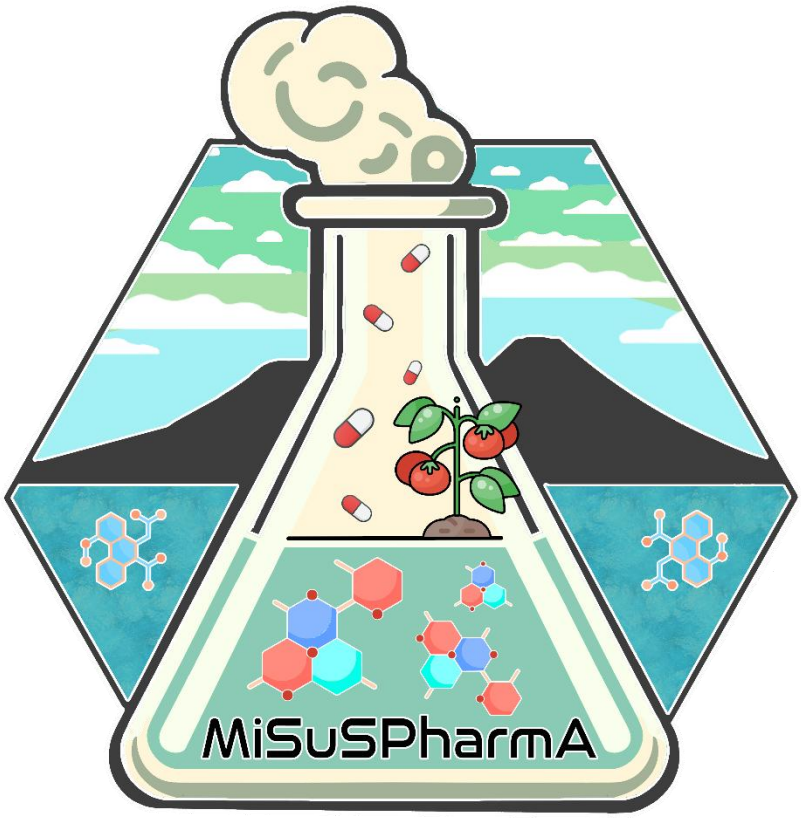


# Mid-Season Sustainable Pharmaceutical Applications Conference

**1st Edition**

**Naples 5-6 May 2025**

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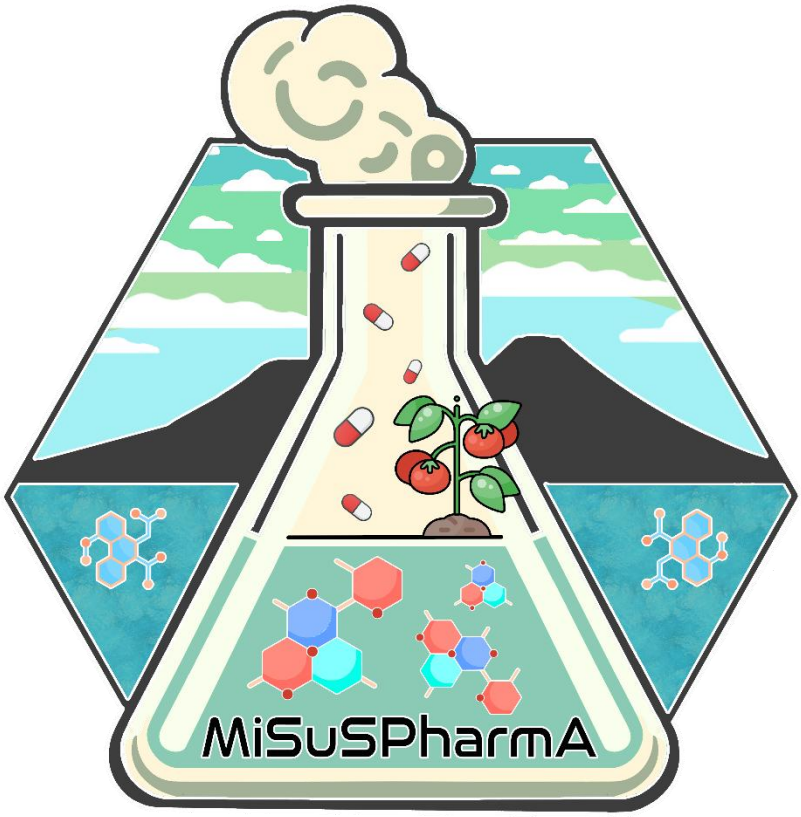


## PLENARY SPEAKERS


# PL01 - SUSTAINABLE PREPARATION OF WHO ESSENTIAL MEDICINES BY MECHANOCHEMISTRY

Evelina Colacino

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**Keywords:** Mechanochemistry, Active Pharmaceutical Ingredients, Rearrangements, Green metrics.

Despite ongoing efforts to lessen the environmental impact of active pharmaceutical ingredient (API) production, the use of organic solvents—responsible for 75% of energy consumption— remains a key component in many manufacturing processes. Solvent-free (or low-solvent) synthesis through mechanochemistry [1,2] offers a solution that adheres to several Green Chemistry Principles [3] and holds great promise as a sustainable technology for the production of value-added chemicals, pharmaceutical fragments, functionalities, and APIs.
This presentation explores case studies that demonstrate the use of mechanochemical processes to produce World Health Organization (WHO) essential medicines at various scales. By applying green chemistry metrics, [4] we highlight how mechanochemistry provides an ecofriendly and cost-effective alternative for the production of green pharmaceuticals, contributing to the larger goal of building a more sustainable planet [5].

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## PL02 - The new deal of plant derived extracellular vesicles in nutraceuticals and cosmetics

Mariantonia Logozzi [1,2], Rossella Di Raimo [1,2], Davide Mizzoni [1,2], Antonella Aloi [1], Giulia Pietrangelo [1], Stefano Fais [1,2*]

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**Keywords:** Extracellular Vesicles, Plant, nutraceutics, cosmetics, delivery, antioxidants

Research in science and medicine is witnessing a massive increase in the literature concerning extracellular vesicles (EVs). From a morphological point of view, EVs include extracellular vesicles of micro and nano size. However, this simplistic classification considers neither the source of EVs, including the cells and species from which EVs are obtained, nor the microenvironmental conditions that can affect EV production. These two factors are of crucial importance for the potential use of EVs as therapeutic agents. In fact, the choice of the most suitable EVs for drug delivery remains an open debate [1], as the use of human-derived EVs may present at least two major problems: (i) autologous EVs from a patient can carry dangerous molecules; and (ii) large-scale industrial production is limited by the need to cultivate a huge amount of cells (cell factories) [1,2] . Plant-derived exosomes (PDEVs) are receiving much attention as a natural source of antioxidants. Previous research has shown that PDEVs contain a range of bioactives and that their content varies depending on the original source of fruits or vegetables, and also on the type of agriculture used (i.e., organic or intensive [2]. In fact, it has been shown that fruits and vegetables from organic farming produce more exosomes, are safer, free of toxic substances, and contain more bioactive substances [3]. More recently, it has the ability of orally administered PDEVs mixes (Exocomplex®) to restore a Redox balance in animals subjected to Redox imbalance by treatment with hydrogen peroxide ($H_2O_2$), has been investigated. The model we used involved mice treated for two weeks with hydrogen peroxide ($H_2O_2$), compared with untreated mice after the period of $H_2O_2$ administration and mice that had received only water during the experimental period. A blood sample was taken at the end of the experiment, along with individual organs at sacrifice. Before treatment, the content of bioactive molecules was determined in samples of PDEs used for in vivo treatment on mice. PDEs were administered orally, starting the day after $H_2O_2$ treatment was stopped [3]. The results first showed that the fruit and vegetable blends (Exocomplex®) had high antioxidant capacity and contained a number of bioactives, including Catalase, Glutathione (GSH), Superoxide Dismutase (SOD), Ascorbic Acid, Melatonin, Phenolic Compounds and ATP. Oral administration of Exocomplex® restored redox balance with reduced serum levels of both reactive oxygen species (ROS) and malondialdehyde (MDA), but also a general recovery of homeostatic condition at the organ level, supporting the future use of PDE for health care. With this study we demonstrated that oral administration of a mix of exosomes obtained from different types of organically derived fruits and vegetables, called Exocomplex®, is able to induce a general antioxidant reaction with a complete recovery of redox balance at the level of the organs examined. Exocomplex®-based products have the potential to represent a new class of supplements characterized by a high level of bioavailability; thus not requiring the use of high amounts of bioactives to achieve a demonstrable effect. This can be achieved by oral administration without the need for more invasive routes of administration. Further studies have shown that a mix of PDEVs is able to completely recover wounds produced in culture of skin-derived fibroblasts of human origin [4]. Moreover, individual antioxidants contained in plant-derived nanovesicles could also be useful in implementing novel therapeutic approaches against serious diseases, as demonstrated in cells of leukemia origin [5]. Lastly, research data are supporting the use of PDEVs as natural delivery systems for molecules of different origin [6].

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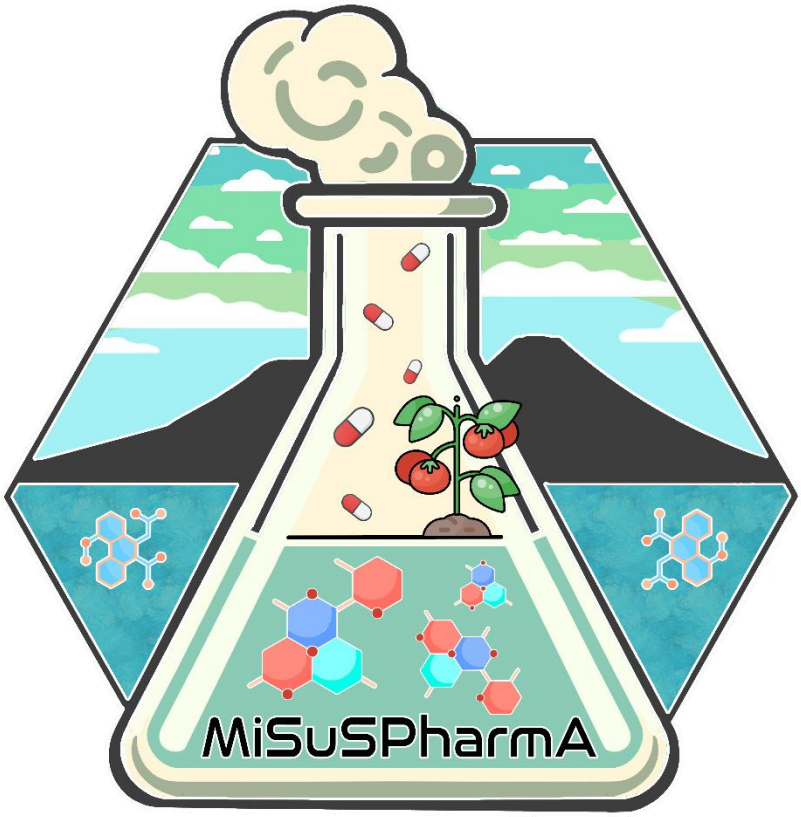


## ORAL PRESENTATIONS

# OC01 - Toward a green Heck reaction protocol to access trisubstituted alkenes, useful pharmaceutical intermediates

Giacomo Rossino[1], Giorgio Marrubini[1], Margherita Brindisi[2], Marc Granje[1,3], Pasquale Linciano[1], Daniela Rossi[1], Simona Collina[1]

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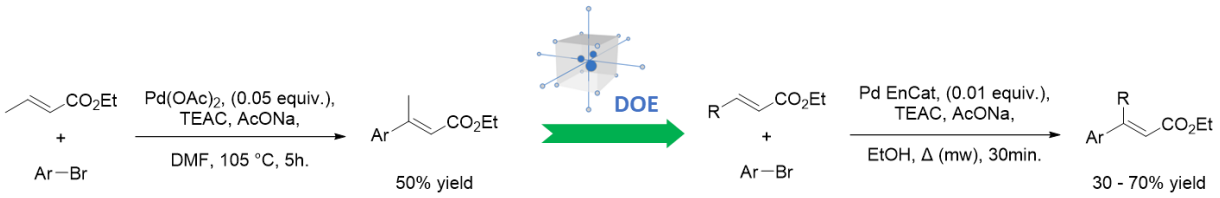
**Keywords:** Heck reaction, trisubstituted alkenes, green solvents, microwaves, design of experiments

The Heck reaction has been extensively utilized to construct various biologically relevant scaffolds and active pharmaceutical ingredients (APIs) [1]. Reactions with terminal alkenes typically give high yields and selectivity, and numerous green adaptations of the original protocol have been developed over the years for these substrates [2]. However, these methods are less effective when applied to internal olefins (which are less reactive and more prone to give byproducts), although these substrates would provide access to trisubstituted alkenes, which are crucial intermediates and privileged structures of pharmaceutical interest [3]. To address this limitation, we developed a Heck reaction protocol under green conditions specifically for the synthesis of trisubstituted alkenes.
Preliminary experiments using a model reaction were guided by a design of experiments (DoE) approach that enabled the identification of optimal conditions, including catalyst loading, equivalents of alkene, base, and solvent. Subsequent experiments refined the process, bringing the reaction to completion while considering additional variables such as time and temperature of the reaction. The resulting protocol employs ethanol as the solvent, microwave (mw) irradiation, and the supported catalyst Pd EnCat®40 (Figure 1). This methodology was applied to a range of aryl bromides and internal olefins to evaluate the substrate scope. Additionally, a straightforward isomerization procedure was developed to convert isomeric byproducts into the desired conjugated E-alkenes. For both the Heck and isomerization reactions, the environmental factor (E factor), costs and yields were compared to the protocols previously employed by us. The newly established approach, recently published, offers a green, efficient, and user-friendly method for synthesizing trisubstituted alkenes via the Heck reaction [4].

Figure 1. Comparison between the old and new protocol for the Heck reaction.




*Difficult catalyst recovery, toxic solvent, long reaction time*

*Supported catalyst, green solvent, short reaction time*

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## OC02 - Continuous Flow Synthesis and Sustainable Joullié-Ugi Chemistry Yield Captopril-Inspired Broad-Spectrum Metallo Beta-Lactamase Inhibitors

Simona Barone[1], Antonella Ilenia Alfano[1], Sveva Pelliccia[1], Pasquale Russomanno[2], Valerio Baia[1], Emilia Cassese[1], Luigi Cutarella[3], Sacha Cancade[4], Mattia Mori[3], Jean Denis Docquier[4], Vincenzo Summa[1], Margherita Brindisi[1]

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**Keywords:** Metallo beta-lactamases, antimicrobial resistance, broad-spectrum MBL inhibitors, flow chemistry, sustainable synthesis

Antibiotic resistance is a pressing global health issue, exacerbated by the emergence of highly virulent and resistant bacterial pathogens, notably the "*ESKAPE*" group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.)*, responsible for a significant portion of hospital-acquired infections and characterized by their ability to evade commonly used antibiotics. The increasing prevalence of metallo-β-lactamase enzymes (MBLs) in bacteria has become a major resistance mechanism, conferring broad-spectrum chemoresistance to β-lactam antibiotics and posing a significant challenge to infection treatment [1, 2]. Since no MBL inhibitors have been approved to date, there is an urgent need to discover novel, safe, and effective compounds with broad-spectrum activity against clinically relevant MBLs. Herein, we report a green and sustainable continuous flow protocol for the synthesis of new MBL inhibitors inspired by the structure of captopril, which displays a weak MBL inhibitory activity. Starting from captopril structure, thanks to our telescoped continuous flow protocol [3], we successfully synthesized a small library of two subseries of compounds bearing a spiroindolinic core, both increasing hydrophobic interactions with MBL active site and selectivity, thus avoiding the unwanted activity on Captopril target, namely ACE enzyme. Synthesized compounds demonstrated *in vitro* low micromolar activity on NDM-1, VIM-2 and IMP-1 MBL isoenzymes and no activity on ACE1-enzyme, thus confirming their selectivity profile. Furthermore, synthesized compounds were evaluated in combination with imipenem against clinical isolates producing MBLs, resulting in a gratifying 4-fold reduction in imipenem minimum inhibitory concentration (MIC). In-depth *in silico* studies, combining docking simulations and molecular dynamics, provided insights into the binding mode of the synthesized compounds within the MBL active site. By adopting a green and sustainable continuous flow protocol, we enabled the efficient and diversity-oriented generation of analogues, facilitating rapid structural optimization, leading us to the development of a novel class of broad-spectrum MBL inhibitors with enhanced efficacy and potency compared to captopril. Their promising *in vitro* activity underscores their potential as effective antibiotic adjuvants. Further chemical optimization, guided by *in silico* studies, will aim to refine their potency and pharmacokinetic properties while preserving their broad-spectrum activity.

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# OC03 - Antibody Drug Conjugates with unconventional payloads: the new frontier of precision medicine in cancer therapy

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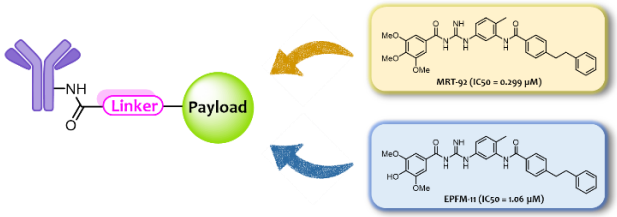
**Keywords:** Precision medicine, ADC, Hedgehog signaling pathway, Smo inhibitors

Precision medicine, also known as personalized medicine, is a cutting-edge method of customizing disease prevention and treatment by considering individual variations in genetics, environment, and lifestyle. The goal of precision medicine is to get the right treatments to the right patients at the right time [1]. Antibody-drug conjugates (ADCs) represent a form of precision medicine, as they enable the selective delivery of therapeutics to target cells and can be tailored to the specific characteristics of a disease. ADCs consist of a monoclonal antibody (mAb) connected to a drug (payload) through a properly designed linker. The mAb is responsible for the selective transport of the drug to the target cells expressing the specific antigen, thus avoiding off-target toxicity of the drug. The chemical linker plays an important role due to its dual ability to be stable in the blood as well as to release drugs into the cells by degrading. We recently demonstrate that is not mandatory to charge the mAb with a cytotoxic payload to obtain effective ADCs [2]. In this study, several ADCs are being developed using Smoothened (SMO) inhibitors as payloads. SMO is a key regulator of the Hedgehog (Hh) signaling pathway, which plays a crucial role in embryonic development and is often dysregulated in various diseases, including cancer. Aberrant activation of Hh signaling is associated with the development of malignant transformation in a variety of human cancers (i.e. glioblastoma, basal cell carcinoma, melanoma), affecting key tumorigenic processes, such as proliferation, invasion and progression of cancer cells, and can be initiated by Patched1/Smoothened-dependent signals [3]. Our group has been involved in the design and synthesis of SMO inhibitors with activities in the μM range for many years [4-6]. **MRT-92** and its derivative **EPFM-11** have been selected for bioconjugation with Cetuximab for cancer treatment through a sustainable Copper-free click reaction (Figure 1) [7].

Figure 1. General Structure of anti-cancer ADC.

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# OC04 - MODULAR SYNTHESIS OF BENZOYLPYRIDINES EXPLOITING A CATALYST- FREE REDUCTIVE ARYLATION STRATEGY

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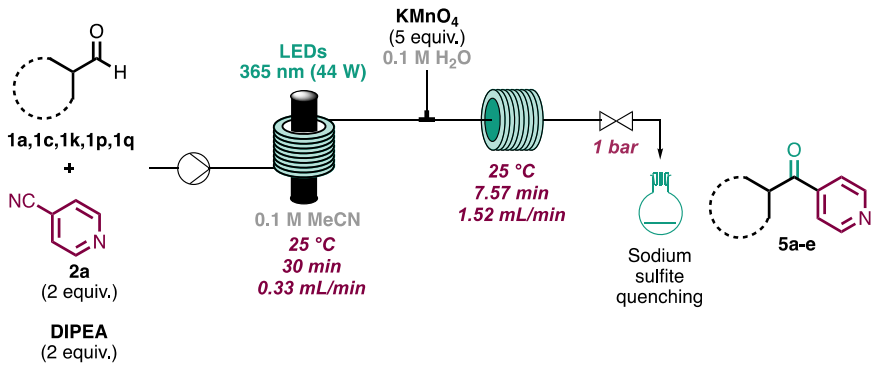
**Keywords:** flow chemistry, green chemistry, photochemistry, arylation

Developments in modern photocatalysis have highlighted the power of Ru- and Ir-based catalysts to bring about a plethora of valuable synthetic processes using visible light [1]. Due to the high cost and potential toxicity, their use in industrial settings is fairly limited. In this context, simple organic molecules with sufficient conjugation continue to play a key role in scaled photochemical reactions and triplet photosensitizers such benzophenone and (thio)xanthone are frequently used examples [2]. While these entities are readily available at low cost, display good solubility and are considered non-harmful, the introduction of electron-donating or -withdrawing substituents, that is critical to modify their photophysical properties, commonly necessitates long and inefficient synthesis routes. To address this challenge, we set out to create an expedited route into electronically differentiated bis-aryl ketones that combine electron-rich benzene systems with electron-deficient pyridyl moieties. Continuous flow processing is employed to provide increased scalability, reaction efficiency as well as reproducibility [3]. Our strategy combines a reductive arylation reaction between aryl aldehydes and cyano-pyridines with an oxidation of the resulting secondary alcohol product to yield the desired bis-aryl ketones via a modular route [4].

Scheme 1. Telescoped flow synthetic pathway.

