerated a waste product. In particular we have obtained oleuropein through a highly sustainable green chemistry extraction protocol. In recent decades, the importance of green chemistry has increased exponentially for several reasons: 1) it avoids the use of dangerous products and organic solvents; 2) It gives new life to waste; 3) It is highly sustainable, reducing pollution and protecting the environment.
The second reason is that, once oleuropein is obtained, we have tested it on in vitro model, and we have demonstrated that it was able to prevent the damage induced by treatment with doxorubicin, involving a cellular organelle: the endoplasmic reticulum.

P42. Antioxidant and neuroprotective properties of an aqueous dry extract from the *S. officinale* aerial parts

Napolitano B[1,2], Percaccio E[1], Másedas G[2,3], Garzoli S[4], Nicotra G[5], Di Giacomo S[1,6], Les F[2,3], López V[2,3], Di Sotto A[1]

[1]*Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy.*
[2]*Department of Pharmacy, Faculty of Health Sciences, Universidad San Jorge, Villanueva de Gállego (Saragossa), Spain*
[3]*Instituto Agroalimentario de Aragón-IA2 (CITA-Universidad de Zaragoza), Saragossa, Spain*
[4]*Department of Chemistry and Pharmaceutical Technologies, Sapienza University of Rome, Italy*
[5]*EPO S.r.l., Milan, Italy*
[6]*Department of Food Safety, Nutrition and Veterinary Public Health, National Institute of Health, Rome, Italy*

Sisymbrium officinale (L.) Scop. (syn. *Erysimum officinale* L., hedge mustard; Fam. Brassicaceae), is a medicinal plant used traditionally to relief respiratory system's disorders, hence its "singers' plant" name [1]. It has been found endowed with myorelaxant, antimicrobial, antimutagenic, antinflammatory and antioxidant activities, likely ascribed to the presence of glucosinolates (mainly glucoputranjivin and isopropyl isothiocyanate) and polyphenolic compounds [1,2]. Glucosinolates are also shown to possess promising chemopreventive and neuroprotective properties [3]. In line with this evidence, in the present study, we investigated the potential neuroprotective and antioxidant properties of an aqueous dry extract of the *S. officinale* aerial parts (EPO S.r.l.; standardized to contain 0.50% w/w glucosinolates), in brain cell models. To this end, murine Neuro-2a neuroblasts and human neuroblastoma SHSY-5Y cells were used. The protective effects were assessed towards the oxidative damage induced by hydrogen peroxide (H_2O_2), tert-butyl hydroperoxide (tBOOH) and amyloid beta peptide (Aβ). Preliminarily, the cytotoxicity of the extract (24 h exposure) was assayed by the MTT assay, to select the nontoxic concentrations to be tested for the cytoprotection; the intracellular concentration of the free peroxide and hydroxyl radicals was measured too [4,5]. Antioxidant in vitro assays, including FRAP (Ferric Reducing Antioxidant Potential), ORAC (Oxygen Radical Antioxidant Capacity), DPPH, xanthine/xanthine oxidase, along with the inhibition of central nervous system (CNS) enzymes (monoaminoxidase A, acetylcholinesterase, tyrosinase), known to be involved in neurodegenerative diseases have been also performed [4]. The extract's tolerability was confirmed in the *Artemia* invertebrate model. A chemical characterization of the extract was obtained by derivatization followed by GC-MS (gas chromatography-mass spectrometry) analysis. The obtained results highlighted that the extract was able to prevent the oxidative stress induced by hydroperoxides in Neuro-2a neuroblasts and by Aβ protein in SHSY-5Y cells. However, mitochondrial activity and cell viability of Neuro2a cells were not affected by the treatments. The *S. officinale* extract has also shown an antioxidant capacity against the free radical DPPH and superoxide anions generated by xanthine/xanthine oxidase (X/X.O.) system, almost reaching and overcoming, respectively, the percentage of inhibition exerted by the standard antioxidant compounds. Conversely, weak, or null inhibitory effects were highlighted against CNS enzymes, compared to the reference compounds. At last, the extract showed to be well tolerated up to the concentration of 500 µg/ml in the *Artemia* invertebrate assay. At the phytochemical analysis, the extract revealed the presence of a rich phytocomplex, characterized by alcohols, sugars, fatty acids and carboxylic acids. Altogether,

the obtained results highlighted the tested *S. officinale* extract is a source of bioactive compounds with potential neuroprotective properties. Further studies could allow to clarify the role of phenolic compounds and glucosinolates in *S. officinale* bioactivities, to develop improved standardized extracts to be exploit for neuroprotective purposes and to confirm these activities *in vivo*.

P43. Essential oils bearing specialized metabolites with potential tyrosinase inhibitory activity: focus on dermal absorption from topical formulations

Sgorbini B, Capetti F, Pavarino M, Cagliero C, Argenziano M, Cavalli R, Bicchi C, Rubiolo P
Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Turin, Italy

Downregulation of tyrosinase is a widely used approach to reduce excessive melanin production and prevent typical age-related skin diseases (e.g., freckles and solar lentigo). Plants are an interesting source of skin-whitening agents. So far, mainly phenolic compounds have been investigated, while terpenoids have been relatively little explored as tyrosinase inhibitors. In this study the tyrosinase inhibitory activity of sixty-nine essential oils (EOs) was first investigated in an *in vitro* enzymatic assay, with Kojic acid serving as a positive control. The chemical composition of the investigated EOs was also characterized by gas chromatography coupled with mass spectrometry. Among the essential oils studied, citral containing EOs (i.e., *Cymbopogon schoenanthus* Spreng (L.), *Litsea cubeba* (Lour.) Pers, *Melissa officinalis* L., and *Verbena officinalis* L.), and the β-myrcene containing EOs (i.e., *Juniperus communis* L. and *Pinus mugo* Turra EOs) were the most promising. The obtained results showed the advantages of using mixtures of compounds/EOs instead of single molecules and underlined the possible additive effect of two monoterpenoids (i.e., β-myrcene and citronellal) on the inhibitory activity of citral.
Since citral is a fragrance that is also listed as an allergen among the 26 fragrances added to Annex III of the Cosmetics Directive by the 7th amendment (2003/15/ EC), this study aimed to blend different essential oils to reduce the amount of citral while maintaining tyrosinase inhibitory activity by exploiting the additive/synergistic effect of essential oils containing β-myrcene and citronellal. Two different formulations (oil/water emulsion and oil solution) containing a mixture of three EOs (i.e. *Litsea cubeba*, *Pinus mugo*, *Cymbopogon winterianus*) and applied to the skin both in non-occlusive and partially occlusive mode were evaluated. The amount of citral and other bioactive compounds (e.g., myrcene, citronellal) from the selected EOs delivered through the skin was evaluated depending on the type of formulation and the mode of application. For this purpose, solvent-free headspace sampling was used in combination with a fast GC-MS analysis using narrow bore columns. The results obtained indicate that an oil/water emulsion is preferable because it releases the bioactive compounds rapidly and minimises their evaporative loss due to their relatively high vapour pressure. In addition, semi-occluded conditions were required to prevent evaporation, resulting in limited availability of the bioactive compounds in the viable skin.

