IOSUD –„DUNĂREA DE JOS” UNIVERSITY OF GALAȚI
Doctoral School for Mechanical and Industrial Engineering

PhD THESIS

THE STUDY OF CHEMICAL COMPOUNDS FROM PLANT SPECIES OF THERAPEUTIC INTEREST

(PhD Thesis Summary)

PhD Student,

Anna Cazanevscaia (Busuioc)

PhD Supervisor,

Prof. chem. Rodica Mihaela Dinică, PhD Habil.

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GALAŢI

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"It is important to make life a dream and a dream a reality." Mary Curie

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Galat

Anna Cazanevscaia (Busuioc)

Content

Introduction

Since ancient times, people around the world have used plants as valuable sources of food and medicine. In Europe, plant chemistry and the isolation of biologically active compounds developed rapidly after the influence of Paracelsus (16th century) who claimed that "*All meadows and pastures, all mountains and hills are pharmacies*" [1], i.e. nature is a supplier of natural products and medicines. After decades of research and evolution, scientific research is reorienting itself to its origins and trying to decipher the power and benefits that nature has to offer.

The world's biggest challenges in recent years are the growth of the human population and the depletion of natural resources that lead to hunger, malnutrition, and poor human health [2]. Other important aspects of today's life are the increase in the number of human diseases, the development of resistance of some diseases to classical treatments, but also the toxic side effects of synthetic drugs [3,4]. The Food and Agriculture Organization of the United Nations (FAO) estimated that ~11% of the world's 7.6 billion people, suffered from chronic undernourishment in 2016 [5]. Most of the malnourished people live in low and sub-middle income regions [5]. Africa has the highest prevalence of undernutrition, but Asia, being the most populous region of the world, has the highest percentage of undernourished people [6]. In the era of climate change and the vulnerability of agriculture and horticulture, underutilized plants are valuable alternatives for their cultivation and use for various purposes.

Today, people grow grains, vegetables, and fruits, using improved varieties and modern agricultural practices, while attempts are made to adapt various species to new growing environments. However, there are many plant species still underutilized or underexploited, including plant species that thrive in extreme environmental conditions and threatened habitats, possessing genetic tolerance to survive harsh conditions and important nutritional, phytochemical, and therapeutic qualities. Adaptation to the cultivation of these species in other geographical areas can contribute to food security, nutrition, and human health. These plants can represent a considerable commercial value and therefore can make a significant contribution to the development of sustainable agriculture [7].

Researchers from different parts of the world are oriented toward research topics of characterization of nutritional and bioactive compounds from various plants. Nutrition is one of the primary determinants of health, and many studies suggest that diets rich in vegetables and legumes can prevent or alleviate a wide range of conditions [8].

Plants are the major components of a balanced human diet and provide essential nutrients such as carbohydrates, proteins, fats, vitamins, and minerals. Research is constantly increasing in exploring different plants, other than traditional vegetables and legumes, to meet global food and medicinal requirements. Many of these plants have been shown to be rich in valuable primary and secondary metabolites [9,10].

Studies conducted in recent decades have shown that many compounds isolated from plants have biologically active properties, and have importance in the prevention of chronic conditions such as cancer, diabetes, and coronary heart disease. Phytochemical compounds have demonstrated numerous activities that include antioxidant, antitumor, antimicrobial, antihypertensive, anti-inflammatory, and hepatoprotective properties [6,11].

Taking into account these aspects, the present study highlights the possibilities of exploring some exotic plant species, adapted to cultivation in Romania, as sources of nutrients and pharmaceutical products.

The doctoral thesis with the title "***Study of chemical compounds from plant species of therapeutic interest***" consists of **nine chapters**.

The **first two chapters** represent the theoretical part of the thesis, which contains the current data from the specialized literature about the studies on the studied plant species, the chemical composition of these species, and their biological properties. **Chapters 3-7** represent the original part of the thesis in which the phytochemical screening and biological properties of some new varieties of Cucurbitaceae species acclimatized in Romania, obtained from a research-development station in Buzău, as well as a species proposed for acclimatization, are presented from the Fabaceae family. Conclusions, future research directions, and dissemination of results are mentioned in **Chapters 8 and 9.**

The main purpose of this doctoral thesis was to characterize, from the point of view of chemical composition, new varieties of plant species adapted to cultivation in Romania from the Cucurbitaceae family: *Momordica charantia* Rodeo, *Cucumis metuliferus* Tempus, *Benincasa hispida* Zefir, *Trichosanthes cucumerina* Felix, *Luffa cylindrica* Elida but also a species of African prevalence, *Casia sieberiana*, and to evaluate their therapeutic properties by analyzing some biological activities.

