S U M M A R Y

of the doctoral thesis entitled:

EVALUATION AND VALORIZATION OF BIOACTIVE MOLECULES FROM INDIGENOUS CUCURBITACEAE

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The developed doctoral thesis covers 7 chapters, two dedicated to the bibliographic study, and 5 chapters dedicated to original research. The chapter devoted to the bibliographic study sought to highlight originally the existing data in the specialized literature for:

1) bioactive compounds existing in the by-products resulting from the processing of the fruits of *Cucurbita sp.* and of other related species as well as the ways of capitalizing them;

2) bioactive compounds, existing in the by-products resulting from the processing of the fruits of *Citrullus sp*., and the methods of valorization.

Chapter 1 includes updated information related to *Cucurbita pepo* var. decora (decorative pumpkins, gourds), *Cucurbita maxima* (pumpkins) and related plants, methods of extracting bioactive compounds from by-products (peels) resulting from the exploitation of the fruits of *Cucurbita sp.* and the respective phyto-compounds highlighted in different varieties of *Cucurbita sp.*.

Chapter 2 includes up-to-date information related to bioactive compounds in the by-products resulting from the processing of *Citrullus sp*. fruits. and the methods of capitalization. Here the focus was mainly on:

a) compounds of interest highlighted in the extracts obtained from the by-products (peels) resulting from the exploitation of *Citrullus lanatus* fruits;

b) methods of obtaining bioactive principles from the by-products (peels) resulting from the exploitation of *Citrullus lanatus* fruits;

c) the biological activities highlighted in the extracts obtained from the biomass (peels) of *Citrullus lanatus.* The information collected and presented originally in this

chapter substantiated the experimental studies carried out in the second part of the thesis, dedicated to original research.

The second part of the thesis had three main objectives, namely:

A) evaluation of the by-products resulting in the form of biomass (peels) from the exploitation of the fruits of three Indigenous *Cucurbitaceae* genera;

B) evaluation of the potential use of biomaterials obtained from the by-products resulting in the form of biomass from the exploitation of the fruits of *Cucurbita sp.* for cosmetic formulations;

C) evaluation of the potential use of biomaterials (extracts) obtained from the by-products (peels) resulting from the exploitation of the fruits of *Citrullus sp*. in agriculture.

In **Chapter 4**, the research carried out to evaluate the by-products resulting in the form of biomass (peels) from the exploitation of indigenous cucurbit fruits, aimed at obtaining biopreparations enriched in bioactive compounds contained in the biomass (consisting of peels) resulting from the fruits of three genera of cucurbits: *Cucurbita sp., Cucumis sp,* and *Citrullus sp..* Biomaterials were obtained in the form of:

a1) extracts in alcoholic solutions;

a2) extracts obtained in propylene glycol;

a3) a crude extract obtained by extraction in chloroform, conditioned in oil obtained from seeds of *Citrullus lanatus*.

For the red/yellow/orange colored extracts, obtained in the three media (alcoholic medium; propylene glycol; chloroform), research was carried out to evaluate the molecular absorption characteristics in the (200-400)nm range, and respectively in the 400-900 nm (color determinations). The total content of polyphenolic compounds was evaluated for all the resulting extracts.

In this stage, depending on the molecular absorption characteristics and respectively on the total content in polyphenols, and the information obtained following the documentation study, the resulting biomaterials were divided into two groups:

I) extracts with the potential to be used in cosmetics, for the production of skin care products;

II) extracts with potential use in agriculture, for plant protection.

In the experimental studies carried out in **Chapter 5**, which aimed to evaluate the potential use of biomaterials obtained from the by-products resulting from the exploitation of the fruits of *Cucurbita sp*. for cosmetic formulations, the preliminary results obtained through in vitro studies showed that:

- all the studied extracts have photoprotective properties;

- the best antioxidant activities are obtained for the crude extract, obtained from the biomass (peels) of *Cucurbita maxima* var. rouge and chloroform, conditioned in oil from the seed of *Citrullus lanatus*, followed by the 16EtOH extract, obtained from biomass (peels) of *Cucurbita maxima* var. rouge and ethanol.

The rest of the extracts obtained and studied in this case have prooxidant activity at the end of the process (60s). It is important to mention that the crude extract obtained from the peels of *Cucurbita maxima* var. rouge in chloroform, conditioned in *Citrullus lanatus* oil, has antioxidant activities, superior to retinol palmitate (50,000 IU) and even to commercial carotenes of cosmetic quality;

- cytotoxicity tests performed *in vitro* on a normal, standardized human cell line, named Primary Umbilical Vein Endothelial Cells, Normal, Human (ATCC PCS-100-010), demonstrated that, for this type of cell line, only one biomaterial is lacking cytotoxicity: namely sample 19 (crude extract conditioned in oil from seeds of *C. lanatus*, obtained by extraction from peels of *Cucurbita maxima* var. rouge and chloroform);

- the experimental data obtained in these studies, indicated sample 19 as a potential candidate for cosmetic formulations intended for skin care, due to the strong antioxidant activity and respectively due to the lack of cytotoxicity, even if the preliminary tests carried out in vitro, indicated a value for photoprotection factor of 34.74%.