P44. Anti-aging activity of natural extract for phytocosmetic applications

Sacilotto A[1], Tinazzi M[1], Giacomini I[1,2], Montopoli M[1,2], Cocetta V[1]

[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy

[2]VIMM - Veneto Institute of Molecular Medicine, Padova, Italy

Skin aging is a biological process characterized by a functional decline evidenced by the formation of wrinkles, loss of elasticity, laxity, and rough appearance. Endogenous factors, as well as external assault, continuously affect the skin leading to structural changes (for example in extracellular matrix components such as collagen and elastin) and inducing physiological-functional alterations that have been shown to be related to increased risk of developing skin pathologies and in the skin aging process itself. Ultraviolet radiation (UV) plays a fundamental role in photo-aging and cellular senescence of skin, as well as in increasing reactive oxygen species (ROS) production.

Considering these aspects, the use of a natural compound with antioxidant and anti-senescent activity appears to be a promising anti-aging candidate. Previous work of our laboratory has already demonstrated a promising anti-aging activity of an extract of Salvia Haenkei (1,2). Therefore, in this work, a different extract (AS) has been used to explore its possible role in skin physiology.

HaCaT human keratinocytes cell line was used as skin model, in which anti-senescent activity was assessed by the beta-galactosidase assay and RT-PCR.

The extract (AS) demonstrated anti-senescence activity, even after induction of senescence by UVB exposure; moreover, results showed antioxidant activity, with a decrease in ROS levels even in the presence of oxidative stress. Even if further studies are needed, preliminary results indicate AS extract as a promising candidate in anti-aging cosmetics.

P45. Screening of extracts from *Crataegus laciniata* Ucria (Rosaceae) for the treatment of skin hyperpigmentation

Mirabile S[1,2], Cacciola A[3], Raimondo FM[4], D'Angelo V[1], Germanò MP[1]

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Stagno D'Alcontres 31, 98166 Messina, Italy.*

[2]*Foundation Prof. Antonio Imbesi, University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy.*

[3]*Department of Clinical and Experimental Medicine, University of Messina, Via Consolare Valeria 1, 98124 Messina, Italy.*

[4]*PLANTA/Centro autonomo di Ricerca, Documentazione e Formazione, Via Serraglio Vecchio 28, 90123 Palermo, Italy.*

Tyrosinase (TYR, EC 1.14.18.1) has been recognized as a key target for the screening of novel bioactive agents for dermatological disorders based on melanin accumulation [1]. The well-known tyrosinase inhibitors such as hydroquinone, kojic acid, arbutin, and phenylthiourea have numerous adverse effects which hampering their long-term application [2]. Due to these safety concerns, the identification and isolation of new compounds from natural sources have attracted increasing interest.

Hawthorn (*Crataegus* spp.) is a large genus of small shrubs and trees belonging to Rosaceae family widely present in North Europe, temperate Asia, Africa and North America including from 150–500 up to 1200 species. In recent years, hawthorn has been demonstrated to be an excellent source of many natural bioactive molecules, which have promising benefits on human health [3].

In the present study, we focused the attention on *Crataegus laciniata* Ucria, a species growing in Sicily, Iberian Peninsula and North Africa (Morocco and northern Algeria). We investigated the inhibitory effects of *C. laciniata* fruit (CFR) and flower (CFL) methanolic extracts on TYR. Results showed that only CFL efficiently inhibits both the monophenolase and diphenolase activity of *Agaricus bisporus* TYR (IC_{50} 67.47 ± 3.86 µg/ml and 103.61 ± 5.46 µg/ml, respectively). In addition, a preliminary phytochemical study demonstrated that CFL is the richest in total phenols (291.08 ± 5.10 mg gallic acid equivalents/g), especially in flavonoids (253.14 ± 7.81 mg rutin equivalents/g).

Moreover, we employed the zebrafish *(Danio rerio)* assay for toxicity assessment as this *in vivo* model presents a high correlation with humans in response to pharmaceutical and cosmetic testing [4]. Early-stage zebrafish embryos at 24 hours post fertilization (hpf) when exposed to varying concentrations of CFL until 96 hpf showed a good survival percentage and the absence of malformations, thus excluding toxic effects.

In conclusion, this work outlines that CFL could be a potential candidate for the treatment of skin disorders due to its favorable safety profiles.

P46. Phytochemical and biological characterization of *Fragaria x ananassa* Duch.

Di Sotto A[1], Percaccio E[1], Di Giacomo S[1,2], Mezzanotte E[1,3], Milana MR[4], Cesa S[5], Ingallina C[5], Acciaro E[6], Cairone F[5], Vitalone A[1]

[1]*Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Italy*
[2]*Department of Food Safety, Nutrition and Veterinary Public Health, National Institute of Health, Rome, Italy*
[3]*Regulatory Affairs Italy – Alfasigma S.p.A. Rome*
[4]*Department of Environment and Health, Italian Institute of Health, Italy*
[5]*Department of Chemistry and and Technology of Drugs, Sapienza University of Rome, Italy*
[6]*"Annalaura Segre" Magnetic Resonance Laboratory, Institute for Biological Systems, CNR, Monterotondo, Italy*

The Favetta strawberry (*Fragaria* x *ananassa* Duch.), also called the strawberry of Terracina (Italy), is a much-loved fruit, with a sweeter flavor than other strawberry varieties, likely associated to its phenolic composition. In the attempt to valorize the local species of Lazio Region and to highlight possible nutraceutical properties of the strawberry of Terracina, we performed a multimethodological phytochemical and biological study. Particularly, the ripe fruits were manually picked, transported within 4 hours to the Italian Institute of Technology of Rome, and stored at -80 °C. Different organic and hydroalcoholic Bligh-Dyer extracts were prepared and dried under a nitrogen flow at the Italian Institute of Health, using a TurboVap® instrument. The hydroalcoholic extracts were subjected to both untargeted and targeted phytochemical analysis. The total amount of polyphenols, tannins and flavonoids were determined by the Folin-Ciocâlteu and aluminium chloride assays, respectively; moreover, the phenolic composition was evaluated by chromatographic analysis. The nuclear magnetic resonance spectroscopy was also made to achieve a comprehensive metabolomic strawberry profile. An in vitro screening of different biological activities, including cytotoxicity in colorectal (Caco-2) carcinoma cells, antioxidant cytoprotective properties towards the oxidative damage induced by tert-butyl hydroperoxide (tBOOH), inhibition of AGE (advanced glycation end-products), and of metabolic enzymes (α-amylase, α-glucosidase, lipase), was also performed.