The **main objectives** of the doctoral thesis with the title: "*Study of chemical compounds from plant species of therapeutic interest*" were:

- ✓ studying the phytochemical profile by optical (UV-Vis), chromatographic (HPTLC, GC, HPLC) and spectral (ICP-MS, IR, and NMR) methods of species from the Cucurbitaceae family adapted to growth and cultivation in Romania, varieties: *M. charantia* Rodeo, *C. metuliferus* Tempus, *B. hispida* Zefir, *T. cucumerina* Felix, *L. cylindrica* Elida and a species of the Fabaceae family, C. sieberiana, procured from Burkina Faso Africa;
- ✓ obtaining some pure compounds from the fruits of *C. metuliferus* and *B. hispida* and their characterization by MS, IR, and NMR spectrometry;
- ✓ analysis of the therapeutic potential of the extracts and juices obtained from the above-mentioned species, analyzed individually and in combination with various *in vitro* methods based on optical methods.

The **specific objectives** of this doctoral thesis were:

- ✓ Harvesting and preparation of plant samples from the Research - Development Station for Vegetables Buzău, Romania, and the Plant Genetic Resources Bank for Vegetables, Floriculture, Aromatic, and Medicinal Plants Buzău;
- ✓ With the help of confocal scanning microscopy, some parts of plants from new varieties of plants adapted to cultivation in Romania were characterized;
- ✓ Obtaining some extracts by conventional and non-conventional methods, from the fruits and leaves of the species that come from Romania, respectively from the bark and leaves of the *C. sieberiana* species;
- ✓ The study of micro- and macronutrients from the fruits and leaves of the studied species using the ICP-MS technique;
- ✓ Qualitative and quantitative evaluation by spectrophotometric methods, of some classes of organic compounds that are part of the secondary metabolites of plants;
- ✓ Extraction, separation, and identification of natural organic compounds from plant samples studied by chromatographic and spectral methods (HPLC/DAD and UHPLC/MS, GC/MS);
- ✓ Separation, isolation, and characterization of ursolic acid from the fruits of C. metuliferus and B. hispida using spectral methods (FT-IR, MS, and NMR);
- ✓ Evaluation of the cytotoxicity of the extracts obtained from the studied species by the MTT method and by the method of plant seed germination;
- ✓ Evaluation of the antioxidant activity of the extracts through various microspectrophotometric methods of free radical inhibition (DPPH, ABTS), reduction of metal ions, and chelation of metal ions;
- ✓ Evaluation of anti-inflammatory activities (proteinase, albumin, and lipoxygenase inhibition study) and by the cell membrane stabilization test;
- ✓ Evaluation of the inhibitory effect on some enzymes involved in various conditions, such as xanthine oxidase involved in gout and α-amylase and β-glucosidase in diabetes;
- ✓ Evaluation of the antimicrobial activity on some pathogenic microorganisms;
- ✓ Obtain and characterize microcapsules with extracts from the plant studied in this thesis.

PART I. CURRENT STATE OF RESEARCH

Chapter 1. Plant species studied of therapeutic interest

1.1. Characterization and importance of species from the Cucurbitaceae family

Plants in the Cucurbitaceae family come from a large group of vegetables, and the most common are pumpkin, melon, and cucumber [50]. Many of these are consumed worldwide, and most plants are also valuable medicinally. The species of this family play an important role in the ecosystems of which they are a part.

Cucurbitaceae are the most diverse family of plants and are cultivated throughout the world in a variety of environmental conditions, even if the optimal conditions are tropical and subtropical they have been easily adapted to cultivation on all continents. More than 300 plant species are used by humans, but only 150 species are extensively cultivated, and 30 of these are crucial for global food production. The main producers of Cucurbitaceae are countries such as India, Turkey, China and the United States. The Cucurbitaceae family has an important economic role because the vegetables and fruits of this family are used in various food products. Edible plants, derived from the Cucurbitaceae family, are a group that includes five genera: Cucurbita, Cucumis, Citrullus, Legenaria and Sechium [61].

Figure 1.1. The origins of species in the Cucurbitaceae family

Among the species of Cucurbitaceae, Momordica charantia, Cucumis metuliferus, Benincasa hispida, Trichosanthes cucumerina and Luffa cylindrica, species all adapted and cultivated in Romania, and an African plant from the Fabaceae family, namely Cassia sieberiana, were analyzed in the doctoral thesis

1.1.1.Momordica charantia

The species Momordica charantia L. is also commonly known as bitter cucumber, bitter gourd, African cucumber [29]. This plant belongs to the genus Momordica, originating in tropical Africa, currently being cultivated almost worldwide in tropical areas, including various parts of the

Amazon, in East Africa, Asia, the Caribbean and even in Europe [30]. The fruits are pendulous, discoid, ovoid or elongated ellipsoid in shape, often narrowed at the ends, which can be white or green and turn orange when ripe. The seeds, which can number from 5 to 30, are rectangular, with subtrident ends, with grooved edges, and are brown or black in color [31].

Momordia charantia is one of the most well-known plants for its antidiabetic properties and is marketed and consumed as a food supplement to regulate blood sugar levels in patients with type II diabetes. It is also used in other conditions such as hypertension, osteoarthritis, cancer, obesity, viral and bacterial infections [63–65].