In **Chapter 6**, the tests were carried out to evaluate the potential use of biomaterials obtained from the by-products resulting from the exploitation of the fruits of *Citrullus sp*. in agriculture. Here were achieved two types of tests:

c1) tests carried out in vitro evaluated the antifungal effect of the extracts obtained from three varieties of *Citrullus lanatus*, and strains of phytopathogenic microorganisms.

c2) preliminary tests carried out on the experimental plots.

In the *in vitro* tests, in the first stage, simple extracts, enriched in the polyphenolic compounds, initially contained in the shells of *C. lanatus*, were tested. The microorganisms used in these studies were the following: *Penicillium expansum* DSM 62841, *Penicillium digitatum* DSM 2731, *Fusarium verticillioides* DSM 62264, *Fusarium culmorum* 46, *Fusarium graminearum* 96, *Fusarium culmorum* 1056 and *Fusarium culmorum* 1471. The results obtained in this phase indicated local/or moderate effects for the species of microorganisms studied.

In the second stage, the tests were performed *in vitro* with extracts (biomaterials) enriched with Cu^{2+} ions or Zn^{2+} ions, (clear liquid biomaterials, enriched with Cu^{2+} ions or Zn^{2+} ions) whose concentration did not exceed 1%. Biomaterials obtained from *C. lanatus* biomass and enriched with copper or zinc ions were tested on the same types of microorganisms. The analysis of the obtained results highlighted a local/moderate antifungal effect for all *Fusarium* species studied. An interesting fact observed here was that the moderate effect highlighted for the biopreparations containing copper (in particular) or zinc, was comparable to the antifungal effect obtained in vitro with an established antifungal agent, called Falcon Pro, usually used in pre-emergent treatments at wheat or barley. This fact has determined us to carry out preliminary tests in the field (on experimental soil plots) to help us better detect the activity of the developed biomaterials. In this aim, *Triticum aestivum* crop, sensitive to *Fusarium* sp., was chosen (Miranda wheat variety) which was pre-emergently treated with the biomaterials

obtained by extraction from the biomass (peels) of *C. lanatus*, enriched with copper or zinc ions. The preliminary tests carried out in the field with the selected biomaterials aimed to evaluate the influence of the treatments carried out on:

1) the normalized vegetation index (NDVI) in four vegetation stages of the wheat crop (BBCH1, BBCH 69, BBCH 75, and at the time of harvesting);

2) the degree of infection (severity of the attack),

3) the average weight of the wheat grains in the ear

4) the copper and zinc content of the wholemeal flour obtained by grinding the wheat grains resulting from the harvested ears. The results obtained from the previously mentioned tests indicated the biomaterial 5EtOH-Cu as the most suitable for plant protection applications. The qualities that recommend the biomaterial 5EtOH-Cu as a potential candidate for obtaining biopreparations intended for plant protection are based on the preliminary results obtained following the preliminary tests carried out in the field, in the year 2023. Following these tests, it was found that under the influence of this biomaterial:

- the values of the normalized vegetation index determined for the Triticum aestivum crop at four vegetation phases, is higher than the untreated infected control variant;

- the degree of infection of wheat ears is a maximum of 3%;

- compared to the infected and untreated control variant, in which an average value of 1.28 g grains/spike is obtained, for the biomaterial 5EtOH-Cu a value of 1.55 g grains/spike is obtained;

- the analysis of the wholemeal flour resulting from the grinding of the wheat grains obtained after harvesting demonstrated that in the case of the treatment with the 5EtOH-Cu variant, the relative concentration of zinc and copper in the wholemeal flour is below the values obtained in the case of the variant considered normal (the witness crop variant, uninfected, untreated).

The results obtained from the studies carried out in the doctoral thesis have been partially published, and most of the results will be published further.

Currently, the doctoral student managed to publish 4 articles from their thesis, in BDI or WOS-indexed journals, as follows: 2 articles published by the PhD student as first author in Scientific Bulletin Series F, Biotechnology, indexed journal BDI (CABI); 1 article published by the PhD student as first author in Scientific Papers. Series A. Agronomy, WOS indexed journal; 1 article published, as first author, in Scientific papers. Series B Horticulture, in the year 2024, WOS-indexed journal.