The obtained drug-extract ratio was 18.6 for the hydroalcoholic extract and 593.0 for the organic one, the latter resulting more concentrated in total polyphenols (232.2 μg TAE/mg vs 2.5 μg TAE/mg), tannins (94.1 μg vs 2.4 μg TAE/mg), and flavonoids (105.5 μg QE/mg and 30.6 μg QE/mg). According to Peterson and Dwyer (1998), the strawberry of Terracina analyzed can be classified as fruits with a high content of flavonoids in the fresh fruits. Comparing the NMR results with data reported in BDA and FooDB databases for other strawberry varieties, the strawberry of Terracina showed a higher content of saturated fatty acids, glucose and fructose, despite a lower amount of monounsaturated fats, linoleic and linolenic acid, sucrose, and amino acids. In regard to the bioactivities, a 50% cytotoxicity was achieved in Caco-2 cells at the highest concentration, while no cytoprotection was highlighted against the damage induced by the pro-oxidant agent tBOOH. The hydroalcoholic extract was also able to inhibit the AGE formation by about 70% with respect to a 20% inhibition induced by the organic one. Moreover, the hydroalcoholic and organic extracts weakly (approximately 30% and 20%, respectively) inhibited the α-amylase and lipase activities. Conversely, both samples significantly interfered with the α-glucosidase enzyme, inducing a 50% and 90% inhibition, respectively. In conclusion, present results highlighted that the strawberry of Terracina is a source of phenolic compounds, especially

flavonoids; moreover, it is endowed with antiglucosidase properties which suggest a potential usefulness as an adjuvant food in hyperglycemia and slimming diets; however, further studies are needed to confirm its potential interest in vivo and to develop standardized extracts to be exploited in the nutraceutical field.

P47. Boosting *Coriandrum sativum* essential oil yield and quality using natural deep eutectic solvents (NADES)

Scandar S, Zadra C, Lanari D, Marcotullio MC
Department of Pharmaceutical Sciences- University of Perugia

Coriandrum sativum L. seeds are a promising secondary metabolite source with potential applications in the pharmaceutical and cosmetic industries.[1] The conventional methods for extracting these metabolites are often associated with low yield, time consumption, and environmental pollution.[2]

In recent years, the use of Natural Deep Eutectic Solvents (NADES) has been gaining attention as a promising green alternative to conventional solvents for the extraction of bioactive compounds from plant material due to their natural origin, biodegradability, and low toxicity.[3]

In this study, we aimed to optimize the use of choline chloride-based NADES as additives for *Coriandrum sativum* seeds essential oil (CEO) preparation.

An initial comprehensive single factor ad-hoc screening study was conducted on choline chloride (ChCl)-based NADES with different hydrogen bond donors (HBD) using various hydrogen bond acceptors (HBD: glucose, glycerol, citric acid and urea), in different HBA-HBD ratios (1:1, 1:2, 1:3, 2:1, 3:1).

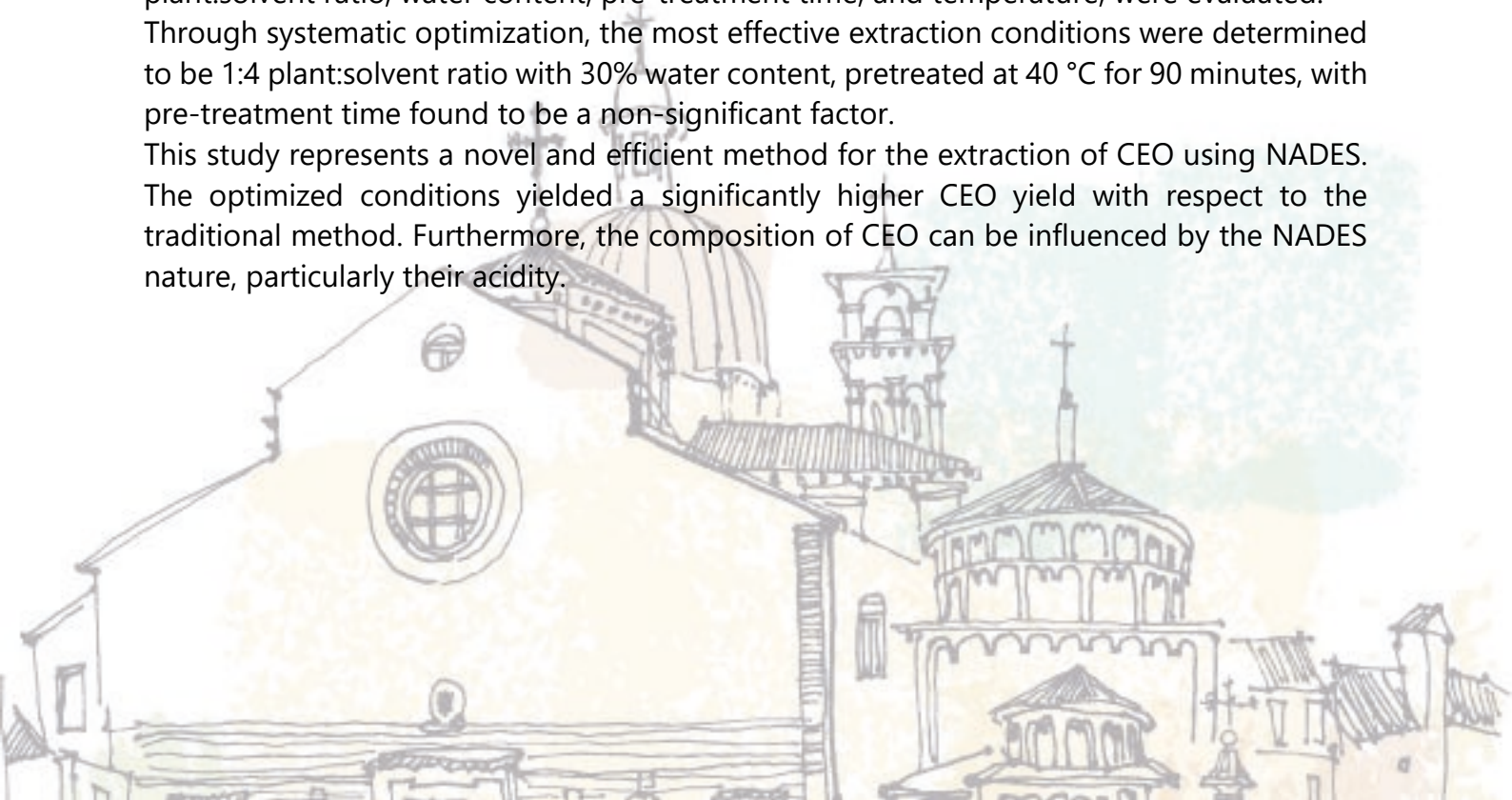
Plant material and aqueous NADES (40% w/w) in a 1:5 ratio were pretreated at 25 °C for 30 min. in an ultrasonic bath and after hydrodistilled for 4h. The results demonstrated that the 1:1 ChCl-Urea NADES outperformed the traditional hydrodistillation method by producing a higher yield of CEO.

To further explore the impact of NADES on CEO composition, GC-MS spectroscopy was utilized to examine the composition of each CEO. The analysis showed that the nature of the NADES played a significant role in determining the composition of the isolated CEO.