1.1.2. Cucumis metuliferus

The spread of Cucumis metuliferus internationally started as early as 1982 in New Zealand when it was cultivated for commercial purposes and easily spread to the market. The fruits are ellipsoid, about 12 cm long, 8 cm in diameter, green in color, and during ripening the color migrates to orange. They occur in two forms, bitter containing cucurbitacins (triterpenoids), which can be toxic, and the non-bitter form which is widely cultivated [101].

Cucumis metuliferus is called Kanda in the traditional medicine of Plateau State of Nigeria, which can be translated as "stop it before it comes" or "a local vaccine", being used as a remedy for many ailments such as peptic ulcer, diabetes , hypertension and HIV viral infections [102]. Farmers in this state use the pulp of the fruit to treat birds suffering from coccidiosis [48]. Some studies have shown that the seeds can be ground into a fine flour that emulsifies in water and by introducing this mixture into the diet produces antiparasitic effects [103].

1.1.3. Benincasa hispida

The species Benincasa hispida (Benincasa cerifera), also called winter melon, ash gourd, ash shield, winter gourd, white pumpkin or wax gourd belongs to the genus Benicasa [119]. Supposed to be a native of Japan and Java, more or less cultivated in India and warm countries. It is a popular vegetable species, especially among the Asian community, being often used both for nutritional and therapeutic purposes, being found in Ayurvedic medicine [120].

Benincasa hispida can be used fresh, but also in a wide variety of food products. In addition, the plant has been used medicinally for various conditions such as gastrointestinal, respiratory, heart disease, diabetes, and urinary diseases [136]. These properties are based on the rich and varied chemical composition in vitamins, proteins and other bioactive chemical compounds

1.1.4. Trichosanthes cucumerina

Snake gourd, viper gourd, snake tomato or long tomato are common names for the plant with the scientific name of Tricosanthes cucumerina. It is a widespread plant in India, Australia, the Pacific Islands and also in West Africa. The fruits of this plant species are usually consumed as vegetables due to their remarkable nutritional value, being a good source of vitamin A, vitamin B and vitamin C [137]. In addition to consumption in fresh or processed form, this species occupies an important place in alternative medicine systems such as Ayurveda and Siddha [25].

1.1.5. Luffa cylindrica

Luffa cylindrica, also known as vegetable sponge, is a plant with valuable fruits in many areas of use. This species represents a natural raw material, cheap, non-toxic, biodegradable and with a high agricultural production of about 8000 fruits per hectare. It is mainly grown in tropical countries such as Brazil, and production can reach 100,000 dozen per year [163].

Its fruits are cylindrical, fusiform with a length of 20-50 cm, diameter of 6-10 cm, green in color when fresh, with a woody consistency, a fibrous (sponge-like) texture and are rich in lignin, cellulose and hemicellulose [165]. They can be eaten when fresh, soft and with matte black, elliptical seeds [166].

Luffa cylindrica is used in various fields such as obtaining medicines, in cosmetics, for shoes, hats, wallpaper, in construction and in materials engineering. In traditional medicine it is used as a remedy for headaches, constipation and other gastrointestinal ailments.

1.2. Characterization and importance of Fabaceae species. *Cassia sieberiana* species

Cassia sieberiana is a plant species more widespread in Africa, belonging to the Fabaceae family, and is a woody shrub commonly called "African laburnum", "flame trees" or "wax trees" [188]. These popular names are due to its large, yellow or red flowers, which appear before the leaves and give it a flame appearance [189]. It is found in the savanna of Sudan, in Nigeria, in Bukina Faso in West and Central Africa, in India and South America [188,190,191]. These plants grow in warm and humid areas, such as tropical and subtropical forests, but can also grow in drier areas [190].

The study of the chemical composition of crude aqueous extracts from leaves, stems, roots and pods revealed the presence of compounds from different classes of compounds such as alkaloids, tannins, flavonoids, anthraquinones, terpenoids, saponins, glycosides and phenols in various concentrations. *C. sieberiana* is traditionally used to treat symptoms of menopause, malaria, hemorrhoids, sexual impotence, stomach pain, leprosy, jaundice, headache, diabetes, infertility, skin diseases, toothache, and HIV [188].

1.3. Partial conclusions

In accordance with the data from the specialized literature, the selected plant species from the families Cucurbitaceae and Fabaceae, have proven therapeutic potential both through the uses of traditional medicine and through research studies carried out in the laboratory.

Momordica charantia, the most studied of the selected species, is particularly known for its antidiabetic properties.

Trichosanthes cucumerina stands out for its benefits in the gastrointestinal tract, Benincasa hispida for its antinociceptive action, Luffa cylindrica for its bronchodilator and hepatoprotective properties, and Cucumis metuliferus for its antimalarial action.

All species have antioxidant, anti-inflammatory, antibacterial and anti-diabetic properties, each with its own unique set of compounds

The species Cassia sieberiana stands out for its valuable therapeutic properties, being used in traditional African medicine.

Chapter 2. Chemical composition of the studied species

2.2. Classes of organic compounds identified in the studied plants

Plants are an important source of chemical compounds used in various commercial products (food, pharmaceutical, chemical, cosmetics) [210]. Plants can be compared to "factories" that under the action of solar energy, consume CO2 and water, and then produce oxygen, energy and basic compounds necessary for the survival of living organisms, such as proteins, carbohydrates, vitamins and lipids [211].