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# OC05 - New Potential Carbonic Anhydrase Inhibitors as Dual-Target Anticancer Agents

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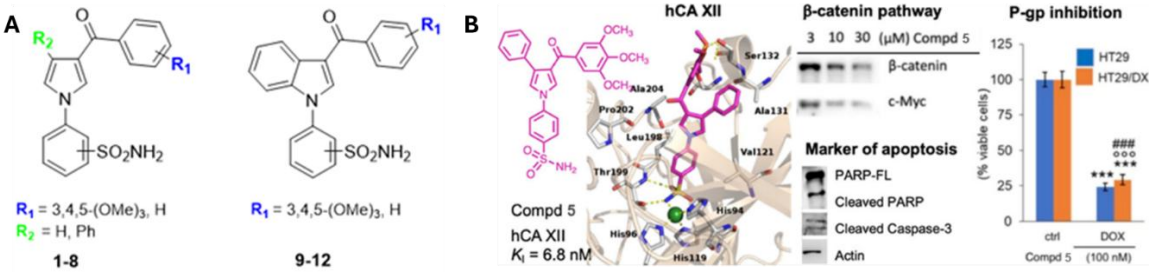
**Keywords:** Cancer, Microwave reaction, carbonic anhydrase, β-catenin, dual target

Inhibiting carbonic anhydrases is nowadays considered a new efficient target in many pathologies, including cancer. Carbonic anhydrases are ubiquitous enzymes that catalyse the reversible hydration of carbon dioxide to produce monohydrogen carbonate and $H^+$ ions. All the different isoforms of human carbon anhydrase (hCA) belong to the α-class. They are distinguished by their location and role in many physiological processes. Their dysregulation has also been found in many pathologies, such as Glaucoma, Parkinson's and cancer disease. In this context, finding new inhibitors selective for specific hCAs isoforms may be a valid new therapeutic approach in the pathologies mentioned above. Starting from previous work on Tubulin polymerization inhibitors [1] we synthesized compounds (1-12) introducing a sulfonamide group on different position of N1 phenyl ring of pyrrole or indole scaffold (figure 1A). These new potential inhibitors [2] were synthesized with a focus on optimizing the main reactions to improve both yield and reaction time. For this reason, we used a microwave reactor, which allowed us to obtain the compounds more rapidly but also with a significant reduction in solvent usage, both for the reaction and for purification, a crucial aspect for progressing toward greener chemistry. These compounds did not significantly inhibit tubulin polymerization but showed interesting activity against isoforms involved in cancer (IX and XII). In particular, derivatives with the sulfonamide group at the para position on the benzene ring exhibited the highest levels of activity, with enzymatic inhibition in the nanomolar range. The most promising compounds were then evaluated across a range of tumor cell lines, demonstrating a strong reduction in cell viability, particularly in colorectal cancer and triple-negative breast cancer cells. Among them, compound **5** showed two notable effects: it effectively inhibited the Wnt/β-catenin pathway and Pgp efflux pumps, both of which play crucial roles in tumor development. Taken together, these results highlight compound 5 as a promising broad-spectrum anticancer agent that targets both hCA and the β-catenin pathway, with particular potential in restoring sensitivity in P-gp expressing cell lines (figure 1B). These findings establish compound **5** as a lead candidate for a new class of dual-targeting anticancer agents and warrant further investigation into its potential for cancer treatment.

Figure 1. (A) Structure of compounds 1-12; (B) Biological activity of compound 5.

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# OC06 - Harnessing the Passerini Reaction for Peptidomimetic Ciprofloxacin Derivatives: A Strategy to Overcome Antibiotic Resistance

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**Keywords:** Multicomponent reactions, Passerini reaction, Antibiotic Resistance, Antibiotics

The critical global health challenge of antibiotic resistance requires novel strategies to restore or enhance the efficacy of current antibiotics. In this study, we developed a series of ciprofloxacin-based peptidomimetics by selective modification at C3 carboxyl group through a multicomponent Passerini reaction [1]. Passerini reaction is a powerful tool in medicinal chemistry, enabling the rapid and efficient synthesis of structurally diverse bioactive molecules reducing solvent and reaction waste. A new alternative protocol for the synthesis of ciprofloxacin-based peptidomimetics was achieved with Passerini reaction by using aqueous surfactant systems. The synthesized derivatives were functionalized at the C3 carboxyl group using the same isocyanide building-block in the Passerini reaction while modifying the aldehyde component. The synthetic strategies applied allowed the rapid analogs synthesis of several derivatives. In particular, a series of aliphatic aldehydes with different chain lengths and aromatic aldehydes were employed to modulate the lipophilicity of the compounds and investigate its impact on antibacterial activity. The synthesized derivatives were evaluated in vitro for antibacterial activity against a panel of LPS-varied Escherichia coli strains and selected ESKAPE pathogens, including *A. baumannii, P. aeruginosa, E. cloacae*, and *S. aureus*. The new compounds showed increased antimicrobial potency, particularly against E. coli mutants. The most active compounds exhibited MIC from 0.22 to 2.19 μM, outperforming standard ciprofloxacin (MIC = 0.62–3.44 μg/mL). Furthermore, the results revealed a strong correlation between the antimicrobial activity and the lipophilicity (LogP) of the compounds which probably influences their ability to penetrate bacterial membranes. Cytotoxicity assays on BALB/c3T3 fibroblast cells confirmed that the derivatives exerted comparable or lower cytotoxic effects than the parent ciprofloxacin. This work highlights the potential of peptidomimetic-based modifications in restoring and expanding the antimicrobial spectrum of ciprofloxacin, offering a promising avenue for combating antibiotic-resistant bacterial infections.

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## OC07 - Moving towards a sustainable development of therapeutic peptides

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**Keywords:** Solid-phase peptide synthesis, green solvents, mixtures, coupling reagents.

The development of greener synthesis processes is a real necessity to transform the industrial landscape, especially the pharmaceutical sector, into a more sustainable reality. Due to the increasing demand from the chemical and pharmaceutical markets for synthetic peptides in the last years, the attention to the greening of their production represents a significant challenge droving researcher toward the introduction of sustainable processes to prepare highly pure, active pharmaceutical ingredients (APIs) [1].
Nowadays the preferred method to obtain peptides is the solid phase peptide synthesis (SPPS), which, unfortunately, does not respect the principles of green chemistry due to the large amount of toxic reagents and solvents used. Because the synthetic procedures do not admit a reduction in the amount of solvent, several attempts have been reported in recent years for replacing DMF with greener solvents [2,3], according to well-known solvent-selection guides.
In this contest, some of the efforts to make peptide synthesis more sustainable in our lab were made. We report a study focused on the replacement of DMF in the fluorenyl methoxycarbonyl (Fmoc) solid-phase peptide synthesis with Dipropylene glycol dimethyl ether (PROGLYDE™, DMM) [4], a well-known green solvent with low human toxicity following oral, inhalation and dermal exposure and easily biodegradable and a mixture anisole/NOP (75:25) [5]. The ability of selected solvents to swell different resins and their capability to solubilize all Fmoc protected amino acids was investigated and model peptides Aib-enkephalin and Aib-ACP were synthesized resulting in favorable outcomes in terms of peptide synthesis efficiency.

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# OC08 - A new sustainable protocol for in-water Suzuki Arylation mediated by sardinian black wool

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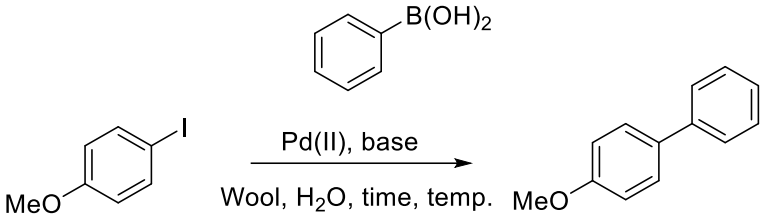
**Keywords**: Sustainability, Green Chemistry, Suzuki, Water, Biomass

The Suzuki-Miyaura reaction is one of the most widely used catalytic reaction for the construction of biaryl moieties. This synthetic method applies to a broad range of compounds in pharmaceutical, agrochemical, and materials science, making it a crucial tool for synthetic chemists [1]. Recent studies have increasingly focused on utilizing water as a solvent in the reaction environment. According to Green Chemistry principles, the excessive use of organic solvents poses significant environmental challenges.
To enhance these methods, various solutions have been explored, including a range of ligands, surfactants, bases, and heterogeneous supports [2]. Here, we report a novel process that employs black Sardinian wool to facilitate the reaction in water, effectively recycling an agricultural waste product that is produced in large quantities and poses management challenges in the zoological sector. After undergoing mechano-chemical treatment, this biomass is applied in the optimization of a new sustainable Suzuki reaction in water on different compounds.

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# OC09 - Towards sustainable drug discovery: AI-driven *de novo* molecular design

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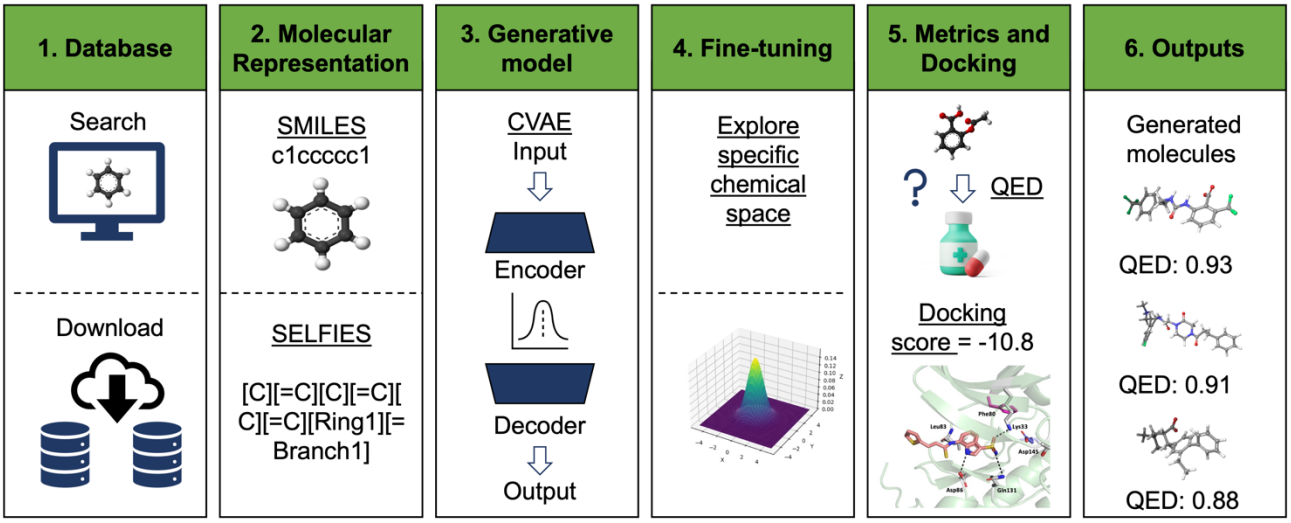
**Keywords:** Drug discovery, generative models, sustainable pharmaceutical research

Drug discovery is a resource-intensive process, requiring innovative strategies to enhance efficiency and sustainability. In this regard, recent advances in deep learning, particularly in de novo drug design, could offer interesting solutions by reducing the need for extensive chemical synthesis and experimental screening [1]. In this work, we introduce a Conditional Variational Autoencoder (CVAE) generative model designed to enhance molecular design by exploring vast areas of chemical space while maintaining key pharmaceutical properties [2]. Utilizing SMILES and SELFIES representations, our model generates novel molecules with optimized properties, validated through drug-likeness, synthetic accessibility, and novelty metrics. We demonstrate its effectiveness by designing molecules targeting CDK2, PPARγ, and DPP-IV, achieving high structural diversity while preserving essential features for target engagement, as demonstrated by docking studies. This proposed model represents a valuable resource for advancing *de novo* molecular design capabilities, also supporting a more sustainable pharmaceutical pipeline.

Figure 1. Workflow for molecule generation using the CVAE model.

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# OC10 - Chitin-Derived Catalysts for Cross-Coupling Reactions: A Mechanochemical Approach

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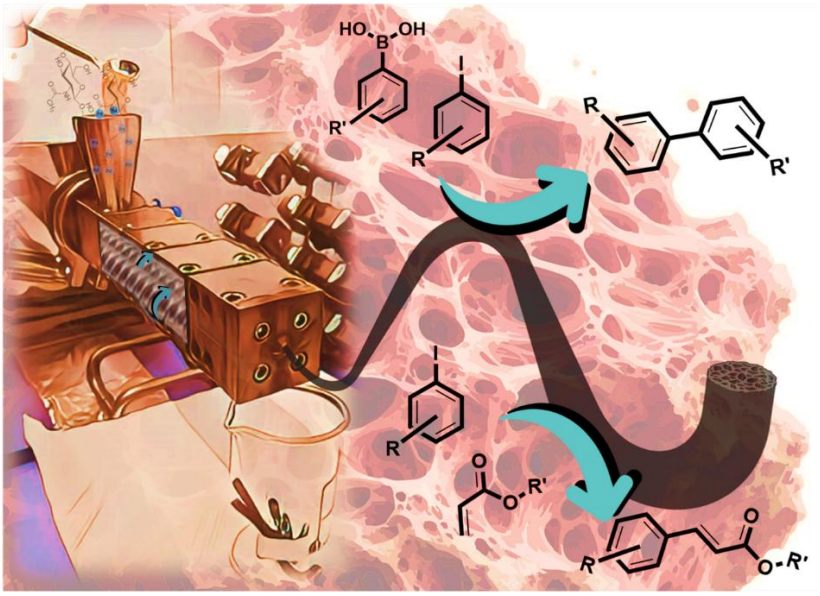
**Keywords:** Mechanochemistry, Chitin, Heterogeneous catalysis, Cross-coupling reactions

In this study, chitin derived from fishery waste was repurposed to develop catalytic materials for cross-coupling reactions. Specifically, mono- and bimetallic nanomaterials based on Pd and Cu, supported on nitrogen-doped carbon, were synthesized using three different methods, including a sustainable mechanochemical approach with a twin-screw extruder (Figure 1) [1]. The catalytic systems were extensively characterized using a multi-technique approach involving XPS, XRD, SEM, TEM, ICP-MS, and $N_2$-physisorption.
Furthermore, the effects of nanoparticle size, N-doping, and their synergistic interactions on catalytic performance were elucidated using Kelvin Probe Atomic Force Microscopy (KPFM), providing valuable insights into charge transfer and metal-support interactions. The catalysts were evaluated in Suzuki-Miyaura, Heck-Mizoroki, and Sonogashira reactions, with a comprehensive parametric study conducted to optimize reaction conditions, including variables such as reaction time, temperature, solvent, base, and catalyst loading. The catalytic performance was assessed under both batch and semi-continuous flow solvent-free conditions. Notably, the Suzuki reaction achieved quantitative yields and an exceptional productivity of 8.7 mol/($g_{Pd}$·h) [2]. Promising results were also obtained for the Sonogashira coupling, highlighting the potential of these materials for sustainable cross-coupling applications.

Figure 1. Schematic representation of mechanochemical catalysts synthesis and their application in cross-coupling reactions.

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## OC11 - Valorization of pomegranate peel: hydrolytic extraction and biological activity on AGS cells

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**Keywords:** pomegranate peel; basic hydrolysis; bioactive compound; AGS cells; antioxidant action

Pomegranate fruit (*Punica granatum* L.) is extensively used in the production of pomegranate juice. The best-known variety, consumed and used for juice production is the Wonderful variety. The production of pomegranate juice, however, leads to considerable amounts of waste mainly consisting of peels. The ongoing exploration of its potential is attracting increasing attention from the scientific community, given its rich composition of bioactive compounds, including ellagitannins such as punicalagins and punicalins, along with ellagic acid derivatives and gallotannins [1].
The first purpose of this work was to study the effects of chemical hydrolysis of dried pomegranate peels on the phenolic profile of the extract using HPLC-DAD and HPLC-DAD-MS techniques. The second aim was the evaluation of the protective properties of these extracts on a human gastric cell line, such as AGS cells, never used on this type of extracts.
The extractions were performed using a traditional decoction method, as well as bicarbonate at different concentrations to promote the partial hydrolysis of high-molecular-weight phenolic structures typical of pomegranate [2]. The goal was to obtain extracts enriched in phenolic derivatives with potentially higher bioavailability and greater bioactivity than their non-hydrolyzed counterparts. The decoction and hydrolysis in the presence of bicarbonate was then applied to several batches of pomegranate peels to evaluate the compositional variability of the final extracts. The proposed procedure is scalable and reproducible and the hydrolyzed extracts were enriched in gallic and ellagic acids, while punicalagins decreased (Figure 1). The total phenols calculated on the dry hydrolysed samples increased compared to those of the extracts from decoction. It should be noted that the variability for the phenolic composition and total phenolic amount of pomegranate peels of different batches was not significant.
The extracts were tested in vitro on the AGS gastric cell line to assess their biological response. At first instance, the effects on cell viability of the extracts at different concentration (5 to 200µg/mL) after 24 and 48h of incubation were evaluated by MTT test and it was observed that only the extracts with the highest phenolic content and administered in high concentration resulted in a decrease in cell viability (Figure 2). These results agreed with the cell cycle cytofluorimetric analysis as all the extracts at the concentration of 50µg/mL after a 24-hour incubation only slightly altered the distribution in the different phases of the cell cycle. At the same concentration no significant effects were observed regarding cell migration and proliferation through the scratch test. Finally, an *in vitro* antioxidant activity, correlated with the phenolic content of the extracts, was observed and therefore experiments are being carried out to evaluate, under oxidative stress conditions, the possible effects of the extracts on the activity of enzymes involved in the main metabolic pathways.
In the future, extraction techniques will be improved and further biological tests performed to better understand the biological effects of these molecules on AGS cells, on which pomegranate phenols have never been tested so far.
*Acknowledgments*. The work was funded by the PRIN project "An integrated multimodal approach for an in vitro evaluation of the nutraceutical potential of botanical extracts (phytocomplexes) from *Olea europaea* L. and *Punica granatum* fruit" (Project code: 2022X3WZAF).

Figure 1. Phenolic profiles of pomegranate peel (Wonderful variety from Emilia Romagna 2022) extracts obtained with hot water and bicarbonate solutions.

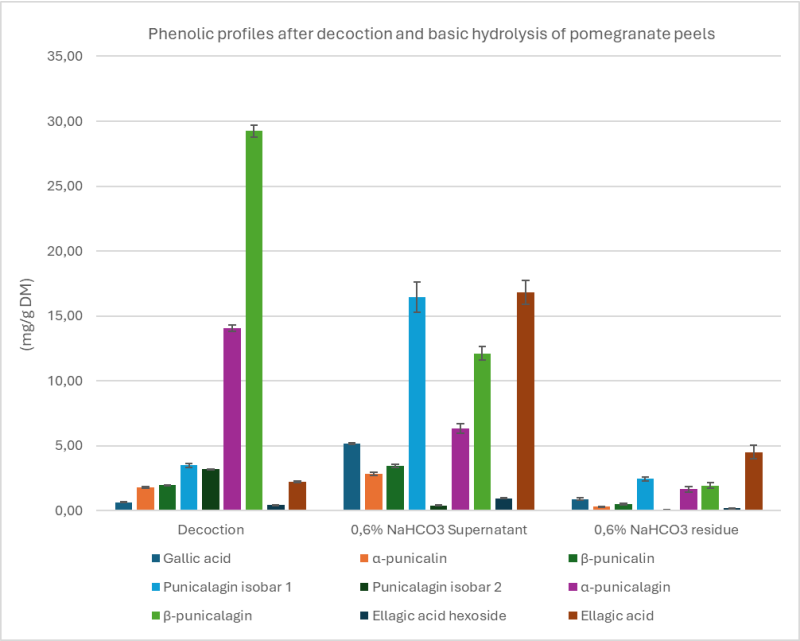
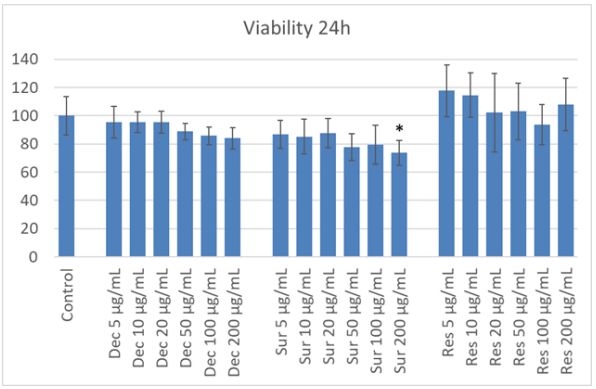
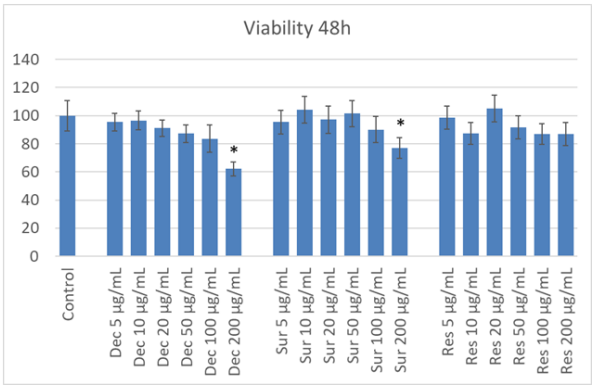



Figure 2. Viability (%) assessed by MTT test after 24 h (top) and 48 h (bottom) incubation with pomegranate peel (Wonderful variety from Emilia Romagna 2022) extracts at different concentrations.

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# OC12 - Sustainable Extraction and Functional Assessment of Pomegranate (*Punica granatum* L.) Polyphenols: A Green Approach for Gut Health and Disease Prevention

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**Keywords:** *In vitro* digestion, *In vitro* fermentation, Antioxidant capacity, Gut microbiota, Short-chain fatty acids

Pomegranate (*Punica granatum* L.) is widely recognized for its health-promoting properties, primarily attributed to its high polyphenol content. It contains bioactive compounds such as ellagitannins and ellagic acid, known for their antioxidant, anti-inflammatory, cardioprotective, anticancer, hepatoprotective, antimicrobial, antiviral, antidiabetic, neuroprotective, and dermatological benefits [1,2]. In this study, a sustainable extraction approach was employed to recover polyphenols from pomegranate by-products, particularly the peel (exocarp, mesocarp, and endocarp). This method maximized bioactive compound recovery while promoting the valorization of agri-food waste, thereby minimizing environmental impact and supporting circular economy principles, aligned with UN Sustainable Development Goal (SDG 12: Responsible Consumption and Production).