In the subsequent phase of the study, the previously established extraction parameters for CEO were optimized using 1:1 ChCl-Urea NADES. Four independent variables, including plant:solvent ratio, water content, pre-treatment time, and temperature, were evaluated.

Through systematic optimization, the most effective extraction conditions were determined to be 1:4 plant:solvent ratio with 30% water content, pretreated at 40 °C for 90 minutes, with pre-treatment time found to be a non-significant factor.

This study represents a novel and efficient method for the extraction of CEO using NADES. The optimized conditions yielded a significantly higher CEO yield with respect to the traditional method. Furthermore, the composition of CEO can be influenced by the NADES nature, particularly their acidity.



P48. Marine macroalgae as a promising source of natural product

Armeli Minicante S[1], Spagnuolo D[2,3], Manghisi A[3], Morabito M[3], Genovese G[3]
[1]*Università IUAV di Venezia*
[2]*Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS)*
[3]*Università degli Studi di Messina*

From the ancient time, marine macroalgae have proven to be a promising source of natural products with various pharmacological and biological activities, including antiviral, anticoagulant, anti-tumoral, antimetastatic, and anti-inflammatory effects (Leandro et al. 2019, Wang et al. 2020). Today more than ever, macroalgae are a precious resource for the extraction of active molecules, and interest on them has increased widely in recent years, also due to the intrinsic eco-sustainability of the algal industry. In our studies, different species of macroalgae were collected from Venice Lagoon, Lake Ganzirri, and the Strait of Messina, in Italy, to investigate the biological activity of sulphated polysaccharides and secondary metabolites. Extracts from *Undaria pinnatifida* (*Ochrophyta*) and *Asparagopsis taxiformis* (*Rhodophyta*) were tested against *Leishmania infantum*, the prevalent agent of leishmaniasis in the Mediterranean, showing a remarkable activity and demonstrating the potential of macroalgae as a relevant source of antiprotozoal products (Genovese et al. 2012, 2013, Vitale et al. 2015, Armeli Minicante et al. 2016) Additionally, marine macroalgae were found to produce a wide variety of bioactive metabolites, which exhibit antiviral, hypocholesterolaemic, hypotensive, antibacterial, anticoagulant, anthelmintic, anticancer, cytotoxic, and antifungal activities (Faggio et al. 2015, Rocha et al. 2018, Álvarez-Viñas et al. 2021). In this context, the biological activity of polysaccharides from *Chaetomorpha aerea* (*Chlorophyta*), *Agardhiella subulata* and *Hypnea cornuta* (*Rhodophyta*) were evaluated for their effects on the adhesion and biofilm formation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Zammuto et al. 2022). Crude extracts showed dose-dependent inhibitory effects on biofilm formation, without exerting any antibacterial activity.
In conclusion, macroalgae demostrated their amazing potential as a source of natural products for the development of biomolecules, which may provide novel drugs. Furthermore, when marine macroalgal biomass collected in dystrophic environments are exploited, the added value of transforming a waste into an economic resource is gained (Spagnuolo et al. 2022).

P49. Optimization of the microwave-assisted hydrodistillation of Carlina acaulis essential oil by fractional factorial design study

Spinozzi E[1], Ferrati M[1], Lo Giudice D[1], Felicioni E[1], Petrelli R[1], Benelli G[2], Maggi F[1], Cespi M[1]

[1]Chemistry Interdisciplinary Project (ChIP) research center, School of Pharmacy, University of Camerino, via Madonna delle Carceri 9/B, 62032 Camerino, Italy

[2]Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 856124 Pisa

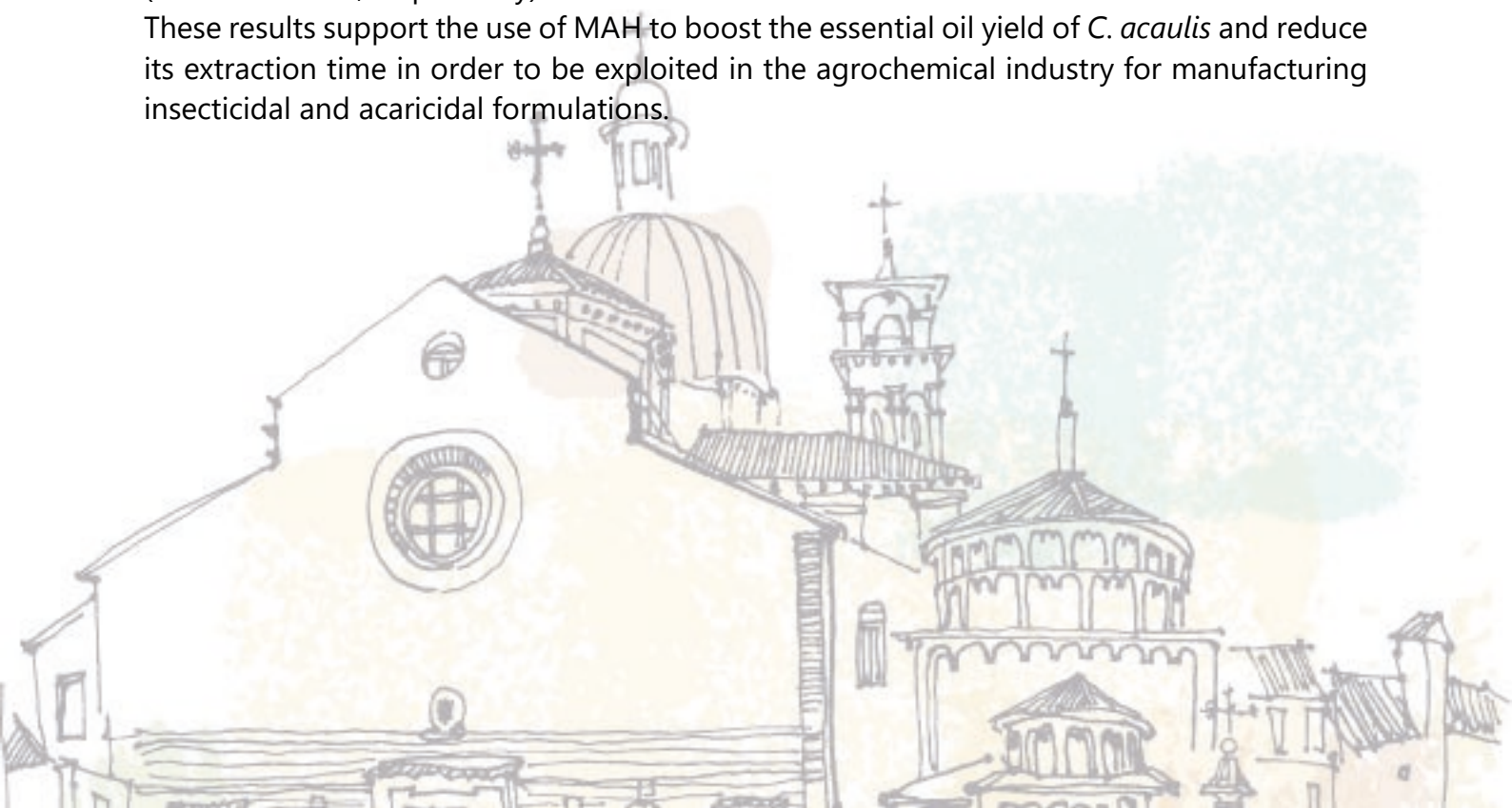
Recent scientific evidence has shown that the essential oil from the roots of *Carlina acaulis* L. (Asteraceae) is a promising insecticidal agent for the management of insect vectors, pests and mites of medical and agricultural importance.[1] These effects are ascribable to its main compound, the polyacetylene carlina oxide.[2]

