PROGEMA Presents

SUPRAMED National Meeting

Thursday, April 28th 2022

Aula A, Section of Botany, Department STEBICEF, Via Archirafi 38

SUSTAINABLE PRACTICES IN MEDICINAL CHEMISTRY:

National Meeting on Environmental Assessment, Valorization Pathways and Green Chemistry Applied to Drug Discovery and Production

BOOK OF ABSTRACT

UNIONE EUROPEA

PON

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Società Chimica Italiana

CONTENTS

COMMITTEES 1
Scientific Committee, Organizing Committee

PROGRAM OVERVIEW 2

ABSTRACTS 5
Plenary Lecture 6
Keynotes 7
Short Communications 10

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SCIENTIFIC PROGRAM

9:00 – 9:15 **Opening Session**

9:15 – 10:00 **Plenary Lecture (PL)**
Chairperson: P. Diana, *University of Palermo*

PL E. Novellino, *Catholic University of the Sacred Heart*
"From Foods to Nutraceuticals"

Waste recovery Circular economy & raw materials/ Green and Sustainable Pharmaceutical Approaches

10:00 – 11:25 **Keynote Lecture (KN) and Short Communications (SC)**
Chairperson: B. Parrino, *University of Palermo*

10:00 – 10:25 **KN1 M. Allegra**, *University of Palermo*
"*Opuntia ficus indica* fruit as a source of indicaxanthin for oxidative stress-dependent, inflammatory-related conditions"

10:25 – 10:40 **SC1 M. Micera**, *Exenia group s.r.l*
"Valorization of agro-industrial waste through supercritical CO_2 and polarity modifiers extraction of bioactive compounds"

10:40 – 10:55 **SC2 U. Perricone**, *Fondazione Ri.MED*
"Computational approaches for natural products profiling"

10:55 – 11:10 **SC3 E. Uliassi**, *University of Bologna*
"Harnessing the therapeutic potential of cashew-nut shell liquid (CNSL) food waste against neurodegeneration"

11:10 – 11:25 **SC4 G. Abruscato**, *University of Palermo*
"Cytotoxic potential of aqueous extracts from rhizomes and leaves of *Posidonia Oceanica (L.) Delile* against HEPG2 cancer cells"

11:25 – 11:45 **Coffee Break**

Green and Sustainable Pharmaceutical Approaches / Bio-Inspired Drug Discovery

11:45 – 13:15 **Keynote Lecture and Short Communications**
Chairperson: C. Ostacolo, *University of Naples*

11:45 – 12:10 **KN2 S. Di Franco**, *University of Palermo*
"Green chemistry for isolation and synthesis of innovative anticancer compounds for the treatment of advanced colorectal cancer"

12:10 – 12:25 **SC5 M. Basilicata**, *University of Salerno*
"Bioassay-guided isolation and identification of a novel vasoactive peptide from enzymatic proteolysis of buffalo milk proteins"

12:25 – 12:40 **SC6 C. Corrado**, *University of Palermo*
"Nobiletin and Xanthohumol counteract the TNFα-mediated activation of endothelial cells through the inhibition of NF-κB signaling pathway"

12:40 – 12:55 **SC7 T. Ciaglia**, *University of Salerno*
"Utilization of fluoroform for difluoromethylation in continuous flow: a concise synthesis of α-difluoromethyl-amino acids"

12:55 – 13:10 **SC8 M. Moschetti**, *University of Palermo*
"Study of antinflammatory effect of selected fraction of grapefruit derived essential oil"

13:10– 13:25 **SC9 I. Restivo**, *University of Palermo*
"Nobiletin and xanthohumol extracted by agri-food industrial waste exert synergistic anti-inflammatory effects"

13:25 – 15:15 Lunch break

Bio-Inspired Drug Discovery

15:15 – 18:00 Keynote Lecture and Short Communications
Chairpersons: S. Cascioferro**,** *University of Palermo*
A. Bertamino, *University of Salerno*

15:15 – 15:40 **KN3 C. Ostacolo**, *University of Naples*
"Food peptidomics: decrypting natural products to hunt the buried pharmaceutical value"

15:40 – 15:55 **SC10 V. Vestuto**, *University of Salerno*
"Effect of Cocoa against 6-hydroxydopamine-induced model of parkinson's disease via endoplasmic reticulum stress inhibition"

15:55 – 16:10 **SC11 C. Pecoraro**, *University of Palermo*
"Metabolomics-assisted discovery of a new anticancer GLS-1 inhibitor chemotype from a nortopsentin-inspired library"

16:10 – 16:25 **SC12 V. Di Sarno**, *University of Salerno*
"Discovery of new potent antinfluenza virus agents: from bovine lactoferrin to tetrapeptide analogues"

16:25 – 16:40 **SC13 S. Raimondo**, *University of Palermo*
"Plant-derived extracellular vesicles as drug delivery systems: a proof of concept study using tangerine vesicles"

16:40 – 16:55 **SC14 S. Musella**, *University of Salerno*
"Spirulina peptide SP6 regulates atherosclerosis progression modulating different metabolic pathways: a multi-omic approach"

16:55 – 17:10 **SC15 G. Pepe**, *University of Salerno*
"Onconutraceutical potential of dietary plant polyphenols"

17:10 – 17:25 **SC16 D. Punginelli**, *University of Palermo*
"Natural peptides from seagrass *Posidonia Oceanica* as scaffolds for synthetic antimicrobial peptides against antibiotic resistance"

17:25 – 17:45 **SC17 E. Salviati**, *University of Salerno*
"A pharmaceutical analysis toolbox to unravel Humulus lupulus chemical and biological profile"

17:45 – 18:00 Closing Remarks

ABSTRACTS

PL

FROM FOODS TO NUTRACEUTICALS

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Popular tradition associates many foods typical of the Italian territory, characterized by a wide biodiversity, with medicinal properties. This category includes simple foods such as vegetables, tomatoes, apples, grapes and spices such as basil.
These foods, especially those to which traditional use attributes additive properties to nutritional ones, have recently been the subject of extensive research.
These studies have scientifically identified and substantiated the effectiveness of the active ingredients contained in them, especially against some pathological conditions of a metabolic type.
The results of these studies have made it possible to classify these foods or part of them, with the term nutraceutical, as they are particularly rich in compounds with a healthy action, the intake of which produces a beneficial therapeutic effect, regardless of their nutritional value.
The formulation of specific nutraceuticals represents a valid example of how a healthy eating style, such as that of the Mediterranean diet, can be transformed from GLOCAL to GLOBAL, thus allowing to benefit from the typicality and health properties of the foods that compose it, even if they are not available in the country of residence.

KN1

OPUNTIA FICUS INDICA FRUIT AS A SOURCE OF INDICAXANTHIN FOR OXIDATIVE STRESS-DEPENDENT, INFLAMMATORY-RELATED CONDITIONS.

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The large amount of waste and by-products from agro-industrial processes represents a great opportunity to develop eco-compatible/sustainable strategies aimed to increase the value of these residues. Indeed, most of these remnants are habitually unused and this leads to their expensive disposal. Conversely, they might well represent an important source of phytochemicals to be recovered and employed within a perspective of a circular and profitable economy.

Interest for phytochemicals has significantly grown over the last decades, as their consumption has been soundly associated to a decreased risk of both the onset and the development of several pathological conditions. Within the agri-food waste, *Opuntia ficus-indica* fruit skin could represent an eco-sustainable, low-cost source of useful molecules for nutraceutical, cosmetic and pharmaceutical purposes. *Opuntia ficus-indica* fruit skin is, indeed, enriched in the betalains pigments, exclusively present within Caryophyllales and in some genera of higher fungi, wherein they replace anthocyanins[1].

Amongst the betalains of *Opuntia ficus-indica* fruit, indicaxanthin has been investigated over the last 20 years for its biochemical, pharmacological and nutraceutical properties. This phytochemical, an adduct of betalamic acid with proline, is highly bioavailable in humans and able to cross the brain-blood-barrier (BBB) in rats. Explored, at first, for its mere antioxidant potential, Indicaxanthin is now regarded as a redox-active compound able to exert significant nutraceutical effects against several targets in a number of experimental conditions. Indeed, thanks to its reducing and amphipathic properties, indicaxanthin has been shown to interfere with cellular, redox-dependent signal transduction pathways in several experimental models of inflammatory-related, oxidative stress-dependent pathological conditions both *in vivo* and *in vitro*. Coherently, significant reducing, anti-oxidative, anti-inflammatory, anti-proliferative, anti-tumoral, spasmolytic, neuromodulatory, neuroprotective and anti-dysmetabolic effects of indicaxanthin have been reported[1]. Interestingly, NF-κB has been proposed as one of the crucial molecular targets that indicaxanthin can interact with, to exert its anti-inflammatory effects.

Along these lines, we here overview the beneficial effects of indicaxanthin on human health and the underlying biochemical mechanisms. Moreover, we suggest its recovery, with green-methodologies, from *Opuntia ficus-indica* fruit skin and its use as a novel nutraceutical agent in combo therapy, i.e. with other therapeutical agents targeting different check points of the disease progression.

References

1. Rahimi, P.; Abedimanesh, S.; Mesbah-Namin, S.A.; Ostadrahimi, A. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 2949–2978.

