SELECTION CALLED FOR THE ASSIGNMENT OF

PhD SCHOLARSHIPS

FUNDED BY PNRR Ministerial Decree 24 April 2024, No. 630

Accademic year 2024/2025 XL Cycle

PhD Programme in: Research Method in Science and Technology

CANDIDATE'S FIRST AND LAST NAME

Francesco Onesimo

RESTRICTED TOPIC: *(see Annex A to the selection call)*

PROJECT TITLE: **Extraction and purification methods and application of oleuropein**

PROPOSED RESEARCH

Introduction of the problem in the context of the research proposed by the candidate:
The research project aims at the isolation and purification of bioactive compounds from the leaves of Olea europaea L. Advanced techniques such as silica gel chromatography and ion exchange will be used, followed by high-performance liquid chromatography (HPLC) with reversed-phase columns, to ensure high-resolution and purity separation.
In parallel, optimized chemical synthesis protocols will be developed to maximize yields and reduce costs. The entire process will be validated by NMR spectroscopy and mass spectrometry to ensure the quality of the compounds obtained. [2]

Relevance of the problem:
The promotion of environmental sustainability plays a role of primary importance, particularly in light of recent scientific discoveries in this area. A key element for the implementation of sustainable strategies lies in the use of agricultural by-products, which are fundamental for the transition to a circular economy model. This approach allows us to operate in synergy not only with the ecosystem, but also with companies engaged in enhancing technological innovation and developing cutting-edge extraction methods. The adoption of these advanced techniques will allow a progressive reduction in the use of organic solvents, contributing significantly to the reduction of the ecological footprint and the overall environmental impact.

Research methodology:
In the literature there are several very well studied methods of oleuropein extraction such as dynamic maceration, Shoxhlet extraction, pressurizing liquid extraction and ultrasound-assisted extraction [3,4]. All these methods have a limit, i.e. they require very long times, large quantities of solvents, and ultimately a high energy expenditure. All of this can carry the risk of degrading some of the key components. Purifying oleuropein from raw extracts is important for high purity, but the techniques we know today as column chromatography have limitations in terms of cost and efficiency.
For example, through HPLC (high-performance liquid chromatography) a degree of purity of up to 95% is obtained, but it has a limitation that this method is not applicable from an industrial point of view.

Objectives and expected results:
The research project aims to improve the process of extraction of oleuropein using more accurate and effective innovative techniques.
Our proposal consists in the use of boronate affinity chromatography (BAC) [5], a technique that overcomes the limitations found in traditional methods. Boronate affinity chromatography (BAC) exploits a specific and at the same time reversible characteristic of this molecule, which allows the interaction between boronic acids and the catechol groups present in oleuropein. This mechanism facilitates selective adsorption of the target of interest. The use of this method makes it possible to significantly reduce the amount of solvent used and to optimize extraction processes. Oleuropein-derived nutraceutical products can provide significant benefits to cardiovascular health, particularly by preventing LDL oxidation. This effect contributes to significantly reducing the risk of developing atherosclerosis. [6]
Oleuropein, suitably modified, could also be applied in the pharmaceutical sector. It is now well documented in the literature how paracetylation can improve the previously mentioned properties. [7] However, in line with the project's sustainability objective, we intend to replace the acetyl group with more environmentally friendly alternatives. In particular, we refer to levulinic acid, a compound easily obtained from lignocellulosic biomass [8]. In addition, functionalization of oleuropein with a fatty acid by the use of an enzyme can be considered in order to obtain a prodrug that can improve problems related to bioavailability, bioactivity and dosage.

Bibliography

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[3] Lama-Muñoz A, Contreras MDM, Espínola F, Moya M, Romero I, Castro E. Content of phenolic compounds and mannitol in olive leaves extracts from six Spanish cultivars: Extraction with the Soxhlet method and pressurized liquids. Food Chem. 2020 Aug 1;320:1266.

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Project abstract (**mandatory**) max 800 characters, including spaces

Chronic degenerative diseases, including cardiovascular, neurological, oncological and metabolic diseases, represent, according to the World Health Organization (WHO), serious threats to human health in our century. In this context, *Olea europaea* L., commonly known as olive tree, has received scientific attention for its beneficial effects, mainly attributable to the presence of a large and complex class of bioactive compounds called polyphenols. [1] Among these, the secoiridoids of Olea europaea L., such as oleuropein, oleocanthal and oleacein, have been the subject of numerous studies for their anti-inflammatory, antioxidant, cardioprotective and neuroprotective properties. Of particular importance is hydroxytyrosol, a potent polyphenol derived from oleuropein, which plays a crucial role in the prevention of oxidation of low-density lipoproteins (LDL), a fundamental process in the pathogenesis of atherosclerosis.