Usually, the essential oils are obtained by classical methods such as hydrodistillation and steam distillation. However, several advanced extraction techniques have been recently introduced among which microwave-assisted hydrodistillation (MAH) has the advantage to boost the essential oil yield and reduce the extraction time with lower energy and water consumption costs.[3] The extraction principle of this technique is based on the absorption of microwave electromagnetic energy by plant cell water through dipole rotation and ionic conductance causing cell membrane disruption and release of active principles.

In this work, we optimized the extraction of *C. acaulis* essential oil using a one-step Design of Experiments (DoE). Suitable mathematical models were used to determine the optimal operative conditions (e.g., extraction time, microwave irradiation power and water/roots ratio) capable of increasing the essential oil yield and the carlina oxide content. Preliminary screening pointed out that only the extraction time had a significant influence on the extraction yield while the content of carlina oxide was not affected by the varied experimental settings as resulted from GC-MS quantitative determination.

For comparative purposes a conventional hydrodistillation under the same conditions of the optimized MAH protocol was performed highlighting a higher efficiency of the latter process in terms of yield (0.65 vs 0.49%, respectively) while the content of carlina oxide was similar (98.6 and 97.9%, respectively).[4]

These results support the use of MAH to boost the essential oil yield of *C. acaulis* and reduce its extraction time in order to be exploited in the agrochemical industry for manufacturing insecticidal and acaricidal formulations.



P50. Anthraquinones: genotoxic until proven otherwise? A study on a substance-based medical device to implement available data for a correct risk assessment

Gasperini S, Lenzi M
Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna

A substance-based medical device (SMD) containing anthraquinones has been the objective of this genotoxicological study. In fact, this class of naturally occurring molecules, i.e., anthraquinones and in particular hydroxyanthracene derivatives (HADs), has been associated with health risks, including genotoxic and carcinogenic effects, even though discordant data on the matter is available. To assess the potential mutagenic effect of the SMD, in this study the "In Vitro Mammalian Cell Micronucleus Test" was performed on human lymphoblastoid TK6 cells according to a flow cytometric protocol. For this purpose, cell cultures were treated with concentrations of the SMD in the range of 0-2 mg/mL for a short treatment time (3 h) both in the absence and presence of an exogenous metabolic activation system, followed by a recovery period in fresh medium (23 h), and for an extended treatment time (26 h) without the exogenous metabolic activation system. At the end of both treatment times, cytotoxicity, cytostasis, apoptosis and micronuclei (MNi) frequency were analyzed in treated cultures and then compared with those measured in concurrent negative control cultures. The SMD did not induce a statistically significant MNi frequency increase under any of the experimental conditions tested. The negative outcome shows that the SMD is non-mutagenic in terms of the ability to cause chromosomal aberrations both in the absence and presence of an exogenous metabolic activation system. Finally, the study ended by analyzing intracellular ROS levels after treatment with the SMD to exclude the pro-oxidant ability, typically linked to DNA damage. Once again, a negative outcome was obtained and, on the contrary, our results demonstrated the ability of the SMD to counteract oxidative stress.

P51. Erucin, a natural hydrogen sulfide (H_2S) donor, improves skeletal muscle (SKM) dysfunction related to obesity

Casale V, Smimmo M, Casillo GM, Bello I, Panza E, Cirino G, Bucci M, Vellecco V
Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via D. Montesano, 49 80131 Naples

H_2S is an endogenous gasotransmitter produced by the reverse transsulfuration-pathway, through the action of three enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-Mercaptopyruvate-sulfurtransferase (3-MST). H_2S is involved in many physio-pathological processes in both central nervous system and peripheral organs and tissues (1). Among the emerging mechanisms of action ascribed to H_2S biological activities, the post-translational modification persulfidation has been identified; proteins that have an accessible cysteine thiol group can be persulfidated leading to a change in their activity (2). This evidence has been further strengthened by the finding that in many pathological conditions, including SKM disorders and obesity, the expression of one or more H_2S-generating enzymes is reduced with consequent impairment of H_2S biosynthesis (3-5). From here, the need to identify new natural H_2S-donors as a potential therapeutic strategy to recover the H_2S deficit. In this context, our attention has been focused on Erucin, a natural isothiocyanate particularly abundant in arugula (*Eruca sativa* Mill., *Eruca vesicaria* L., etc.). Erucin exerts several beneficial actions, including anti-inflammatory, vasorelaxing, hypoglycemic, and anti-obesity effects due to its capacity of releasing H_2S (6). Based on this evidence, the present study aims to evaluate the protective role of Erucin in SKM dysfunction related to obesity by using both *in vitro* and *in vivo* settings.

In vitro studies were performed on murine SKM myotubes (C2C12). Cells were treated with Erucin (3μM; 2h) and then stimulated with Sodium Palmitate (SP, 300μM; 6h), a saturated-free fatty acid to mimic the hyperlipidemic conditions *in vitro*. In a separate set of experiments, SP-exposed cells were treated with a selective Sirtuin-1 (SIRT-1) inhibitor (EX-527; 100μM; 30min) followed by the addition of Erucin. Then, the expression of H_2S-generating enzymes, as well as the SKM-specific inflammatory markers: tumor necrosis factor 1-α, and inducible nitric oxide synthase (TNFα, iNOS) were evaluated by RT-PCR. SIRT-1 persulfidation was also evaluated. *In vivo* studies were performed on db/db mice, a genetic murine model of obesity. Mice were treated with Erucin (3mg/kg) *by gavage* for 4 weeks, then locomotor tests rotarod, and weight tests were performed. Evaluation of H_2S generating enzymes expression on quadriceps of db/db mice was also evaluated.

SP-exposed C2C12 displayed a significant increase of iNOS and TNFα, coupled with downregulation of 3-MST/H_2S signaling. Treatment with Erucin recovered the increased expression of both iNOS and TNFα, to control values. Pre-treatment of SP-exposed cells with EX-527 significantly reduced the beneficial effect exerted by Erucin on iNOS and TNFα expression. The persulfidation assay showed that SIRT-1 was persulfidated in normal conditions and this signal was lost in SP-exposed cells. Notably, Erucin treatment induced SIRT-1 persulfidation in SP-exposed cells. Finally, *in vivo* experiments showed that db/db mice displayed a significant reduction in SKM performance compared to healthy mice, coupled with a reduction of 3-MST expression. Erucin treatment significantly ameliorated the impaired locomotor activity observed in db/db mice.