KN2

GREEN CHEMISTRY FOR ISOLATION OF INNOVATIVE ANTICANCER COMPOUNDS FOR THE TREATMENT OF ADVANCED COLORECTAL CANCER

Di Franco, S.;[a] Turdo, A.;[b] Gaggianesi, M.;[a] Veschi, V.;[a] Modica, C.;[a] Lo Iacono, M.;[b] Todaro, M.;[b] and Stassi, G.[a]

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Colorectal cancer (CRC) represents the second leading cause of cancer-associated death worldwide[1]. Its mortality is mainly caused by patient refractoriness to common anti-cancer therapies and consequent metastasis formation. Given the known toxic side effects of chemotherapy, new efficient treatment approaches are urgently needed to effectively improve patient outcomes. In this context, limited therapeutic options are currently available for advanced colorectal cancer (CRC), which is driven and supported by a specific cell subset endowed with stem-like features, called cancer stem cells (CSCs)[2]. New natural treatment approaches showed capabilities to selectively target the CSC subpopulation by rendering them targetable by standard cytotoxic compounds. Herein, we show the anti-cancer properties of the polymethoxyflavones and prenylflavonoids, extracted from Citrus sinensis and Humulus lupulus[3], and of neo-synthetic bis(indolyl)thiazole alkaloid analog, nortopsentin 234 (NORA234)[4], in the specific targeting of CRC stem cells (CR-CSCs). Together, our study highlights the importance of natural compounds as efficient remedies to aid oncological therapies, thus providing a rational basis to develop an effective strategy for the treatment of patients with advanced CRC.

References

[1]Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. *Wiley Online Library*. **2022**, *72*(1), 7-33.

[2]Zeuner, A.; Todaro, M. ; Stassi, G. ; De Maria, R. *Cell Stem Cell*. **2014**. *5*(6), 692-705.

[3]Turdo, A.; Glaviano, A.; Pepe, G.; Calapà, F.; Raimondo, S.; Fiori, M.E.; Carbone, D.; Basilicata, M.G.; Di Sarno, V.; Ostacolo, C.; Parrino, B.; Cascioferro, S.; Pecoraro, C.; Di Franco, S.; Bellavia, D.; Gaggianesi, M.; Veschi, V.; Lo Iacono, M.; Ganduscio, G.; Pantina, V.D.; Mangiapane, L.R.; Bongiorno, M.R.; Alessandro, R.; Todaro, M.; De Maria, R.; Diana, P.; Campiglia, P.; Stassi, G. *Cancers*. **2021**. *13*(16), 3927.

[4] Di Franco, S.; Parrino, B.; Gaggianesi, M.; Pantina, V.D.; Bianca, P.; Nicotra, A.; Mangiapane, L.R.; Lo Iacono, M.; Ganduscio, G.; Veschi, V.; Brancato, O.R.; Glaviano, A.; Turdo, A.; Pillitteri, I.; Colarossi, L.; Cascioferro, S.; Carbone, D.; Pecoraro, C.; Fiori, M.E.; De Maria, R.; Todaro, M.; Screpanti, I.; Cirrincione, G.; Diana, P.; Stassi, G. *iScience*. **2021**. *24*(6), 102664.

KN3

Food peptidomics: decrypting natural products to hunt the buried pharmaceutical value

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In recent years, peptidomics is gaining ever-growing relevance in different scientific domains. The emerging field of food peptidomics is defined as the whole pool of peptides generated during food processing, storage and, especially, during digestion. Food peptidomics procedures have been successfully applied in understanding food protein digestion, in the identification of peptide biomarkers and food-derived bioactive peptides. Identification of bioactive peptides is of particular interest in medicinal chemistry, allowing the discovery of new biologically active molecular entities, improving the structure-activity relationships concerning drug/target interaction, and driving the rational design of small molecules modulating the same targets. In this presentation, a description of the most relevant results obtained in peptidomics by our research group during the last years is given. In particular, the peptidomics workflow used to identify the encrypted peptides from spirulina platensis and buffalo milk dairy products will be described along with the pharmacological studies used for their biological characterization. Finally, the efforts made to translate these results into medicinal chemistry campaigns will be outlined.

References

1. Carrizzo, A., Conte, G.M., Sommella, E., Damato, A., Ambrosio, M., Sala, M., Scala, M.C., Aquino, R.P., De Lucia, M., Madonna, M, Sansone, F, Ostacolo, C, Capunzo, M, Migliarino, S, Sciarretta, S, Frati, G, Campiglia, P, Vecchione, C. Novel Potent Decameric Peptide of Spirulina platensis Reduces Blood Pressure Levels Through a PI3K/AKT/eNOS-Dependent Mechanism. (**2019**) Hypertension 73(2):449-457.
2. Carrizzo, A.; Basilicata, M.G.; Pepe, G.; Sørensen, K.K.; Ciccarelli, M.; Sarno, V.D.; Damato, A.; Venturini, E.; Borrelli, A.; Musella, S.; Abate, M.; Pietro, P.D.; Ostacolo, C.; Campiglia, P.; Vecchione, C. A Novel Vasoactive Peptide "PG1" from Buffalo Ice-Cream Protects from Angiotensin-Evoked High Blood Pressure. Antioxidants (**2021**) 10, 441
3. Pepe, G., Basilicata, M.G., Carrizzo, A., Adesso, S., Ostacolo, C., Sala, M., Sommella, E., Ruocco, M., Cascioferro, S., Ambrosio, M., Pisanti, S., Di Sarno, V., Bertamino, A., Marzocco, S., Vecchione, C., Campiglia, P. β-Lactoglobulin Heptapeptide Reduces Oxidative Stress in Intestinal Epithelial Cells and Angiotensin II-Induced Vasoconstriction on Mouse Mesenteric Arteries by Induction of Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Translocation. Oxid Med Cell Longev. (**2019**) 2019, 1616239
4. Basilicata, M.G., Pepe, G., Adesso, S., Ostacolo, C., Sala, M., Sommella, E., Scala, M.C., Messore, A., Autore, G., Marzocco, S., Campiglia, P. Antioxidant Properties of Buffalo-Milk Dairy Products: A β-Lg Peptide Released after Gastrointestinal Digestion of Buffalo Ricotta Cheese Reduces Oxidative Stress in Intestinal Epithelial Cells. Int J Mol Sci. (**2018**) 19(7),1955.
5. Basilicata, M.G., Pepe, G., Sommella, E., Ostacolo, C., Manfra, M., Sosto, G., Pagano, G., Novellino, E., Campiglia, P. Peptidome profiles and bioactivity elucidation of buffalo-milk dairy products after gastrointestinal digestion. Food Res Int. (**2018**) 105, 1003-1010.

VALORISATION OF AGRO-INDUSTRIAL WASTE THROUGH SUPERCRITICAL CO_2 AND POLARITY MODIFIERS EXTRACTION OF BIOACTIVE COMPOUNDS

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Agricultural and agro-industrial activities generate significant quantities of different organic waste every year. It is estimated that 2.4 billion tons of waste were produced in Europe in 2018, and only 55% of these was involved in recovery processes of useful materials. The remaining 45% was treated as waste, with all the related bureaucratic and economic problems[1]. This linear economic model, characterized by similar volumes of industrial waste and final product, is no longer sustainable, in fact, many countries of the European Union are working to replace it with a circular approach, which is based on the reuse of waste through green chemistry and engineering principles, in order to obtain useful bioactive molecules[2]. One valid approach could be the extraction with supercritical fluids, such as carbon dioxide.
The extraction with this green solvent has several advantages, compared to the more traditional types: in particular, the solvent is not toxic for the environment, as well as for the operators, in addition it is recovered from the atmosphere and exploited for extraction purposes[3]. This means that, at the end of the process, the CO_2 separated from the extracted molecules is stored for subsequent extraction processes. The extraction is carried out at high pressures, low temperatures and in the absence of oxygen and therefore harmful oxidative conditions for the extracted molecules are avoided. On the other hand, being a non-polar compound, supercritical CO_2 is not suitable for the extraction of polar molecules. To overcome this problem, through the synergistic activity of Separeco s.r.l. and Exenia group s.r.l., an extraction plant was designed and built also equipped with a co-solvent pump useful for changing the polarity of the solvent and thus obtaining even the most polar molecules. The co-solvents used were mainly water and ethanol.
After numerous extractive tests carried out on a Exenia plant and modified for experimentation even on more polar molecules and also for the separation of cuticular waxes (interesting molecules rich in long-chain alcohols and alkanes) directly from the extract, 3 recovery vegetable matrices were selected, rich in active substances of different polarity: nettle leaves[4], grape pomace[5] and olive pomace[6]. First, the matrices were ground and subsequently subjected to the extraction process divided into sequential extractions, initially with only supercritical CO_2 to obtain the lipophilic fraction and, subsequently, extractions with water and ethanol as co-solvents. Although the lipophilic fraction was rich in interesting waxes and unsaturated fatty acids, the most promising results were obtained on the hydrophilic fraction, where there was a marked presence of polyphenolic substances, even equal to 40 mg/g. For the analysis of these substances, a spectrophotometric Folin-Ciocalteu assay was performed. Results were obtained at 765 nm.
In addition, the vegetation water was removed from the fresh olive pomace through a complex system of centrifuges and ultracentrifuges, in order to obtain an aqueous solution rich in nutrients. This by-product can be used as an algal culture medium especially for ubiquitous microalgae such as *Nannochloropsis oculata*, after proper COD and BOD abatement procedure. To do this, Exenia group s.r.l. and Separeco s.r.l. collaborated in the design of an electrolytic cell and a modular photobioreactor, as well as a correct formulation of stock solutions (rich in macro and micro elements) to maximize algal growth within this aqueous recovery solution.