Plant metabolism consists of numerous chemical reactions catalyzed by enzymes, which take place in various plant cell structures and the production of a variety of chemical molecules from the category of primary metabolites [212]. They play a major role in maintaining the viability of the plant (proteins, sugars, vitamins and lipids). In addition to primary metabolites, a series of compounds such as terpenes, phenolic compounds, nitrogen compounds (alkaloids, cyanogenic glycosides, non-protein amino acids) and sulfur compounds are synthesized in plants, compounds that are synthesized in the chemical processes of secondary metabolism [213]. They are biosynthesized by plants and specific to each individual species, often manifesting functions characteristic of some plant organs or tissues, they can only be identified in a certain period of growth and development within a species, or they can be activated only in certain stress factors such as the attack of microorganisms, insects or the lack of nutrients [214]. Secondary metabolites are considered compounds of interest for various reasons, such as their structural diversity and their potential as drug candidates [213].

In general, secondary metabolites are produced by biosynthetic modifications of primary metabolites, including methylation, glycosylation, and hydroxylation. Plants contain highly specific secondary metabolites, sometimes highly toxic at high concentration, and represent a valuable library of bioactive compounds with broad activity in the context of human cells, bacteria, fungi and parasites [215]. For economic reasons, the study of secondary metabolites has been the subject of research in recent years, contributing to the development of several fields of phytochemistry. Statistical data highlight the fact that over a thousand new chemical compounds from different plant species are identified annually. According to literature data, approximately 25% of drugs used to prevent and treat various ailments contain compounds of plant origin [211,216].

2.3. Partial conclusions

Deciphering the chemical composition of the studied plant species is topical, even if many of the compounds have been identified and are already known.

In this chapter, the primary and secondary metabolites of the plants, and especially of the species of the Cucurbitaceae and Fabaceae families studied in this work, were highlighted, as well as their importance in the life of the vegetable kingdom.

According to data from the literature, the analyzed species have a specific chemical composition, with valuable compounds from the classes of saponins, terpenoids and alkaloids. Thanks to these biologically active compounds, these species have a valuable therapeutic potential.

PART II PERSONAL CONTRIBUTIONS

Chapter 3. Characterization of plant material and obtaining extracts from the Cucurbitaceae and Fabaceae plant families

3.3. Cultivation, sampling and characterization of plant material

Cultivation and sampling of plant material

The plant material analyzed in this study is represented by the fruits and leaves of the species: Momordica charantia variety Rodeo, Cucumis metuliferus variety Tempus, Benincasa hispida variety Zefir, Luffa cylindrica Elida and Trichosanthes cucumerina variety Felix (Figure 3.1), acclimatized in Buzău, Muntenia region, Romania (Southeastern Europe). To carry out the studies proposed in this paper, green and mature fruits were collected, as well as leaves from the period 2019-2021 from SCDL and BRGV, Buzău, Romania. The selected species were acclimatized using the seeds of endemic plants from Tibet, Kalahari Desert, Nepal and China (Hubei University) from 1996-2019 as starting material.