Given emerging evidence on the influence of pomegranate polyphenols on gut microbiota and overall health, this study aimed to: (1) chemically characterize this standardized pomegranate extract using UHPLC-HRMS; (2) evaluate the impact of fermentation on polyphenol bioaccessibility; and (3) investigate its effects on gut microbiota functionality through *in vitro* digestion and fermentation with fecal material from diverse donors (adults and children, both healthy and affected by obesity and celiac disease).

The UHPLC-HRMS analysis identified 59 bioactive compounds, with gallotannins and ellagitannins being the most abundant. Fermentation significantly increased total phenolic content (TPC), while antioxidant capacity decreased. Notably, pomegranate extract modulated gut microbiota by enhancing short-chain fatty acid (SCFA) production across all subject groups, indicating potential prebiotic effects. These findings suggest that sustainably extracted pomegranate polyphenols may contribute to gut health and the prevention of dysbiosis-related diseases (supporting UN SDG 3: Good Health and Well-being).

This research underscores the importance of green and sustainable bioprocessing strategies in pharmaceutical and nutraceutical applications, aligning with global efforts toward environmentally responsible innovations in life sciences.

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# OC13 - Investigation of *Hippophae rhamnoides* Extract on Intestinal Function Regulation: A Sustainable Approach to Digestive Health

Maria Vittoria Morone[1], Elisabetta Caiazzo[2], Hammad Ullah[2], Daniele Giuseppe Buccato[2], Lorenza Francesca De Lellis[2], Luca Borromeo[2], Danaé S. Larsen[3], Alessandro Di Minno[2,4], Armando Ialenti[2], Maria Daglia[2]

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**Keywords:** vegetable extract; gastrointestinal motility; constipation; intestinal transit regulation; aquaporins

Gastrointestinal disorders, including altered intestinal motility (constipation and diarrhea), are prevalent conditions linked to unhealthy lifestyle and dietary habits. These disorders significantly impact quality of life, as proper digestive health is essential for nutrient absorption, energy production, immune function, and mental and skin health. Synthetic laxatives are widely used to manage these conditions (i.e. bisacodyls and sodium picosulphate). However, drug long-term use can lead to adverse effects such as dependency, electrolyte imbalances, and altered gut microbiota. Moreover, as synthetic laxatives are excreted unchanged in urine and feces, they are a source of water contamination [1]. As a result, there is growing interest in natural alternatives, including prebiotics, probiotics, and botanical extracts, which are perceived as safer and more sustainable options for maintaining digestive health. *Hippophae rhamnoides* L. (Sea Buckthorn) extract, in particular, has been traditionally recognized for its ability to regulate intestinal transit [2]. Rich in bioactive compounds such as flavonoids, carotenoids, phenolic acids, vitamins (C and E), and essential fatty acids, *H. rhamnoides* offers a combination of antioxidant, anti-inflammatory, and mucosal-protective properties. Aquaporin-3 (AQP3), a transmembrane protein involved in water transport across the plasma membrane, plays a key role in intestinal fluid balance and is essential for digestive function [3].
This study focused on the potential of *Hippophae rhamnoides* L. extract, which has been traditionally recognized for its ability to regulate intestinal transit. In more details, we investigated whether *H. rhamnoides* extract could modulate AQP3 expression in HT-29 cells exposed to non-cytotoxic concentrations of the extract (10-100 µg/mL).The extract used in this study was standardized to isorhamnetin and produced by EPO S.r.l., an Italian company that prioritizes sustainable extraction methods, reducing environmental impact by adopting greener production practices (aligning with UN Sustainable Development Goal (SDG 12): Responsible Consumption and Production).
Western blot analysis was employed to evaluate its impact on AQP3 protein levels, while the extract's chemical composition was assessed using UHPLC-HRMS/MS. The chemical profile of the extract revealed a complex array of bioactive compounds, including flavonoids, sphingolipids, glycerophosphocholines, triterpenoids, and various glycerolipids. Notably, treatment with *H. rhamnoides* extract at 100 µg/mL for 24 hours led to a significant increase in AQP3 protein expression compared to the control.
In conclusion, our preliminary findings provide scientific evidence supporting the traditional use of *Hippophae rhamnoides* for regulating intestinal function, with potential applications for improving digestive health in a sustainable and environmentally responsible manner (aligned with UN SDG 3: Good Health and Well-being). Further studies are needed to fully elucidate its mechanisms of action and confirm its clinical benefits.

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# OC14 - Evaluation of a Polyphenol- and Micronutrient-Rich Supplement for Managing Prolonged Fatigue: A Sustainable Approach to Well-being

Lorenza Francesca De Lellis[1], Hammad Ullah[1], Daniele Giuseppe Buccato[1], Alessandra Baldi[1], Maria Vittoria Morone[2], Luca Borromeo[1], Alessandro Di Minno[1,3], Roberto Sacchi[4], Maria Daglia[1]

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**Keywords:** Prolonged fatigue; natural remedies; adaptogenic plant extracts; physiological effects

Prolonged fatigue is a physiological condition characterized by persistent exhaustion that disrupts daily activities and reduces quality of life. Nutritional deficiencies and chronic inflammation are key contributing factors, highlighting the need for holistic and sustainable solutions. While synthetic drugs are often used to manage fatigue (such as CNS stimulants, antidepressants, dopaminergic agents, and corticosteroids), long-term use may be associated with side effects and limited efficacy in addressing underlying causes. In addition, most of these drug categories are included in the surface water Watch List (WL) [1] under the Water Framework Directive, which is a mechanism for monitoring emerging pollutants and substances (among which synthetic drugs) that may pose a significant risk at Union level as they are dispersed in the aquatic environment through urine and feces, both in their original form and as metabolites. As a result, there is growing interest in natural alternatives, such as adaptogenic plant extracts, which may offer a safer and more sustainable approach. Increasing evidence suggests that polyphenol- and micronutrient-rich diets may help alleviate fatigue-related symptoms by modulating inflammation [2,3]. However, effective and consumer-friendly interventions remain limited. This study assessed the anti-fatigue potential of a commercial food supplement containing *Eleutherococcus senticosus* extract (known for its anti-inflammatory and adaptogenic properties), combined with vitamins B and C, in healthy individuals with prolonged fatigue. A real-life, post-market survey was conducted on 42 participants who received 250 mg of *E. senticosus* extract daily for 14 days. The potential effects of the food supplement were determined by using validated questionnaires including Fatigue Severity Scale (FSS) to assess fatigue levels and Short Form Health Survey-12 (SF-12) to assess quality of life. Assessments were conducted at baseline (T0), after 7 (T1) and 14 days (T2) of supplementation, and following a 7-day post-treatment period (T3). Results showed a significant reduction in FSS scores and an improvement in the physical component of SF-12, with sustained benefits during follow-up. No statistically significant changes were observed in the mental component of SF-12. These findings suggest that this supplement may serve as a promising, non-pharmacological strategy for prolonged fatigue management.
By leveraging plant-based bioactives and essential micronutrients, this approach aligns with the principles of sustainable health and well-being (UN Sustainable Development Goal (SDG 3): Good Health and Well-being) and responsible consumption (UN SDG 12: Responsible Consumption and Production). Further research is warranted to elucidate its mechanisms of action and optimize its formulation for enhanced efficacy.

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## OC15 - Essential oil (EO)-based formulations active against oral cavity pathogens

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**Keywords:** Essential oils; Antimicrobial activity; Oral pathogens; mouthwashes; toothpaste.

Essential oils (EOs) have gained attention for their antimicrobial activity, making them promising alternatives to conventional treatments for bacterial and fungal infections affecting the oral cavity. This study aimed to develop and evaluate novel EO-based formulations with antimicrobial and antifungal properties for the treatment of oral infections, addressing the increasing global challenge of antibiotic resistance. To explore their potential use as mouthwash and toothpaste, EO-based formulations have been designed, optimized and tested to enhance stability and efficacy. A total of four mouthwashes were produced, each formulated with essential oils of *Mentha x piperita*, *Thymus vulgaris*, *Syzygium aromaticum* and *Coriandrum sativum*. Additionally, a toothpaste was developed maintaining the same active ingredient composition as the mouthwashes [1]. The mouthwash formulation was prepared by dissolving EOs in a suitable emulsifier to ensure a stable and homogeneous solution. The toothpaste formulation was prepared by incorporating EOs into a gel base containing hydrocolloids. The antimicrobial and antifungal effects of the EO-based mouthwashes were evaluated through a series of *in vitro* assessments, using the broth micro-dilution (BMD) testing to evaluate the minimum inhibitory concentration (MIC) and minimum cytocidal concentration (MCC) assays against common oral pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Candida albicans* [2]. All microorganisms were ATCC (American Type Culture Collection) strains and tests were performed following the EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines [3]. Stability tests on the mouthwashes involved gas chromatography-mass spectrometry (GC-MS) analysis of the EOs in the formulations, evaluation of physical appearance through shelf-life testing, monitoring of globule size in the dispersed phase using Dynamic Light Scattering (DLS) and ζ potential measurement. Moreover, pH assessment of the formulations was performed. The BMD assay demonstrated that the *T. vulgaris* EO was effective against *S. aureus* (0.50 ± 0.01 % v/v), *S. pyogenes* (0.10 ± 0.03 % v/v), and *C. albicans* (0.25 ± 0.01 % v/v), while the EO of *C. sativum* showed effectiveness against *S. aureus* (0.031 ± 0.01 %v/v), *S. pyogenes* (0.05 ± 0.04 % v/v), and *C. albicans* (0.50 ± 0.04 v/v). The MCC assay showed that the EO of *T. vulgaris* was effective against *S. pyogenes* (0.16 ± 0.08 % v/v), and *C. albicans* (0.50 ± 0.01 % v/v), followed by the *C. sativum* EO, which was effective against *S. aureus* (0.125 ± 0.01 % v/v), *S. pyogenes* (0.08 ± 0.09 % v/v), and *C. albicans* (0.50 ± 0.03 % v/v). The chemical stability test with GC-MS confirmed that the formulations remained stable over 30 days. Physical stability assessments using DLS indicated that particle sizes ranged from 13.38 nm to 51.20 nm, maintaining colloidal stability over time. Zeta potential measurements showed a consistent negative charge, ensuring minimal aggregation. The pH of the formulations remained within the range compatible with oral health (6.2–7.6). The palatability survey, conducted with 26 volunteers, demonstrated high acceptance, with most of participants providing positive feedback on both taste and texture. The study confirms the effective antimicrobial and antifungal efficacy of the EO-based formulations tested, which might be promising natural alternative formulation for the application in dentistry field as oral hygiene products.

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# OC16 - Unleashing the potential of plant cell culture extracts for sustainable cosmetic bioactives

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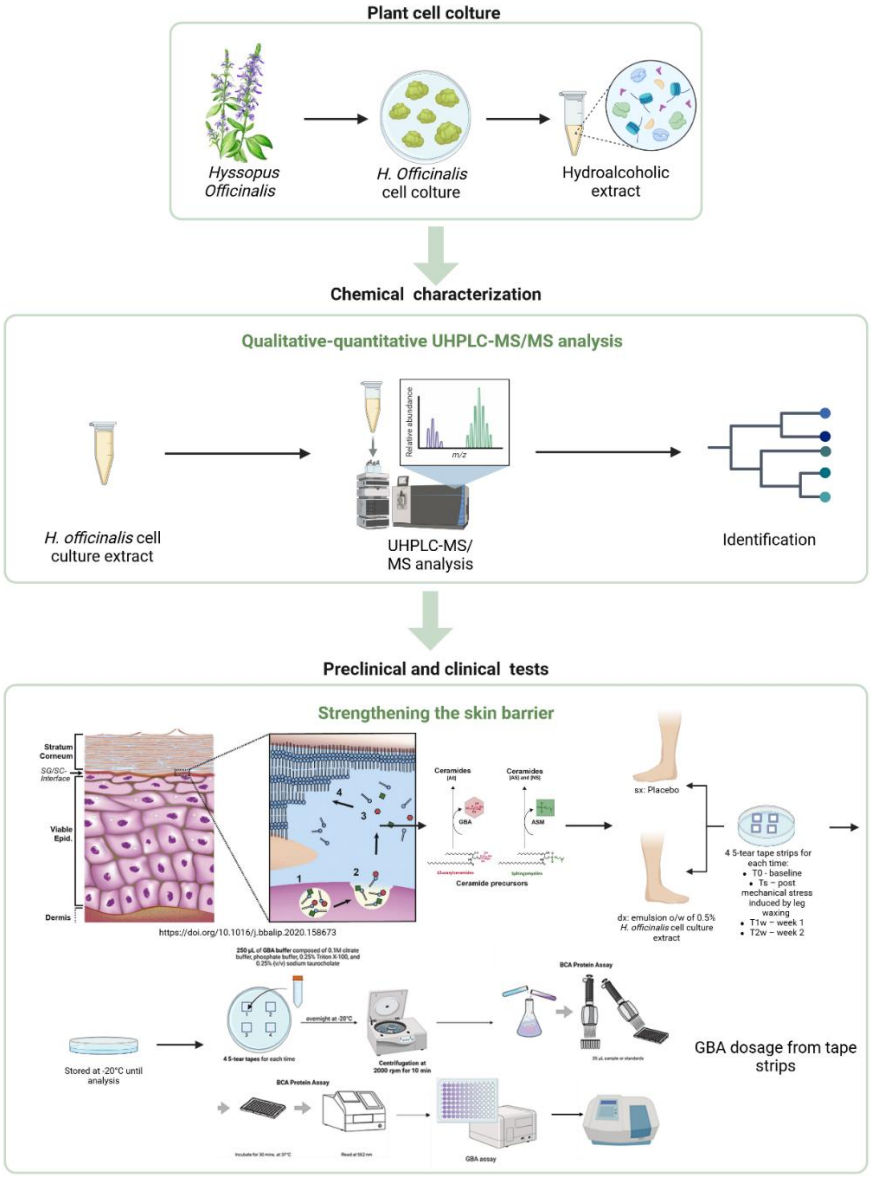
**Keywords:** Plant cell cultures, *Hyssopus officinalis*, skin barrier, biofactories, sustainability

The cosmetics industry is undergoing a significant transformation, driven by the increasing demand for sustainable and eco-friendly ingredients. Consumers are seeking products that combine efficacy with environmental responsibility, which has led to the growing use of natural cosmetic actives. However, traditional cultivation of medicinal plants, which provide these ingredients, faces challenges such as variable yields, inconsistent quality, and contamination risks [1]. In this context, plant cell cultures offer a controlled, stable, and sustainable alternative. These cultures allow for the large-scale production of bioactive metabolites, overcoming the limitations of conventional plant cultivation [2]. Due to their totipotency, plant cell cultures function as efficient "biofactories," producing valuable secondary metabolites. These compounds, often synthesized in small amounts and unevenly distributed across plant tissues, can now be produced in a more targeted and optimized manner, leading to enhanced yields and consistent quality—critical factors for the modern cosmetic industry [2].
In this study, we focused on plant cell cultures of *Hyssopus officinalis* (*Lamiaceae*), a species known for its antioxidant, anti-inflammatory, and wound-healing properties [3]. A hydroalcoholic extract was obtained from these cultures and assessed for its potential in skincare. While *H. officinalis* has a long history in traditional medicine, its applications in cosmetics remain largely unexplored. Our research aimed to evaluate the effects of *H. officinalis* cell culture extract on skin barrier function, hydration, and antioxidant protection, highlighting its potential as a novel, sustainable bioactive ingredient for cosmetics.
Using a multidisciplinary approach that integrates *in vitro*, *ex vivo*, and clinical studies, we characterized *H. officinalis* cell culture extract bioactive profile [4]. The extract was able to promote skin hydration and barrier function by stimulating ceramide production via key enzymes such as β-glucocerebrosidase [5]. These effects were observed *in vitro*, *ex vivo*, and *in vivo*, using tape strips of volunteers subjected to mechanical stress. In addition to barrier enhancement, *H. officinalis* cell culture extract helps protect essential skin structural proteins, such as involucrin and filaggrin [6].
In conclusion, *H. officinalis* cell culture extract positively impacts key markers of skin health by promoting ceramide synthesis and improving epidermal cohesion. These findings highlight the innovative potential of plant cell cultures as a sustainable and high-quality source of cosmetic ingredients, representing a step toward a more environmentally responsible future in the cosmetics industry.

Figure 1. Workflow of the study design.

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# OC17 - Upcycling Fennel Residues: Humic Substances as Innovative Bioactive Agents for Sustainable Cosmetics

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**Keywords:** Humic substances, fennel residues, bioactive cosmetics, antioxidants, anti-inflammatory, circular economy

The cosmetic industry is increasingly shifting towards sustainability by integrating bioactive ingredients derived from natural and renewable sources [1]. Among these, humic substances (HS) have emerged as promising multifunctional compounds due to their antioxidant, anti-inflammatory, and skin-regenerating properties [2]. Our study investigates the potential application of HS extracted from composted fennel (*Foeniculum vulgare*) residues as active agents in sustainable cosmetics. In this work, fennel residues were subjected to controlled composting conditions to promote the natural degradation of organic matter and enhance the formation of humic-like substances. After reaching maturity, the composted material was processed using an alkaline extraction method to isolate HS. The extracted HS were then purified and chemically characterized to determine their structural composition and bioactive potential. A combination of advanced spectroscopic techniques, including Nuclear Magnetic Resonance (NMR), was employed to analyze the molecular structure of HS. These techniques provided insights into the presence of functional groups such as carboxyls, phenols, and quinones, which are known for their biological activity. Additionally, Thermochemolysis GC-MS was used to identify specific bioactive compounds within the HS fraction, including polyphenolic derivatives and other antioxidant molecules. To assess the potential of fennel-derived HS in skin care applications, a series of bioactivity tests were conducted. The antioxidant capacity of HS was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging assays. These methods measure the ability of HS to neutralize free radicals, which are major contributors to oxidative stress, skin aging, and inflammation. The results demonstrated that HS exhibited strong radical-scavenging activity, comparable to conventional antioxidants used in cosmetics. In addition to antioxidant activity, the antimicrobial potential of HS was examined through Minimum Inhibitory Concentration (MIC) determination against common human pathogenic bacterial strains. The study focused on both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Notably, HS showed significant antimicrobial efficacy, with a predominant inhibitory effect against *S. aureus*, a key contributor to skin infections and acne. These findings suggest that HS could serve as a natural preservative and active ingredient in cosmetic formulations, reducing the need for synthetic antimicrobial agents. Noteworthy, in vitro bioscreens performed with HS in preclinical human skin models show a good safety profile and biocompatibility. To support these in vitro findings, the extracted HS was incorporated into an oil-in-water (O/W) emulsion and evaluated in a randomized, double-blind, placebo-controlled clinical trial. The results showed multiple skin benefits, including a significant increase in hydration, improved skin barrier function, and enhanced viscoelastic properties, particularly greater elasticity and firmness. This study highlights the potential of upcycled fennel-derived HS as innovative bioactive agents for sustainable cosmetics. The findings underscore the strong antioxidant and antimicrobial properties of HS, as well as their ability to enhance skin hydration and elasticity in vivo. By transforming agricultural waste into high-value cosmetic ingredients, this approach contributes to the development of eco-friendly skincare solutions while promoting a circular economy. Future research should focus further on stability testing, cellular responses in vitro, elucidating the mechanisms underlying the skin benefits of HS, optimizing extraction methods for large-scale production, and exploring additional applications in cosmeceuticals, such as anti-acne treatments and wound healing formulations.

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## OC19 - Supramolecular Red-Light-Photosensitized Nitric Oxide Release Within Biocompatible Nanocarriers

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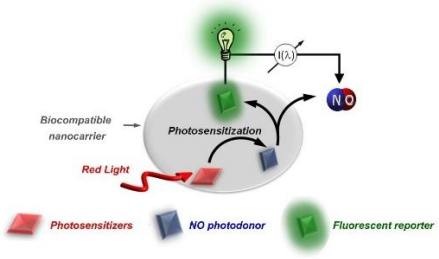
**Keywords:** Nitric Oxide, Photosensitizers, Red light, Fluorescence, Biocompatible Nanocarriers

Nitric oxide (NO) is an inorganic free radical that plays a multifaceted role in the regulation of many physiological and pathophysiological processes [1] and is emerging as a promising unconventional therapeutic agent to tackle severe diseases, including cancer [2]. However, the strict dependence of its biological effects on concentration and site of generation [2] requires precise spatiotemporal control over its delivery. Light activation of appropriate NO photoprecursors represents an optimal strategy, making the development of precursors and methods for triggering NO release within the therapeutic window highly desirable [3]. In this contribution, we demonstrate that NO release from a blue-light activatable NO photodonor (NOPD) with a self-fluorescence reporting can be triggered catalytically by the much more biocompatible red light exploiting a supramolecular photosensitization process (figure 1), with an improvement of about 300 nm toward longer and more biocompatible wavelengths [4,5]. Different red-light absorbing photosensitizers (PS) have been co-entrapped with the NOPD within different biocompatible nanocarriers such as PEG-PCL nanoparticles, Pluronic® micelles, microemulsions and branched cyclodextrin polymers. The intra-carrier photosensitized NO release, involving the lowest, long-lived triplet state of the PS as the key intermediate and its quenching by the NOPD, is competitive with that by molecular oxygen. This allows NO to be released with good efficacy, both under aerobic and anaerobic conditions. Moreover, the process is accompanied by the formation of a green, fluorescent photoproduct which acts as a reporter for the real-time monitoring of NO release. Therefore, the adopted general strategy provides a valuable tool for generating NO from an already available NOPD, otherwise activatable with the poorly biocompatible blue light, without requiring any complex chemical modification, and instead using biocompatible and non-toxic materials, as well as avoiding the use of sophisticated and expensive irradiation sources.

Figure 1. Schematic of red-light photosensitization mechanism for the NO release with fluorescent self-reporting.