References

1.Londoño N., Cano A., Cabezas H. Current Opinion in Chemical Engineering 34, **2021**: 100738.
2. Geissdoerfer M., Savaget P., Bocken N.M.P.,Hultink E.J. Journal of Cleaner Production 143, **2017**: 757–768.
3. Silva, Rui P.F.F., Rocha-Santos T.A.P.,Duarte A.C. Trends in Analytical Chemistry 76 **2016** 40–51.
4.Kornélia K., Vatai G.,Békássy-Molnár E. PeriodicaPolytechnica Chemical Engineering 59, **2015**: 168–73.
5.Pedras, B.M., Regalin G., Sá-Nogueira I., Simões P., Paiva A., Barreiros S. The Journal of Supercritical Fluids 160 **2020**: 104793.
6.Gullón P., Gullón B., Astray G., Carpena M., Fraga-Corral M., Prieto M.A., Simal-Gandara J. Food Research I. 137 **2020**109683.

SC2

COMPUTATIONAL APPROACHES FOR NATURAL PRODUCTS PROFILING

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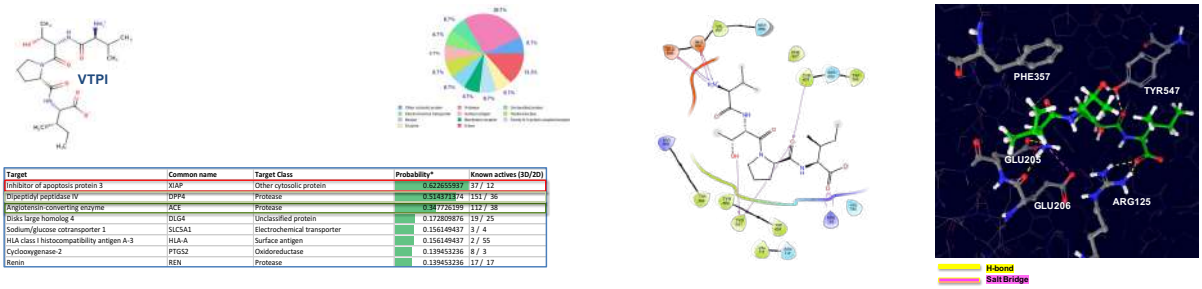
The molecular informatics group of the Ri.MED Foundation participated in the PROGEMA project applying computational chemistry techniques that could help in the profiling of natural compounds. In detail, the work was focused on the main components of the hops and orange blend (LUPARA) and on the peptides derived from the spirulina algae. Techniques based on molecular fingerprints and ligand similarity towards annotated databases allowed us to identify possible biological targets for the molecules of interest to PROGEMA[1] (Fig.1). This preliminary ligand-based profiling part was integrated with a structure-based pharmacophore profiling run on the whole PDB database, with a specific focus on the human kinome. The combination of the previously described technique with other structure-based computational approaches, allowed us to make an hypothesis on the binding for the predicted targets[2,3] (Fig.1). Once the binding, hence the protein-ligand interaction were identified, computational model constraints were created and helped in the prioritization of new molecules on the selected targets. The methods here described represent a useful tool for the design of new molecules of therapeutic interest on the predicted and possibly biologically validated targets. Here we present the results of in silico application of main LUPARA and Spirulina peptides profiling.



Target	Common name	Target Class	Probability*	Known actives (3D/2D)
Inhibitor of apoptosis protein 3	XIAP	Other cytosolic protein	0.622655937	37 / 12
Dipeptidyl peptidase IV	DPP4	Protease	0.514371174	151 / 36
Angiotensin-converting enzyme	ACE	Protease	0.347726199	112 / 38
Disks large homolog 4	DLG4	Unclassified protein	0.172809876	19 / 25
Sodium/glucose cotransporter 1	SLC5A1	Electrochemical transporter	0.156149437	3 / 4
HLA class I histocompatibility antigen A-3	HLA-A	Surface antigen	0.156149437	2 / 55
Cyclooxygenase-2	PTGS2	Oxidoreductase	0.139453236	8 / 3
Renin	REN	Protease	0.139453236	17 / 17

Figure 1. Profiling in silico of Spirulina active peptide with ligand-based (left) and structure-based (right) approaches

1. Daina, A.; Michielin, O.; Zoete, V., *Nucleic Acids Res.*, **2019**, 47(W1), W357–W364;
2. Friesner, R.A.; Murphy, R.B.; Repasky, M.P.; Frye, L.L.; Greenwood, J.R.; Halgren, T.A.; Sanschagrin, P.C.; Mainz, D.T., *J. Med. Chem.* **2006**, 49, 21, 6177–6196
3. Wolber G.; Langer T., *J. Chem. Inf. Model.* **2005** *45* (1), 160-169

HARNESSING THE THERAPEUTIC POTENTIAL OF CASHEW-NUT SHELL LIQUID (CNSL) FOOD WASTE AGAINST NEURODEGENERATION

Uliassi, E.;[a] Ramos, G. A.;[b] Miranda, C. O;[b] Oliveira, A. S.;[b] Bartolini, M.;[a] Naldi, M.;[a] Liparulo, I.;[a] Bergamini, C.;[a] Wu, L.;[c] Fraser, P.E.;[c] Abreu, M.;[d] Kiametis, A.S.;[d] Gargano, R.;[d] Hu, D. V.;[e] Sahin, C.;[e] Silveira, E.R.;[f] Prchal, L.;[g] Soukup, O.;[g] Korábečný, J.;[g] Cummins, C. L.;[e] Romeiro, L.A.S.;[b] and Bolognesi, M.L.[a]

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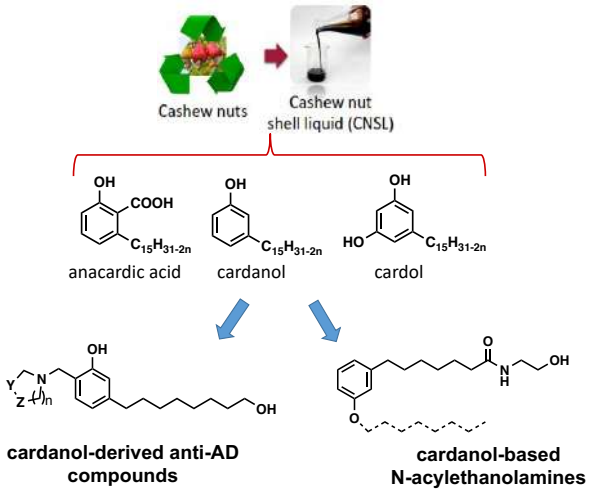
The use of food industry waste as starting material to synthesize novel biologically active compounds to be further optimized into novel drugs has been gaining momentum in drug discovery.[1] As part of our efforts to develop sustainable drugs, we explored the possibility of using cashew nut shell liquid (CNSL) as a broadly available and inexpensive starting material.[2] CNSL is an inedible oil and a byproduct of cashew nut food processing, with a high content of phenolic lipids. The rational modification of their structures has emerged as a successful medicinal chemistry approach to develop novel lead candidates against neurodegenerative diseases.[2] Particularly, we have investigated whether cardanol, a phenolic CNSL component, could serve as a scaffold for designing and synthesizing novel cardanol-derived compounds as potential disease-modifying treatments for Alzheimer's disease (AD).[3] Furthermore, we have been exploiting the neuroprotective potential of CNSL components by developing cardanol-based N-acylethanolamines for tackling chronic neuroinflammation as a feature common to a wide range of neurodegenerative disorders. Overall, these results suggest that CNSL is a promising raw material for developing sustainable disease-modifying treatments for currently incurable neurodegenerative diseases.



References

1. Brun, N.; Hesemann, P.; Esposito, D. *Chem. Sci.* **2017**, *8*, 4724– 4738.
2. Uliassi, E.; de Oliveira, A.S.; de Camargo Nascente, L.; Romeiro, L.A.S.; Bolognesi, M.L. *Molecules* **2021**, *26*, 5441.
3. de Andrade Ramos, G.; Souza de Oliveira, A.; Bartolini, M.; Naldi, M.; Liparulo, I.; Bergamini, C.; Uliassi, E.; Wu, L.; Fraser, P.E.; Abreu, M.; Kiametis, A.S.; Gargano, R.; Silveira, E.R.; Brand, G.D.; Prchal, L.; Soukup, O.; Korábečný, J.; Bolognesi, M.L.; Romeiro, L.A.S. *RSC Med Chem.* **2021**, *12*, 1154-1163.

CYTOTOXIC POTENTIAL OF AQUEOUS EXTRACTS FROM RHIZOMES AND LEAVES OF *POSIDONIA OCEANICA (L.) DELILE* AGAINST HEPG2 CANCER CELLS.