In SKM dysfunction associated with obesity, the 3-MST/H_2S pathway is reduced, and this event accounts for impaired SIRT-1persulfidation. Erucin, by releasing H_2S, replaces SIRT-1 persulfidation, exerting a protective role on obesity-induced SKM disorders.

P52. Phytochemical investigation and antibacterial activity of *Satureja bachtiarica* Bunge aerial parts

Rahmani Samani M, D'Urso G, Masullo M, Piacente S
University of Salerno, Department of Pharmacy

Satureja bachtiarica Bunge is a perennial and aromatic plant of Lamiaceae family, distributed in the Zagros mountain of Iran. It is used as traditional medicinal herb and spice [1,2]. For the essential oil of *Satureja* spp., a wide range of biological and pharmacological properties including antioxidant, anti-inflammatory, antispasmodic, antibacterial, antiviral, and anti-nociceptive have been reported [3]. Little is known about the polar constituents of this species. For this reason, a phytochemical investigation of the aerial parts has been carried out along with the evaluation of the antibacterial activity. The air-dried aerial parts of *S. bachtiarica* were extracted by maceration at room temperature using EtOH-H_2O 7:3 v/v. The obtained extract was subjected to *n*-butanol /water partition to remove free sugars. An analytical approach based on LC-HRMS/MS analysis by using a Linear Ion Trap-Orbitrap hybrid mass spectrometer with electrospray ionization, in negative ion mode was carried out. The metabolite profile obtained guided the isolation of 38 compounds, of which the structures have been unambiguously elucidated by NMR analysis. Different classes of natural compounds have been identified, mainly belonging to monoterpenes, flavonoids, organic acids, indoles, phenylpropanoids, phenolics, lignan, coumarin, and biphenyls. A biphenyl derivative, never described before, has been characterized.

The antimicrobial activity of the extract and of main isolated compounds occurring in the extract was evaluated against Gram-positive and Gram-negative bacteria. Moreover, the ability of compounds to inhibit the formation of biofilms by these bacteria and to affect the metabolism of microbial cells present within the biofilms has been evaluated.

P53. Phenolic profile and biological activities of hydroalcoholic extracts from leaves, flowers and stems of *Sinapis pubescens* L. subsp. *pubescens* (Brassicaceae) wild from Sicily (Italy)

Arena P[1,2], Miceli N[1], Marino A[1], Davì F[1,2], Cavò E[1], Spadaro V[3], Raimondo FM[4], Cacciola F[5], Laganà Vinci R[1], Mondello L[1,6,7], Taviano MF[1]

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy*

[2]*Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy*

[3]*Department STEBICEF/Section of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy*

[4]*PLANTA/Research, Documentation and Training Center, Via Serraglio Vecchio 28, 90123 Palermo, Italy*

[5]*Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, Via Consolare Valeria, 98125 Messina, Italy*

[6]*Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy*

[7]*Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, via Àlvaro del Portillo 21, 00128 Rome, Italy.*

In recent years, species belonging to the *Brassicaceae* family that grow spontaneously in Sicily (Italy) are being studied by our research team to discover new potential sources of bioactive compounds. As part of a project aimed at the investigation of the specific and intraspecific taxa of *Sinapis* L. that grow wild in Sicily, the present study is focused on *Sinapis pubescens* L. subsp. *pubescens* (hairy mustard), an edible species not studied so far.

Specifically, the hydroalcoholic extracts obtained from the aerial parts (leaves, flowers, and stems) of *S. pubescens* subsp. *pubescens* were investigated for their phenolic profile and biological properties. The quantitative determination of total polyphenols, flavonoids, and condensed tannins was attained by spectrophotometric methods; the phenolic profile of the extracts was characterized by means of HPLC-PDA/ESI-MS analysis, which led to the identification of 55 compounds. The comparison of the polyphenolic compounds detected in the flowers, leaves, and stems highlighted consistently different qualitative-quantitative profiles.

The antioxidant properties of the extracts were investigated by *in vitro* methods based on different mechanisms: the primary antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and reducing power assays, while the secondary antioxidant properties were determined by the ferrous ion chelating activity assay. The aerial parts of *S. pubescens* subsp. *pubescens* were found to be a valuable source of antioxidant compounds; particularly, the leaf extract showed higher primary antioxidant properties than the others, both radical scavenging activity (IC_{50}= 0.53 ± 0.02 mg/mL) and reducing power (15.11 ± 0.14 ASE/mL), which appear to be related to the total polyphenol content. On the other hand, the flower extract showed greater secondary antioxidant properties, namely Fe^{2+} chelating activity (IC_{50}= 0.49 ± 0.02 mg/mL), which seem to be related to the flavonoid content. The antimicrobial properties were investigated against bacteria and yeasts by standard methods; none of the extracts showed antimicrobial activity against the strains tested. Finally, the extracts resulted non-toxic after a preliminary toxicity evaluation by the *Artemia salina* lethality bioassay (LC_{50} > 1000 µg/mL), indicating their potential safety.

The present study substantially improves the knowledge of *S*. *pubescens* subsp. *pubescens* hitherto unexplored, indicating a promising use of wild species, included in the genus *Sinapis,* as new sources of active metabolites which could provide health benefits.

P54. Investigation of the anti-inflammatory activity of oleocanthal and oleocanthalic acid in an *in vitro* model of neuroinflammation

Rinaldi I[1], Barbalace MC[1], Freschi M[1], Malaguti M[1], Digiacomo M[2], Giusti L[3], Lucacchini A[2], Angeloni C[1], Hrelia S[1]

[1]Department for Life Quality Studies, Alma Mater Studiorum, University of Bologna, Italy
[2]Department of Pharmacy, University of Pisa, 56126 Pisa, Italy
[3]School of Pharmacy, University of Camerino, Italy

The increase in life expectancy is leading to a consequent rise in the incidence of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, which mainly affect the elderly population. These conditions share several features, such as abnormal protein deposition, oxidative stress, impaired mitochondrial function, and neuroinflammation. The latter plays a crucial role in the pathogenesis of these diseases, and it involves the activation of microglia, the resident immune cells of the brain with an important role in maintaining CNS (central nervous system) homeostasis. In neurodegenerative diseases, microglia are excessively activated, leading to the overproduction of pro-inflammatory mediators such as cytokines, reactive oxygen species, and nitric oxide (NO), causing neuronal death.

Extra virgin olive oil (EVOO) has been widely associated with beneficial properties, mainly attributed to its phenols, which exert antioxidant and anti-inflammatory activities. Among them, oleocanthal (OL) is known for its several demonstrated health benefits. Of note, it has a strong anti-inflammatory "ibuprofen-like" activity since it inhibits the cyclooxygenase enzymes.