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## OC20 - Nanoparticles based on inulin and polyesters for the delivery of siRNA and Docetaxel to solid tumors

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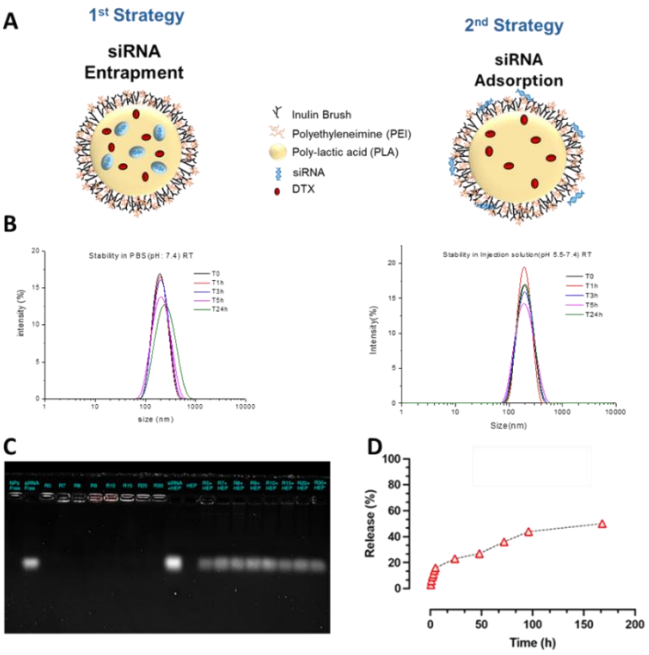
**Keywords:** inulin; combined therapy, siRNA, cancer

We propose a cutting-edge triblock copolymer-based nanosystem ((inulin (INU)- polyethyleneimine (PEI)- polylactide (PLA), named IPP-NPs) designed for the co-delivery of siRNA and docetaxel (DTX) (Fig. 1A), harnessing the synergistic potential of gene silencing and chemotherapy within a single engineered platform [1].
The nanosystem is structured with an INU backbone that provides stealth properties, effectively evading immune recognition and clearance. PEI plays a crucial role for siRNA complexation through electrostatic interactions, whereas PLA ensures efficient hydrophobic drug encapsulation [2]. IPP-NPs were fabricated through the emulsion/solvent diffusion technique, thus producing monodisperse NPs (100–200 nm, PDI 0.1) with tunable surface properties. Zetasizer analysis have been also used to follow the physical stability of NPs dispersed in biologically relevant media and pharmaceutical vehicles suitable for i.v. administration (Fig. 1B), before and after lyophilization with hydroxypropyl-β-cyclodextrin as a cryoprotectant. NPs exhibited remarkable stability under physiological and cell culture conditions, maintaining structural integrity and long-term stability after lyophilization. The localization of siRNA (adsorbed vs. entrapped) influenced the zeta potential, shifting from negative to positive depending on formulation parameters. In both cases, siRNA was completely loaded into NPs (1 μg of siRNA can be completely complexed with 30 μg of NPs) (Fig. 1C) and released in a sustained manner up to seven days (Fig. 1D).
Finally, DTX was encapsulated with full efficiency at a 5% w/w ratio, demonstrating robust drug loading capacity of NPs. In preclinical evaluations using triple-negative breast cancer (MDA-MB-231) cells, IPP-NPs showcased excellent biocompatibility, exhibiting no detectable cytotoxicity at concentrations up to 50 μg/mL over 72 hours. Ongoing investigations aim to elucidate the cellular uptake and transfection efficacy of NPs in different cancer cell lines.
These findings demonstrate IPP-NPs as a new platform for co-delivering RNA and chemotherapeutics. This strategy paves the way for next-generation combination cancer therapies, offering enhanced efficacy and a promising new frontier in oncological treatment paradigms.

We acknowledge the grant CN00000041 "National Center for Gene Therapy and Drugs based on RNA Technology" (E63C22000940007); the "My First AIRC grant 2023" (29369) and "Progetti di ricerca di Rilevante Interesse Nazionale (PRIN)" (20223FJTWF).

Figure 1. NP structures (A); Stability in PBS and NaCl 0.9% (B); siRNA complexation and Heparin displacement assay (C); siRNA release from NPs (D).

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# OC21 - Halloysite-BioBased Nanocomposites: A Green Solution for Antibacterial, Anticorrosion, and $CO_2$ Conversion Applications

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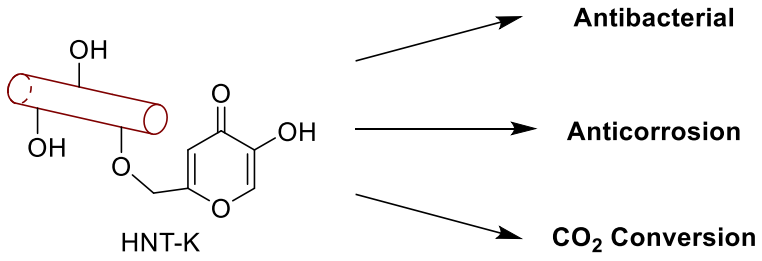
**Keywords:** $CO_2$, Kojic acid, Halloysite, Sustainable material, Chelating agent

The growing need for sustainable and multifunctional materials has driven research towards naturally derived nanostructures with diverse technological applications. Halloysite nanotubes (HNTs), aluminosilicate clay minerals with a unique tubular morphology, have emerged as an eco-friendly and versatile platform for advanced material design. In this study, HNTs were functionalized with kojic acid (K), a natural chelating agent, to develop a bio-based material with broad applicability in antimicrobial, anticorrosion, and catalytic processes (Figure 1).
The resulting HNT-K composite demonstrated remarkable antibacterial activity against multiple pathogens, attributed to its iron-chelating capability. This makes it a promising candidate for antimicrobial applications and targeted drug delivery [1]. Beyond biological applications, incorporating an ionic liquid enhanced the composite's anticorrosion by effectively preventing iron oxidation while maintaining environmental sustainability. This functionality positions it as a valuable component for protective coatings in industrial settings.
The study further explored the hybrid material's catalytic potential for $CO_2$ conversion. Functionalization with copper ions enabled efficient $CO_2$ reduction via photocatalytic methane production and epoxide-based carbonate fixation, achieving high conversion efficiencies under mild conditions [2]. Notably, integrating xanthopterin, a biomimetic light-harvesting pigment derived from *Vespa orientalis*, further improved $CO_2$ photoconversion efficiency and selectivity, enhancing the material's potential for sustainable carbon migration.
This research underscores halloysite-based materials as a compelling, eco-friendly solution for addressing global challenges, including bacterial resistance, metal corrosion, and $CO_2$ emissions. By leveraging natural and renewable resources, this work paves the way for next-generation sustainable technologies with broad industrial and environmental impact.

Figure 1. Structure of HNT-K composite.

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## OC22 - Automation for scaling nanoparticle production and characterization

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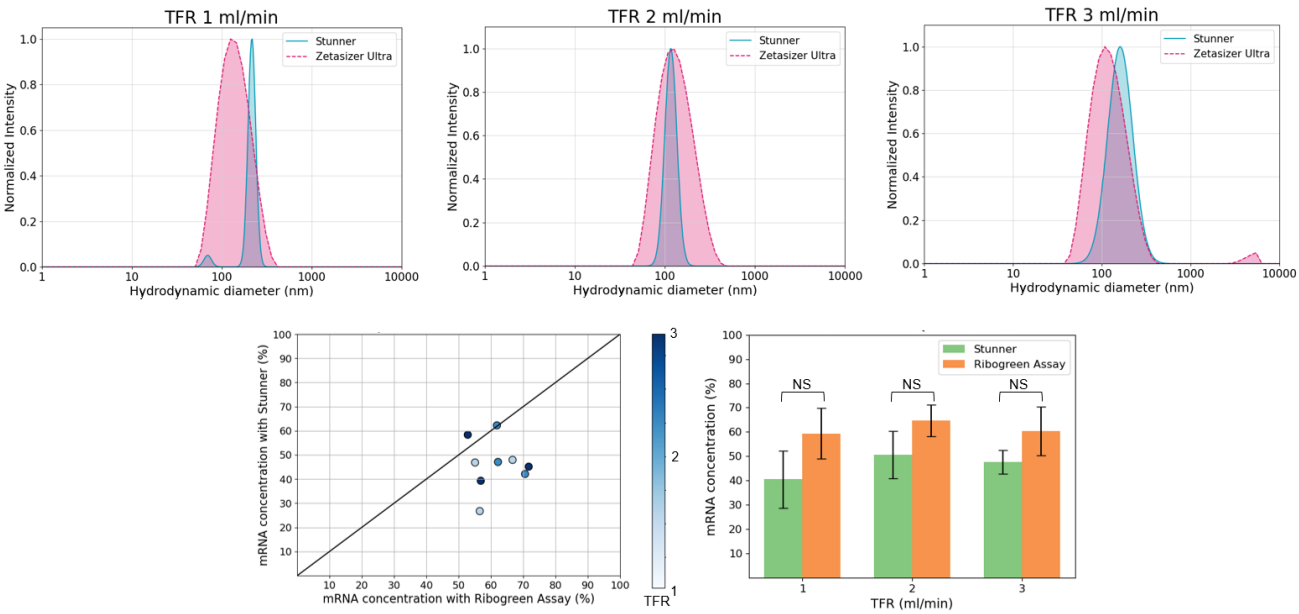
**Keywords:** lipid nanoparticles, automation, mRNA delivery, quality profile, nanoparticle characterization

The intracellular delivery of genetic information to target cells has been a major challenge in gene therapies. The success of lipid nanoparticle (LNP)-based mRNA vaccines, such as Comirnaty® and Spikevax®, during the SARS-CoV-2 pandemic has significantly boosted interest in LNPs for RNA delivery [1,2]. Meanwhile, the increasing role of automation in laboratory techniques has led to more efficient methodologies for nanoparticle production and characterization [3]. However, conventional methods for LNP characterization are often time-consuming, costly, and lack high throughput, thus limiting their efficiency in development and scalability [4]. While previous studies have utilized nanoscale mixing technologies to produce LNPs and analyze their colloidal properties with high-throughput instruments [5], our work demonstrates how high-throughput instruments can overcome the limitations of the conventional methods, especially in the context of LNP characterization. We leveraged advanced tools, such as the Stunner (Unchained Labs) plate reader dynamic light scattering instrument, to improve LNP analysis, providing rapid and precise data on their quality profile. We show that the Stunner is a reliable and rapid tool for analyzing LNP formulations containing mRNA, produced via semi-automated microfluidics, and provides consistent results when compared to conventional methods, such as the Zetasizer Ultra for size and dispersity and Ribogreen Assay for mRNA loading efficiency.

We investigated the reliability of the Stunner for analyzing colloidal properties using standard polystyrene Nanosphere™, comparing the results with those obtained from the Zetasizer Ultra with multi-angle scattering. Likewise, we analyzed the colloidal properties of the LNP-mRNA formulations which showed good agreement between the two instruments. To verify the reliability of the instrument in quantifying the mRNA loading yield, we compared the Ribogreen Assay and Stunner. We evaluated the limit of detection (LOD) and limit of quantification (LOQ) for both approaches, and the RiboGreen assay had a higher LOD and LOQ (LOD = 0.005 µg/ml for Stunner vs 0.03 µg/ml and 0.03 µg/ml for RiboGreen without and with Triton, respectively; LOQ = 0.01 µg/ml for Stunner vs 0.1 µg/ml and 0.08 µg/ml for RiboGreen without and with Triton, respectively). We then measured the loaded mRNA yield into the LNPs using both instruments and found that the Stunner tends to underestimate the quantity, however this difference was not found to be statistically significant.

These results highlight the potential of automation in nanoparticle characterization. The Stunner demonstrates a rapid and reliable approach for analyzing LNP formulations, offering valuable insights for optimizing and scaling RNA-based therapeutics. Automation in nanoparticle production and characterization presents a promising avenue for overcoming current limitations, with significant implications for advancements in nanotechnology and biotechnology applications.

Figure 1. The three images at the top show the size distribution of LNPs at different total flow rates (TFRs) of 1, 2, and 3 mL/min at the back scatter angle, comparing data from the Stunner and the Zetasizer Ultra. The image at the bottom left compares the mRNA concentration in LNPs measured with the Stunner (%) versus mRNA concentration in LNPs measured with the Ribogreen Assay (%) for the three types of LNPs. The image at the bottom right is a bar chart showing the mRNA concentration (%) in the different LNPs, comparing Stunner and Ribogreen.

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# OC23 - NMR Spectroscopy for Structural Insights into RBC-Encapsulated Enzymes: A Sustainable Strategy for Drug Delivery in Green Chemistry

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**Keywords:** NMR, HOS, RBCs, Drug Delivery, Therapeutic Enzymes

Green Chemistry is revolutionizing the pharmaceutical sector by promoting the use of eco-friendly processes and materials to reduce environmental impact and enhance therapeutic efficiency. In drug delivery, various technologies, such as biocompatible nanoparticles, degradable polymers, and eco-friendly synthesis processes, have demonstrated improved drug bioavailability and reduced side effects. However, an even more advanced and sustainable solution is the use of red blood cells (RBCs) as drug carriers, offering numerous advantages from both environmental and therapeutic perspectives [1]. Unlike polymeric and liposomal nanoparticles, which often require synthesis processes involving organic solvents, high energy consumption, and materials that can generate toxic residues, RBCs are fully biocompatible, biodegradable, and renewable [2]. They can be loaded with drugs, therapeutic enzymes, and carrier proteins through mild and non-toxic methods, without the need for chemical stabilizers or synthetic coatings. This drastically reduces the release of pollutants into the environment and minimizes the risk of adverse reactions in the body. Therapeutic enzymes are used in the treatment of various diseases, including enzyme replacement therapy for genetic deficiencies and the selective metabolism of tumor precursors. However, their efficacy is often limited by poor in vivo stability, immunogenicity, and inactivation by antibodies. Traditionally, PEGylation has been employed to extend the half-life of therapeutic enzymes by shielding them from the immune system and plasma proteases [2]. However, the widespread use of PEG has led to adverse reactions, including hypersensitivity and anaphylaxis, necessitating the search for safer alternatives [1]. Encapsulation of enzymes within RBCs represents a highly promising alternative to PEGylation, offering significant benefits in terms of biocompatibility, biodegradability, and stability. From a pharmacological and therapeutic perspective, RBCs protect enzymes from immune system recognition and plasma proteases, enabling prolonged and controlled drug release, reducing side effects, and enhancing therapeutic efficacy. Among the most studied drugs in this context, L-Asparaginase, used in the treatment of acute lymphoblastic leukemia (ALL), has shown promising results when encapsulated in RBCs (GRASPA), [3] with clinical studies confirming its improved stability and safety compared to conventional formulations [1]. So far, the characterization of RBC-encapsulated enzymes has mainly focused on their enzymatic activity. However, evaluating the preservation of their higher-order structure (HOS) is essential for optimizing encapsulation and storage conditions. In this regard, solution-state NMR spectroscopy serves as a valuable tool for analyzing protein structure in confined environments. In this study, we demonstrate how NMR characterization of three RBC-encapsulated proteins provides insights into the preservation of their HOS, along with a semi-quantitative assessment of encapsulation efficiency. The use of RBCs in drug delivery thus represents a greener, safer, and more effective alternative to conventional technologies, contributing to the transition toward sustainable pharmaceuticals without compromising therapeutic efficacy.

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# OC24 - Peptide-based soft materials to access higher contrast efficiency for safer Fe(III)-complexes in MRI scans

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**Keywords:** peptides, MRI, contrast agents, supramolecular chemistry

Gadolinium-Based Contrast Agents (GBCAs) have represented the main resource for Magnetic Resonance Imaging (MRI) since the mid-1980s, significantly enhancing tissue differentiation and detection of pathological states. Despite their extensive use, concerns about the long-term toxicity of GBCAs have arisen, particularly because autopsy studies reveal trace amounts of Gadolinium remaining in the body long after the administration [1]. This has driven research efforts to find safer, Gadolinium-free alternatives, particularly focusing on the potential of endogenous paramagnetic metal ions such as Fe(III), which are naturally regulated in the body [2]. However, since no water molecules directly coordinated to the metal center can be present in Fe(III) complexes, as they can promote iron reduction resulting in the formation of Reactive Oxygen Species (ROS), the relaxivity of these complexes is generally low. For this reason, soft materials like Peptide-based Hydrogels and Nanogels (PBHs and PBNs) have been proposed to encapsulate the Fe(III) complex Fe-Deferasirox (Fe-DFX) as the generation of supramolecular CAs can allow to increase the efficiency of the contrast, in terms of relaxivity value. A lysine-containing peptide sequence (Fmoc-AIVAGK-$NH_2$) was chosen as building block for the PBH formation, as the presence of the positive charge can promote the encapsulation of the negatively charged complex. Fe-DFX loaded PBN were also obtained, starting from the macroscopic hydrogel, through green top-down procedures.

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# OC25 - Electronic interaction-enhanced NO photorelease and photothermal conversion in Green-Synthesized N-doped carbon dot nanoconjugates

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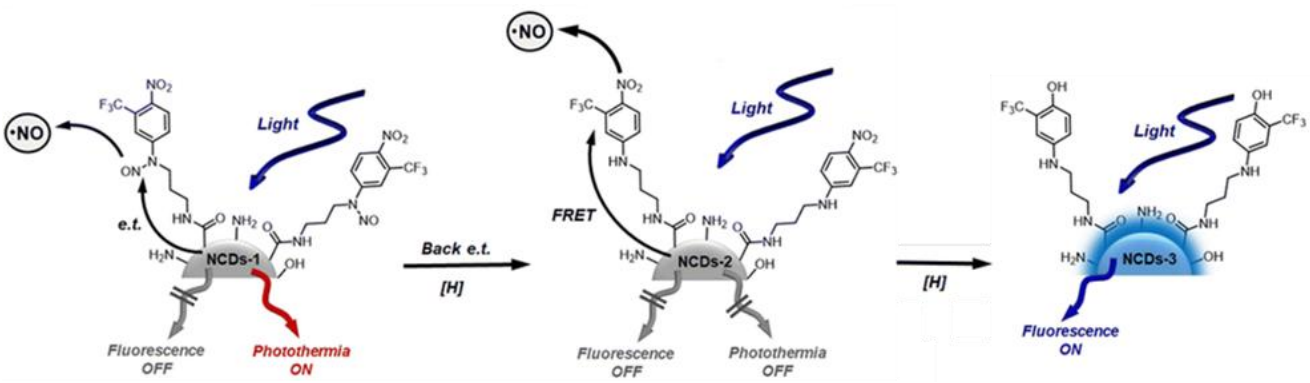
**Keywords:** Nitric Oxide, Carbon Dots, Photodynamic Therapy, Photothermal Therapy

A novel, water-soluble nanoconjugate, named NCDs-1, has been engineered by covalently integrating a nitric oxide (NO) photodonor, capable of releasing two NO molecules in a stepwise manner, with blue-emitting nitrogen-doped carbon dots (NCDs) [1]. The synthesis of the NCDs follows a green and biocompatible hydrothermal approach, utilizing citric acid and urea in water, ensuring an environmentally friendly and non-toxic process.
The nanoconjugate, approximately 10 nm in size, exhibits a unique absorption band, not present in the individual components, indicating strong electronic interactions in the ground state. Upon blue light excitation, NCDs-1 shows a nearly tenfold increase in NO generation efficiency compared to the free photodonor, likely due to efficient photoinduced electron transfer between the NCDs core and the NO donor. The NO molecules are released in two stages, with the second release coinciding with the restoration of the NCDs blue fluorescence, serving as a real-time optical marker for monitoring NO production.
Importantly, NCDs-1 also enables NO release under the much more biocompatible green light, expanding its operational light range and enhancing its safety for the potential therapeutic use in living systems. Additionally, NCDs-1 demonstrates significant photothermal conversion efficiency, absent in bare NCDs. This capability is valuable for photothermal therapy (PTT), used for treating conditions like cancer and bacterial infections, with minimal damage to surrounding tissue. The combination of NO release and photothermal effects opens new avenues for multimodal therapies.
Ongoing experiments focus on optimizing the NCD synthesis to shift their absorption towards the green wavelength range, improving NO release efficiency under green light while maintaining a green chemistry approach for enhanced therapeutic applicability.

Figure 1. Schematic representation of the stepwise release of two NO molecules from NCDs-1, along with the simultaneous restoration of fluorescence and activation of PTT upon exposure to visible light.

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## OC26 – Seeing More by Combining Views: A Broader Look at Nanoparticles

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**Keywords:** Nanoparticles, Nanoparticle Tracking Analysis, Dynamic Light Scattering, Field-Flow Fractionation

Accurate characterization of nanoparticles is crucial for advancing drug delivery systems. This presentation emphasizes the value of combining orthogonal techniques — Nanoparticle Tracking Analysis (NTA), Dynamic Light Scattering (DLS), and Field-Flow Fractionation (FFF) — to gain a fuller, more reliable understanding of nanosystem properties. By integrating these methods, we can overcome individual limitations and achieve a broader, more precise view of nanoparticle behavior.

# OC27 - Legumes-based surfactants: a sustainable approach for pharmaceutical, cosmetic, and food industries

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**Keywords:** natural surfactant, lentils, purification, surface tension, emulsifying ability

Surfactants are a relevant class of chemical compounds widely used across various industrial sectors, including chemical, pharmaceutical, cosmetic, and detergent industries. Their relevance for numerous processes and applications mainly lies in their ability to reduce surface/interfacial tension between immiscible phases, such as oil and water, acting as stabilizers for dispersed systems. Synthetic surfactants, primarily derived from petroleum-based sources, still dominate industrial production. Common examples include sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES), extensively used in household cleaning agents and personal care products. However, several concerns related to their toxicity toward living organisms and high environmental impact have raised the interest in developing more sustainable alternatives.