Abruscato, G; Lazzara,V; Punginelli, D; Mauro, M; Vazzana, M; Arizza, V; Luparello, C.

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The water environment is a reservoir of bioactive compounds in terms of primary and secondary metabolites produced by aquatic species which exert a wide range of therapeutic effects in humans.[1-4] Thus, they represent novel promising prevention and/or treatment agents and beneficial supplements for the formulation of functional food and food-packaging material. With the aim to identify novel substances endowed with anticancer action, cell-free aqueous extracts were prepared from the green and beached leaves and the rhizomes of the marine seagrass *Posidonia oceanica (L.) Delile*. HepG2 hepatocarcinoma (HCC) cells were used as an *in vitro* cell model to test the potential anti-liver cancer ability of the preparations by analyzing their effects on cell viability and locomotory behavior, cell cycle distribution, apoptosis and autophagy modulation, mitochondrial function and redox state, as already reported.[5-7] All the samples, apart from those obtained from *P. oceanica*'s beached leaves, were able to affect cell viability in a dose-response manner and the IC_{50} at 24 h was calculated and used as exposure concentration in the subsequent assays. Cell cycle impairment, the increase of the percentage of cells in the sub-G_0 cycle phase and the accumulation of annexin-V^+/PI^- cells at early times of exposure indicated the apoptosis-promoting effect of both leaf and rhizome extracts. In addition, the intracellular accumulation of acidic vesicular organelles, hallmarks of the autophagic process which is required by HepG2 cells to sustain their survival and active growth, decreased after both treatments although to a lesser degree in the presence of rhizome extracts. Interestingly, only the leaf extract was shown to induce mitochondrial dysfunction through the dissipation of the transmembrane potential and to maintain over 24 h a steady downregulation of intracellular reactive oxygen species which are acknowledged redox-active signaling messengers necessary for cells' biological activities. As expected from the observed derangement of HepG2 cells' healthy state, the treatments were responsible for an early block of cell locomotory behavior in wound healing assays, thereby suggesting that the preparations may be also potential suppressors of HCC metastatic attitude. Collectively, the results so far obtained strongly suggest the potential anti-HCC ability of the preparations which appears to be stronger for green leaf than rhizome extracts and merits further investigation addressed to the isolation of the substance(s) responsible for the cytotoxic effect and to the opening of new interesting scenarios for its future biomedical and nutraceutical applications.

References

1. Lazzara, V.; Arizza, V.; Luparello, C.; Mauro, M.; Vazzana, M. *Mar. Drugs*. **2019**, *17*, 617.
2. Mauro, M.; Lazzara, V.; Punginelli, D.; Arizza, V.; Vazzana, M. *Dev. Comp. Immunol*. **2020**, *108*, 103669.
3. Luparello, C.; Mauro, M.; Lazzara, V.; Vazzana, M. *Molecules* **2020**, *25*, 2471.
4. Punginelli, D.; Schillaci, D.; Mauro, M.; Deidun, A.; Barone, G.; Arizza, V.; Vazzana, M. *Dev. Comp. Immunol*. **2022**, *126*, 104258.
5. Luparello, C.; Ragona, D.; Asaro, D.; Lazzara, V.; Affranchi, F.; Celi, M.; Arizza, V.; Vazzana, M. *Biology* **2019**, *8*, 76.
6. Luparello, C.; Ragona, D.; Asaro, D.; Lazzara, V.; Affranchi, F.; Arizza, V.; Vazzana, M. *J. Mar. Sci. Eng.* **2020**, *8*, 261.
7. Luparello, C.; Branni, R.; Abruscato, G.; Lazzara, V.; Drahos, L.; Arizza, V.; Mauro, M.; Di Stefano, V.; Vazzana, M. *EXCLI J*. in press.

BIOASSAY-GUIDED ISOLATION AND IDENTIFICATION OF A NOVEL VASOACTIVE PEPTIDE FROM ENZYMATIC PROTEOLYSIS OF BUFFALO MILK PROTEINS

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Arterial hypertension is the most important risk factor for cardiovascular diseases, myocardial infarction, heart failure, renal failure and peripheral vascular disease.[1] Several studies highlighted that both endothelial dysfunction and oxidative stress have been implicated in the development of arterial hypertension.

In order to prevent and counteract arterial hypertension, intake of food-derived antioxidants may protect from oxidative stress and tissue damage. In this contest, milk dairy products are important dietary components that contribute to the total intake of antioxidants as they release bioactive peptides during gastrointestinal digestion. In this study we identified in gastro-intestinal digest of buffalo ice-cream, a novel antioxidant pentapeptide "QKEPM", namely PG1.[2] In particular, after investigated *in vitro* its bioaccessibility, bioavailability and biotrasformation, the effects of PG1 peptide have been investigated in *ex vivo* by vascular reactivity studies performed on mice mesenteric arteries and *in vivo* on Angiotensin II-treated mice.

This α_{S1}- casein peptide owns potent vascular activity in counteract the effects of angiotensin II-evoked vasoconstriction and high blood pressure levels. Its effects are mediated by the inhibitory effect on AT1 receptor leading to a downregulation of p-ERK½/Rac1-GTP and consequent reduction of oxidative stress (Figure 1). These results strongly candidate PG1, as a novel bioactive peptide for the prevention and management of hypertension, thus expanding the armamentarium of preventive strategies aimed at reducing the incidence and progression of hypertension and its related cardiovascular complications.

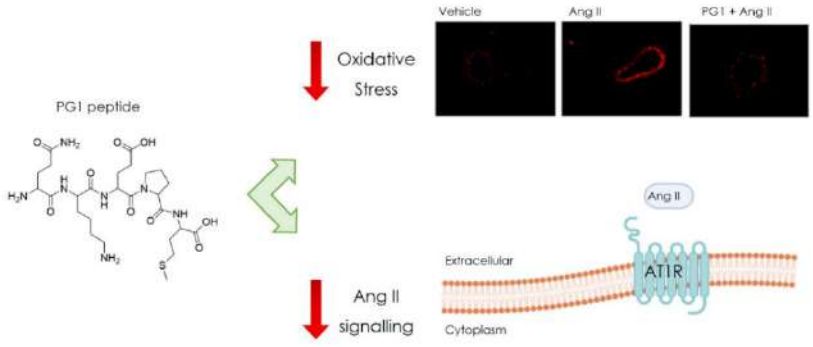

Figure 1. Molecular mechanism of PG1 peptide against Angiotensin-Evoked High Blood Pressure.

References

1. Weycker, D.; Nichols, G. A.; O'Keeffe-Rosetti, M.; Edelsberg, J.; Khan, Z. M.; Kaura, S.; Oster, G. *Am. J. Hypertens*. **2007**, *20*, 599-607.

2. Carrizzo, A.; Basilicata, M. G.; Pepe, G.; Sørensen, K. K.; Ciccarelli, M.; Sarno, V. D.; Damato, A.; Venturini, E.; Borrelli, A.; Musella, S.; Abate, M.; Pietro, P. D.; Ostacolo, C.; Campiglia, P.; Vecchione, C. *Antioxidants*. **2021**, *10*, 441.

NOBILETIN AND XANTHOHUMOL COUNTERACT THE TNFα-MEDIATED ACTIVATION OF ENDOTHELIAL CELLS THROUGH NF-kB SIGNALING PATHWAY

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Angiogenesis, the formation of new blood vessels from the endothelium of the existing vasculature, is a fundamental process in tumor growth, progression, and metastasis.
Recently, several natural compounds received increasing attention for their anti-proliferative, pro-apoptotic, and anti-angiogenic activities in tumor cells [1,2]. In particular, polyphenols, able to affect the activity of multiple pathways and mediators involved in the carcinogenesis process, were suggested as valuable inhibitors of cancer cell growth and angiogenesis[3]. Flavonoids are the most abundant polyphenols in the diet and many reports have shown that the anti-tumor activity is exerted both on tumor cells and on the surrounding microenvironment in particular demonstrating anti-angiogenic, and angiopreventive activities[4].
Xanthohumol (XCF), the major prenylated flavonoid of the hop plant (Humulus lupulus L.), and Nobiletin (NCF), a polymethoxylated flavonoid from red orange (Citrus sinensis), are among the chemopreventive natural compounds with the ability to target the tumor vasculature. In sight of this, we studied the role of XCF and NCF in modulating endothelial cell functions in vitro. In particular, we investigated the mechanism by which these compounds can counteract the effects of TNFα on human umbilical vein endothelial cells (HUVEC). In our experiments, HUVEC has been treated with xanthohumol and nobiletin singularly or in combination before stimulation with TNFa, a powerful inducer of endothelial cells activation[5,6]. We used a subtoxic dosage of 10μg/ml of the two compounds[7]. Firstly, through ELISA assay, we have demonstrated that the pretreatment with the two compounds and their mix on HUVEC is able to significantly reduce the TNFα-mediated release of VEGF, an essential growth factor for vascular endothelial cells. The results obtained from the 3D invasion and matrigel assays demonstrate that pre-treatment with all compounds inhibits HUVEC invasive ability and reduces the number of branching points induced by TNFα. These results let us hypothesize that these compounds can affect neo-angiogenesis. As well known, activated endothelial cells provide cancer cells with a gateway during metastasis formation, here we revealed that pre-treatment with all compounds inhibits the adhesion of colon cancer cell line adhesion on activated HUVEC monolayer. Therefore, we have analyzed the molecular mechanism by which xanthohumol and nobiletin exert their effects on the adhesion capability of tumor cells. Our data showed that the effects of these compounds is mediated by the inhibition of NF-κB pathway, which regulates the adhesion of tumor cells to the vascular endothelium promoting the expression of two adhesion molecules: VCAM-1 and ICAM-1.
Nowadays, a promising strategy for treatment of cancer is antiangiogenic therapy which is directed against the tumor supplying blood vessels. Our results could suggest a rationale for future development of these compounds as potential chemopreventive supplements to target diseases which involve angiogenesis.

