## TEZĂ DE ABILITARE

## Contribuții la studiul fitochimic al plantelor medicinale

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## THE ABSTRACT OF THE THESIS

## Contributions to the phytochemical study of medicinal plants

This habilitation thesis represents my scientific, professional and academic work that I carried out after obtaining the degree of Doctor of Pharmacy (Pharmaceutical Sciences). In 2001, I defended my PhD thesis entitled "Study of phytopharmaceutical products obtained from Saccharomyces cerevisiae (OMEC no.3570 of 19.04.2002), coordinated by Prof. Csedő Carol at UMF. Tîrgu Mureş.

The teaching, professional and research activities I carried out at the University of Medicine and Pharmacy of Tg. Mureş, Faculty of Pharmacy, from 1996 until today, at the Department of Pharmacognosy and Phytotherapy, Department of Specialty Pharmaceutical Sciences. In 1996 I started my professional activity as a full-time PhD student at this discipline. Since 2001, after my thesis, I started my teaching activity at the department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy.

Main professional and academic achievements are presented in the first chapter, with more than 20 years of postdoctoral work.

Scientific achievements are contained in four main chapters:

- Instrumental methods in the investigation, determination and analysis of chemical compounds in plant products;
  - Microbiological methods with volatile oils and plant products
  - Other studies on the analysis of plant products
- Study of macro- and microelements, B vitamins and antioxidants in yeast cells during storage.

The main research direction was phytochemical study of medicinal plants, determination and analysis of some chemical compounds in plant products of pharmaceutical interest.

In the phytochemical study I dealt with the class of polyphenols. Polyphenols are the active compounds that are gaining more and more ground in the analysis of natural constituents, as they are compounds used in the prevention of many diseases. Polyphenols include simple phenols, flavonoids, anthocyanins. Quantitative determination methods have been applied for the determination of these constituents, but complementary methods such as high performance liquid chromatography (HPLC) are available and welcome. These methods have the advantage of low solvent and reagent consumption and reduced analysis time. They are efficient in the determination of several compounds and by coupling them with mass spectrometry new and novel compounds can be determined from different plant products. In this chapter the analytical

applications of mass spectrometry for polyphenolic compounds in medicinal plants and spectrophotometric methods are described in two chapters. The analytical applications were done by high-performance liquid chromatography (HPLC) using the coupling to different high-performance detectors (MS, DAD-ESI-TOF, DAD-ESI-MS/MS), which makes fast and accurate analysis possible. Such determinations have been made on plant products of bark, leaves, flowers and fruit harvested from *Syringa vulgaris*, the fruits of *Ribes nigrum*, *Ribes rubrum*, *Ribes uva crispa* and *Prunus spinosa* and medicinal plants from modern phytotherapy with a use in veterinary medicine. In the field of phytochemistry these techniques are widely used, allowing the separation, identification and quantification of the numerous components in an extract. They offer excellent separation efficiency with high sensitivity.

The spectrophotometric methods were applied to determine the concentrations of flavonoids, anthocyanins, polyphenols in the mentioned medicinal plants and in addition to the listed plants, *Calluna vulgaris* and *Punica granatum* were determined.

Spectrophotometric determinations were also applied to the evaluation of antioxidant capacity by ABTS and DPPH assays. These are methods based on electron transfer.

With high-performance liquid chromatography techniques we performed the determination of carotenoid compounds in the flowers of *Telekia speciosa*, a species found in the spontaneous flora of Romania, being a rich source of natural colorants.

The pharmacologic activity of plant products depends very much on these concentrations and assay values or conversely, these values determine the therapeutic value of plant products.

Nowadays, volatile oils in the field of aromatherapy are being given special attention and are being put to remarkable use. The volatile oils of different species of *Thymus (Thymys vulgaris, Thymus serpyllum, Thymus glabrescens, Thymys pulegioides)* collected from the Medicinal Plant Garden of our university have been studied. These volatile oils obtained by us under laboratory conditions by Neo-Clevenger methods were further studied by gas chromatography and by coupling with mass spectrometry the different constituents of Thymus volatile oils were obtained. These volatile oils are of pharmacologic importance.

In another chapter I dealt with the microbiological study of volatile oils and plant products, scientific research related to antibacterial and antifungal activities. These microbiological activities were tested by agar diffusion and tube dilution methods and the minimum inhibitory concentration of the different plant products was determined. These microbiology activities are of pharmaceutical importance, because new antibacterial and antifungal sources are being sought in addition to the existing synthetic ones.

Another chapter is devoted to the method of obtaining microscopic preparations, describing hitherto undescribed microscopic preparations in plants and organs. Also in this chapter we have described ethnobotanical studies of ethnobotanical interest, when medicinal plants are charted in order to introduce other less known medicinal plants in phytotherapy.

In the final chapter we describe the research carried out immediately after the completion of the PhD thesis. The experience gained in the PhD thesis culminated in patent applications and patents in 2003 and 2004. These patents are about obtaining yeast cells enriched with various micronutrients useful to the human body, having high bioavailability to the human body. These yeast products enriched with iron, zinc, copper and selenium are products of pharmaceutical interest, the results of which I published the first ISI article, during my postdoctoral period. We chose these micronutrients because they have a well-determined, important role in the human body. Selenium is a micronutrient commonly found in vitamin products, but less studied in our country, with which we obtained very good results. In addition to the importance of trace elements, I also looked at the concentration of water-soluble vitamins, vitamin B1 and B2. After enrichment of yeast cells we determined and monitored the concentration of these vitamins after 6 and 12 months of storage. These studies complemented the information on yeast preparations during storage. Of the four micronutrients in selenium-enriched yeast cells, the loss of vitamin B was negligible. For selenium-enriched yeast cells, we used antioxidants such as vitamin C and tocopherol to enhance the physiological effect and monitored their concentration during storage.

Yeast-based preparations, micro-nutrient enriched yeasts are natural sources. In addition to their micronutrient content, they are also sources of B vitamins. The ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) is a modern and accurate method for the determination of micro-nutrient concentrations. For the determination of vitamins B1 and B2 we applied a microbiological method. The concentration of vitamins C and E was determined by HPLC.

The final chapter is devoted to prospects for a teaching and scientific career. In terms of teaching, it is essential to update specialist knowledge, to continuously improve the analytical programme of courses and practical work in Pharmacognosy and Phytochemistry, and in Phytotherapy and other subjects in the curriculum. I would like to continue with the introduction of new phytochemical determinations in the practical work, I would like the continuous modernization of the infrastructure, equipment and apparatus of the Pharmacognosy and Phytochemistry discipline in order to improve the quality of the teaching process. Another goal is the publication of articles in ISI or BDI indexed journals and the publication of specialist books in the field of pharmacognosy. The academic activity anticipates involvement in various activities for the benefit of the academic community in Târgu Mureş.

On the scientific side, I intend to apply for and win grants or projects as a project leader/member in order to complete some promising phytochemical research I have started.

The research direction is the continuation of the current directions: the study of medicinal plants, the study of chemical compounds responsible for different actions and uses. Another direction is the study of medicinal plants with an application in veterinary medicine, to search for new formulations in different animal (poultry) diseases and to test new formulations with the help of colleagues in veterinary medicine. In the future I would also like to try to obtain and apply nano nutraceuticals with promising use in the pharmaceutical field.