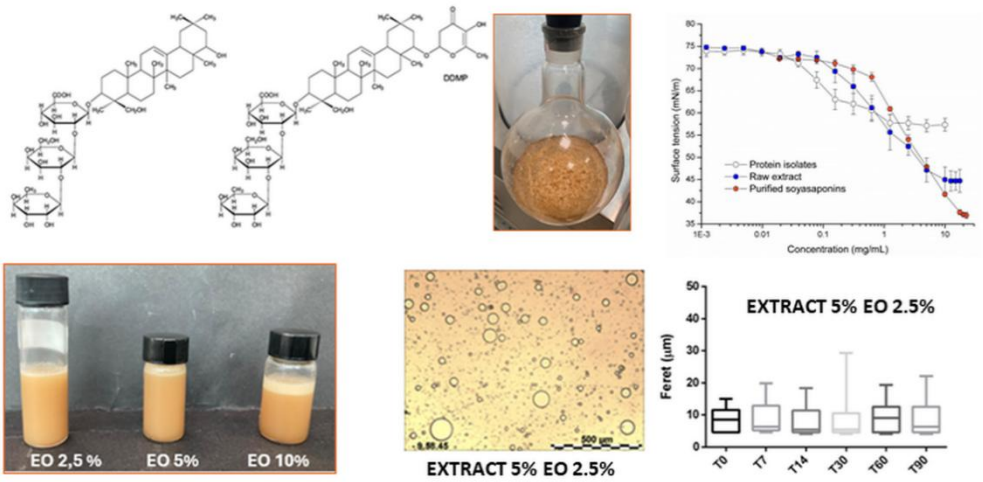
Natural surfactants offer significant advantages over their synthetic counterparts. Sourced from plants, they are generally more sustainable, biodegradable, and environmentally friendly, reducing pollution risks and enhancing biocompatibility. Various plant-derived compounds exhibit surfactant-like properties, such as saponins and proteins from legumes, which have been demonstrated to be able to facilitate oil-water emulsification and contribute to emulsion stabilization [1,2]. Specifically, saponins are a large variety of natural amphiphilic compounds, widely distributed as secondary metabolites in plants. Chemically, they are glycosides, which are composed of sugar chains, chemically linked to a non-polar part, having a steroidal or triterpene backbone. Legumes represent the main dietary and food sources of saponins [3].

This study aimed to extract, purify, and characterize the surface and emulsifying properties of soyasaponins extracted by edible legumes such as lentils. Raw extracts from legumes were obtained using ethanol/water mixtures at a different polarity and, then, freeze-dried. Total soyasaponins (including soyasaponin I and VI) and protein contents were analytically determined by UV-vis spectroscopy and HPLC-MS/MS. Protein and soyasaponins were purified from the raw extract using isoelectric precipitation and size-exclusion chromatography, respectively. Surface properties of the raw extract and each isolated amphiphilic component (soyasaponins and proteins) were investigated by tensiometry. Additionally, their potential applications as emulsifiers were explored by evaluating the emulsifying ability in relation to several natural or synthetic oils, and the stabilizing effect towards biphasic formulations, such as emulsions and creams.

The highest soyasaponin content was determined in lentils, using a 70:30 ethanol: water solution as extraction mixture. Aqueous solutions of the raw extracts and purified soyasaponins showed a lowering of the surface tension up to 40-45 mN/m at concentrations above their apparent critical micelle concentration ($CMC_{app}$), which is comparable with that of synthetic surfactants (i.d. polysorbate 80). All formulations (emulsions and creams) prepared with the raw extracts appeared to be well emulsified, without any visible instability phenomenon and remarkable changes in droplet size, up to three months from the preparation. Shorter stability times (few weeks) were observed for formulations prepared using the purified soyasaponins, highlighting the relevance of the presence of the proteins in the physical stabilization of emulsions and creams (Figure 1).

Our findings suggest that these natural surfactants hold promise for pharmaceutical, cosmetic, and food-related applications, aligning with the growing demand for sustainable and skin-friendly alternatives in modern industry.

Figure 1. Overview of the results obtained from the extraction and characterization of soyasaponins, proteins, and their mixtures from lentils: surface tension measurements, emulsifying ability, and emulsion droplets size and stability.

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# OC28 - From Biowaste to Innovation: Designing Composite Lignin-Zein Nanoparticles from Artichoke Byproducts

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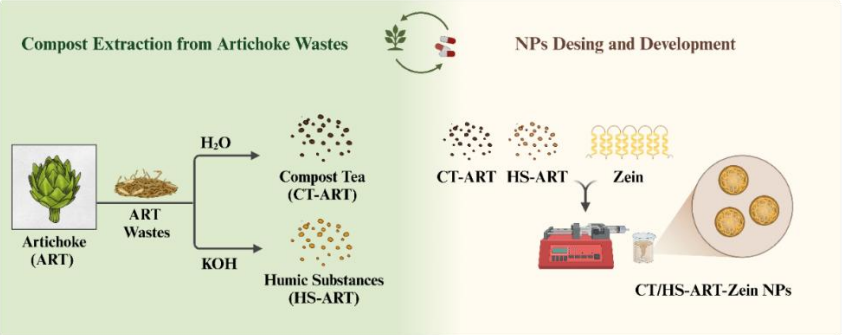
**Keywords:** Biowastes, Lignin, Zein, Composite nanoparticles

The circular economy concept is increasingly driving the development of innovative value-added materials by converting biowastes into useful resources across various industries, particularly in the pharmaceutical sector [1]. Compost tea (CT) and humic substances (HS), derived from agrifood waste, have been widely studied for their beneficial properties [2] and represent a potential source of lignin, the most abundant polymer of plant origin. Lignin exhibits several beneficial properties, including biocompatibility, biodegradability, and inherent redox and antimicrobial activities. Furthermore, it holds great potential for the development of sustainable nanomaterials, such as lignin-based nanoparticles (NPs), whose formulation remains challenging due to lignin complex chemical structure and strong tendency to aggregate [3].
Our research presents an innovative strategy for producing lignin-based nanoparticles (NPs) directly from aqueous extracts of composted artichoke (ART) waste, utilizing these sustainable resources to advance eco-friendly NPs production. The dissolved organic fractions from compost waste were analyzed and the optimal conditions for developing lignin-based NPs from CT-ART and HS-ART materials, through controlled nanoprecipitation, were identified. To improve the properties of the NPs, composite systems were successfully generated by combining lignin from CT-ART and HS-ART with zein (Fig.1). Characterization techniques, including Dynamic Light Scattering, Scanning and Transmission Electron Microscopy, and Differential Scanning Calorimetry, underscored the association between the components and the critical role of zein in reducing compost-based NP size down to ~200 nm, improving simultaneously the monodispersity (PDI ~0.1) and the stability of the dispersion. Additionally, freeze-drying investigations allowed the production of long-term stable dry powders which successfully retained their original colloidal properties upon redispersion in water. Finally, the optimized NPs, both pre- and post-freeze-drying, exhibited robust antioxidant activity and effective antimicrobial properties against tested microorganisms.
These findings highlight the potential of biowaste and natural polymers, such as zein, in the production of stabilized compost-based NPs, offering promising applications in the nutraceutical and pharmaceutical sectors, aligning with the principles of the "waste-to-wealth" approach.

Figure 1. Schematic representation of compost extraction from artichoke wastes and composite NPs production.

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# OC29 - Tailoring the Properties of Biodegradable Films: A Study of Chitosan, PVA, and Fish Gelatin Blends

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**Keywords:** Biodegradable films, chitosan, poly(vinyl alcohol), gelatin

The urgent need for sustainable alternatives to plastic-based packaging is driving interest in biomaterials like chitosan and gelatin. These biopolymers possess desirable properties for edible food packaging, including biodegradability, edibility, film-forming ability, and biocompatibility. The availability of food-grade chitosan and gelatin, produced under established regulations and standards, further supports their application in this field. The development of food packaging materials with appropriate mechanical strength and low permeability is crucial [1]. This research focused on fabricating and characterizing ternary films based on chitosan (CH), poly (vinyl alcohol) (PVA), and fish gelatin (FG) using a casting method. A series of films with varying CH/PVA/FG blend compositions (50/50/0, 40/40/20, 35/35/30, 30/30/40, and 25/25/50) were prepared to investigate the influence of blend ratio on film properties relevant to food packaging. Incorporation of 20% FG into CH/PVA films enhanced both toughness and elasticity. The optimal CH/PVA/FG ratio of 40/40/20 yielded films with a tensile strength (TS) of 41.93 ± 3.24 MPa and an elongation at break (EAB) of 133.13 ± 13.23%. Analysis of the ternary films revealed water vapor permeability (WVP) values between 0.686 and 0.818 g mm/kPa h $m^2$. As the proportion of fish gelatin in the film matrix increased, a corresponding increase in WVP was observed. Conversely, water solubility decreased by up to 23% with higher FG content. Water absorption, however, showed a dramatic increase, reaching up to 874% with increasing FG concentration. Furthermore, a clear trend was observed in the ternary films: increasing FG concentration led to improved ultraviolet-visible light barrier properties, increased opacity, and enhanced thermal stability. The enhanced compatibility observed in the ternary films is attributed to hydrogen bonding interactions between fish gelatin, chitosan, and poly (vinyl alcohol), as evidenced by FT-IR spectroscopy. XRD analysis corroborated the miscibility of the polymer blends. SEM and AFM analyses provided insights into the resulting surface morphology. The resulting films exhibited improved overall functionality, demonstrating the benefits of this blending approach [2].

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# OC30 - Kefiran-Containing Electrospun Nanofibers as Alternative Filters for Surgical Face-Masks

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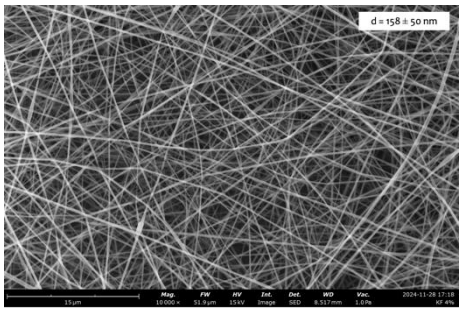
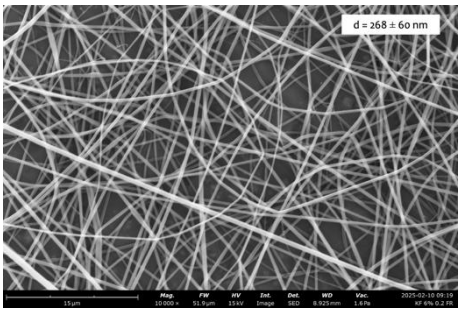
**Keywords:** kefiran, electrospinning, nanofiber, cross-linking, mechanical properties

Surgical face-masks are essential personal protective equipment that safeguard wearers from biological contaminants. Most current face-masks are composed of non-biodegradable plastic materials and their filtration efficiency declines over time due to microbial contamination and particles accumulation. To address these issues, the work aimed to produce kefiran-containing nanofibers (NFs) *via* electrospinning as alternative long-lasting filters for surgical face-masks. NFs are characterized by high porosity and high surface area-to-volume ratio, improving both filter filtration and breathability [1]. Kefiran (KF) is a biodegradable polysaccharide extracted from kefir grains, endowed with antimicrobial properties [2]. The addition of cross-linkers, i.e. citric acid (CA), was also considered to obtain vapour resistant fibrous membranes. Kefir grains (Kefiring, Italy) were cultured in milk for 7 days. KF was extracted by dissolving kefir grains in boiled water (1:10 v/v), followed by centrifugation (10,000 RCF, 20 min, 20°C) and collection of the supernatant. Ethanol was added to the supernatant (1:2 v/v) and the mixture was stored at 4 °C overnight. KF was separated by centrifugation (10,000 RCF, 20 min, 4°C) and dissolved in water (80°C); the process was repeated twice before freeze-drying [2]. KF extract was characterized in terms of: i) morphology (Scanning Electron Microscopy, SEM), ii) melting and degradation points (Differential Scanning Calorimetry, DSC), iii) chemical composition (Fourier-transform infrared spectroscopy, FT-IR) and iv) degree of purity, by determining protein content through a Bicinchoninic Acid Protein Assay. Finally, KF molecular weight was estimated by Size Exclusion Chromatography (SEC). Solutions with increasing KF concentration (0.1- 6% w/v) were prepared in water (80°C) and their viscosity was measured to calculate the critical entanglement concentration (CEC), that is the lowest concentration at which KF chain entanglement occurs. Aqueous KF solutions (4 and 6% w/v) were prepared and elettrospun. KF-NFs morphology and size were investigated by SEM, while mechanical and viscoelastic properties by Dynamic Mechanical Analysis and Texture Analyzer. Cross-linked KF-NFs (cKF-NFs) were prepared by heating in a lab oven NFs prepared starting from aqueous solutions 6% w/v KF and CA increasing concentrations (1.5- 3% w/v). cKF-NFs were characterized as mentioned above. KF was successfully extracted from kefir grains as confirmed thorugh FT-IR analysis; KF degree of purity was equal to 99,67% and its average molecular weight was 2348.60 ± 12.30 kDa. DSC analysis identified KF melting point (80°C) and KF degradation temperature (< 200°C), confirming data reported in literature. Freeze-dried KF extract showed a porous architecture and were freely soluble in water. KF solutions rheological analysis showed an increase in viscosity with increasing KF concentration, with a CEC of 0.26% w/v. KF-NFs were successfully produced by electrospinning. SEM images (Figure 1) confirmed that homogeneous, bead-free KF-NFs were obtained and, as expected, NFs diameter increased with KF concentration. Since the resulting KF-NFs completely dissolved in water, KF-NFs cross-linking was performed by using CA as cross-linker to obtain vapour resistant NFs. Cross-linking improved KF-NFs mechanical properties and allowed KF-NFs to swell after water contact. Highly pure KF was extracted from kefir grains and thoroughly characterized. Moreover, KF-NFs were successfully produced. Cross-linking ensured the production of swellable cKF-NFs with optimal mechanical properties. Ongoing studies are focused on producing cKF-NFs using different crosslinkers, such as tannic acid. Once the most promising cKF-NFs are obtained, a characterization in terms of water vapor permeability, breathability and antimicrobial activity against *Staphylococcus aureus* and *Escherichia Coli* will be conducted.

Figure 1. SEM images at 10,000X magnification and diameters (mean values ± SD; n=200) of KF-NFs obtained from KF solutions at 4% w/v (on the right) and 6% w/v (on the left).

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# OC31 - Biobased hydrophobic films based on cocoa shell cellulose and with natural waxes for sustainable packaging

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**Keywords**: Waste valorization; Cocoa shell cellulose; Hydrophobic films; Biobased materials; Sustainable packaging

The circular manufacturing of biodegradable and biobased materials using agri-food byproducts is being promoted in bio-sector applications. Herein, the development of biobased and environmentally friendly films was carried out using cocoa shell (CS)-cellulose. The cellulose was first extracted by NaOH/$NaClO_2$-based treatments, resulting in pure-white crystalline cellulose with 25.32% yield. The cellulose obtained was functionalized to carboxymethyl cellulose 'CMC' with high degree of substitution, using a one-pot alkalization and esterification process. The films were fabricated by CS-CMC using a combination of plasticizers: glycerol and sorbitol (30%, *w/w* to CS-CMC). Poor hydrophobicity of cellulose films seriously limits their practical applications, and therefore, the newly designed films were spray-coated with different concentrations of beeswax-to-carnauba wax (100:0, 75:25, 50:50, 25:75, and 0:100) ratios. The films were analyzed using different advanced instrumental techniques including FTIR, SEM/EDX, XRD, TGA/DSC, and mechanical analysis. The films coated with natural waxes exhibited improved hydrophobicity, water vapor transmission rate (WVTR), mechanical strength, and physical properties. Amongst all, the films coated with 25% beeswax and 75% carnauba wax exhibited optimal material properties such as hydrophobicity (WCA>125°), tensile strength (3.58 ± 0.01 MPa), Young's modulus (7.12 ± 0.11 GPa), elongation at break (39.30 ± 1.24%), and puncturing strength (17.95 ± 0.07 N/mm$^2$). Morphological analyses indicated that the produced films were less transparent with higher surface roughness that created air-pockets to improve the hydrophobicity of bioplastic films. FTIR films further supported uniform coating of natural waxes on the CS-CMC based surfaces. Further, excellent biodegradability with high antioxidant, antimicrobial, and cytocompatibility potential were reported suggesting sustainable food packaging applications.

# OC32 - Design and characterization of cyclodextrin-porous matrices for effective antimicrobial delivery

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**Keywords:** Cyclodextrin, nanosponge, inclusion complex, antimicrobial agents

Nowadays, fungal and bacterial skin infections represent a global therapeutic challenge, posing a serious threat due to the increasing prevalence of multi-resistant bacterial strains [1]; There is an urgent need of antimicrobial topical treatments which can effectively eradicate fungal and bacterial skin infections, minimizing side effects and risk of developing resistant strains. Oleic acid (OA) is a free fatty acid characterized by antimicrobial properties and by a poor water solubility [1]; the use of cyclodextrins (CDs) is proposed as a solubility-enabling strategy. Nanosponges (NS) have emerged as an innovative material composed of small particles with nanometer-wide cavities [2]. CD-based nanosponges (CDNS) appear as hyper-cross-linked materials that can be obtained by direct reaction between native CDs with a cross-linking agent[2]. This study aims to obtain an inclusion complex (IC) between CD and OA (CD-OA-IC). Naked CDNSs and OA loaded CDNS (CDNS-OA-IC) were prepared. Finally, CDNS-OA-IC will be integrated into a porous matrix formed by the interaction of CDNSs with hydrophilic polymers (e.g., chitosan) for topical application to treat bacterial and fungal skin infections. IC was synthesized by the co-precipitation method. OA ethanolic solution was added to CD dispersion according to 1:10 v/v ratio. A physical mixture (PM) was also prepared by blending OA and CD in the same molar ratio. For the preparation of naked CDNSs a dispersion of CD, citric acid monohydrate, and sodium phosphate monobasic monohydrate were freeze-dried and transferred to an oven at 160 °C for 2 h. CDNS-OA-ICs were prepared with different OA: CDNSs w/w ratios. To investigate the formation of CD-OA-IC, CDNSs, and CDNSs-OA-IC, a physico-chemical characterization was performed through scanning electron microscopy (SEM), fourier-transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). Finally, *in vitro* studies will be carried out to evaluate CD-OA-IC and CDNS-OA-IC antibacterial and antifungal properties. The morphology of CD-OA-IC shows differences when compared to those of CD alone and PM (Fig.1). Modifications in both shape and morphology by comparison with the native materials can be attributable to IC formation. FTIR analysis revealed the chemical bonds present in CD-OA-IC compared with the native materials and PM. The characteristic peaks of OA disappear in the spectrum related to CD-OA-IC, suggesting the formation of CD-OA-IC (Fig.2). A s for DSC analysis, in CD-OA-IC the absence of OA peaks, present in PM, suggested OA complexation with CD (Fig. 3). XRPD profiles provide evidence for the formation of CD-OA-IC. The diffraction profiles of CD-OA-IC differ notably from PM. The peaks at 5° and 10.2° in CD-OA-IC are not found in PM, while PM displays a strong peak at 14°, nearly absent in CD-OA-IC. SEM images revealed that the obtained CDNS-OA-IC has a porous structure (see red square box in Fig.5). FT-IR, XRPD and TGA-DSC analysis also confirm the obtaining of CDNS-OA-IC (data not shown). Future studies will be focused on the evaluation of the antibacterial and antifungal properties of both CD-OA-IC and CDNS-OA-IC.

Figure 1. SEM micrographs of (A) CD, (B) PM, and (C) CD- OA-IC.

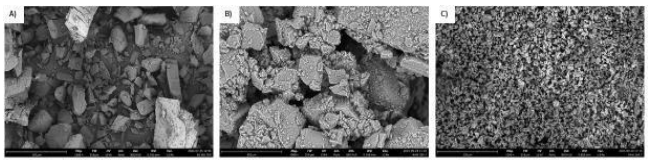


Figure 2. FTIR spectra of CD, OA, PM and CD-OA-IC.

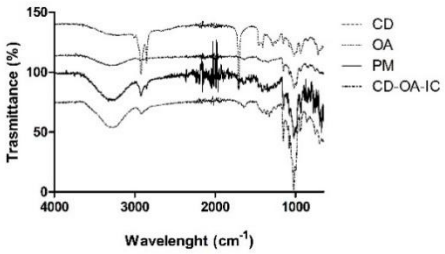



Figure 3. DSC thermograms of (A) OA, (B) CD, (C) PM, and (C) CD-OA-IC.

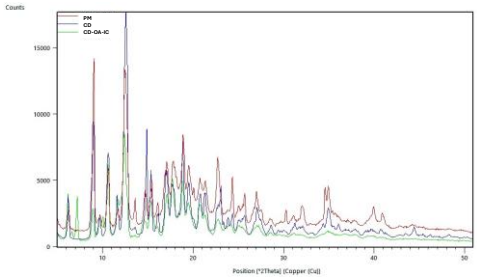



Figure 4. XRPD patterns of PM, CD, and CD-OA-IC.

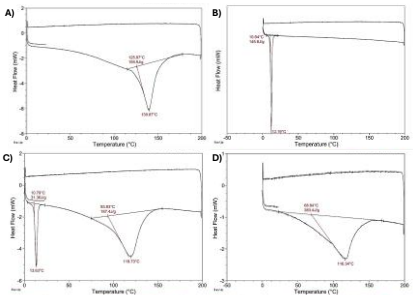
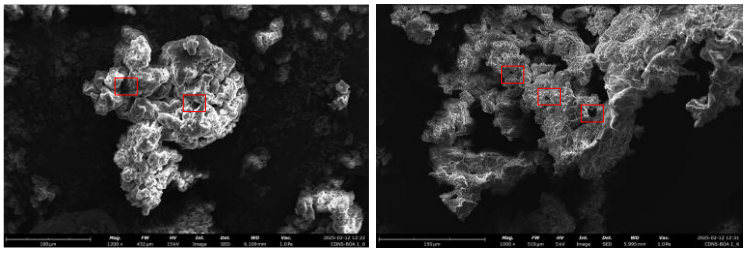



Figure 5. SEM micrographs of CDCN-OA-IC.