References

1. Rossi, T.; Gallo, C.; Bassani, B.; Canali, S.; Albini, A.; Bruno, A. *Pol Arch Med Wewn* **2014**, 124, 713-722.
2. Shuman Moss, L.A.; Jensen-Taubman, S.; Rubinstein, D.; Viole, G.; Stetler-Stevenson, W.G. *Carcinogenesis* **2014**, 35, 1556-1563.
3. Calis, Z.; Mogulkoc, R.; Baltaci, A.K. *Mini Rev Med Chem* **2020**, 20, 1475-1488.
4. Tosetti, F.; Noonan, D.M.; Albini, A. *Int J Cancer* **2009**, 125, 1997-2003.
5. Yoshimatsu Y.; Kimuro S.; Pauty J.; Takagaki K.; Nomiyama S. et al. *PLoS One* **2020**, 15(5):e0232356.
6. Chen L.; Deng H.; Cui H,.;Fang J.; Zuo Z,;Deng J.; Li Y.; Wang X.; Zhao L. *Oncotarget* **2018**, 9(6):7204-7218.
7. Turdo, A.; Glaviano, A.; Pepe, G.; Calapa, F.; Raimondo, S.; Fiori M.E.; Carbone, D. et al. *Cancers (Basel)* **2021**, 13, 3927.

UTILIZATION OF FLUOROFORM FOR DIFLUOROMETHYLATION IN CONTINUOUS FLOW: A CONCISE SYNTHESIS OF α-DIFLUOROMETHYL-AMINO ACIDS

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The synthesis of organofluorine compounds has grown because of the wide scope of their applications in biological and material sciences in recent years. Currently, the selective incorporation of fluorine atoms or fluorine containing moieties into small molecules to modulate their biological properties has become a conventional strategy in drug design.[1,2]

One of the most versatile reagents for direct difluoromethylation is chlorodifluoromethane (CHF_2Cl, Freon 22).[3] However, CHF_2Cl is a strong ozone depleting gas and it is controlled under the Montreal Protocol. In view of these considerations several alternative difluoromethane sources have been developed, among the most attractive is fluoroform (CHF_3, Freon 23). Fluoroform is generated as waste-product during the synthesis of chlorodifluoromethane; it is a nontoxic and ozone-friendly gas, which was recently used for the direct difluoromethylation of a variety of substrates utilizing strong lithium bases.[4, 5, 6] Herein we describe a scalable, continuous flow synthesis route to Cα-difluoromethyl amino acids using fluoroform as the reagent. Cα-difluoromethyl amino acids are potent and selective irreversible inhibitors of their respective α-amino acid decarboxylases.[7] This class of compounds exhibit a broad spectrum of biological activities, such as antibacterial, antihypertensive, cancerostatic, and cytotoxic activities. Actually, D,L-α-difluoromethylornithine (eflornithine) is in medical use for the treatment of African sleeping sickness as well as of Pneumocystis carinii pneumonia, the most frequent opportunistic infection associated with acquired immunodeficiency syndrome (AIDS).[7]

Preliminary successful batch experiments encouraged us to develop a continuous flow protocol for this reaction using methyl diphenylacetate as model substrate for the method optimization. The developed setup was applied to different starting materials such as esters, malonates and aminoacids. [8]

The general reaction conditions were suitable for a variety of substrates and the protocol appears to be appealing for industrial applications.

References

1. Dolbier, W. R. *J. Am. Chem. Soc*. **2000**, 122 (42), 10492–10492.
2. Okazoe, T. *Physical and biological sciences*. **2009**, 85 (8), 276-89.
3. Hine, J.; Porter, J. *J. Am. Chem. Soc*. **1957**, 79, 20, 5493–5496
4. Iida, T.; R. Hashimoto, K.; Aikawa, S.; Ito, K.; Mikami, K. *Angew. Chem. Int. Ed*., **2012**, 51, 9535–9538.
5. Aikawa, K.; Maruyama, K.; Honda, K.; Mikami, K. *Org. Lett*. **2015**, 17, 4882–4885.
6. Aikawa, K.; Maruyama, K.; Nitta, J.; Hashimoto, R.; Mikami, K. *Org. Lett*. **2016**, 18, 3354–3357
7. Smith, D.; Davies, S.; Nelson, M.; Youle, M.; Gleeson, J.; Gazzard, B. *AIDS*. **1990**, 4(10):1019-21.
8. Köckinger, M.; Ciaglia, T.; Bersier, M.; Hanselmann, P.; Gutmann, B.; Kappe, C.O. *Green Chem*., **2018**, 20, 108-112.

STUDY OF ANTINFLAMMATORY EFFECT OF SELECTED FRACTION OF GRAPEFRUIT DERIVED ESSENTIAL OIL

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Introduction:

The development of chronic inflammation is caused by the accumulation of toxins in the body, which have an impact on numerous diseases. Many studies confirm that inflammation is promoted by micro-organisms (bacteria, viruses, fungi and parasites), chemical substances, physical agents, tissue death. To date, natural compounds, are known to be an effective remedy in preventing chronic inflammation. In particular, Grapefruit (*Citrus paradisi;* family: *Rutaceae*) peel is rich in phenolics and flavonoids and possess several health benefits, including anti-inflammatory properties. Grapefruit essential oil (GEO) is known for its healthy properties; less consideration is given to the biological properties of the fractions obtained from GEO (Cfr-GEO). The aim of this study is to evaluate the ability of the Cfr-GEO in counteracting the LPS-induced inflammation in a human macrophage cell line. In order to translate the benefical properties of Cfr-LEO towards an industrial application, we tested the properties of this smelling/flavouring agent also as spry-dry and drinks enriched in Cfr-GEO formulations.
The data obtained demonstrate that Cfr-GEO alone or in different formulations exerts a protective effect against inflammatory stimuli and lay the basis for the development of foods/drinks aimed at preventing or alleviating chronic inflammatory conditions. In the future, the identification of the actor responsible for the healthy effects observed could lead to the more targeted formulation of foods/drinks solutions with beneficial properties.

Materials and Methods:

In this study, human monocyte THP-1 cell line was used and was purchased from ATCC (Manassas, VA, USA). In order to evaluate the protective effects of Cfr-GEO or spry-dry GEO or drink enriched in GEO; pretreatment for 2h with Cfr-GEO (doses 0.005%, 0.01%, 0.02 %) and subsequent treatment with LPS for 6h (1000 ng/ml) was performed.

Findings:

The anti-inflammatory activity was tested by dosing specific cytokines by RT-PCR and ELISA. We demonstrated that the pretreatment with Cfr-GEO is able to counteract the effects induced by LPS such as the induction of the expression of pro-inflammatory cytokines IL-6, TNF-a, IL-1β.

Conclusion & Significance:

The data obtained demonstrate that Cfr-GEO exerts a protective e effect against inflammatory stimuli and lay the basis for the development of foods/drinks aimed at preventing or alleviating chronic inflammatory conditions. The anti-inflammatory activity of Cfr-GEO maybe a result of a complex interaction among its various chemical compounds which might produce a synergistic effect, highlights the properties of the single compounds. The identification of the actor responsible for the healthy effects observed could lead to the more targeted formulation of foods/drinks solutions with beneficial properties.

NOBILETIN AND XANTHOHUMOL EXTRACTED BY AGRI-FOOD INDUSTRIAL WASTE EXERT SYNERGISTIC ANTI-INFLAMMATORY EFFECTS

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The citrus and beer industry, following the processing of oranges [Citrus sinensis (L.) Osbeck] and the use of hop buds (Humulus Lupulus, L.), produces more than one million tons of waste product *per annum*. The recovery of phytochemicals from this low-cost raw material can represent a eco-sustainable strategy of enhancement and development of nutraceutical products, cosmetics and pharmaceuticals. Turdo e coll [1] have recently isolated from extracts of waste product of blood oranges and hop buds, selected Nobiletin (NCF) and Xanthohumol (XCF) enriched-fractions, respectively, and evaluated their anti-cancer potential.

In this study, we assessed the anti-inflammatory activity of NCF and XCF on human intestinal epithelial cells (Caco-2 differentiated cells) stimulated for 24 hours by IL-1β (25 ng/ml), as an *in vitro* model of intestinal bowel disease (IBD).

