

EXECUTIVE SUMMARY

of Phd thesis entitled:

“RESEARCH ON THE ANTIMICROBIAL ACTIVITY OF EXTRACTS OBTAINED FROM NATIVE PLANTS”

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The plants represent an inexhaustible, cheap and handy resource, having multiple purpose in our everyday lives. In the same time, plants are an underexploited resource of antimicrobial agents and worthy of the full attention of researchers. It was already demonstrated the antibacterial potential of secondary metabolites from plants, both in monotherapy and as synergists or enhancers, along with other antibacterial agents. The use of phytochemicals and plant extracts as antimicrobial agents is a research topic which receives increasingly more attention lately. Modern research on herbal antimicrobial agents find their applicability in areas such as veterinary medicine, food preservation and plants protection.

The aim of the doctoral research was to study the antimicrobial activity of native plant extracts seeking to build a portfolio of scientific results obtained by pre-competitive research, based on which could be developed applied research projects focused on areas such as veterinary and human medicine, plant protection and cosmetic and food industry.

The thesis is divided into two sections, namely, the first one- bibliographic study and the second – the own research. These two sections comprise six chapters, as follows: three chapters of documentary and three chapters of experimental research (one of which is the Conclusions). The thesis comprises a total of 67 figures from which 57 are original, and a total of 23 tables from which 15 are original.

Chapter I of the thesis includes bibliographical study which presents information from literature. By describing some general considerations is illustrated the overall context of existing research in the field, and a brief historical examples of the progress of current research on the use of plant extracts, to exploit their antimicrobial and antioxidant action, both internationally

and nationally. Therefore it is presented the international state of the art being highlighted the effects of plant extracts on microorganisms of medical interest, the use of plant extracts in food industry and the use of plant extracts with antimicrobial effects in plant protection. Nationally, is described on the one hand, the antibacterial and antifungal activity of plant extracts and on the other hand, their significance for plant protection.

Chapter II, contains a short preview of the biologically active compounds from plants, their classification and the role they fulfill in the defense mechanism of the plant, with particular emphasis on compounds of interest influencing the antimicrobial and antioxidant activity, namely: terpenoids, polyphenols and alkaloids.

Next, in Chapter III are presented several methods of extraction of bioactive compounds from plants with a brief review on conventional and unconventional methods. Also, this chapter includes an analysis of the extraction parameters, with the possibility of their optimization in order to obtain higher yields, depending on the compounds of interest.

Starting from the fourth chapter, entitled materials and methods, is presented the second part of the thesis, the experimental research. Chapter IV contains a detailed description of materials and methods necessary in the experimental design, the selected vegetal material, the microorganisms used in antimicrobial assays and the preparation of the selected plant extracts. There are also described the techniques for assessing the antimicrobial and antioxidant activity of plant extracts and methods of evaluation of biologically active compounds. Finally, it is detailed the testing methodology of the effects of plant extracts on vegetal organisms.

Chapter V entitled Results and discussion provides a snapshot of the results obtained from the research on antimicrobial activity plant extract obtained from twelve species of native plants, namely: *Arctium lappa* (burdock), *Artemisia absinthium* (wormwood), *Datura stramonium* (jimson weed), *Equisetum arvense* (horsetail), *Glycyrrhiza glabra* (licorice), *Humulus lupulus* (hops), *Rosmarinus officinalis* (rosemary), *Sambucus ebulus* (danewort), *Sambucus nigra* (elderberry), *Tagetes patula* (marigold), *Taraxacum officinale* (dandelion), *Xanthium strumarium* (common cocklebur). This chapter is divided into 8 sections, grouped either according to the plant material studied, either by microorganisms used or possible applications of the antimicrobial potential of the plant extracts.

Extending the phenomenon of antibiotic resistance of pathogenic bacteria is an important issue affecting human health and the possibility of effective treatment of infections caused by such strains. Therefore, the concerns of specialists for identification and use of natural antimicrobial compounds, derived from plant material are increasing. Based on these considerations, a first aspect examined in this thesis was to highlight the antibacterial activity of plant extracts, which resulted in subsection entitled „*The antibacterial activity of medicinal*

plant extracts". Were used alcoholic and aqueous extracts from different plants of the local flora, being tested against bacterial strains pathogenic or potentially pathogenic to humans or animals: *Escherichia coli*, *Pseudomonas fluorescens*, *Enterococcus faecalis*, *Bacillus cereus* and *Staphylococcus aureus*. Moreover, for the extracts that showed the best antibacterial activity it was performed quantitative determination of total polyphenol content and flavonoid content. The results were used for the comparison of plant extracts obtained by different extraction methods. Among the studied extracts, alcoholic extracts from *G. glabra* root (licorice) prepared by ultrasonication, showed the strongest antibacterial effects.