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# OC33 - Materials containing plant drugs obtained by the Sol-Gel method for biomedical use

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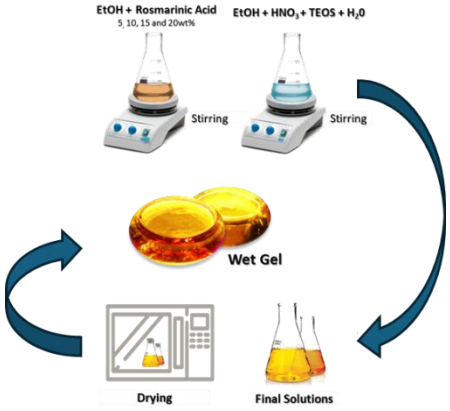
**Keywords:** Sol-gel method, silica-based biomaterials, rosmarinic acid, biomedical applications

Implanted biomedical devices can induce adverse responses in the human body, such as infections and inflammation, which can cause failure of the implant. Natural products are today one of the main sources of new pharmaceutical molecules that can prevent infections and/or inflammations. One method used for the synthesis of a wide range of materials, including biomaterials is the Sol-Gel technique. This method involves the formation of a sol (a colloidal dispersion of solid particles in a liquid) which is subsequently transformed into a gel (a cross-linked three-dimensional solid structure) through polymerization or cross-linking processes. In the context of biomaterials, the sol-gel technique has been employed to produce materials with specific characteristics suitable for various biomedical applications, such as tissue regeneration, drug delivery and tissue engineering [1]. The aim of this work is the sol-gel synthesis of biomedical implants with anti-inflammatory properties. Different weight percentages (5, 10, 15, 20$_{wt}$%) of rosmarinic acid were incorporated into the silica matrix (Figure 1). Rosmarinic acid is a phenolic compound present in several plants, including rosemary (from which it takes its name), sage and lemon balm. It is known for its antioxidant, anti-inflammatory, antimicrobial and antiviral properties [2]. These properties make it useful in various contexts, such as natural medicine, cosmetics and even the food industry, where it is used as a natural preservative. Rosmarinic acid is the subject of numerous studies to fully understand its beneficial effects on human health. The interactions between different organic and inorganic phases in the hybrid materials were studied using Fourier transform infrared spectroscopy (FTIR). The controlled release was monitored at different time intervals with UV-vis spectroscopy. The biocompatibility of $SiO_2$ and $SiO_2$/RA were evaluated, indicating that these synthesized materials did not induce cell toxicity. Furthermore, the antimicrobial activity of $SiO_2$ and $SiO_2$/RA was tested against *Escherichia coli* (E. coli) and antioxidant activity was evaluated by the FRAP method

Figure 1. Flowchart of the Sol-Gel process to obtain biomaterials.

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# OC34 - Green valorization of olive pruning / defoliation residues for innovative food ingredients GOING – Green Olive Pruning

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**Keywords:** Circular economy, Olive leaf valorization, Extraction techniques, Bioactive compounds, Cellulose

Establishing a local supply chain from agricultural waste involves transforming by-products into valuable resources, thereby reducing environmental impact and enhancing economic resilience [1]. In regions where olive farming is a key industry, the efficient utilization of waste presents significant economic opportunities. Traditionally, the olive supply chain has focused primarily on oil production, with by-products often discarded or repurposed as animal feed. However, advancements in food science and sustainability have revealed that these by-products contain bioactive compounds that can enhance food quality, prolong shelf life, and offer health benefits. Olive processing generates solid, liquid, and mixed waste, which can be repurposed through innovative approaches aligned with sustainability and circular economy principles. Olive leaves (*Olea europaea* L.), a major by-product of tree pruning, account for 25% of the dry weight of pruning residues, with Italy alone producing approximately 750,000 tons of olive leaf waste annually. Beyond their environmental impact, these leaves hold therapeutic potential due to their abundance of anti-inflammatory and antioxidant compounds, including flavonoids, pentacyclic triterpenes, and phenolic secoiridoids. The GOING project focuses on the valorization of olive leaf by-products, particularly from the indigenous 'Caiazzana' cultivar in Caiazzo, Campania, along with other cultivars such as 'Corolea,' 'Frantoio,' 'Itrana,' and 'Leccino.' The study aims to identify and quantify specialized metabolites in olive leaf extracts using three environmentally friendly extraction techniques: Ultrasound-Assisted Extraction (UAE), Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE) [2]. Among these, the 'Caiazzana' cultivar exhibits a distinctive chemical profile, particularly high levels of polyunsaturated fatty acids (PUFAs), including α-linolenic acid, known for its anti-inflammatory and cardiovascular benefits. It also has a unique flavonoid profile, rich in luteolin and its glycosides, which contribute to strong antioxidant activity and hold potential neuroprotective and anti-cancer properties. Of the extraction methods tested, PLE proved most effective for polyphenol recovery, while SFE excelled in extracting carotenoids and fatty acids. UAE produced complex extracts, though high sugar content necessitated additional fractionation to accurately quantify bioactives. A dual ultrasound method, employing alcohol followed by sequential solvents of increasing polarity, yielded an antioxidant-rich fraction (OlP) containing 67% glycosylated flavonoids. This fraction was subsequently incorporated into a starch:glycerol:sorbitol film, which demonstrated moisture-absorbing and natural plasticizing properties, making it applicable across food, cosmetic, and pharmaceutical industries [3]. Additionally, preliminary research suggests that 'Caiazzana' olive leaf cellulose could serve as a sustainable alternative to conventional cellulose sources. The valorization of olive leaf bioactives and cellulose strengthens the circular economy, transforming agricultural waste into high-value resources. Integrating these processes into olive oil production can reduce the sector's environmental footprint while generating new revenue streams. This approach is particularly relevant in Campania, where olive oil production plays a vital agricultural and economic role, leading to significant olive leaf by-product generation. By optimizing the underutilized potential of olive leaves, this research paves the way for the development of sustainable, eco-friendly industrial applications. Future efforts will focus on collaborating with local stakeholders to further expand and refine the valorization process, fostering regional sustainability and economic growth.

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## OC35 - Characterization of the Chemical Profile of Kissabel Red Flesh Apples Stored in Biodegradable Packaging by Applying NMR: Impacts on Quality and Shelf-Life

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**Keywords:** food, red-flesh apples, bio-packaging, metabolomics, NMR

Fruits and vegetables are highly perishable and prone to post-harvest losses, which is one of the major obstacles to making these nutritious commodities available to consumers. Furthermore, packaging is one of the most important post-harvest operations, with a significant role in controlling postharvest damages and physical, chemical, and biological contaminants. However, conventional food packaging greatly contributes to an exponentially growth of packaging waste and pollution globally. Eco-sustainable bio-packaging in food wrapping represents an alternate option to reduce negative environmental impact, keeping the product's long shelf-life and consumers' health.
Minimally processed apples are highly susceptible to tissue softening, enzymatic browning, and microbiological development, thus hindering their commercial marketing. Within the Agritech project [1], the present study aimed at the chemical characterization by NMR methodology of red-fleshed apple slices (Kissabel), evaluating the effect of coating and biofilm on sample storage. The analysis included an in-depth investigation of the storage of Kissabel apple under controlled temperature and packaging conditions for 21 days. In particular, the research was conducted on hydrophilic and lipophilic metabolites, the functionality of different types of packaging and the impact of treatment using an untargeted approach based on NMR spectroscopy. This methodology made it possible to characterize the chemical composition of specific metabolites and to quantify their concentration changes over time, analysing the influence of treatment and packaging type. Kissabel' apples (R201®) were supplied by Consorzio Melinda S.c.a. (Cles (TN) - Val di Non - Italy). Half of the samples were coated with a bilayer coating based on carboxymethylcellulose, alginate, citric and oxalic acid by the dipping method. Then, the apples as such (K) and the coated apples (KT) were cut into slices of approximately 100 g and packed using two commercial biodegradable films (biofilms) of vegetable origin: PLA (polylactic acid obtained from maize starch) and ECO (composed of maize starch, cassava and eucalyptus). All packed samples were then stored for 21 days ($t_{21}$) at 5°C and monitored at 14 days ($t_{14}$). The apples were subsequently freeze-dried, ground into powder, and extracted according to the Bligh-Dyer procedure. The hydroalcoholic and organic extracts obtained were brought to dryness and resolubilized in appropriate deuterated solvents for NMR spectroscopic investigation. The analysis of the samples was performed by combined interpretation of one-dimensional ($^1$H) and two-dimensional ($^1$H-$^1$H TOCSY, $^1$H-$^{13}$C HSQC, $^1$H-$^{13}$C HMBC) NMR experiments. The $^1$H NMR spectral assignments of the extracts were performed using 2D experiments and literature data from other matrices analyzed under the same experimental conditions [3]. Sugars, organic acids, amino acids, polyphenols, fatty acids, sterols, phospholipids and galactolipids were identified and quantified in the extracts. In order to investigate the functionality of the two biofilms, the metabolite profile of the untreated (K) and treated (KT) apples packed in ECO and PLA at time 14 and 21 days was compared with that of the samples at time zero ($t_0$). Analyzing the quantification data at the three times ($t_0$, $t_{14}$, $t_{21}$), overall, ECO and PLA biofilms were effective in slowing down the loss of quality of Kissabel apples during storage, preserving the cell structure and reducing spoilage. However, the ECO biofilm showed a better ability to maintain a favourable internal storage environment, reducing water loss. This resulted in optimal preservation of the freshness and visual appearance of the fruit. Metabolomic analysis by NMR revealed significant changes in the chemical profiles of the apples, confirming the effectiveness of the packaging and more so the coating in prolonging the shelf-life of the product.

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[1] The research was conducted as part of the Agritech project and received funding from the European Union European Next-Generation EU (NATIONAL REPRESENCE AND RESILIENCE PLAN (PNRR) - MISSION 4 COMPONENT 2, INVESTMENT 1.4 - D.D. 1032 17/06/2022, CN000022).
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# OC36 - From Waste to Value: Recovery of Bioactive Compounds and Cellulose from Tomato By-Products for Functional Food Applications

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**Keywords:** tomato waste, cellulose, functional food, SFE, UAM, UHPLC-HR-MS analysis

The recovery of high-value compounds from supply chain by-products is a topic of growing interest [1]. Waste generated from fruit and vegetable production and processing represents a potential source of bioactive and nutraceutical compounds [2]. Among these, tomato by-products stand out for their high antioxidant content, making them promising candidates for use as functional ingredients [3,4]. Additionally, extracting cellulose from tomato stems aligns with circular economy principles [5]. This study explores the valorization of tomato supply chain waste as a source of bioactive compounds for fortified food products. Tomato by-products were first lyophilized to preserve thermolabile bioactives and then subjected to a dual extraction process. Carotenoids were selectively extracted from the peels using supercritical $CO_2$ extraction (SFE), while the remaining material was further processed via ultrasound-assisted maceration to recover polyphenols. Simultaneously, lyophilized stems underwent extraction and purification to isolate cellulose, achieving an estimated yield of 30%. The recovered cellulose was intended for use as a functional additive in food formulations. Spectroscopic analyses (UV-Vis and ATR-FTIR) confirmed the presence of carotenoids in the lipophilic extract and verified the structural integrity of the isolated cellulose. High-resolution mass spectrometry (UHPLC-HR-MS) revealed that the SFE extract was enriched in carotenoids and free fatty acids, while the hydroalcoholic extract contained abundant hydroxycinnamic acids and glycosylated flavonoids. The cellulose fraction was further characterized by thermogravimetric analysis (TGA) to assess its thermal stability, X-ray diffraction (XRD) to confirm its high crystallinity, and scanning electron microscopy (SEM) to reveal its uniform fibrous morphology. Additionally, antioxidant assays (DPPH, ABTS, TBARS) and cytotoxicity tests on immortalized cell lines were conducted to evaluate the bioactive extracts. These extracts were then incorporated into a fortified food product—homemade pasta—where 10% of the flour was replaced with cellulose extracted from tomato waste. The dough was further enriched with both the SFE extract and the hydroalcoholic extract. In vitro gastrointestinal digestion was performed following the INFOGEST static protocol. The digestion process preserved the characteristic three-peak absorption spectrum of carotenoids, while lipid profiling via UHPLC-HR-MS indicated increased linoleic acid levels and a stable free fatty acid composition, suggesting that the polyphenolic extract played a protective role against oxidative degradation. This study highlights the potential of tomato waste as a valuable source of bioactive compounds with applications in the food industry and its contribution to the circular economy. Enhancing tomato by-products can improve the nutritional and antioxidant profile of fortified food products without compromising lipid stability. Future studies should focus on assessing the bioavailability of these bioactives after intestinal absorption and their impact on metabolism in vivo. Additionally, optimizing extraction methods and applying microencapsulation strategies could enhance the stability and efficacy of bioactive compounds, expanding their potential use in the nutraceutical sector. Finally, extending this approach to other agri-food chains could foster the development of sustainable functional ingredients, reducing waste and driving innovation in high-value product creation.

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# OC37 - Exploring the Metabolomic Profile Along the *Riccio Tomato* Supply Chain: In-Depth Chemical Characterization and Assessment of Biological Activity for Food Safety

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**Keywords:** *Riccio tomato*; sustainability; metabolomics; FT-ICR mass spectrometry; HPLC-MS/MS

Among Italian landraces, *Riccio tomato,* an ancient beneficial tomato cultivar [1], was recently re-proposed by local farmers for the high sustainability of its cultivation. Particularly suited for growing on clay soils, this landrace has adapted well to hill land, demonstrating drought resistance and cryptogamic diseases. The reduced water intake can result in a low intake of contaminants, since water has proven to be a source of heavy metals and pesticide residues [2]. Thus, this variety can potentially improve tomato quality, which emerges as a priority issue for consumer safety. In this regard, the present study carried out in the frame of the ONFOODS consortium (Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security – Working ON Foods) [3], is aimed at appraising food safety and genuineness along the food supply chain from production to retail. Whole tomatoes (T), tomato sauce (TS), and tomato waste (TW) extracts, harvested in two different years (2022 and 2023) were subjected to a multimethodological analytical protocol comprising untargeted (NMR, FT-ICR MS) and targeted (HPLC MS/MS, UV-Vis, ICP-OES) chemical methods and biological (antioxidant, chelation, and cytoprotection activity) tests. The analyses revealed that compared to T22, T23 exhibited higher levels of sugars, total soluble solids, titratable acidity, and an intense red coloration, indicating enhanced lycopene and carotenoid content. Metabolomic profiling highlighted significant variations in amino acids, sugars, and organic acids between samples harvested in 2022 and 2023, while organic acid levels remained almost similar. Spectrophotometric and HPLC-MS studies suggested that pedoclimatic factors can influence phenolic compounds synthesis, with significant differences observed in hydroxycinnamic acid derivatives and flavonoids. Tomato by-products (peel and seeds) demonstrated notable antioxidant activity, providing valuable bioactive compounds for potential nutraceutical applications. Regarding heavy metals and pesticides, their levels were found below safety limits, underscoring the minimal risk of contamination from processing. Overall, this comprehensive analysis highlighted the chemical and nutritional profile of *Riccio tomatoes*, turned out to be a highly promising relaunched cultivar, thus contributing to preserve natural resources with implications for both food quality and safety.

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# OC38 - Exploring *Cucumis prophetarum* as a Natural Insulin Sensitizer: A Promising Ally Against Type 2 Diabetes

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**Keywords:** *Cucumis prophetarum* L.; decoction; UHPLC-HRMS; flavone C-glycosides; cytotoxicity; antidiabetic effect

The growing prevalence of type 2 diabetes mellitus (T2DM) has become a major global health concern, driven by sedentary lifestyles, poor dietary habits, and an aging population. T2DM is characterized by insulin resistance and progressive beta-cell dysfunction, leading to chronic hyperglycemia and an increased risk of severe complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy. While current pharmacological treatments, such as insulin sensitizers, insulin secretagogues, and SGLT2 inhibitors, effectively manage blood glucose levels, they are often associated with side effects like weight gain, gastrointestinal disturbances, and hypoglycemia. Additionally, some patients experience treatment resistance, necessitating alternative therapeutic strategies. Given these challenges, there is a growing need for novel, sustainable, and less invasive approaches to T2DM management. Natural compounds derived from medicinal plants have gained increasing attention due to their potential insulin-sensitizing, anti-inflammatory, and antioxidant properties, which may help regulate glucose metabolism with fewer adverse effects. Research into plant-based therapies aligns with the rising interest in nutraceuticals and functional foods, offering complementary or preventive solutions for metabolic disorders. In this context, *Cucumis prophetarum* L., a climbing plant belonging to the *Cucurbitaceae* family and traditionally used in folk medicine for skin infections, gastrointestinal disorders, and cancer, has emerged as a promising candidate. However, while the medicinal properties of its fruits are well known, the therapeutic potential of its leaves, stems, and roots remains largely unexplored. This study investigates the insulin-sensitizing properties of decoctions obtained from these less commonly utilized plant organs. The research evaluates their cytotoxicity on HepG2 and L6 cells and characterizes their chemical composition using UV–Vis and ATR-FTIR spectroscopic techniques, alongside ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HR-MS/MS). Results indicate that leaf and stem decoctions are rich in apigenin *C*-glycosides, while root extracts predominantly contain raffinose and cucumegastigmane II. Cytotoxicity assays confirm that all extracts exhibit low toxicity. To assess their insulin-sensitizing effects, the study analyzes key proteins involved in insulin signaling in insulin-resistant L6 myoblasts. The findings reveal that stem and root decoctions (300 µg/mL) significantly enhance the phosphorylation of IRS-1 (Tyr612), GSK3β (Ser9), and AMPK (Thr172) compared to palmitic acid-treated cells. The leaf decoction increases IRS-1 (Tyr612) and AMPK (Thr172) levels while reducing p-GSK3β (Ser9), and the root decoction also decreases p-mToR (Ser2448), suggesting a regulatory role in lipid metabolism. These results suggest that *Cucumis prophetarum* decoctions may alleviate insulin resistance by modulating key metabolic pathways, making them promising candidates for further research as natural antidiabetic agents. Moreover, the study highlights the biological relevance of plant organs not conventionally used in traditional medicine and opens new perspectives for their valorization in nutraceutical applications and functional food development, supporting a more sustainable approach to diabetes management.

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## OC39 - Valorization of Olive Oil By-Products: A Comprehensive Study of Phenolic Profiles, Antioxidant Activity, and Prebiotic Characteristic

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**Keywords:** Olive by-products, phenolic compounds, prebiotic potential

The olive oil industry produces large quantities of plant by-products, especially olive pomace and vegetative waters, which impose environmental and disposal concerns due to their high organic load [1]. These by-products are often underexploited, leading to potential environmental alarms [2]. Notable bioactive compounds in such waste are phenolic substances, including secoiridoid derivatives, which are peculiar phenolics of olive products [3]. Secoiridoids are a class of phytochemicals that exhibit significant bioactive properties, including potent antioxidant, anti-inflammatory, and anti-diabetic effects [4]. This study focuses on evaluating the prebiotic characteristic of samples as raw material and their corresponding hydroalcoholic extracts from selected olive waste material, including olive pomace (OP, OPE) and olive stone (OS, OSE) of the production of monovarietal high-quality extra virgin olive oil, and also the oils themselves, i.e., high-quality monovarietal extra virgin olive oil (EVOO)(OLH, OLHE), and low-quality monovarietal industrial EVOO (OLL, OLLE), by analyzing their phenolic profiles, their total phenolic content and antioxidant activity to establish their use as sustainable, functional foods and/or new pharmaceutical applications. In the study to be presented, the prebiotic characteristics of olive oil by-products were assessed through an in-vitro fermentation assay utilizing the probiotic strains *Lactiplantibacillus plantarum* IMC509™ and *Lacticaseibacillus rhamnosus* IMC501™. All treatments exhibited positive growth curves, indicating favorable conditions for probiotic proliferation. Notably, fermenting fluids derived from *L. plantarum* cultures treated with olive pomace (OP), olive pomace extract (OPE), olive stones (OS), and olive stone extract (OSE) demonstrated a significant increase in the production of short-chain fatty acids (SCFAs), specifically acetate (C2) and valerate (C5), compared to control sample at the final incubation time. Meanwhile, C5 production at the final time of incubation was observed in *L. rhamnosus* cultures treated with the raw material (OP and OS), revealing the potential prebiotic characteristic of these by-products. Additionally, fermenting fluids exhibited an increase in total phenolic content and antioxidant capacity at the end of fermentation for both probiotic strains, specifically in OP and OPE samples. Phenolic profile analysis by HPLC-DAD/MS revealed that pomace contained higher amounts of phenolics than stones, e.g., 3.4 times the content of hydroxytyrosol, 4.8 times of 3,4-DHPEA-EDA (dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol), and 4.6 times of verbascoside. The extracts displayed a high total phenolic content, with OPE containing 1745 ± 1 ppm GAE and OSE containing 1379 ± 1 ppm GAE. Mineral composition analysis via ICP-MS indicated high potassium levels (35.2 ± 0.6 mg/g) and calcium (3.7 ± 0.1 mg/g) in pomace compared to stone, demonstrating the nutritional richness of such by-products.

These findings support the potential of olive oil-derived by-products to serve as a source for functional foods and nutraceuticals, addressing environmental issues while contributing to health-focused applications.