Our results showed that pre-incubation of cells for 1 h with non-cytotoxic doses of either NCF or XCF (2.5-20 µg/mL), concentration-dependently reduced IL-1β-induced nitric oxide (NO) release. Estimation of the combination index, following Chou-Talalay method [2], indicated that NCF and XCF in combination (1:1, w:w) synergistically inhibited IL-1β-induced NO production. In addition, ELISA results showed that NCF/ XCF combination resulted in a suppression of the IL-1β-induced prostaglandin E_2 release, stronger than that exerted by NCF or XCF alone. Accordingly, immunoblotting analyses demonstrated that NCF/XCF combination significantly decreased IL-1β-induced protein levels of inducible NO synthase and cyclooxygenase-2. Finally, NCF/XCF combination prevented IL-1β-stimulated NF-κB activation, increased nuclear translocation of Nrf2, enhancing the expression levels of detoxifying and antioxidant defence enzymes, such as heme-oxygenase-1 (HO-1) and glutathione peroxidase. Accordingly, the NCF/XCF combination prevented redox state changes of the cells induced by IL-1β, by inhibiting ROS formation and restoring intracellular GSH levels. Again, the effects of combined treatment with NCF plus XCF were increased compared to those produced by NCF or XCF alone.

In conclusion, our results show that the combination of Nobiletin and Xanthohumol, synergically increases the anti-inflammatory effect of the single compounds in an *in vitro* model of IBD and provide a mechanistic basis of the observed action involving the inhibition of NF-kB signaling and stimulation of the Nfr2/ HO-1 pathway. In emphasizing the advantage of recovery of bioactive phytochemicals from waste material, our results encourage the formulation of nutraceuticals containing Nobiletin and Xanthohumol to enhance their healthy effects.

[1] Turdo A. Glaviano A., Pepe G., Calapà F., Raimondo S, Fiori M.E., Carbone D. et al, *Cancers* **2021** 13(16):3927.
[2] Chou, T.C. Cancer Res. **2010** 70, 440.

EFFECT OF COCOA AGAINST 6-HYDROXYDOPAMINE-INDUCED MODEL OF PARKINSON'S DISEASE VIA ENDOPLASMIC RETICULUM STRESS INHIBITION

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The endoplasmic reticulum (ER) is essential for intracellular homeostasis of Ca^{2+}, controlling synthesis, and folding of proteins. During cellular stress, variations in ER homeostasis and its functioning occurs due to accumulated misfolded proteins in the ER. This condition is referred as ER stress. Therefore, a cascade of signaling events termed unfolded protein response (UPR) is activated as adaptative response to mitigate the ER stress condition.[1,2]

Activating transcription factor 6 (ATF6), protein kinase R-like ER kinase (PERK), and inositol- requiring enzyme 1 (IRE1) are the three main transmembrane proteins initiating UPR signaling in ER stress response. In normal conditions they are maintained in an inactive state. When ER stress is triggered, they are activated through dissociation from GRP78/binding protein (BIP), the master regulator chaperone of UPR, which aids proper folding of misfolded proteins.[3]

A large body of evidence suggests that natural products target the ER stress signaling pathway, exerting a potential action in cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases like Alzheimer's and Parkinson's.[4,5]

Polyphenols-rich cocoa has been shown many benefits on human health including antioxidative and anti-inflammatory effects. The antioxidant action of cocoa is due to its high content in flavonoids, such as epicatechin, catechin, and proanthocyanidins, naturally occurring in this matrix.[6]

The goal of our study was to investigate whether the known antioxidant properties of cocoa have any influence on Parkinson's disease through modulation of unfolded protein response (UPR) examining molecular mechanisms of endoplasmic reticulum stress activated-pathways.

Our findings demonstrate, for the first time, the ability of cocoa to specifically targets PERK sensor, with significant antioxidant and antiapoptotic activities as crude and fractioning extract in 6- hydroxydopamine-induced SH-SY5Y cell model of Parkinson's disease.

References

1. Lin, J.H., Walter, P., Yen, T.S.B. *Annual Review of Pathology: Mechanisms of Disease*. **2008**, 3, 399-425.
2. Corazzari, M., Gagliardi, M., Fimia, G.M., Piacentini, M. *Frontiers in Oncology*. **2017**, 7 (APR), 78.
3. Wang, M., Wey, S., Zhang, Y., Ye, R., Lee, A.S. *Antioxidants and Redox Signaling*. **2009**, 11 (9), 2307-2316.
4. Remondelli, P., Renna, M. *Frontiers in Molecular Neuroscience*. **2017**, 10, 187.
5. Martucciello, S., Masullo, M., Cerulli, A., Piacente, S. *International Journal of Molecular Sciences*. **2020**, 21 (6), 1905.
6. Oracz, J., Żyżelewicz, D. *Antioxidants*. **2020**, 9 (12), 1230, 1-4.

METABOLOMICS-ASSISTED DISCOVERY OF A NEW ANTICANCER GLS INHIBITOR CHEMOTYPE FROM A NORTOPSENTIN-INSPIRED LIBRARY

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Among the various hallmarks of cancer, deregulation of cellular metabolism plays a crucial role in sustaining survival, proliferation, migration, and invasiveness.[1] A plethora of studies have correlated glutaminase (GLS), the enzyme responsible for glutamine hydrolysis, to a wide range of tumors.[2,3] In nature, there are two isoforms of GLS: GLS-1 and GLS-2, encoded by human glutaminase genes. Although both isoforms have been extensively related to cell proliferation, GLS-2 exhibits contrasting functions in tumorigenesis.[4] On the contrary, GLS-1 has shown coherent implications in the progression of different types of tumors.[5] Few chemotypes of GLS-1 inhibitors have been described, and only one candidate, IPN60090, is currently in phase 1 clinical trials.[6]
In order to discover new tools for cancer treatment, considerable efforts have been made to identify and develop new molecules based on natural compound scaffolds. Among the marine compounds, nortopsentin, a marine bis indolyl alkaloid isolated from deep-sea sponge *Spongosorites ruetzleri*, inspired the synthesis of several compounds with antiproliferative activity in the micromolar-submicromolar range.[7] Herein we report the synthesis of nortopsentin derivatives bearing an indolyl-thiazolyl-7-azaindole scaffold, in which different aminoalkyl chains at the indole nitrogen atom were inserted and showed excellent results in preclinical studies. Biological analysis provided a deep characterization of the anticancer properties of the new thiazole derivatives, highlighting their selective toxicity against cancer cells and their role in suppression of cell migration, clonogenic potential, and matrix metalloproteases activity. Moreover, using a cell-based metabolomic protocol, it was identified the ability of a new thiazole derivative to inhibit the enzymatic activity of GLS-1, confirmed by *in vitro* enzymatic assays and molecular modeling studies.

References

1. Hanahan, D.; Weinberg, R. A. *Cancer Discov.* **2022**, *12(1)*, 31–46.
2. Masisi, B. K.; El Ansari, R.; Alfarsi, L.; Craze, M. L.; Jewa, N.; Oldfield, A.; Cheung, H.; Toss, M.; Rakha, E. A.; Green, A. R. *Cancers*. **2021**, 3963.
3. Zhou, Q.; Zhan, H.; Lin, F.; Liu, Y.; Yang, K.; Gao, Q.; Ding, M.; Liu, Y.; Huang, W.; Cai, Z. *Biosci. Rep.* **2019**, BSR20182372.
4. Wang, Z.; Liu, F.; Fan, N.; Zhou, C.; Li, D.; Macvicar, T.; Dong, Q.; Bruns, C. J.; Zhao, Y. *Front Oncol.* **2020**, *10*, 589508.
5. Lampa, M.; Arlt, H.; He, T.; Ospina, B.; Reeves, J.; Zhang, B.; Murtie, J.; Deng, G.; Barberis, C.; Hoffmann, D.; Cheng, H.; Pollard, J.; Winter, C.; Richon, V.; Garcia-Escheverria, C.; Adrian, F.; Wiederschain, D.; Srinivasan, L. *PloS one.* **2017**, *12(9)*, e0185092.
6. Zhou, W. X.; Chen, C.; Liu, X. Q.; Li, Y.; Lin, Y. L.; Wu, X. T.; Kong, L. Y.; Luo, J. G. *Eur. J. Med. Chem.* **2021**, *210*, 112980.
7. Cascioferro, S.; Attanzio, A.; Di Sarno, V.; Musella, S.; Tesoriere, L.; Cirrincione, G.; Diana, P.; Parrino, B. *Mar. Drugs.* **2019**, *17,* 35.