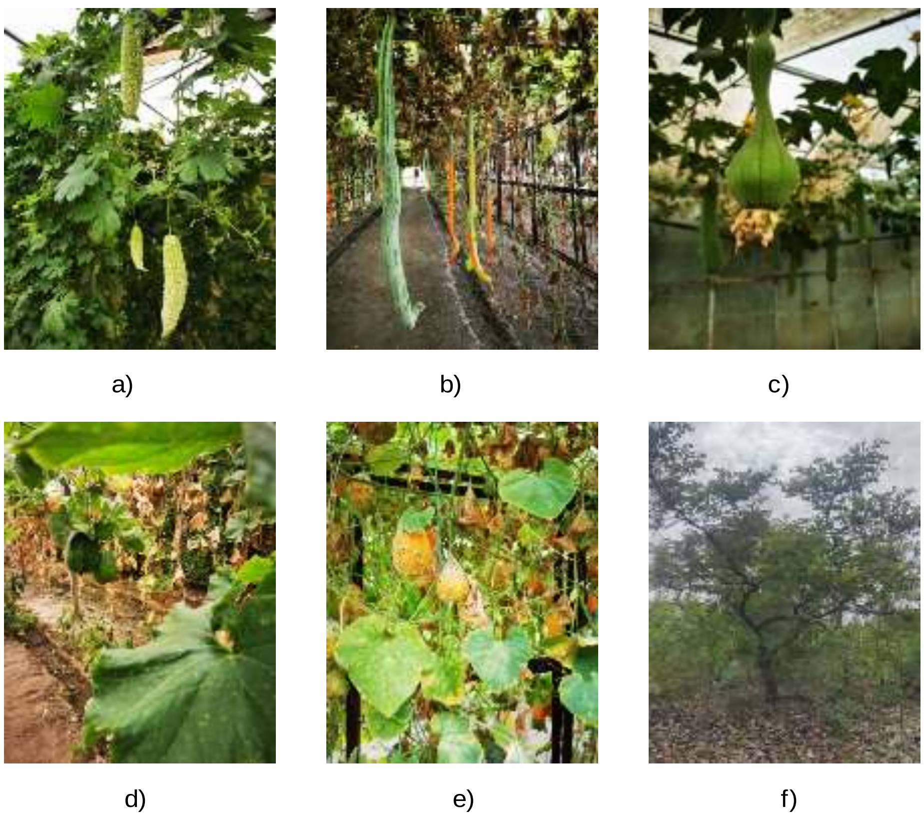


Figure 3.1. Cultures of the varieties from Romania: a) *M. charantia* Rodeo; b) *T. cucumerina* Felix; c) *L. cylindrica* Elida; d) *B. hispida* Zefir e) *C. metuliferus* Tempus and f) *C. sieberiana* from Dienderesso forest, Burkina Faso

3.4. Extraction of biologically active compounds from the studied species

The biological system of plants consists of a wide range of primary and secondary metabolites, from which the biological properties and activities result. In order to exploit the chemical composition of plants and obtain compounds of interest, in the last 50 years, extraction technologies have seen a continuous evolution. Thus, ecological methods have been developed that use reduced amounts of synthetic and organic chemicals, shorter working time, improved yield and quality of the extract. In this context, new extraction strategies have been designed to overcome some of the disadvantages of traditional extraction methods [368]. Thus appeared the terms "green technology" or "green chemistry methods" that address new extraction methods due to the fact that they use less energy and reduced volumes of organic solvents so that they are not toxic to the environment [369]. In this study, one of the green chemistry methods was used, namely ultrasound-assisted extraction, but also traditional methods such as decoction and extraction with reflux solvents.

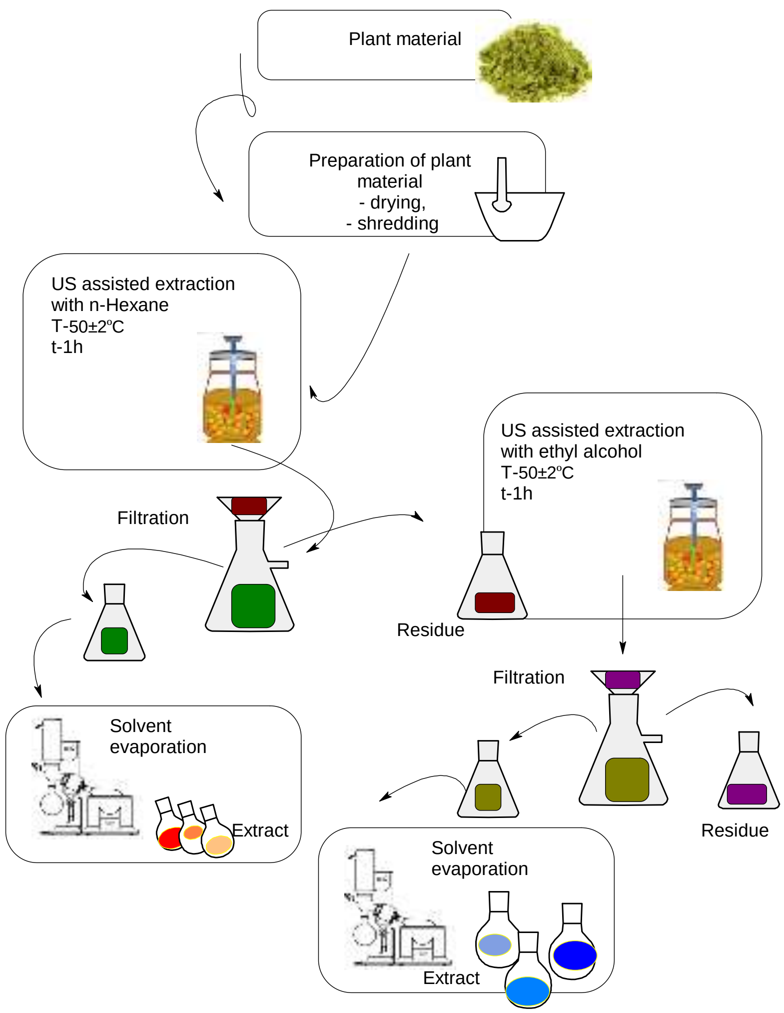


Figure 3.4. Scheme of ultrasound-assisted extraction. T=temperature, t=time.

The extraction was carried out in two stages with solvents of different polarities to separate the lipophilic compounds from the hydrophilic ones (Figure 3.4.).

