

**"VICTOR BABEȘ" UNIVERSITY OF
MEDICINE AND PHARMACY FROM TIMIȘOARA
DOCTORAL SCHOOL
PHARMACY DOMAIN**



**PHYSICOCHEMICAL AND BIOLOGICAL
INVESTIGATIONS ON BIOACTIVE COMPOUNDS
AND PHARMACEUTICAL FORMULATIONS BY
ADVANCED INSTRUMENTAL ANALYSIS**

ABSTRACT

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The habilitation thesis, entitled "Physicochemical and Biological Investigations on Bioactive Compounds and Pharmaceutical Formulations by Advanced Instrumental Analysis," displays my academic path over the past nine years (2014-2023) following the defense of my PhD thesis in June 2014 (OMS 441/ 18.08.2014).

The habilitation thesis is divided into 4 main chapters. The first chapter provides a concise exposition of my scientific accomplishments, while the second and third chapter outline my academic achievements and professional activity. Last, the fourth chapter illustrates my academic and scientific perspectives. The scientific accomplishments have resulted from the conducted studies and multidisciplinary research undertaken in the field of pharmaceutical sciences, which comprise four primary areas of focus, divided in four subchapters.

The first subchapter is focused on mass spectrometry (MS) based high resolution investigations of biomolecules and xenobiotics. Mass spectrometry (MS) exhibits exceptional characteristics in terms of its sensitivity, detection limit, analysis speed and a wide range of applications in diverse domains such as glycomics, proteomics, metabolomics, drug development, and forensic research. The investigations conducted during the doctoral programme created a suitable groundwork for this specific research topic. Throughout my PhD studies, my research primarily focused on the improvement, development and application of contemporary glycomic technologies, encompassing the combination of high-resolution mass spectrometry (HRMS) with automated infusion methodologies and nanotechnology. I applied the same type of methodologies to the high throughput screening and sequencing of various types of biomolecules and xenobiotics such as complex mixtures of brain gangliosides, *in vivo* metabolites of triterpenes such as betulinic acid (BA) and semisynthetic derivatives of phytocompounds such as isoflavones and triterpenic acids.

The second subchapter of my scientific accomplishments was dedicated to the assessment of phytochemical screening and biological evaluation of a wide variety of total plant extracts and secondary metabolites possessing pharmacological activity. The plant extracts investigated in this study encompassed Lemon balm, different species of *Artemisia*, and Peppermint. The primary emphasis focused on various kinds of phytocompounds, specifically triterpenes and polyphenols. The plant extracts were subjected to phytochemical analysis mainly using high-performance liquid chromatography combined with mass spectrometry (LC-MS), which is acknowledged as the most reliable method for profiling phytochemicals in this type of biological matrix. LC-MS profiling enabled the identification of correlations between the phytochemical composition of the analyzed samples and their demonstrated biological activities by various *in vitro* and *in vivo* techniques.

The third subchapter of my scientific achievements was dedicated to the investigation of nano-scale delivery systems for natural, semi-synthetic and synthetic

molecules, designed for the improvement of their pharmacological properties. Phytocompounds and plant-based products have a substantial impact on the domain of drug discovery due to their varied structures and capacity to engage with a broad spectrum of biological targets. Nonetheless, the clinical effectiveness of both natural substances and synthetic pharmaceuticals is impeded by a greater number of obstacles. These challenges include insufficient water solubility or low lipophilicity, inappropriate molecular size, sluggish dissolution rate, elevated metabolic rate, or rapid clearance. This research area emphasized the incorporation of these compounds into different delivery systems, which have shown promise in significantly enhancing their efficacy by improving their stability and bioavailability, as well as optimizing their biological activities. Among the various strategies that we applied was the design of gold nanoparticles, cyclodextrins, liposomes, polyurethane nanoparticles and cocrystals for the conjugation of various phytocompounds such as triterpenic acids or consecrated synthetic pharmaceuticals.

Lastly, the fourth subchapter of my scientific accomplishments centered on the development of synthetic and semi-synthetic derivatives exhibiting anti-cancer properties. Conventional chemotherapy strategies are accompanied by a number of limitations, including the manifestation of significant adverse effects and the emergence of treatment resistance. Hence, it is imperative to identify novel antitumor compounds that exhibit efficacy and selectivity, by specifically targeting signaling pathways in cancer. The introduction of heterocyclic 1,2,4 or 1,2,3- triazole scaffolds is fundamental to the advancement and synthesis of a diverse array of pharmacologically significant drug candidates possessing anti-cancer attributes, which exhibit efficacy against multiple categories of tumor cell lines. In this context, 5-mercapto-1,2,4-triazole derivatives selectively targeting the PI3K/AKT pathway and S-substituted 1H-3-R-5-mercapto-1,2,4-triazoles designed to target MEK1, ERK2, PDK1 and VEGFR2 pathways were synthesized and subsequently assessed in terms of their efficacy as anti-cancer agents. In addition, we conducted grafting of triazoles onto a range of triterpenic acids such as betulinic acid, oleanolic acids and ursolic acids, resulting in the synthesis of semi-synthetic derivatives. The cumulative findings from the biological evaluation indicated that the substitution with triazoles presented notable benefits in relation to the overall antiproliferative capacity. Consequently, triazole derivatives show promise as structural frameworks for the development of novel heterocyclic triterpenoids with anticancer properties.