DISCOVERY OF NEW POTENT ANTINFLUENZA A VIRUS AGENTS: FROM BOVINE LACTOFERRIN TO TETRAPEPTIDE ANALOGUES

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Influenza is a serious threat to health in all parts of the world and among the viruses that bring out this infection, influenza A viruses (IAV) are the only influenza viruses known to cause influenza pandemics [1] .The control and treatment of influenza depends mainly on chemical or biochemical agents and, natural products and their structural analogues have historically made a major contribution to pharmacotherapy.[2] Nevertheless, natural products also present challenges for drug discovery and, at present, the development of antiviral drugs represents a crucial strategy in the control and prevention of seasonal and pandemic influenza. Over the years, the anti-influenza virus effect of bovine lactoferrin (bLf) and the role of its tryptic fragments was widely investigated in antiviral activity.[3] Recently, through a truncation library, we identified the tetrapeptides, Ac-SKHS-NH_2 (**1**) and Ac-SLDC-NH_2 (**2**), derived from bLf C-lobe fragment 418–429,[4] which were able to bind hemagglutinin (HA) and inhibit cell infection in a concentration range of femto- to picomolar. So, in this work, we performed a systematic SAR study through the development of ala-scan peptides starting from the most promising tetrapeptides identified previously. This study allowed us to assess the importance of the side chain on peptide bioactivity. We carried out binding affinity measurements by microscale thermophoresis (MST) and surface plasmon resonance (SPR), as well as hemagglutination inhibition (HI) and virus neutralization (NT) assays on synthesized peptides. Computational studies were performed to identify possible ligand–HA interactions. All applied methods agreed upon the identification of a novel potent tetrapeptide, Ac-SAHS-NH_2, able to bind hemagglutinin with high affinity and inhibit influenza virus hemagglutination and cell infection at femtomolar concentration. This small sequence, with high and broad-spectrum activity, can represent a valuable starting point for the design of small molecules. The work carried out opens the way to new perspectives for the development of new anti-influenza drugs, especially in a context in which the emergence of new and drug-resistant viruses highlights the need for new antiviral approaches and strategies.

References

1. Hale, B.G.; Albrecht, R.A.; García-Sastre, A. F. *Microbiol.* **2010**, 5, 23–41.
2. Atanasov, A. G. et al.. *Biotechnol. Adv.* **33**, 1582–1614 (2015).
3. Superti, F.; Agamennone, M.; Pietrantoni, A.; Ammendolia, M.G *Viruses* **2019**, 11, 51.
4. Scala, M.C.; Sala, M.; Pietrantoni, A.; Spensiero, A.; Di Micco, S.; Agamennone, M.; Bertamino, A.; Novellino, E.; Bifulco, G.; Gomez-Monterrey, I.M.; *Sci. Rep*. **2017**, 7, 10593.

PLANT-DERIVED EXTRACELLULAR VESICLES AS DRUG DELIVERY SYSTEMS: A PROOF OF CONCEPT STUDY USING TANGERINE VESICLES

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Extracellular vesicles (EVs) are lipoproteic particles released by organisms belonging to different kingdoms, and research in recent years has indicated they are mainly involved in cross-kingdom interaction. The content of EVs consists of various biomolecules, such as proteins, nucleic acids, sugars, and lipids. Extracellular vesicles from plants (PDEVs) have recently attracted attention for the therapeutic opportunities that they offer[1]. EV-like structures have been purified from different citrus fruits[2-4] , grapes[5], and tomatoes[6]; their anti-inflammatory, anti-oxidant, and anti-tumor properties have been described[1,7]. Furthermore, plant-EVs possess several characteristics that make them suitable as drug delivery systems, such as the ability to cross biological barriers, stability, and safety[8,9]. Here we will investigate the possibility to use tangerine-derived EVs (TEVs) as a vehicle for the delivery of anti-tumor siRNAs.
TEVs have been isolated EVs from tangerine juice by differential centrifugation, filtration steps and ultracentrifugation. The vesicles were characterized by dimensional (Nanosight), morphological (Transmission Electron Microscope) and molecular (Western Blot) analyses. Co-incubation, sonication and electroporation approaches have been tested to load siRNAs in TEVs. Glomax and confocal microscopy assays have been carried out to evaluate the loading efficiency and cell internalization.
Preliminary results demonstrated that TEVs range in size from 150 to 200 nm and contain the Heat Shock Protein 70. Metabolomics analysis revealed the presence of several lipids and metabolites characteristic of the citrus fruit. Through electroporation protocol, we obtained an average oligonucleotide loading efficiency of 12%. TEVs loaded with the siRNA are internalized by colon cancer cells leading to the decrease of the target.
In conclusion, the data obtained from these preliminary investigations confirm that it is possible to load TEVs with siRNA; future functional studies will allow us to define if they can serve as a drug delivery vehicle in colon cancer treatments.

References

1. Urzi O, Raimondo S, Alessandro R. *Int J Mol Sci*. **2021**;22.
2. Raimondo S, Naselli F, Fontana S, Monteleone, F.; Lo Dico, A.; Saieva, L.; Zito, G.; Flugy, A.; Manno, M.; Di Bella, M. A.; De Leo, G.; Alessandro, R. *Oncotarget*. **2015**, 6:19514-27.
3. Stanly C, Moubarak M, Fiume I, Turiak L, Pocsfalvi G. *Int J Mol Sci*. **2019**, 20.
4. Bruno SP, Paolini A, D'Oria V, Sarra A, Sennato S, Bordi F, Masotti A. *Front Nutr*. **2021**;8:778998.
5. Ju S, Mu J, Dokland T, Zhuang X, Wang Q, Jiang H, Xiang X, Deng ZB, Wang B, Zhang L, Roth M, Welti R, Mobley J, Jun Y, Miller D, Zhang HG. *Mol Ther*. **2013**;21:1345-57.
6. Bokka R, Ramos AP, Fiume I, Manno M, Raccosta S, Turiák L, Sugár S, Adamo G, Csizmadia T, Pocsfalvi G. *Foods*. **2020**;9.
7. Alfieri M, Leone A, Ambrosone A. *Pharmaceutics*. Apr 6 **2021**;13.
8. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang HG. *Mol Ther*. **2010**;18:1606-14.
9. Zhuang X, Teng Y, Samykutty A, Mu J, Deng Z, Zhang L, Cao P, Rong Y, Yan J, Miller D, Zhang HG. *Mol Ther*. *Feb* **2016**;24:96-105.

SPIRULINA PEPTIDE SP6 REGULATES ATHEROSCLEROSIS PROGRESSION MODULATING DIFFERENT METABOLIC PATHWAYS: A MULTI-OMIC APPROACH

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The importance of natural compounds as possible adjuvant or side approaches to pharmacological therapy for the prevention and treatment of multifactorial cardiovascular dis-orders has experienced a boost in the last years. Bioactive compounds, especially those of plant or marine origin, have been recognized of several healthy properties and have found widespread adoption in nutraceuticals and functional foods.[1] Along with traditional phytochemicals, bioactive peptides from food and other natural matrices have generated attention as novel therapeutic compounds against different pathologies such as diabetes, hypertension and dyslipidemia, which define the metabolic syndrome.[2] The interest towards natural molecules is related to low or absent side effects since they are easily metabolized by endogenous enzymes. In addition, bioactive peptides can display very high specificity for their biological targets. In this scenario, bioactive Microalgae have emerged as a rich source of peptides with different properties, such as for the prevention of cardiovascular disease.[3] We recently characterized a novel decameric peptide from the gastro-intestinal digest of Spirulina platensis, named SP6 (GIVAGDVTPI, allophycocyanin _-chain, f:95–104), which showed very potent in vivo anti-hypertensive activity by exerting endothelium-dependent vasodilation via a PI3K (phosphoinositide-3-kinase)/AKT (serine/threonine kinase Akt) pathway, converging on nitric oxide (NO) release.[4] Since hypertension and atherosclerosis are comorbid conditions often found in combination, the aim of this study was the evaluation SP6 in modulating the atherosclerosis progression in a mouse model of high fat diet ($ApoE^{-/-}$). For this purpose, an integrated multi-omics-mass spectrometry-based approach was used. Polar metabolites and lipids were extracted from 20 μL of plasma and analyzed by a combined mass spectrometry approach. Multivariate statistical analysis revealed that different pathways were positively modulated by SP6, including several markers of atherosclerotique plaque progression, such as sphingomyelins, glycerophospholipids, aminoacids, and TCA intermediates. These preliminary results pave the way to further extension of SP6 as potential adjuvant for atherosclerosis management.

References

1. W.Y. Huang, S.T. Davidge, J. Wu Crit. Rev. *Food Sci. Nutr*. 53 (**2013**) 615–630.
2. K.G. Alberti, P. Zimmet, J. Shaw, IDF *Lancet* 366 (**2005**) 1059–1062.
3. E. Sommella, *et* al. *Molecules* 23 (**2018**) 1152.
4. A. Carrizzo, *et* al. *Hypertension* 73 (**2019**) 449–457.