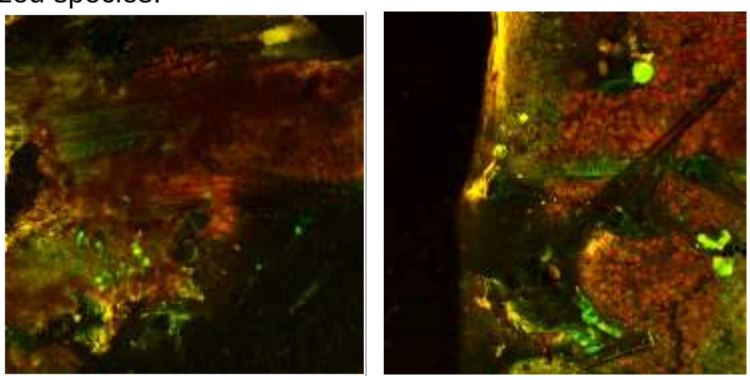
All the obtained extracts were weighed and stored in well-sealed vials at a temperature of -20±2°C, being used for subsequent analyses. Also, for the studied extracts, the value of the total extractable components (TEC%) was determined.

3.5. Results and discussion

Cultivation and sampling of plant material

Characterization of plant material by CLSM microscopy

A plant material can be characterized by several methods, one of them being the visualization of the internal structure of the tissue with the help of modern methods and devices such as CLSM microscopy. Thus, it was possible to visualize in section some samples of leaves, green and ripe fruits of some analyzed species.



a. b.

Figure 3.10. CLSM images of tissue from a.-longitudinal, b.- transverse sections of C. metuliferus leaves (original pictures)

In Figure 3.10. you can see the images made with the help of CLSM, which highlighted in the leaf sections the compact assimilating parenchyma that is rich in chloroplasts, but also secretory channels full of bioactive compounds. Conductive vessels have slightly thickened secondary walls with spiral ornamentation. Long, pluricellular or glandular epidermal hairs can also be observed, shorter, with a single-cell foot and the basal gland.

3.6. Partial conclusions

The plant materials formed from the leaves and fruits of new varieties such as M. charantia Rodeo, C. metuliferus Tempus, T. cucumerina Felix, B. hispida Zefir and L. cylindrica Elida adapted to cultivation and cultivated in Romania were taken for the study from Research - Development Station for Vegetable Cultivation (SCDL) Buzau. The species C. sieberiana was brought from Africa from the Hauts-Bassins region, from the forest of Dienderesso, Burkina Faso.

Part of the analyzed plants were characterized with the help of CLSM microscopy, where the tissues and healthy plant cells of the fruits and leaves were highlighted through transversal and longitudinal sections.

Several extraction methods of biologically active compounds have been selected, some of which have also been studied for various biological properties such as antimicrobial, anti-inflammatory or antioxidant ones. Extractions were made by conventional and non-conventional methods from the fruits and leaves of the species studied in this research. Among the conventional methods, decoction, maceration and Soxhlet extractions were performed with different solvents. Ultrasonically assisted sequential extraction using several solvents with different polarities has been shown to be one of the best non-conventional extraction methods. The highest values of total extractable components (TEC) were observed in the case of samples obtained by ultrasound-assisted extraction, the values being in a range of 44.3 - 61.5%.

Chapter 4. Analysis of the chemical composition of the studied species by various methods

4.4.3. Quantification of organic compounds by spectrophotometric methods from the studied plant species

4.4.3.1. Total polyphenol content assay

In recent years, the number of studies on the physiological activities of plant compounds has increased greatly. Many of these studies have emphasized the beneficial effects of polyphenols in terms of their antioxidant, antiproliferative and anti-inflammatory properties [397] (Figure 4.9).

Figure 4.10 shows the results obtained for the determination of the total content of phenolic compounds (TPC) of the tested samples. A significant concentration of polyphenolic compounds was identified in the juice of C. metuliferus (18.97 ± 1.25 mg Eq AG / mL juice), and a higher concentration showed the juice of M. charantia (27.64 ± 1, 89 mg Eq AG / mL juice). In one of the studies on the juice obtained from different varieties of the M. charantia species, total polyphenol content values between 8.15 ± 0.51 and 11.47 ± 0.49 mg EqAG/g SU were identified [28], values lower compared to the data obtained in this study. The two varieties of B. hispida and T. cucumerina showed a lower content of polyphenols, 10.32 ± 1.06 mg EqAG / mL juice and 10.253 ± 0.56 mg EqAG / mL juice, respectively. These results may be due to phenolic compounds such as gallic acid, caffeic acid, tannic acid, p-coumaric acid and trans-ferulic acid [398].