Recently, the increased requirements of consumers for "bio" products from plants that were not treated with the "traditional" chemical compounds led to the evaluation of alternatives. Therefore, the experiments were carried out to evaluate the inhibitory effects against *Rhizoctonia solani*. Choosing *Rhizoctonia solani* species for testing was due to the fact that it is polyphagous, attacking and causing serious damage to many species of the family Solanaceae (potato, tomato, eggplant, peppers), and causing death of plants in the seedling stage. The results of evaluating the effect of different concentrations of plant extracts on mycelial growth of *R. solani* can be found in Chapter V.2 of the thesis, "Research on the in vitro effectiveness of herbal extracts against *Rhizoctonia solani* infection" From the results obtained, it was observed that marigold, licorice and elderberry extracts possess a high in vitro antifungal activity against *R. solani*.

After some **Preliminary research on antifungal activity of plant extracts against *Pythium* sp.**, (Chapter V.3) and more specifically by comparative testing of methods for evaluating the antifungal activity of plant extracts, the poisoned food method was selected as the most effective in achieving clear results. Thus, were evaluated a number of extracts obtained from flowers of marigold (*Tagetes patula*), licorice root (*Glycyrrhiza glabra*), leaves of burdock (*Arctium lappa*), aerial parts of horsetail (*Equisetum arvense*) and elderberries (*Sambucus nigra*). Extracts from marigold (*T. patula*) and licorice (*G. glabra*) showed a high antifungal activity against *Pythium* sp. even at low concentrations.

Furthermore, the attention has turned to "**Research on antifungal activity of several plant extracts against *Alternaria alternata***" (section V.4). *Alternaria* sp. may affect a large number of plant species of economic interest (tomatoes, wheat, sorghum, barley, sunflower, rapeseed, cotton, etc.), thereby causing significant crop losses. In the experiments reported were used alcoholic and aqueous extracts from various organs of plants from four species, two medicinal plants and two weeds (*Artemisia absinthium*, *Rosmarinus officinalis*, *Datura stramonium*, *Xanthium strumarium*) with superior results of the ethanolic extract from *X. strumarium*, at all concentrations tested. Choosing as raw material the weed species for obtaining products derived from plants fungicides can lead to an organic method of disease control, while

providing a solution to weed management and facilitating economic uses for these unwanted species.

In the next chapter, *Tomato late blight control with extracts obtained from Artemisia absinthium*, the purpose of the study was to compare the antifungal activity of wormwood samples from different geographic areas of our country. The results showed that alcoholic extracts obtained from wormwood has an inhibitory effect on the growth of *P. infestans* mycelium, regardless of the harvesting place of the vegetal material. Although the antimicrobial activity showed different intensity, there was no statistically significant differences of the effect produced by the three wormwood extracts originating from different geographical areas. This fact highlights the availability of further development of a product based on plant extracts, which can be prepared and used regardless of the source of raw material.

All previous experiments have shown that extracts from different plant species are capable of inhibiting, the *in vitro* development of mycelium of phytopathogenic fungi. Considering these results, in another series of experiments was conducted a complex investigation of the effects of antimicrobial (antibacterial and antifungal) and antioxidant activity of extracts from *Sambucus ebulus* (danewort) and *Humulus lupulus* (hops).

Using plant extracts into practice is conditional upon obtaining various information related to their chemical composition, to their mechanisms of action and, not least, of any cytotoxic effects. It is also presented the biological activity of extracts obtained from (*S. ebulus* - fruit, *H. lupulus* - flowers, *S. nigra* - fruit, *T. patula* - flowers, *X. strumarium* - fruit), with superior results for hops extract.

In the last chapter, in order to have a better picture of the effects of plant extracts that have proven antifungal properties, they have been tested directly on plants in several experimental versions:

- ✓ Highlighting the antifungal effects (against *Phytophthora infestans*) by testing on detached leaves;
- ✓ Establish the impact of plant extracts on the germination process
- ✓ Demonstration of the protective effect of seeds treatment, on the soil, with plant extracts against *Rhizoctonia solani* infection

The results indicate that the extracts of marigold and hops could be used as biocontrol agents for the prevention of infections with *R.solani*, at soil level and *Phytophthora infestans* on the leaves.

Chapter VI - CONCLUSIONS includes the general findings of experiments and emphasis the original contributions to the study of antimicrobial activity of extracts obtained from native plants and possible practical applications in industry.

The problem addressed and the solutions proposed in this paper provide a high degree of novelty. This resulted from the valorization of some plant species less studied (aromatic and medicinal plants, cultivated plants or spontaneous flora) by practical applications other than those for which they have been already recognized.

The impact of this doctoral research is supported by the fact that the results have applicability, usability and economic efficiency being directed toward designing viable and environmentally friendly solutions for the design of therapeutic products that can be used in medicine and plant protection.

This doctoral research has also led to increased visibility of Romanian research through participation in scientific meetings and dissemination of results in doctoral papers and articles published in scientific journals.