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CONTRIBUTIONS IN THE FIELD OF NATURAL AND SYNTHETIC COMPOUNDS WITH APPLICATIONS IN HEALTH AND ENVIRONMENTAL PROTECTION

ABSTRACT

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The habilitation thesis "Contributions in the field of natural and synthetic compounds with applications in health and environmental protection" is structured in two sections, the first refers to the most important results from the author's research activity, from the period immediately after PhD graduation, and the second presents the evolution of the professional career, as well as the directions of scientific research that will be approached in the near future.

The main part of the thesis consists in the presentation of the data obtained as a result of research aimed to study natural compounds, polyphenols, anthocyanins, glucosinolates and synthetic ones, o-hydroxybenzamide derivatives, in terms of obtaining, characterizing and evaluating their biological activity, development efficient and ecological synthesis and characterization methods, as well as the development of hybrid biomaterials from biological waste.

Particular attention was paid to obtaining new o-hydroxybenzamide derivatives, thus continuing and developing the research direction followed during the Ph.D. Novel compounds, not mentioned in the consulted scientific literature, ethyl, methyl esters, hydrazides, hydrazones of salicylamides, N-(3-bromo-phenyl)-2-hydroxy-benzamide, 5-chloro-2-hydroxy-N-phenylbenzamide, 5-bromo-2-hydroxy-benzamide, N-(2-bromo-phenyl)-2-hydroxy-benzamide, N-(2-chlorophenyl)-2-hydroxybenzamide and N-(4-chlorophenyl)-2-hydroxybenzamide, were obtained. Along with the use of classical synthesis using conventional heating, eco-friendly methods have also been developed for the synthesis of these classes of compounds, the synthesis in the microwave field being the best choice in this case due to clearly superior yields, shorter reaction times and economy of solvent. The compounds thus synthesized were characterized using modern analytical methods such as infrared spectroscopy, nuclear magnetic resonance, mass spectrometry, elemental analysis, confirming their identity. Since the encapsulation of drugs in cyclodextrins increases the chemical and physical stability of the drug and improves the distribution of the drug through biological membranes and due to the low solubility in aqueous media of hydroxybenzamide derivatives, the complexation of ethyl esters obtained starting from N-(2-chlorophenyl)-2- hydroxybenzamide and N-(4-chlorophenyl)-2-hydroxybenzamide with β-cyclodextrin using the kneading method was achieved. The complexes obtained in the solid phase were characterized by specific methods such as X-ray diffraction, scanning electron microscopy, thermogravimetric analysis, infrared spectroscopy and ultraviolet-visible spectroscopy. For the liquid phase study of the complexation of hydroxybenzamide

derivatives with cyclodextrins, the ethyl ester of [2-(2-bromophenylcarbamoyl) phenoxy]acetic acid was chosen as a representative compound, UV-Vis absorption spectroscopy being used to demonstrate the formation of the complex. Using the Benesi-Hildebrand equation, the ester:cyclodextrin stoichiometric ratio of the complex was determined to be 1:1. Through molecular modeling it was proven that the most stable configuration of the complex is the one in which the benzamidic part of the ester is found in the cavity of the cyclodextrin, a fact that is consistent with the data obtained from the ^{1}H-NMR spectra.

The synthesized compounds were also tested to evaluate their biological activity. The antioxidant activity of the new derivatives of N-(2-bromo-phenyl)-2-hydroxy-benzamide, 5-bromo-2-hydroxy-benzamide and 5-chloro-2-hydroxy-N-phenyl-benzamide was evaluated using chemical (DPPH, TEAC, FRAP) and electrochemical (cyclic voltammetry) methods, proving their antioxidant character. The antifungal potential of N-(2-bromo-phenyl)-2-hydroxy-benzamide derivatives against *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Saccharomyces cerevisiae* was also demonstrated. The antimicrobial potential of N-(2-bromo-phenyl)-2-hydroxy-benzamide and N-(4-bromo-phenyl)-2-hydroxy-benzamide derivatives was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shighella flexneri*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Candida parapsilopsis*, *Candida albicans*, the most sensitive microorganisms to the action of 2-bromo-substituted derivatives being *Candida albicans* and *Streptococcus pyogenes*. Among the 4-bromo-substituted derivatives, only anilide showed antimicrobial activity, inhibiting the bacteria *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Antibacterial activity of N-(2-chloro-phenyl)-2-hydroxy-benzamide and N-(4-chloro-phenyl)-2-hydroxy-benzamide derivatives as well as some inclusion complexes in β-cyclodextrin of esters, was tested against five bacterial strains, Gram-positive and Gram-negative. Compounds derived from N-(2-chlorophenyl)-2-hydroxybenzamide showed higher activity against Gram-positive bacterial strains, with MIC values ranging from 0.125-0.5 mg/mL. Lower or no antibacterial effect was shown by the N-(4-chlorophenyl)-2-hydroxybenzamide series (MIC ≥0.5 mg/mL). The most sensitive to the action of chloro-substituted salicylanilide derivatives were found to be *Streptococcus pyogenes* and *Streptococcus mutans*. The effect of cyclodextrin complexation on the antibacterial activity of the uncomplexed compound was studied for the two ethyl esters, starting from the premise that the encapsulation of antimicrobial compounds in cyclodextrins should lead to an adequate control of the drug release rate, so