During olive oil shelf-life, phenolic compounds may be modified by different processes. In particular, OL is progressively oxidized to oleocanthalic acid (OA) and this conversion is accelerated by inappropriate storage conditions. To date, the biological effects of OA are completely unknown, and the anti-inflammatory activity of OL has not been characterized in the brain yet. On these bases, the aim of this study was to investigate the potential anti-inflammatory activity of OL and OA in an in vitro model of neuroinflammation.

BV-2 microglial cells were treated with OL and OA at different concentrations and after 2 h activated with LPS 100 ng/ml for 24 h. NO production, analyzed with Griess assay, was significantly reduced by OL, but not by OA, compared to cells exposed only to LPS. Furthermore, OL significantly decreased the gene expression of pro-inflammatory factors, namely iNOS, NLRP3, COX2, IL-1β, IL-6 and TNF-α and up-regulated anti-inflammatory mediators such as IL-4 and CD206, compared to LPS. Conversely, OA significantly reduced only the expression of IL-1β and COX2, although to a lesser extent than OL. In addition, the protein levels of iNOS and NLRP3, analyzed by immunoblotting, were significantly reduced by OL compared to LPS, but not by OA. Interestingly, OL was able to completely inhibit p38 MAPK phosphorylation induced by LPS suggesting that the reduction of neuroinflammation triggered by OL could be, at least partially, due to the modulation of this signaling pathway.

In conclusion, our data clearly demonstrate that OL exerts a strong anti-inflammatory activity in the brain, by efficiently counteracting microglia activation. This confirms the beneficial role of EVOO consumption for human health. Nonetheless, the strong biological activity of this compound is lost when it's oxidized to OA. This emphasizes how crucial is to store EVOO

in appropriate conditions and highlights the importance of consuming EVOO as fresh as possible to benefit from the health effects of this product.

P55. Evaluation of the anti-adipogenic and lipolytic activity of *Ficus carica* L. and γ-oryzanol

Tirelli E[1], Pucci M[1], Chiocchio I[2], Mandrone M[2], Mac Sweeney E[1], Mastinu A[1,3], Memo M[1,3], Poli F[2], Uberti D[1,2], Abate G[1,3]

[1]Università degli Studi di Brescia, Dipartimento di Medicina Molecolare e Traslazionale (DMMT), Sezione di Farmacologia

[2]Università di Bologna, Dipartimento di Farmacia e Biotecnologie (FaBiT)

[3]NutriFun4Health, Center for Research and Services on Nutraceuticals and Functional Food for Health, Università degli Studi di Brescia

Due to the high prevalence of obesity and type 2 diabetes, the identification of new compounds capable of acting on adipocyte differentiation is of paramount importance. Adipose tissue is highly dynamic and the largest organ in humans involved in lipid storage and mobilization based on energy requirements. It consists of several cell types, including mature adipocytes, which can increase in number and size. Decreasing proliferation and adipogenesis at the early stage of adipocyte differentiation may represent potential targets for preventing or treating obesity and, in turn, diabetes. Therefore, the purpose of this study was to test the anti-adipogenic and lipolytic activities of two botanicals, *Ficus carica* L. (FC) and γ-Oryzanol (ORY), which are known as traditional medicines owing to their numerous pharmacological properties.

Murine pre-adipocytes were used for this purpose. 3T3-L1 cells were differentiated into mature adipocytes and then treated for 48 h with different concentrations of FC and ORY phytoextracts to assess cell viability and lipolytic activity. We subsequently analyzed the expression of specific genes involved in adipocyte differentiation and lipid metabolism, including PPAR-γ, C/EBP-α, Adiponectin, FABP4, and FAS. In addition, the composition of the FC phytoextract was characterized by phytochemical analysis.

FC and ORY were tested for cytotoxicity using an MTT assay. The results showed good viability and biocompatibility for both compounds. Then, using Red O, FC and ORY lipolytic activities were evaluated and a dose-dependent decrease in lipid accumulation in mature adipocytes treated with FC and ORY was observed. Treatments of 50 and 80 µg/ml for FC and of 15 and 30 µM for ORY were then chosen to better investigate their role in adipogenesis. In particular, FC phytoextract and ORY negatively affected adipocyte differentiation and modulated lipid metabolism, mainly by acting on the gene expression of C/EBP-α and PPAR-γ, which are important regulators of the adipogenic process and lipid metabolism, which in turn modulates the expression of downstream genes such as adiponectin. Finally, FC, phytoextracts and ORY were unable at any of the concentrations used to modulate the gene expression of FAS, an adipogenic enzyme involved in the de novo lipogenesis.

Our data demonstrate that both compounds are able to exert a lipolytic effect, reducing adipocyte differentiation and modulating lipid metabolism via the activation of PPAR-γ and its target genes such as FABP4 and Adiponectin. Further studies are needed to investigate the efficacy and safety of these compounds from a drug therapy perspective.

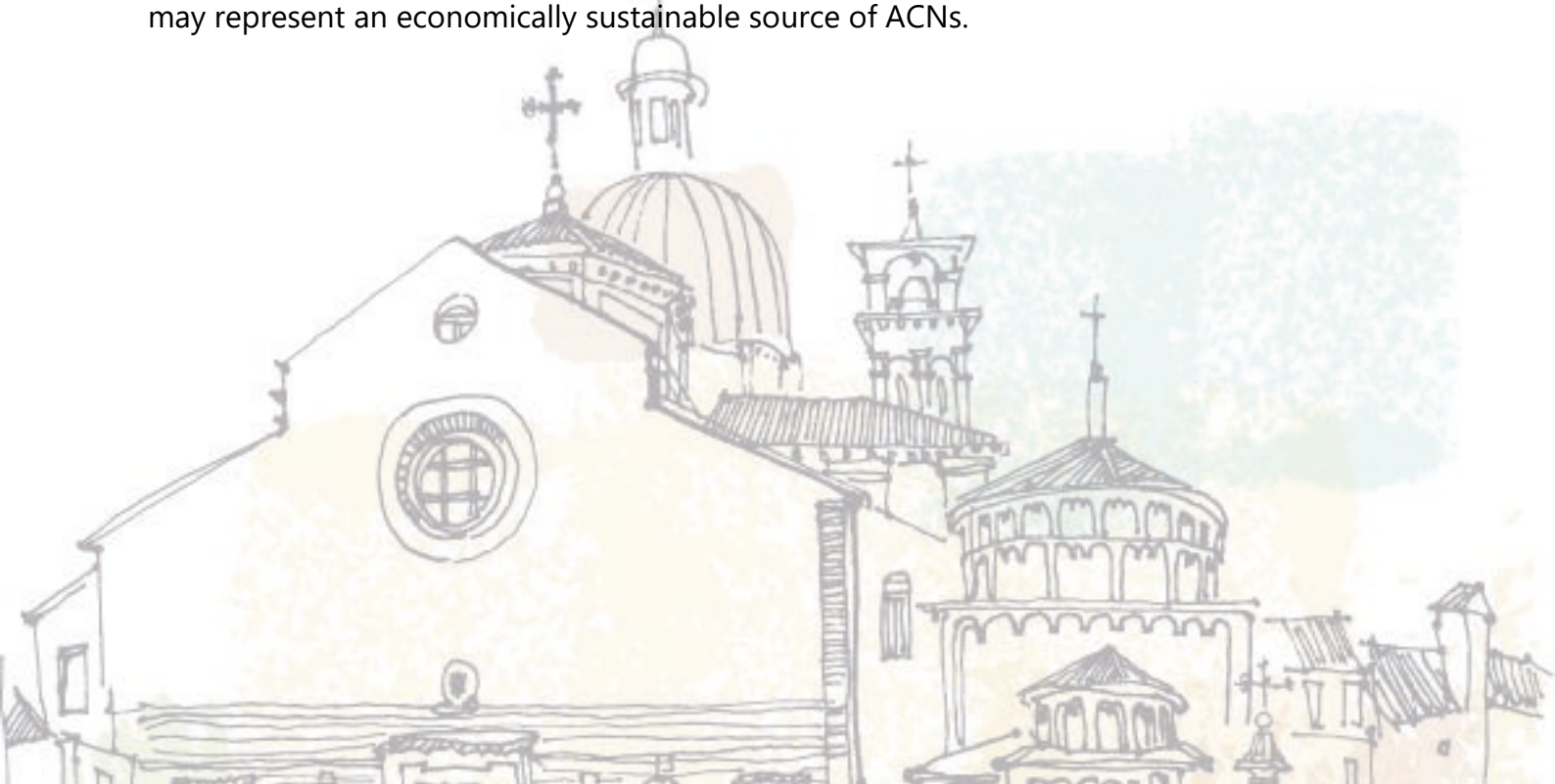
P56. Purple corn anthocyanins as a nutraceutical approach to prevent the progression of multiple sclerosis and its associated symptoms: preventive effect against neuroinflammation