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# OC40 - Grape Pomace Bioactives in Gummy Jellies: A Sustainable Path to Wellness

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**Keywords:** sustainability, *Vitis vinifera* L. pomace, functional food, polyphenols, gummy jellies

The transition toward more sustainable production models is a priority in today's economic and environmental landscape, aligning with the goals of the 2030 Agenda for Sustainable Development. The agri-food sector, particularly the wine industry, plays a crucial role in this transformation, generating bioactive-rich by-products that can be repurposed into valuable resources, thereby reducing waste and creating added value. Among these, grape pomace—the primary solid residue of winemaking—represents a significant opportunity for valorization. If not properly managed, its release into the environment can lead to ecological issues due to the presence of phytotoxic and slow-degrading organic compounds. However, thanks to its high content of polyphenols, fibers, lipids, and other secondary metabolites, grape pomace can be recovered to develop nutraceutical, cosmetic, agricultural, and energy products, supporting a more circular economy. In this context, the present study proposes a strategy for the recovery and valorization of red grape pomace, promoting a sustainable approach to the production of gummy jellies enriched with bioactive compounds. After freeze-drying and pulverization, the pomace underwent aqueous extraction by decoction, followed by semi-purification via gel filtration chromatography with Amberlite XAD-4, aiming to enrich the polyphenolic fraction and remove potential interferences. The extract was chemically characterized using UHPLC-HRMS, while the total polyphenol content was quantified via the Folin-Ciocalteu method. Several tests were conducted to ensure the safety and efficacy of the product. The bioavailability of bioactive compounds was assessed through simulated in vitro digestion following the INFOGEST protocol, with HPLC-DAD analysis determining the polyphenolic fraction effectively absorbed in the intestine. In parallel, a cytotoxicity evaluation was performed on human intestinal epithelial Caco-2 cells, a widely used model for studying the absorption and safety of bioactive compounds. The extract's antioxidant activity was confirmed by DPPH and ABTS assays, further demonstrating the potential of grape pomace as a source of functional ingredients.
The gummy jellies, enriched with grape pomace extract, were formulated using agar-agar as a natural gelling agent, fructose as a sweetener, lemon juice as a color stabilizer and flavor corrector, and grape pomace extract as a natural colorant and source of bioactives. The final product exhibited a soft texture, intense red color, pungent aroma, and a sweet yet slightly sour taste. To assess product acceptability, a sensory panel test was conducted with a selected group of volunteers, evaluating color, aroma, texture, and overall taste. The results indicated high consumer acceptance, suggesting that gummy jellies could serve as an innovative alternative to traditional dietary supplements. This research demonstrates that gummy jellies enriched with polyphenols extracted from grape pomace represent an innovative, safe, and bioavailable solution for health and well-being promotion. The integration of bioavailability and cytotoxicity assessments in human gut cell models provides essential validation of the functional potential of the product. Moreover, the recovery of grape pomace aligns perfectly with a circular economy model, contributing to waste reduction and the development of new industrial and territorial opportunities. This study highlights how wine industry by-products can be transformed into high-value resources for nutraceuticals and functional nutrition, ultimately supporting the 2030 Agenda goals and fostering a more resilient, responsible, and sustainable production system.

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# OC41 - Exploring antioxidant activity and polyphenolic profile of *Fagopyrum esculentum* seeds

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**Keywords**: buckwheat, antioxidant activity, extraction, polyphenols

Buckwheat (*Fagopyrum esculentum*) is an alternative pseudocereal crop belonging to the *Polygonaceae* family. Buckwheat seeds with high nutritional values, are known as a gluten-free dietary source of amino acids, vitamins, starch, dietary fiber and essential minerals. In addition, they possess higher antioxidant activity in comparison with frequently used cereals as oats, rice and barley mainly due to the presence of polyphenols plant secondary metabolites with unique beneficial properties for human health.
The aim of this study was the investigation of the main antioxidant polyphenolic compounds in buckwheat seeds and its fractions namely brown hull, endosperm white flour and related mixture or whole grain flour. The optimization of the phenolic extraction procedure was performed by using ultrasound-assisted extraction (UAE). The determination of total phenolic content (TPC) aimed at the selection of the most suitable extraction parameters. The total phenolic content (TPC) of buckwheat extracts was evaluated by both traditional Folin–Ciocalteu assay (F–C assay) and the novel spectrophotometric Fast BB Blue assay (FBBB) while the total flavonoid content (TFC) of these extracts was measured by the $AlCl_3$ method. The antioxidant activity was determined by FRAP (Ferric reducing antioxidant power) and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assays, and the radical scavenging activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The quali-quantitative analyses of the phenolic profiles were performed by using HPLC-MS and HPLC-DAD. The HPLC method was validated according to the ICH (International Conference of Harmonization) guidelines.
A 1:20 matrix/solvent ratio (EtOH:$H_2O$, 1:1) for 15 minutes by using an ultrasonic bath (100 W, 37 kHz) three times led to the highest TPC yield and was selected as the extraction procedure. According to the antioxidant and anti-radical assays, the hull fraction reported highest values 3.69 mg GAE/mL and 1.17 mg GAE/mL (Gallic Acid Equivalent), respectively.
HPLC-MS analysis allowed the identification of 25 phenolic compounds in the whole grain flour, belonging to different chemical classes: phenolic acids (gallic acid, caffeic acid, protocatechuic acid), flavanols (rutin, catechins and procyanidins) and flavones (orientin, luteolin and vitexin). The HPLC-DAD analyses showed a variable composition in terms of polyphenolic profiles among buckwheat seed brown hull, white endosperm flour and the related mix of whole grain flour.
The analytical method developed and validated showed to be a reliable and effective tool for the analysis of the phenolic profile of buckwheat seed fractions and whole grain flour. The analytical method could be applied to selecting *F. esculentum* varieties highly rich in polyphenol for functional food and nutraceutical applications.

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# OC42 - Green Extraction Strategies for the Sustainable Valorisation of Tomato ByProducts

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**Keywords:** tomato waste, carotenoids, polyphenols, SFE, UAM, UHPLC-HR-MS analysis

Agri-food residues, though an environmental burden, can become valuable resources if properly valorized. Rich in bioactive compounds such as carotenoids, polyphenols, lipids, polysaccharides, and fibers, their recovery is often hindered by extraction difficulties [1]. In line with bioeconomy principles, the sustainable transformation of these compounds into high-value products is a key aspect of the circular economy. Various extraction techniques are commonly employed to recover bioactive compounds from agro-industrial residues, each offering specific advantages depending on the target molecules. This study explores the valorization of tomato by-products through low-impact extraction techniques and the characterization of recovered bioactives, aiming to define an optimal strategy for their selective extraction. Supercritical $CO_2$ extraction (SFE) presents significant advantages over conventional solvent-based methods [2], as it eliminates the need for toxic organic solvents and enables selective extraction by adjusting temperature and pressure. Optimization of this green and environmentally friendly technique was carried out using a prototype system, with extraction yield (g extract/g matrix) as a key parameter. The addition of co-solvents, such as sunflower seed oil or ethanol [3], improved the recovery of lipophilic compounds, although requiring additional purification steps. Other extraction methods were also investigated, including ultrasound-assisted maceration (UAM) and microwave hydrodiffusion and gravity (MHG) extraction. UAM was performed using sequentially n-hexane and methanol as extractants [4], while MHG exploited in-situ water to release active hydrophilic compounds. The obtained extracts were characterized via spectroscopic (UV-Vis, ATR-FTIR) and chromatographic (UHPLC-HRMS) techniques. SFE and UAM in n-hexane, despite differences in extraction yield, proved highly effective for lipophilic compounds such as carotenoids (mainly lycopene and β-carotene) and free fatty acids (palmitic, palmitoleic, oleic, stearic). Conversely, UAM-based alcoholic extract and MHG extract favored the recovery of polar molecules such as polyphenols, providing complementary advantages: UAM was more efficient in extracting hydroxycinnamoyl compounds, while MHG favored jasmonic acid derivatives. Moreover, both extracts exhibited a comparable flavonoid content, further highlighting their similar capacity to recover these bioactive compounds despite differences in extraction selectivity [5]. These findings emphasize the potential of integrating multiple extraction techniques to maximize bioactive compound recovery from tomato by-products, fostering their sustainable application in nutraceutical and functional food industries. The complementary use of SFE, UAM, and MHG enables the selective extraction of both lipophilic and hydrophilic compounds while minimizing environmental impact. Ultimately, this study underscores the critical role of green extraction strategies in advancing the circular economy in the agri-food sector, offering innovative pathways to transform waste into high-value resources and promote a more sustainable industrial model.

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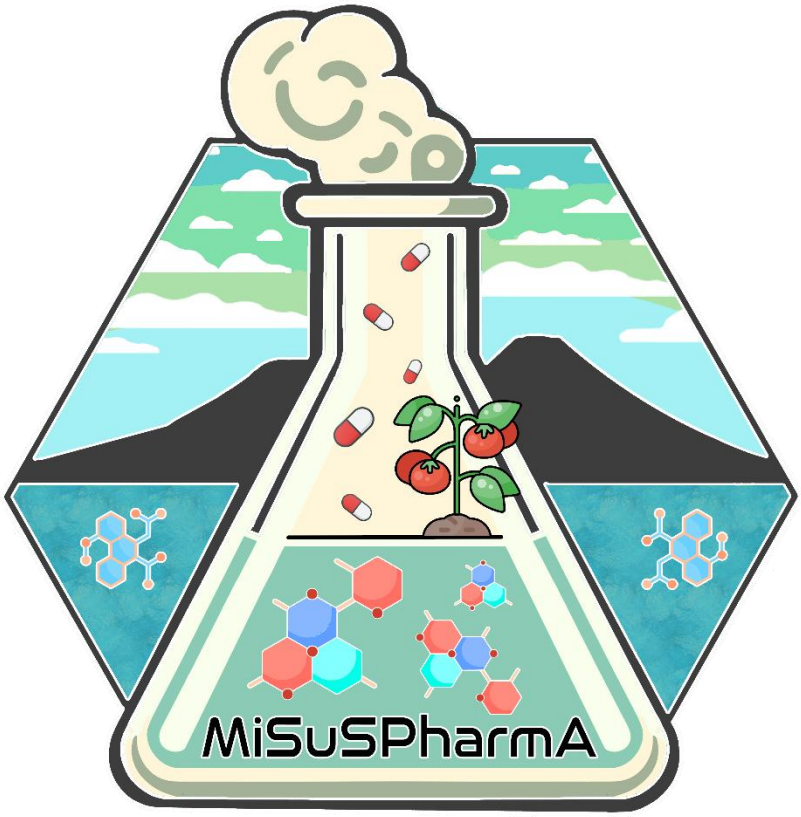


## POSTER PRESENTATIONS


# P01 - The Co-crystal Platform: a modular approach to mechanochemical co-crystallization

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**Keywords:** Co-crystals, Mechanochemistry, Raman spectroscopy, Differential Scanning Calorimetry, Structural characterization

Among the many solid forms, compounds can exist as co-crystals, crystalline solids composed by at least two different chemical entities sharing the same lattice.
Pharmaceutical co-crystals show many advantages, such as ease of co-administration in a single dose, thus ameliorating the patient's compliance [1], and increase of the water solubility of lipophilic APIs [2].
Mechanochemical procedures have proved to be a valid alternative for the production of high quality cocrystals [3], as well as a powerful screening tool applicable to large libraries of compounds, since efficiency and speed are satisfyingly high. A typical, widely used lab-scale instrument is the ball mill, which allows to get high quality results and high yields in just a few minutes, with little to no usage of solvents.
Here, a workflow for the screening and characterization of co-crystals is presented. The platform is structured as follows: the selection of candidates is driven by their potential pharmaceutical application; then, a knowledge-based choice of suitable co-formers is performed, based on functional group compatibility and propensity to form supramolecular synthons. The chosen pairs are subjected to standard mechanochemical procedures, primarily Liquid-Assisted Grinding (LAG), during which a screening of solvents is also performed.
The obtained samples are then fully characterized by means of Raman spectroscopy, Differential Scanning Calorimetry, Thermogravimetric Analysis, XRPD and solid-state NMR. If the sample passes all the check, it is directed to the second-line R&D, where scaling up and technology transfer occur. However, if the sample proves not to be a co-crystal, the designed feedback loop allows to change the synthetic routes, until a co-crystal is obtained. Should the changes in the early stages of the second layer of the platform not produce a new phase, the negative result will be stored for future refinements of the choice parameters.
Embedded in the characterization module is the coupled DSC-Raman method, which provides insights on the thermal phase changes at a molecular level.
The platform's modular architecture is well-suited for the integration of machine learning and AI systems, which would act as powerful aids for the selection of input candidates [4].
The described workflow allowed to discover several new co-crystals; here a representative species, namely CpdB, is briefly described. The co-crystalline nature of CpdB was confirmed by all the aforementioned techniques. Interestingly, by means of coupled DSC-Raman it was found that the new phase undergoes a thermal transition between the co-crystal and a salt form. Comparison between the experimental spectrum and those calculated at the B3LYP/6-311G** level of theory for model API anions allowed to assess the protonation state of the anionic API in the salt form.

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## P02 - Ferroptosis Induction by a New Pyrrole Derivative as potential anticancer agents

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**Keywords:** Cancer, Microwave reaction, ferroptosis, microtubule inhibitors, heterocycle

Ferroptosis is being increasingly investigated as a non-apoptotic, iron-dependent, regulated form of cell death. The accumulation of reactive oxygen species (ROS) and the peroxidation of extra-mitochondrial lipids are the main initial hints of ferroptosis. Ferroptosis-inducing agents are an emerging class of non-apoptotic, iron-dependent compounds for anticancer chemotherapy. Different microtubule inhibitors, such as paclitaxel and vinblastine, have been shown to promote ferroptosis in tumor cells, in this context starting from different work of my research group on tubulin polymerization inhibitors [1] we synthesized a series of new aroyl diheterocyclicpyrrole (ARDHEP) derivatives like ferroptosis inducing agents (Figure 1). These compounds were synthesized with a microwave reactor to optimize the yield and the reaction time. These microwave reactions allowed us to use a very small quantity of solvent, two or three millilitres, reducing the environmental impact. In fact, we are trying to reduce or eliminate the use of solvents and toxic substances in accordance with greener chemistry. Among them, the new derivatives were first tested in enzymatic essay, most compounds inhibited tubulin polymerization with $IC_{50}$ values in the submicromolar range of concentrations. The most promising derivative, compound 12 (Figure 2), characterized by a thiazole and a furan ring respectively in position 1 and 4 of the pyrrole scaffold exhibited the most potent in vitro anticancer activity against breast cancer (BC), triple-negative breast cancer (TNBC), and colorectal cancer (CRC) cell lines. This interesting result was then confirmed by in vivo studies showing significant efficacy in a HCT116 CRC xenograft model (Figure 3). Compound 12 reduced the expression levels of ferroptosis inhibitory factors glutathione peroxidase 4 (GPX4) and ferritin heavy chain (FTH), and it increased levels of ferroptosis promoting factors cyclooxygenase 2 (COX2) and acyl-CoA synthetase long-chain family member 4 (ACSL4) [2].

Figure 1. Structure of compounds 1-20.

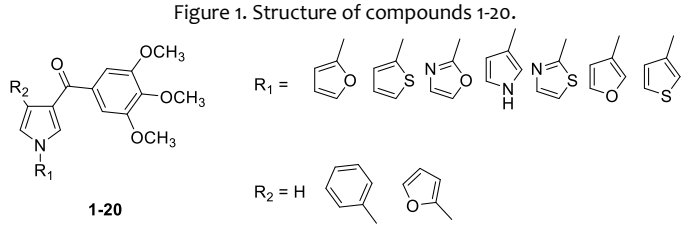



Figure 2. Structure and binding mode of compound 12.

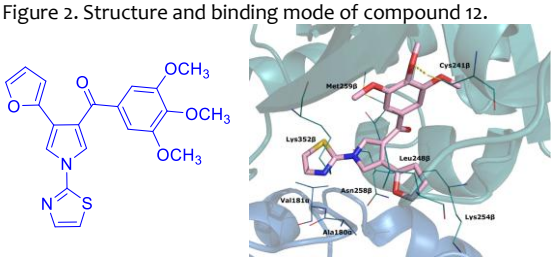
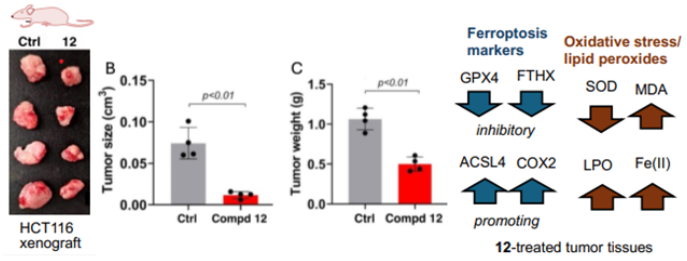



Figure 3. Biological activity of compound 12.

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## P03 - A pH-responsive crosslinker platform for Antibody-drug conjugate targeting delivery

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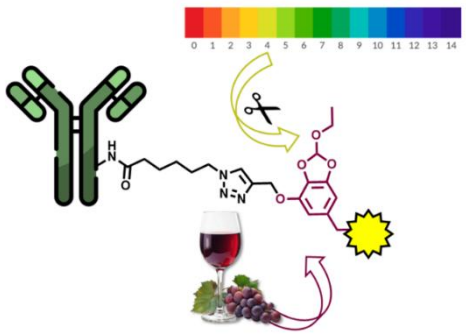
**Keywords:** Antibody, Bioconjugate, Drug-delivery, Linker

Antibody–drug conjugates (ADCs) are targeted bioconjugate constructs that integrate the potency of cytotoxic drugs with the selectivity of monoclonal antibodies. ADCs represent a significant step toward sustainable and precision therapies by enabling targeted drug delivery, minimizing off-target effects, and reducing overall drug dosage, thereby enhancing treatment efficacy while lowering environmental and systemic toxicity. Moreover, by enhancing drug selectivity and efficacy, ADCs contribute to a more efficient use of pharmaceutical resources, lowering the required dosage and decreasing the environmental footprint of drug manufacturing and disposal. With the last of 16 ADCs approved by the Food and Drug Administration (FDA) for cancer treatment in January 2025, and more than 100 ACDs in clinical trials, this technology represents a very innovative and efficient approach in the selective drug delivery field [1] . They are currently among the most promising drug classes in oncology, with efforts to expand their application in other fields.

ADCs consist of a monoclonal antibody (mAb) connected by a properly designed linker, to a potent therapeutic agent known as payload. The linker plays a pivotal role as it needs to ensure the stability of the bioconjugate in plasma while releasing the payload once the ADC is internalized into target cells. Linkers can be classified as cleavable and uncleavable: in the first case the payload is released upon the lysosomal degradation of the whole ADC, in the second case the linker is sensitive to specific conditions of the intracellular environment which trigger its cleavage and the subsequent elimination of the payload [2]. Lysosomes maintain cellular homeostasis by generating a highly acidic environment of pH 4.5 – 5.0[3], this condition encouraged the development of pH-responsive drug-delivery systems such as pH-labile ADCs.

We here report a new pH-responsive linker based on a 1–6 self-immolative *p*-hydroxybenzyl alcohol scaffold, which is non-toxic and easily accessible synthetically from gallic acid, a natural tannin component found in several plants and available in large quantities at a low price. It bears a orthoester moiety that is hydrolyzed under acidic conditions, triggering a 1,6-elimination mechanism for the release of the payload [4].




Various primary and secondary amine-, alcohol- and phenol-containing molecules can be bound by the formation of carbamates, carbonates or ethers. Besides this, orthogonal reactivity to these bonds is possible by introducing azides, alkynes, tetrazines or other fragments for click chemistry to allow easy conjugation with macromolecular supports. We proved that our linker is suitable for conjugation of the cytotoxic drugs Doxorubicin (a primary amine) and Combretastatin A4 (a phenol) with an antibody carrier. The release kinetics of the linker-payload systems were evaluated and the resulting ADCs exhibited improved cytotoxic activity in cancer cells compared to the unconjugated antibody and to the linker-payload fragment.

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# P04 - Synthetic Progress Toward cladosporols A and B

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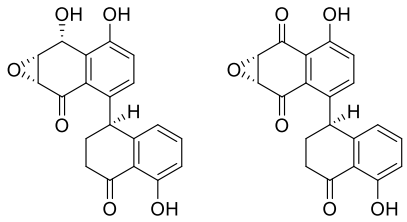
**Keywords:** Total Synthesis, Natural Products, Antifungal agents

Fungal infections represent a dangerous threat for the agri-food sector, affecting all the nutritionally relevant crops [1]. It has been estimated that each year more than 30% of the annual crop production is lost due to this cause, representing a huge economic and social issue [2]. Furthermore, the continued use of fungicides sharing the same mode of action has led to the development of resistance mechanisms, requiring the exploration of novel solutions [3]. To broaden the spectrum of promising chemotypes, we are investigating the potential of cladosporol derivatives. First isolated in 1992, this class of molecules is known to possess antifungal properties, inducing cell wall malformations via glucan synthase inhibition [4, 5]. Despite their biological relevance, a total synthesis has never been reported in more than 30 years, limiting their full potential. To address this gap, we are currently working on the development of a synthetic route for the obtainment of the bi-dihydronaphthalene backbone, shown in Figure 1, from natural substrates. In detail, we aim to obtain it by dimerization of commercially available juglone, a naphthoquinone derivative present in walnut plants such as *Juglans regia*, via lithiation reaction [6]. The achievement of this milestone will potentially lead us to the first synthesis of cladosporol compounds; moreover, the intermediates obtained during our study will be tested against fungal pathogens to evaluate the biological potential of simplified cladosporol analogues.

Figure 1. Structure of cladosporol A and B.