that they can be used more efficiently. The obtained results confirmed the possibility of using ethyl esters in complexed form, with all the related advantages.

Another important research direction pursued after PhD graduation was the study of some natural extracts from plants in order to determine the composition in active principles and to evaluate the biological activity.

Thus, ethanolic extracts rich in polyphenolic compounds were obtained by maceration, and demonstrated antioxidant effects evaluated by chemical methods, DPPH, TEAC, FRAP, inhibitory effects on reactive oxygen species in human erythrocytes subjected to oxidative stress induced with H_2O_2 studied by flow cytometry and antitumor activity on A375 – human melanoma and B164A5 – moraine melanoma cancer cells evaluated by the MTT proliferation assay and the "*wound healing*" assay. A375 cells were found to be more susceptible to stimulation with plant extracts than B164A5 cells, and the highest rate of cell inhibition was observed after stimulation of A375 cells with willow, linden, and sage extract. The strongest antimigratory effect is attributed to the blueberry extract which also showed the lowest cytotoxicity determined on healthy HaCat human keratinocytes.

Extracts from fruits rich in anthocyanins belonging to the *Prunus* genus were obtained by ultrasonication, and their composition was determined by high-performance liquid chromatography and confirmed by mass spectrometry. This study highlights the variation in phenolic and anthocyanin content, as well as the different antioxidant capacities found in various fruits of the *Prunus* genus. Sour cherries, bitter cherries and sweet cherries proved the highest antioxidant activities determined by the DPPH, TEAC, FRAP methods, the total phenol content determined by the Folin-Ciocâlteu method, being strongly correlated with their antioxidant activity.

Ethanol extracts rich in anthocyanins were obtained by ultrasonication from blueberries, blackberries and mulberries and were characterized in terms of antioxidant activity (DPPH method), anthocyanin content (pH differential method) and phenolic compounds (Folin-Ciocâlteu method). Blueberry extract demonstrated the strongest antioxidant activity and the highest content of anthocyanins and phenols. The composition of the extracts was determined by HPLC and confirmed by mass spectrometry. The antihyperglycemic effect of the extracts was tested in rats to whom diabetes was artificially induced by i.p. administration of streptozotocin. All the studied extracts showed antihyperglycemic effects, blackberry and mulberry extracts showing the most pronounced

antihyperglycemic activity, with the maintenance of glucose levels after 5-6 weeks of experiments at 270 mg/dL and 155 mg/dL, respectively.

Another study aimed to investigate the stability of ethanolic extracts of anthocyanins from blueberries, blackberries and mulberries with and without the addition of antioxidants, ascorbic acid (AA) and butyl hydroxyanisole (BHA), considering that heat treatment can influence the stability of anthocyanins in food. Variations in anthocyanin content (pH differential method) and antioxidant activity (FRAP method) were determined during storage for 2 weeks at 60°C and 4 months at room temperature. At room temperature, anthocyanins showed better stability but underwent very rapid degradation at high temperature. Untreated extracts were more stable than those treated with antioxidants, and in addition, anthocyanins in ascorbic acid-treated extracts showed faster degradation. The negative effect of ascorbic acid on the stability of anthocyanins at room temperature and that the addition of BHA has no significant influence, sometimes even negative one (mulberry extract) was also highlighted. The antioxidant activity shows only a slight decrease compared to the anthocyanin content during heat treatment.

The electrochemical behavior of some beries extracts was followed, cyclic voltammetry being used as a method to estimate the antioxidant activity. The antioxidant capacities of the extracts were also evaluated by the DPPH method and correlated with their oxidation potential.

The electrode potential of cyanidine was determined by both experimental (cyclic voltammetry) and theoretical (computational) methods. Among the 6 investigated cyanidine conformers, the most stable structure was chosen, comparison with experimental results shows a calculation error of 0.05 V for electrode potential, which can be attributed to the underestimation of the calculated free energies. The result of the study demonstrates the usefulness of computational methods in predicting the antioxidant character of some active principles.