ONCONUTRACEUTICAL POTENTIAL OF DIETARY PLANT POLYPHENOLS

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Nowadays, high attention has been addressed to natural components in fruits and vegetables with potential antioxidant, anti-inflammatory, anticarcinogenic and antimutagenic properties. The consumption of dietary polyphenols in cancer therapy is mainly aimed at chemoprevention to reduce the drug resistance, to the identification of synergistic effects with anticancer drugs in order to decrease drug concentrations and, consequently, the side effects associated with current cancer treatments. In fact, chemotherapy protocols including surgery, pharmacotherapy, radiotherapy, and biologically based therapies, have unintended side effects, which compromise both health and well-being of patients. In this regard, dietary polyphenols have been widely demonstrated to be able to not only reduce oxidative and inflammatory stress typical of the onset and development of cancer, but also to counteract the side effects associated with drug therapy. This new topic is known as "onconutraceutics".
In this regard, we investigated the anti-cancer properties of the polymethoxyflavones and prenylflavonoids extracted from *Citrus sinensis* and *Humulus lupulus*, respectively.[1] The natural biofunctional fractions, singularly and in combination, reduced the cell viability of CRC stem cells (CR-CSCs) and synergized with 5-fluorouracil (5-FU) and oxaliplatin (FOX) chemotherapy. These phenomena were accompanied by a reduced S and G2/M phase of the cell cycle and upregulation of cell death-related genes.
In addition to anticancer action, dietary polyphenols are able to reduce drug side effects of current chemotherapeutic protocol. In this regard, we studied the effects of pomegranate (*Punica granatum L.*) juice extract (PPJE) on the oxidative and inflammatory state in 5-FU-treated human skin keratinocytes (HaCaT) and intestinal epithelial cells (IECs).[2,3] 5-FU is a pyrimidine analogue used as an antineoplastic agent to treat multiple solid tumors. Despite its use and efficacy, it also has important side effects in healthy cells related to its pro-oxidant and pro-inflammatory potential. Our results showed that PPJE significantly inhibited reactive oxygen species release and increased the cellular antioxidant response, as indicated by the increased expression of cytoprotective enzymes, such as heme oxygenase-1 and NAD(P)H dehydrogenase [quinone] 1. PPJE also inhibited nitrotyrosine formation and 5-FU-induced inflammatory response, as indicated by the reduced cytokine level release. Moreover, PPJE inhibited nuclear translocation of p65-NF-κB, a key factor regulating the inflammatory response. In 5-FU-treated HaCaT and IEC-6 cells PPJE also inhibited apoptosis and promoted wound repair.
These results suggest a potential use of dietary polyphenols as natural remedies to aid oncological therapies and to reduce the side effects of chemotherapy protocols.

References

1. Turdo, A.; Glaviano, A.; Pepe, G.; Calapà, F.; Raimondo, S.; Fiori, M. E.; Carbone, D.; Basilicata, M. G.; Di Sarno, V.; Ostacolo, C.; Parrino, B.; Cascioferro, S.; Pecoraro, C.; Di Franco, S.; Bellavia, D.; Gaggianesi, M.; Veschi, V.; Lo Iacono, M.; Ganduscio, G.; Pantina, V. D.; Mangiapane, L. R.; Bongiorno, M. R.; Alessandro, R.; Todaro, M.; De Maria, R.; Diana, P.; Campiglia, P.; Stassi, G. *Cancers*. **2021**, *13*, 3927.
2. Rapa, S. F.; Magliocca, G.; Pepe, G.; Amodio, G.; Autore, G.; Campiglia, P.; Marzocco, S. *Antioxidants.* **2021**, *10*, 203.
3. Pepe, G.; Rapa, S. F.; Salviati, E.; Bertamino, A.; Auriemma, G.; Cascioferro, S.; Autore, G.; Quaroni, A.; Campiglia, P.; Marzocco, S. *Antioxidants.* **2020**, *9*, 699.

NATURAL PEPTIDES FROM SEAGRASS *POSIDONIA OCEANICA* AS SCAFFOLDS FOR SYNTHETIC ANTIMICROBIAL PEPTIDES AGAINST ANTIBIOTIC RESISTANCE

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Discovering novel antimicrobial agents against multi-drug resistant bacterial pathogens represents an important challenge to overcome the continuous widespreading of antibiotic resistance worldwide. We have given attention to marine plants as unconventional sources of antibiotics, focusing in particular on antimicrobial activities of polypeptide-enriched extracts from beached rhizomes, green and brown leaves of Mediterranean seagrass *Posidonia oceanica* L. (Delile) against some relevant pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*). The antimicrobial activity of the extracts was tested both in bacterial planktonic cells both in biofilm structured bacteria, due to the relevant role of microbial communities in antibiotic resistance. The crude extracts obtained from *P. oceanica* have shown to be active, inhibiting the growth of four selected pathogens and the formation of biofilm of *S. aureus*. Starting from biological activities, polypeptide-enriched extracts were further investigated by high-resolution mass spectrometry (RP-HPLC/nESI-MS/MS) and database search in order to detect the presence of novel natural peptides, responsible of the observed activity *in vitro.*

Database search has allowed the characterization of fourteen peptides in *P. oceanica* extracts, mostly related to bacterial proteins included in investigated database. Moreover, several detected peptides have shown similarities with already described antimicrobial peptides (AMPs) isolated from bacteria, animals and plants. Three synthetic peptides derived from original sequences identified in green leaves and rhizome extracts have been tested *in vitro* and one of them have shown a significant antibiofilm activity against *E. coli*.

With the aim to design new and more efficient synthetic antimicrobial and antibiofilm peptides (SAAMPs), bioinformatic analysis has been performed using online servers, to improve natural peptides features and to select derivative molecules according to favourable chemical-physical and structural properties. Biological activities, cytotoxic, hemolytic and allergenic potential were also predicted too.

Overall, bioinformatic analysis has showed that natural peptides identified in *P. oceanica* extracts may represent a new platform for *ab novo* design of effective antimicrobial and antibiofilm peptides, making them promising candidates for the treatment of infections caused by drug-resistant pathogens.

A pharmaceutical analysis toolbox to unravel Humulus lupulus chemical and biological profile

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Humulus lupulus L. (Hop) is well known for the various therapeutical and nutraceutical properties, related to the complex secondary metabolite's composition. Nevertheless, there is the need of novel strategies to enrich the comprehension of the chemical and biological properties of this multiactivity phytocomplex. For this purpose, in this study we will report the integration and application of advanced analytical techniques suitable for the detailed characterization of hop phytochemicals, the evaluation of their biological properties by conventional and omic approaches, and finally elucidate the fate of key molecules after assumption.
Online comprehensive two-dimensional liquid chromatography coupled to tandem mass spectrometry (LC × LC-MS/MS) was employed to unravel the hop phytocomplex metabolite profile, resulting in higher peak capacity values and increased number of detected metabolites with respect to 1D-LC-MS methods [1] Furthermore, to understand the metabolic fate of bitter acids fraction, *in vitro* and *in vivo* metabolism was investigated by mouse liver microsomes and in biofluids. A sensitive and accurate UHPLC-HRMS method was developed and validated, and, as a result, novel I and II phase metabolites were characterized, showing for the first time several conjugated metabolites [2]. The possible use of hop secondary metabolites as natural immunomodulators and adjuvants in chemotherapy protocols has been evaluated. After class specific-fractionation, three different fractions of hop secondary metabolites were obtained and cytofluorimetric analysis revealed that a fraction mainly composed by Humulinones and Cohulupone derivatives, was able to affect Natural Killer compartment, enhancing the cytolytic activity of NK cells against leukemia cell line K562 through selective activation of NKp44 activating receptors [3].
Finally, mass spectrometry-based metabolomics was applied to evaluate the ability of specific hop fractions rich in β-acids and prenylflavonoids to modulate Dendritic Cells metabolism after stimulation with lipopolysaccharide (LPS). Untargeted analysis revealed that hop fraction modulate several key pathways linked to pro-inflammatory and glycolytic phenotypes, including the oxidative and non-oxidative steps of pentose phosphate pathway (PPP), citrulline-arginine and arginine-succinate pathways, as well as purine-pyrimidine metabolism [4]. This integrated platform shows the potential of analytical based approaches for the deep comprehension of natural products in the fields of medicinal chemistry and nutraceutics.

References

1. Sommella, E.; Pagano, F.; Salviati, E.; Chieppa, M.; Bertamino, A.; Manfra, M.; Sala, M.; Novellino, E.; Campiglia, P. Chemical profiling of bioactive constituents in hop cones and pellets extracts by online comprehensive two-dimensional liquid chromatography with tandem mass spectrometry and direct infusion Fourier transform ion cyclotron resonance mass spectrometry. *J. Sep. Sci.* **2018**, 41, 1548–1557.

2. Salviati, E.; Sommella, E.; Carrizzo, A.; Di Sarno, V.; Bertamino, A.; Venturini, E.; Vecchione, C.; Campiglia, P. Characterization of phase I and phase II metabolites of hop (*Humulus lupulus* L.) bitter acids: *In vitro* and *in vivo* metabolic profiling by UHPLC-Q-Orbitrap. *J. Pharm. Biomed.,* **2021**, 201, 114107.

3. Salviati, E.; Ciaglia, E.; Sommella, E.; Montella, F.; Bertamino, A.; Ostacolo, C.; Parrino, B.; Rubino, R.; Vecchione, C.; Puca, A.; Novellino, E.; Campiglia, P. Immunomodulatory activity of Humulus lupulus bitter acids fraction: enhancement of natural killer cells function by NKp44 activating receptor stimulation. *J. Funct. Food*, **2019**, 61, 103469.

4. Sommella, E.; Verna, G.; Liso, M.; Salviati, E.; Esposito, T.; Carbone, D.; Pecoraro, C.; Chieppa, M.; Campiglia, P. Hop-derived fraction rich in beta acids and prenylflavonoids regulates the inflammatory response in dendritic cells differently from quercetin: Unveiling metabolic changes by mass spectrometry-based metabolomics. *Food Funct.*, **2021**, 12, 12800 - 12811.