Magni G[1], Marinelli A[2], Riboldi B[1], Romano S[3], Narbad A[3], Nicoletti C[4], Di Lorenzo C[1], Ceruti S[1], Petroni K[2]

[1]*Dept. Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy*
[2]*Dept. of Biosciences, Università degli Studi di Milano, Milan, Italy*
[3]*Quadram Institute Bioscience, Norwich, UK*
[4]*Dept. of Molecular and Developmental Medicine, Università degli Studi di Siena, Siena, Italy*

Anthocyanin (ACNs) are bioactive constituents of fruits and vegetables that provide health benefits and are able to prevent pathologies as cardiovascular and age-related neurodegenerative diseases, and metabolic disorders. In fact, ACNs have specific anti-inflammatory, antioxidant properties and neuroprotective effects [1]. The administration of ACN-enriched purple corn extracts is able to reduce trigeminal-associated pain in various pathological conditions, also thanks to the modulation of microglia reactivity [2]. Aim of this study was to examine whether ACN-rich purple corn dietary supplement has positive effects on trigeminal pain associated to multiple sclerosis (MS) and on the onset and progression of MS.

Eleven days before the induction of experimental autoimmune encephalomyelitis (EAE) [3], Dark Agouti rats were assigned to drink water, yellow or purple corn extracts. The development of EAE and TG pain was evaluated and fecal samples were collected at significant time points for the analysis of microbiota composition and ACN metabolites. After sacrifice, central nervous system tissues were collected for qPCR, ELISA and WB analyses. Our results suggest that purple corn extract positively influence the progression of EAE motor symptoms and reduce associated TG pain by modulating glia activation, pro-/anti-inflammatory mediators and autophagy. These results suggest a possible application of purple corn extract as a safe preventive or adjuvant nutraceutical to be administered to MS patients, in order to reduce drug dosage and associated side effects and to significantly improve their quality of life. Furthermore, being an agricultural waste component, corn cobs may represent an economically sustainable source of ACNs.



P57. New dinorneolignans from *Cedrela odorata* and other analogues with potential antiangiogenic properties

D'Angelo V[1], Braca A[2], De Leo M[2], Parisi V[3], De Tommasi N[3], Germanò MP[1]
[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences University of Messina, Viale Stagno D'Alcontres 31, 98166, Messina, Italy*
[2]*Department of Pharmacy, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy*
[3]*Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 152, 84084 Fisciano (SA), Italy*

Lignans and neolignans are known dietary polyphenols found in plants. Numerous studies reveal that these compounds can be promising chemotherapeutic/chemopreventive agents. Their anticancer activity can occur through the induction of apoptosis, inhibition of cell proliferation, and the hindering of metastasis and angiogenesis [1]. In the course of our screening project on antiangiogenic specialized metabolites, a pool of lignan and neolignan derivatives isolated from different plant sources were investigated. Plant materials were extracted with solvents of increasing polarity, including *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH 9:1, and MeOH. The compounds were isolated using different chromatographic techniques such as flash chromatography by Biotage Isolera, Sephadex LH-20, and RP-HPLC and their structures were identified using 1D and 2D NMR (COSY, HSQC, HMBC, ROESY), ECD, and HRESIMS data. Two new di*nor*neolignans were obtained from *Cedrela odorata* L. (Meliaceae) stem bark and characterized as 9-acetyltoonin C and *treo*-guaiacylglycerol-β-methyl vanillate ether. Other known lignans and neolignans were obtained from two conifers belonging to *Podocarpus* genus (Podocarpaceae) [2, 3] and from *Salvia macilenta* Boiss (Lamiaceae).

All compounds were investigated for their antiangiogenic activity using zebrafish (*Danio rerio) in vivo* model. Among the isolates, *treo*-guaiacylglycerol-□-methyl vanillate ether and *erythro*-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-oxyneolignan induced the best antiangiogenic response at 10 μM on the release of endogenous alkaline phosphatase by endothelial cells as a marker of embryo vessel growth.

In conclusion, this study suggests the potential role of isolated lignan derivatives as new therapeutic agents able to modulate angiogenesis.

1° Congresso intersocietà sui prodotti vegetali per la salute: il ruolo delle piante medicinali nella medicina moderna

Con il contributo di:

AVD

Schwabe Pharma Italia
From Nature. For Health.

Sial

BIOS LINE
Natura che funziona

NORSAN

ERBAMEA
Piante officinali e integratori alimentari

fidia

ERBENOBILI
SPAGYRIC LAB

LABOMAR

LOIMA

GUACCI SPA
Distribuzione Farmaceutica dal 1946

cosmesi di laboratorio
HELAN
GENOVA
L'efficacia nelle erbe

STRINGHETTO

tecniche nuove

ORDINE DEI FARMACISTI DELLA PROVINCIA DI NAPOLI

FONDAZIONE
Ordine dei Farmacisti
della Provincia di Napoli

federfarma napoli
Associazione Sindacale dei Titolari di Farmacia della Provincia di Napoli

1° Congresso intersocietà sui prodotti vegetali per la salute: il ruolo delle piante medicinali nella medicina moderna