Considering the advantages of complexing anthocyanins with cyclodextrins, the interactions between six anthocyanins (cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, malvidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin-3-O-rutinoside, malvidin-3-O-rutinoside) and cyclodextrins were investigated by computational techniques (Gaussian 09W software). The results showed that anthocyanidin-3-O-rutinosides are favored for obtaining inclusion complexes with cyclodextrins, mainly due to the larger number of OH groups involved in the formation of hydrogen bonds and that β-cyclodextrin represents the optimal choice as a host molecule.

Ethanol extracts from vegetables rich in glucosinolates (white cabbage, broccoli, black radish and cauliflower) obtained by microwave irradiation, were studied in order to evaluate the antioxidant effect (DPPH method), total phenol content (Folin-Ciocâlteu method) and their HPLC profile and to evaluate their antimicrobial capacity against a range of pathogens, including resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* or carbapenem-resistant *Pseudomonas aeruginosa*. The studied extracts demonstrated good antioxidant capacities, the best values being obtained in cauliflower and broccoli, which also present the highest content of polyphenols. Bacterial strains were moderately sensitive to cauliflower and broccoli extracts showing greater sensitivity to cabbage and black radish extracts for which bactericidal effects were also highlighted. The minimum bactericidal concentration values for these two extracts were higher than the minimum inhibitory concentration values, exerting a more pronounced inhibitory effect on Gram-negative bacteria. Tested dilutions showed no antifungal activity.

Another study presented in this thesis concerns the development of fast and efficient methods (HPLC-DAD and GC-MS) for the detection and quantification of the residues of some fungicides, boscalid, tebuconazole, iprodione and imazalil, in commercial vegetables and fruits. Residues of imazalil have been found in clementines and lemons. The concentration of imazalil in lemons was quantified, by both methods, as higher than the maximum residue limit (MRL). Iprodione was detected only by the HPLC method in white and red potatoes but in amounts lower than the MRL. The boscalid and tebuconazole were not found in the analysed vegetables and fruits.

The presence of pesticides was traced in the soil and some corn, wheat and rape crops samples, from Banat - Moravița area. Rapid and sensitive HPLC-DAD methods were developed for the simultaneous detection of residues of several pesticides, imidacloprid, amidosulfuron, bromoxynil and deltamethrin. The presence of imidacloprid was observed in the studied soils, and the residual amounts determined were between 0.13-0.21 μg/g. Amidosulfuron, bromoxynil and deltamethrin were not detected in soil, corn and wheat samples. The amount of bromoxynil found in rape seeds and stems was 0.25 and 1.29 μg/g, respectively. In maize and wheat (Dealu Mare), imidacloprid concentration values exceeded the MRL values.

Another study aims to transform some biological waste into biocomposite materials with possible use in medicine. Hydrothermal extraction with water under sub- and super-critical conditions and microwave field extraction were used to obtain hydroxyapatite from

bovine bones. The most effective extraction method was found to be microwave assisted extraction, and the compound obtained was pure hydroxyapatite (FTIR and X-ray diffraction), with a Ca/P ratio (TEM-EDX) closer to standard. The use of microwaves has also been shown to be beneficial in the case of obtaining hydroxyapatite by synthesis, leading to obtaining a nanostructured product of high purity. Type I collagen was obtained by extraction from pig skin and characterized by the spectrophotometric method of hydroxyproline determination and denaturation temperature. Biomimetic collagen-hydroxyapatite and chitosan-hydroxyapatite membranes were obtained by ultrasonication and characterized by SEM images.

Another direction of research addressed in the thesis refers to the valorization of polyethylene terephthalate (PET) waste. The hydrothermal depolymerization of PET waste under sub- and supercritical conditions and its hydrolytic depolymerization in alkaline solution using microwave irradiation were studied to obtain pure terephthalic acid in high yields. Compared to conventional heating methods, these processes achieved a significant reduction in the reaction time required for the total depolymerization of PET, as well as higher yields of terephthalic acid.

The present thesis presents only the results already published, without referring to those in the process of publication or which are not fully completed.

In addition to the scientific activity, to which the author dedicated more space, the thesis also presents the professional and academic activity carried out at the “Vasile Goldiș” Western University of Arad and at the National Institute of Research and Development for Electrochemistry and Condensed Matter, Timișoara.

The thesis ends with the university career development plan both on a scientific and didactic level, with the author's proposals for future research, respectively university, postgraduate or master's courses, emphasizing her ability to guide PhD students.