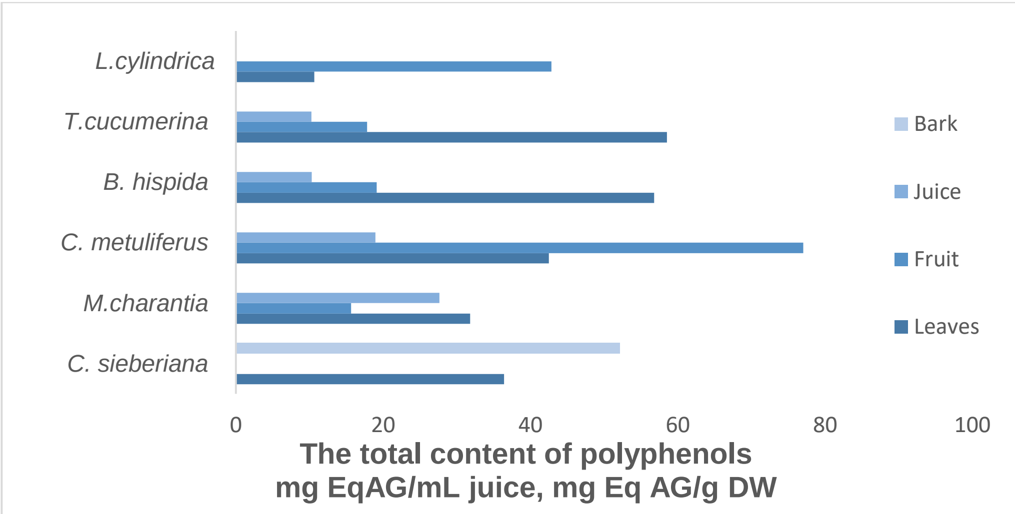


Figure 4.10. The total content of polyphenols in the juices, leaves and fruits of the varieties analyzed values represent the mean of three separate experiments and error bars with standard deviation.

The cultivar analyzed in this study presented a total polyphenol content of 15.63 ± 0.333 mg EqAG / g SU (Figure 4.10). Compared to the juice, the extract of M. charantia (bitter cucumber) is poorer in compounds from the class of polyphenols, valid only for this species. The leaves presented a chemical composition richer in polyphenols, as was also observed in the case of B.

hispida and T. cucumerina samples. lower polyphenols from the leaves and fruits of L. cylindrica compared to other species [402].

The numerous related studies in this field, of identification and dosage of plant phenolic compounds, indicate the current worldwide interest in this subject. In order to determine which polyphenols are most suitable for application in various food, cosmetic or pharmaceutical products that have not yet been exploited, further studies using modern analytical techniques are needed.

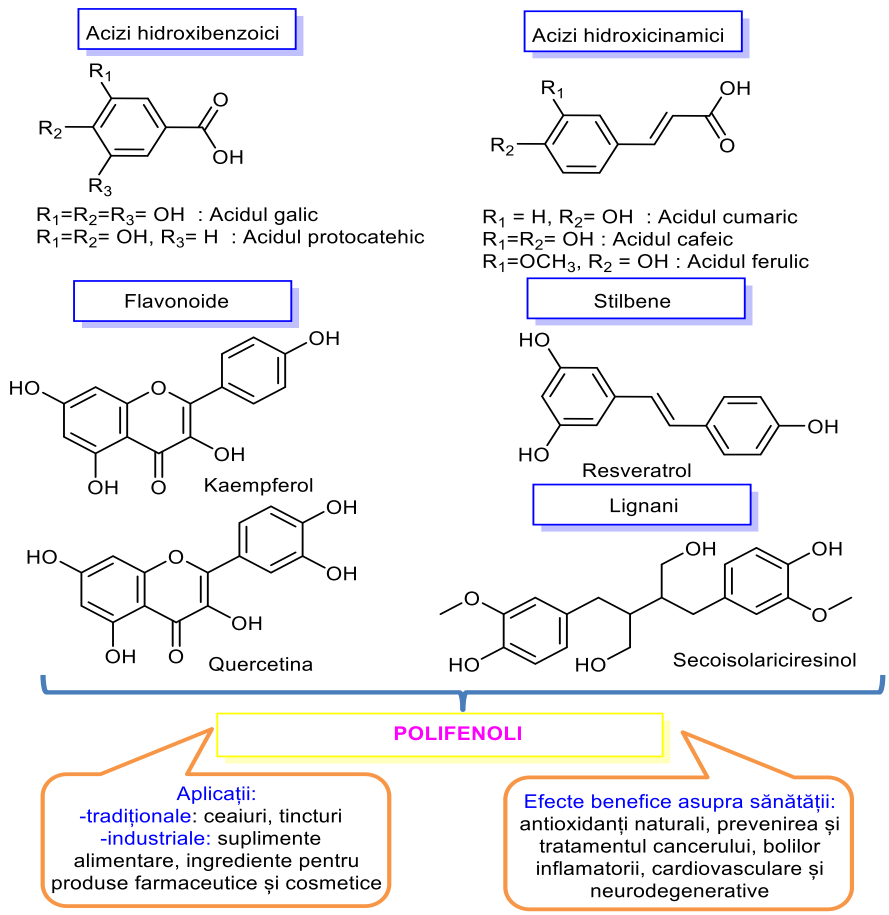


Figure 4.9. The main subclasses of polyphenols present in the studied species of Cucurbitaceae and Fabaceae, possible health benefits and their applications [24–30]

4.5. Partial conclusions

Following the qualitative phytochemical screening, it was demonstrated that all the analyzed plant species contain various classes of compounds of therapeutic interest, some results obtained being correlated with the data presented in the specialized literature.

As a result of the quantification of the various classes of compounds, the differences between the analyzed plant species as well as between the various anatomical parts of the same species were highlighted.