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# P05 - Firsts steps towards a safer and greener synthesis of MF63

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**Keywords:** MF63, mPGES-1, Cyanation, Copper, Potassium hexacyanoferrate (II) trihydrate

Prostaglandin $E_2$ ($PGE_2$), a bioactive derivative of Arachidonic Acid (AA), has been found to play a role in multiple inflammatory diseases. It is mainly produced by cyclooxygenases (COX-1 and COX-2) followed by the inducible microsomal enzyme prostaglandin E synthase-1 (mPGES-1). The COX/mPGES-1/$PGE_2$ pathway has also been found to be induced in several forms of cancer, where the increased COX-2 and mPGES-1 expression leads to decreased survival rate. $PGE_2$ has also showed to possess a pro-tumorigenic role via several mechanisms as mice lacking mPGES-1 display slower growing tumors, decreased angiogenesis, less metastasis and increased infiltration of cytotoxic T cells [1]. Due to its role in inflammatory diseases as well as in cancer, selective inhibitors of mPGES-1 might represent a new treatment approach without any potential side effects resulting from the inhibition of other prostaglandins including $PGI_2$ and $TXA_2$. MF63 represents a promising drug with a potent in vivo mPGES-1 inhibitory activity showing an $IC_{50}$=0.9 nM in a preclinical LPS-induced hyperalgesia model in guinea pig [2]. MF63 synthesis (**Errore. L'origine riferimento non è stata trovata.**) starts with an oxidation reaction performed on commercially available 3-chlorophenantrene by means of chromium (VI) oxide yielding the corresponding quinone **1**. Compound **1** is then treated with commercially available 2,6-dibromobenzaldehyde yielding the phenanthrene imidazole scaffold compound **2**, which is then reacted in the presence of copper (I) cyanide to obtain MF63 [2]. Considering the hazards associated with cyanide salts, we attempted to modify MF63 synthesis by performing a safer and greener cyanation reaction either on compound **2** or on 2,6-dibromobenzaldehyde involving the non-toxic cyanide source potassium hexacyanoferrate (II) trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$) (**Errore. L'origine riferimento non è stata trovata.**).

Figure 2. MF63 synthesis.

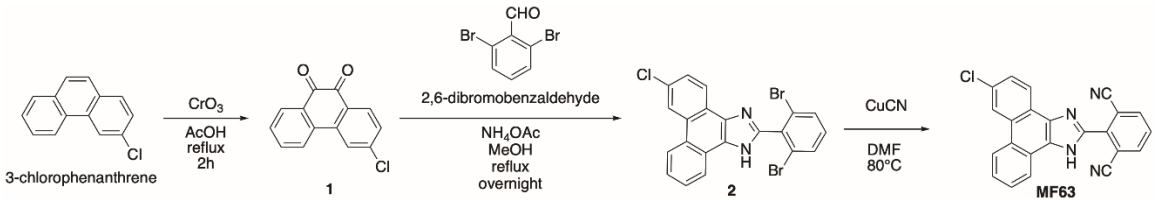
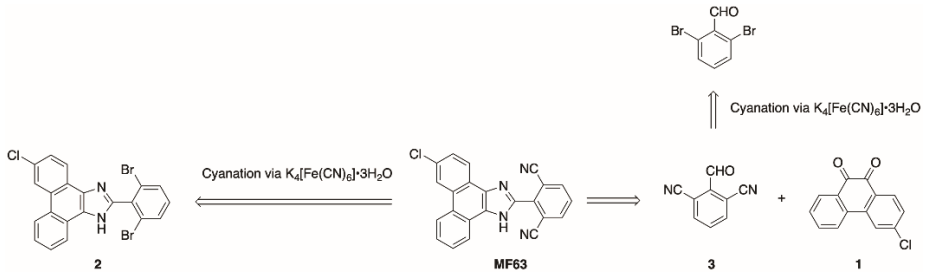



Figure 3. New cyanation approaches.

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## P06 - Covalent reversible peptidomimetic Inhibitors of SARS-CoV-2 $M^{pro}$: A Fast-Track Approach Using Multicomponent Reactions

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**Keywords:** SARS-CoV-2, $M^{pro}$, Covalent Reversible Inhibitors, Peptidomimetics, MCRs

Multicomponent reactions (MCRs) represent a powerful synthetic approach in drug discovery, enabling the rapid assembly of structurally diverse molecules in a single step. Their efficiency and versatility make them particularly suitable for drug discovery applications, including those targeting viral enzymes.
The Main protease ($M^{pro}$) of SARS-CoV-2, a cysteine protease, is a validated antiviral target due to its essential role in the viral life cycle and its high conservation across coronaviruses [1]. Leveraging its specific cleavage of polypeptides after a glutamine residue and its catalytic mechanism, we employed MCRs as a fast and flexible strategy to develop novel $M^{pro}$ inhibitors. Specifically, the Passerini reaction–amine deprotection–acyl migration (PADAM) oxidation pathway [2] was used to synthesize small peptidomimetic compounds equipped with a ketoamide warhead, acting as reversible covalent inhibitors. The new inhibitors were designed with γ-lactam in the P1 position as a cyclic glutamine mimetic, proline in P2 to confer a β-turn conformation, and various heteroaryl groups in the P1' and P3 positions [3].
Biochemical assays demonstrated $IC_{50}$ values in the nanomolar to low micromolar range against both SARS-CoV-2 and MERS-CoV $M^{pro}$. In cell-based antiviral assays, the inhibitors displayed low micromolar $EC_{50}$ values with no detectable cytotoxicity.
Furthermore, X-ray co-crystallography of protease–inhibitor complexes elucidated key molecular interactions, providing valuable insights for further optimizations of this new class of broad-spectrum CoVs inhibitors.

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## P07 - Synthesis and antimicrobial activity of ferulic acid dimers and derivatives

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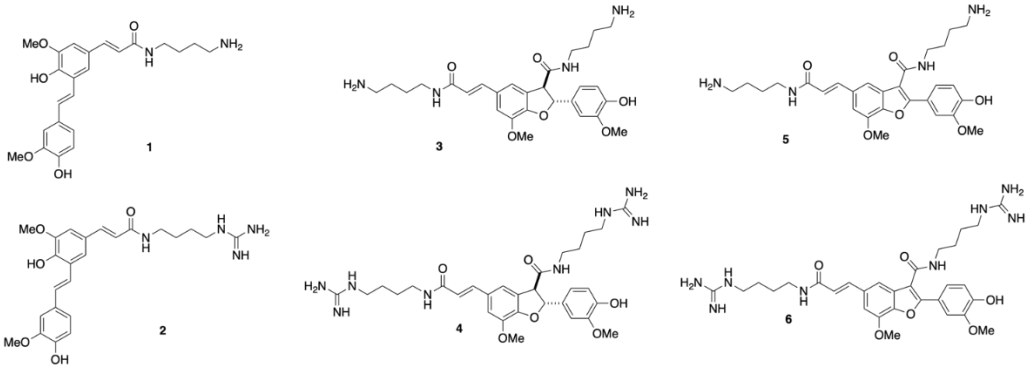
**Keywords:** phytoanticipins, antimicrobial agents, siderophores, hordatines, hybrid compounds.

The pressure of growing human population and global warming on the food system, along with the devastating impact of plant diseases, are major treats for food production. In particular, fungi, bacteria and viruses are responsible for about 30% of crop losses annually. Although pesticides are a powerful weapon for crop protection, their effectiveness is decreasing also due to their excessive use and misuse in agriculture. The discovery and development of novel compounds for crop protection is therefore fundamental [1, 2]. Plants have developed different defense mechanisms against pathogens attacks, such as the biosynthesis of specialized metabolites know as phytoalexins and phytoanticipins. Chemical scaffold modifications of these natural molecules could provide a valid strategy to obtain more effective compounds [3- 5]. We designed and synthesized novel and highly active nature-inspired antifungal agents to be used as biofungicides for crop protection. Among phytoalexins, hordatines scaffolds and derivatives have been synthesized and tested to evaluate the antifungal and antibacterial activity. A chemo-enzymatic reaction was exploited to generate natural and non-natural ferulic acid dimers which have been functionalized with putrescine and agmatine. Their biological activity was evaluated *in vitro* on mycelium growth of 3 strains of phytopathogenic fungi (*Botritis cinerea*, *Pyriculariae oryzae* and *Fusarium culmorum*), on *P. oryzae* conidia and on common strains of Gram-positive and negative bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica Enteritidis* and *Pseudomonas aeruginosa*). The tested compounds did not inhibit fungal mycelium growth, but at a concentration of 500 μM they showed inhibition of *P. oryzae* spore germination. At a lower concentration (200 μM), they did not inhibit germination but still showed 100% inhibition of appressorium formation, an essential step in virulence of *P.oryzae* and infection process. Synthesized compounds were also tested against a panel of Gram-positive and Gram-negative bacteria showing moderate to good antimicrobial activity.

Figure 4. Ferulic acid dimers and derivatives.

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## P08 - Development of Bioconjugates for the treatment of anaplastic thyroid carcinoma

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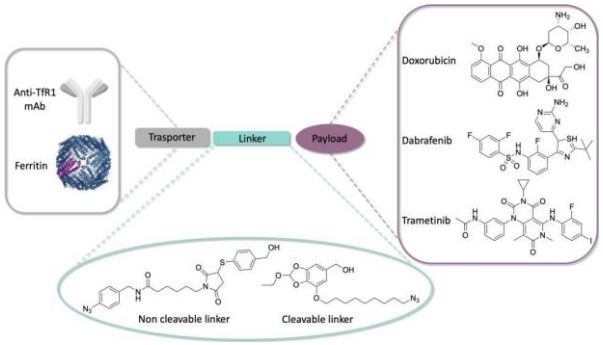
**Keywords:** Anaplastic Thyroid Carcinoma, Precision Medecine, Antibody Drug Bioconjugate

Anaplastic thyroid carcinoma (ATC) is a rare, but extremely aggressive malignancy which contributes up to 30-40% of thyroid cancer-specific mortality [1]. Standard therapy with surgery and radioactive iodine ablation is not successful with a tumour related death of 6 months. This project aims to synthetize different linker-payload systems for the conjugation with proper targeting agents in order to give selectivity to the tumour mass.
The targeting agents chosen for the development of bioconjugates are monoclonal Antibodies (mAb) and Ferritin. Ferritin is a globular protein that has an enhanced cellular internalization in many tumors such as ATC [2]. mAbs are molecules that could selectively bind specific target such as receptors overexpressed on tumor cells. As described in Scheme 1, three different drugs, here called payloads, have been selected for the bioconjugation: *i)* Doxorubicin, a cytotoxic drug often used to establish general activity of bioconjugates; *ii)* Dabrafenib and Trametinib, BRAF inhibitors applyed in thyroid cancer therapy, and suffering for the development of tumour resistance and off-target toxicity. The linker is a molecule that connect the payload to the transporter and consent the release of the drug on the target. Those bioconjugates need to be internalized and release the drug after the internalization to carry out their therapeutic effect. In this context, cleavable linkers and non-cleavable linkers [3,4], developed inside the research team have been used [3]. The bioconjugation of the linker payload systems to prefuntionalised cysteines of the transporter protein by a Copper free Click Chemistry reaction [5] is ongoing.

Scheme 1.

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# P09 - Zein/HP-β-CD Nano-in-Micro-Particles as a Novel Platform to Enhance Cannabidiol Oral Delivery

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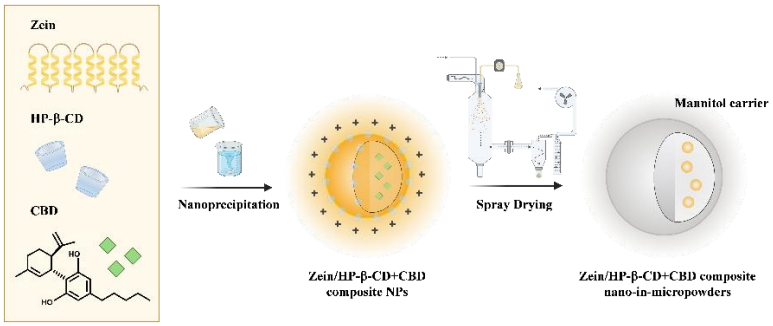
**Keywords:** Zein, HP-β-CD, Cannabidiol, Composite nanoparticles, Micropowders

Nutraceuticals have gained significant attention for their role in preventive healthcare, performance enhancement and immune system support. Recently, research has not only focused on their health benefits but also on optimizing their delivery in the body [1]. Nanotechnology represents an effective strategy for manipulating and enhancing nutraceutical activity, tackling the technological limitations hindering their development and efficient oral delivery [2]. As a result, growing efforts have been directed toward designing advanced delivery systems that maximize the nutritional potential of these bioactive substances. Cannabidiol (CBD) is an active compound that shows major anti-inflammatory effects; unfortunately, CBD application in the food and pharmaceutical sectors are limited due to low bioavailability, extensive first-pass metabolism, poor water solubility and sensitivity to oxidation [3].

In this study, we present the development of an innovative oral delivery platform for CBD, leveraging the combined properties of zein, a natural prolamin extracted from corn endosperm, and 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD). The system was designed using nano/microprocessing techniques, with nanoparticles (NPs) initially formulated through a liquid-liquid dispersion method and subsequently converted into a solid product (Nano-in-Micro particles) via spray drying using mannitol as inert carrier. This emerging technology enhances the stability and handling of the final product. The developed systems were thoroughly characterized using a broad panel of techniques, including dynamic light scattering (DLS), zeta potential (ζ) analysis, ultraviolet-visible (UV-Vis) spectroscopy, scanning and transmission electron microscopy (SEM/TEM) and analytical sieving. HP-β-CD played a key role in the formulation process, functioning as both a stabilizing agent for NPs and a host molecule, forming inclusion complexes with zein. Additionally, the NPs effectively encapsulated CBD, ensuring high encapsulation efficiency. The final powder product demonstrated excellent stability, ease of handling and prolonged shelf life. Current research efforts are now directed at evaluating the performance of this delivery platform within the gastrointestinal (GI) tract, including its interaction with body fluids and oral mucosa, biodegradation process and in vivo transit time. Although HP-β-CD has yet to receive approval for food applications, its limitation is primarily related to cost rather than safety concerns. The zein/HP-β-CD delivery system developed in this work represents a novel and promising approach for the efficient delivery of CBD, with potential applications in dietary supplements and drug delivery systems.

Figure 1. Schematic representation of the formulation process.

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# P10 - Development of a Stable Nanoemulsion for Buccal Delivery of Cannabis Extracts: Improving Bioavailability and Patient Compliance

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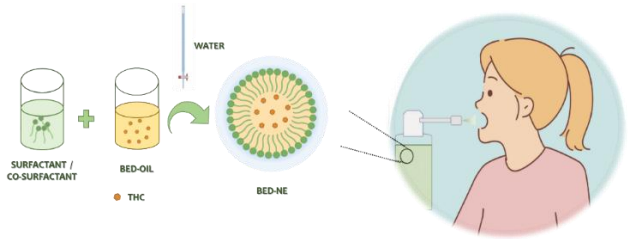
**Keywords:** Cannabis extract, nanoemulsion, Buccal delivery, Bioavailability Enhancement

Over the past few decades, there has been a significant increase in scientific interest regarding cannabis and its bioactive compounds, driving research into their therapeutic potential across a wide range of diseases. Extensive studies have highlighted the effectiveness of cannabis oil extracts in treating conditions such as cancer, diabetes, inflammation, epilepsy, and neurodegenerative disorders. However, traditional oil-based cannabis extracts, though widely utilized for oral administration, come with several inherent challenges, including low bioavailability, slow absorption, limited stability, and undesirable taste and odor profiles [1]. Furthermore, the instability of these extracts, which are particularly sensitive to light, heat, and oxidation, can compromise their therapeutic efficacy over time, presenting significant obstacles to their clinical application. In order to overcome these challenges, we present a novel nanoemulsion (NE) formulation for buccal delivery, incorporating Cannabis Bedrocan® extract in MCT (medium-chain triglyceride) oil (50% w/w), designed to overcome the limitations of traditional oil-based formulations. This innovative approach ensures more effective buccal administration of Cannabis Sativa L. extracts, addressing critical issues such as improved dose control, enhanced solubilization of THC in the buccal medium, and increased stability of THC over time compared to conventional oil extracts [2,3]. A preliminary study was conducted to optimize formulation conditions, including the selection of surfactants and excipients, to ensure stability and homogeneity of the nanoemulsion for buccal use. The resulting THC-loaded NE demonstrated outstanding physical stability, maintaining an optimal hydrodynamic diameter and polydispersity index for up to 60 days. Further stability tests, including dilution studies in biological fluids, confirmed the absence of any physical changes such as creaming or coalescence, underscoring the formulation's robustness. In vitro release studies revealed a rapid and controlled release of THC from the nanoemulsion, suggesting a faster onset of action compared to conventional oil-based formulations, which typically exhibit slower absorption rates. Moreover, the NE can be conveniently administered via the oromucosal route using a commercially available spray, providing reproducible and consistent doses of nanoemulsion with optimized therapeutic properties. In conclusion, the THC-loaded nanoemulsion offers a promising and innovative solution for the buccal delivery of cannabinoid extracts, overcoming many of the challenges associated with traditional oil-based systems. This formulation not only enhances the stability and bioavailability of THC but also provides a safer, more efficient, and faster-acting therapeutic alternative. This work lays the groundwork for the development of advanced cannabinoid formulations with improved therapeutic efficacy and greater patient compliance.

Figure 1. Composition of NE and administration pathway.

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# P11 - Radish microgreens as a sustainable source of bioactive compounds with nutraceutical potential

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**Keywords:** Biofactories, Microgreens, Radish, Glucosinolates, Antioxidant activity

Biofactories are biological platforms for the sustainable production of bioactive compounds with potential nutraceutical applications. The main biofactory models include microgreens, cell cultures, and cell suspensions. Specifically, microgreens are "tender immature greens produced from seeds of vegetables and herbs having fully developed cotyledons with or without the emergence of a rudimentary pair of first true leaves" [1]. They showed higher concentrations of bioactive compounds, such as glucosinolates, compared to mature plants [2]. Glucosinolates (GSLs) are anionic sulfur-containing secondary metabolites found almost exclusively in the Brassicaceae family, including cabbage, rapeseed, and radish. Recently, these compounds have gained attention for their human-promoting effects, including antimicrobial, antifungal, antihypertensive, and anti-inflammatory potential [3]. In this scenario, the main aim of the current work was the optimization of microgreen growth conditions of different radish cultivars, Raphanus sativus L. cv. Saxa (SA), White Tip (WT), Daikon (DA), Red Rubin (RR), China Rose (CR), and Tango (TA), to maximize GSLs production and investigate their nutraceutical potential. The seed germination was carried out using specific perforated trays, in darkness at 23±1°C for 4 days. The sprouts obtained were transferred in a hydroponic system, under 5000K light illumination, supplied with nutrient solutions, and harvested after 3, 6, or 9 days of cultivation. Microgreens obtained underwent a double extraction protocol both in acidified water (1% HCOOH) at 80°C for the selective extraction of GSLs, and in hydroalcoholic solvent (MeOH/H2O 80/20 + 1% HCOOH) for polyphenols recovery. The determination of GSLs concentration in the water extracts was performed using an opportunely validated HPLC-HESI-MS-MS method. The total polyphenol content (TPC) was performed on the hydroalcoholic extracts by spectrophotometry, according to Folin–Ciocalteau's assay, using gallic acid as the reference standard. The total anthocyanin content (TAC) was determined according to the spectrophotometric pH-differential method, using cyanidin-3-glucoside as the reference standard. In addition, the antioxidant activity of the polyphenolic extracts was evaluated by spectrophotometric FRAP, DPPH, and ABTS assay, using Trolox as the reference standard. Our results indicated that the GSLs quali-quantitative distribution in radish microgreens was influenced by both cultivar type and harvest time. The maximum GSLs yield was obtained after 6 days of cultivation for most cultivars, except for WT and CR, which showed the maximum concentration of total GSLs at T1. Glucoraphasatin was the most abundant compound in most cultivars, exhibiting the highest concentration in the CR cultivar at T1 (123.77 mg/g DW), followed by glucoraphenin and glucoraphanin. In terms of antioxidant potential, TA exhibited the TPC (17.21 mg GAE/g DW), TAC (11.91 mg C3G/g DW), and antioxidant activity, with the lowest $IC_{50}$ values for DPPH and ABTS (0.67 and 0.88 mg/ml, respectively). In conclusion, 3 days-CR microgreens and 6 days-SA, RR, and TA microgreens, with their high GSLs content, valuable antioxidant activity, and short cultivation period, represent a useful source for the sustainable and standardized production of bioactive compounds with potential applications in nutraceutical industries.

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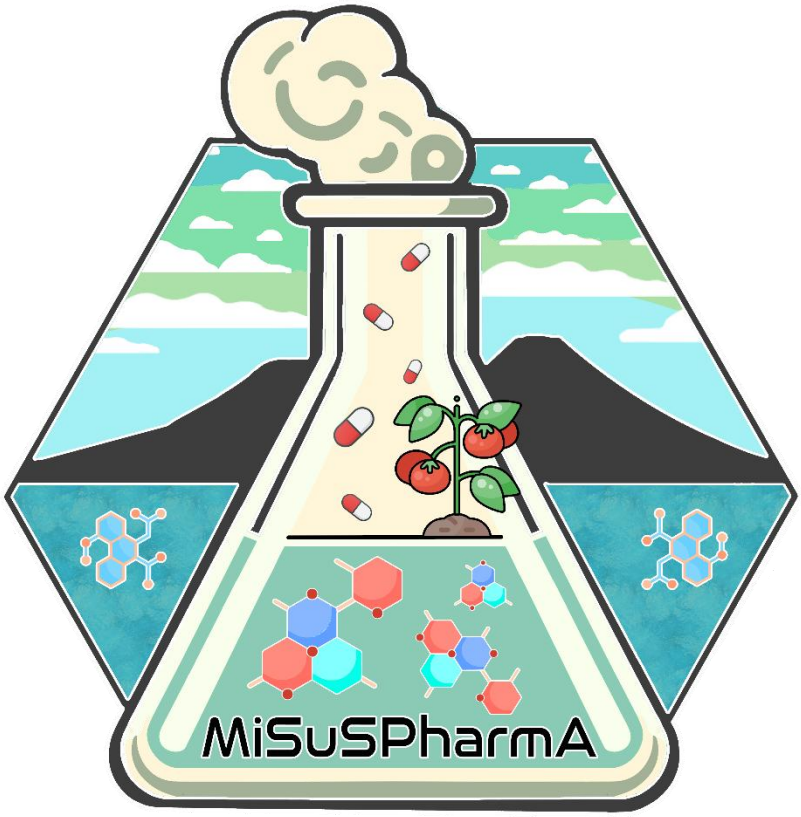


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