Following the completion of my doctoral studies in June 2014, I engaged in research activities that have yielded the publication of 39 in extenso articles. Among these articles, 36 were published in ISI indexed impact factors journals. Moreover, I was the principal author for a number of 13 ISI articles. Nonetheless I participated in the publication of 3 book chapters published by international publishing houses and over 60 meeting abstracts published in the volumes of scientific conferences, out of which 3 were published in ISI journals. The scientific articles published since the beginning of my scientific career collected a number of 651 citations and a Hirsch-

index of 16, according to WOS. Moreover, 16 of my co-authored articles were awarded by UEFISCDI. Throughout my scientific career, I actively participated in eight national research grants and internal UMFVBT grants within which I held various roles, including PhD student, post-doctoral researcher and project responsible.

The second chapter was dedicated to my academic achievements. I started my academic career in September 2014 as a university assistant at "Victor Babes" University of Medicine and Pharmacy of Timisoara, Faculty of Pharmacy, Department II, discipline of Pharmaceutical Chemistry. After two years, in September 2016, I was validated as university lecturer and in September 2020 as assistant professor, both positions at the same discipline, Pharmaceutical Chemistry respectively. In 2022 I competed for a position as an assistant professor within the same faculty and department, at the discipline of Pharmacology- Pharmacotherapy where I carry out my work until now. This chapter presents my academic activity primarily by lecturing university courses and practical laboratories addressed to romanian and french series of bachelor cycle within the Faculty of Pharmacy. Moreover, this chapter presents my activity in various types of commissions for admission and license examination and competition commissions for academic positions. Furthermore, my activity as member in the board of the Department II within the Faculty of Pharmacy is presented, along with my membership of Ethics board of "Victor Babes" University of Medicine and Pharmacy of Timisoara. During the UMFVBT Days, in December 2018, I was honored with a special recognition for my academic accomplishments in the didactic activity. This recognition was conferred upon me in the form of a prize for the activity I successfully completed.

The third chapter focused on my professional accomplishments. I completed my bachelor's degree at "Victor Babes" University of Medicine and Pharmacy of Timisoara, Faculty of Pharmacy in 2007. During January 2008-December 2010 I was a resident in Clinical Pharmacy at Timiș County Emergency Clinical Hospital in Timișoara. I became specialist pharmacist in Clinical Pharmacy (OMS 1535/2010) after passing the specialist exam in the October 2010 session at the Faculty of Pharmacy, UMFVBT. Since 2016 I am principal pharmacist in Clinical Pharmacy (OMS 985/2016) after passing the principal exam in the June 2016 session at the Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy of Bucharest. Currently I am principal pharmacist in Clinical Pharmacy at the Municipal Emergency Clinical Hospital in Timisoara.

The fourth chapter was dedicated to my academic and scientific perspectives. The primary aim of the proposed academic development plan is to enhance the progression of my professional growth within the field of Pharmacology-Pharmacotherapy, Department II, Faculty of Pharmacy, UMFVBT. This plan comprises two essential elements: the development plan for teaching activities and the development plan for research activities. In terms of the advancement of my

pedagogical endeavors in the field of Pharmacology-Pharmacotherapy, my goal is to make a significant contribution to the enhancement and modernization of Pharmacology and Pharmacotherapy courses, as well as the editing of academic course materials.

With regards to furthering my scientific trajectory, my objective is to augment the current research pursuits undertaken by my affiliated research group, as well as introduce new areas of investigation. I am interested in participating in both national and international grant competitions with the aim of securing funding for my research endeavors. Concurrently, my objective is to disseminate the results of my research by publishing scientific articles in prestigious international journals and presenting them at national and international scientific conferences. I am interested in maintaining and fostering ongoing collaborative efforts with colleagues within the Faculty of Pharmacy, as well as cultivating external collaborations. Furthermore, my objective is to develop new partnerships with colleagues within UMFVBT, as well as with other national and international academic institutions and research organizations. Additionally, I seek to establish collaborative relationships with local pharmaceutical companies. Not least, my objective is to involve a diverse group of students, encompassing individuals who are pursuing advanced degrees such as master's and doctoral programs, and who possess a keen interest in research, in all of my projects. I aspire to engage in collaborative activities with young individuals who possess a strong inclination towards scientific pursuits, while actively participating in the ongoing exploration of technological advancements..

The final section of the habilitation thesis is dedicated to the references that served as the foundation for the development of this thesis, together with the list of the main scientific publications.