For the first time, juices and extracts from various parts of the plant were studied comparatively for several species of the Cucurbitaceae family and for the species C. sieberiana.

The total content of phenolic compounds in the studied species was higher for the fruits of C. metuliferus (77.04 ± 0.45 mg EqAG/g SU) and in the leaves of T. cucumerina (58.55 ± 0.55 mg EqAG/ g SU).

According to data from the literature and the analyzes performed in this study, the highest content of β-carotene was presented by bitter cucumber fruits (103.65 ± 0.70 μg EqβC/g SU), followed by C. metuliferus, and for T .cucumerin the lowest concentration was observed (3.75 ± 0.21 mg EqβC/g SU).

It has been proven that the fruits have a low content of vitamin C compared to the leaves of each species. The extracts from the leaves of T. cucumerina and C. metuliferus were highlighted with a high content of vitamin C (19.65±0.01 mgAA/10 g SU, and 16.86 ± 0.06 mgAA/10 g SU, respectively).

Chapter 5 Separation of compounds of therapeutic interest by spectrometric and chromatographic methods

5.2. Analysis of the chemical composition of the extracts from the plant materials studied by the TLC/HPTLC technique

HPTLC (High-Performance Thin-Layer Chromatography) i.e. "High-Performance Thin-Layer Chromatography" represents a technique in which the stationary phase is a solid one and can be represented by a chromatographic plate on which silica gel, aminoethylated cellulose, alumina or various polyamides etc. and the mobile phase is liquid and can be a solvent or a mixture of solvents in various proportions, the mobile phase depending on the chemical compound(s) to be isolated from the mixture.

5.3.2. Results and discussion

In the extracts of C. metuliferus - pulp, C. metuliferus - external skin of fruits, B. hispida - external skin of fruits, B. hispida - pulp, L. cylindrica, T. cucumerina and M. charantia compounds were identified from class of saccharides after their separation by HPTLC chromatography (Figure 5.2). In extracts from the peel of C. metuliferus fruits, mannose (Rf=0.43) and raffinose (0.09) were identified, in extracts from B. hispida fruits galactose (Rf=0.33), mannose, and a unidentified compound (Rf=0.27), in the extracts from the fruits of L. cylindrica xylose (Rf=0.55) and an unidentified compound ((Rf=0.13), in the extracts of T. cucumerina maltose (Rf=0 ,22) and in the extracts of M. charantia maltose and an unidentified compound (Rf=0.73) (Figure 5.2).

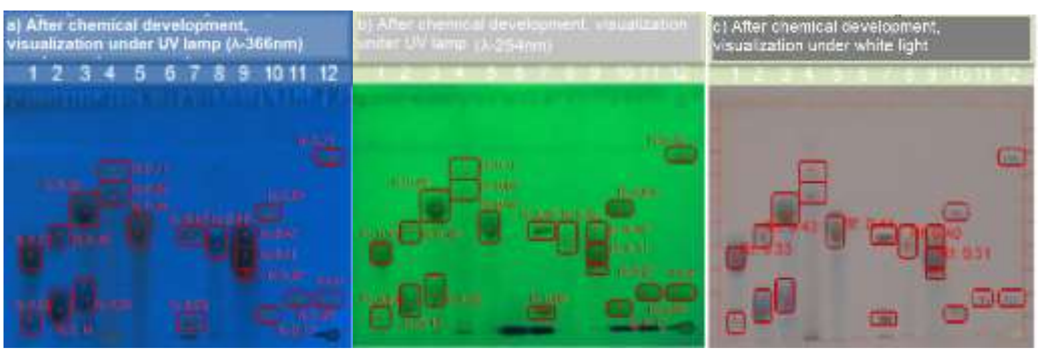


Figure 5.2. HPTLC separation of: pure compounds (1) galactose+raffinose, (2) mannose+lactose, (3) maltose+xylose, (4) deoxyribose+ribose, (5) arabinose, and plant species extracts: (6) *C. metuliferus* - outer skin of fruit, (7) *C. metuliferus* - pulp, (8) *B. hispida* - outer skin of fruit, (9) *B. hispida* - pulp, (10) *L. cylindrica*, (11) *T cucumerina*, (12) *M. charantia*

5.4. Analysis of the chemical composition of the extracts from the plant materials studied by HPLC-DAD and UHPLC-MS

In this study, 22 compounds of interest were identified by HPLC-DAD analysis in juice samples obtained from the fruits of the analyzed plant species, namely gallic acid, neochlorogenic acid, caffeic acid, p-coumaric acid, trans-ferulic acid , (+)-catechin hydrate, (-)-epicatechin, procyanidin A2, procyanidin B2, rutin, quercetin-3-D-galactoside, kaempferol-3-glucoside, hyperoside, naringenin, kaempferol, quercetin, cinnamic acid, carnosol, carnosic acid, ursolic and oleanolic acids. Quantitative analysis (expressed in µg/g juice), of the compounds identified in the four species belonging to the Cucurbitaceae family and cultivated in Romania (*M. charantia, C. metuliferus, B. hispida and T. cucumerina*).

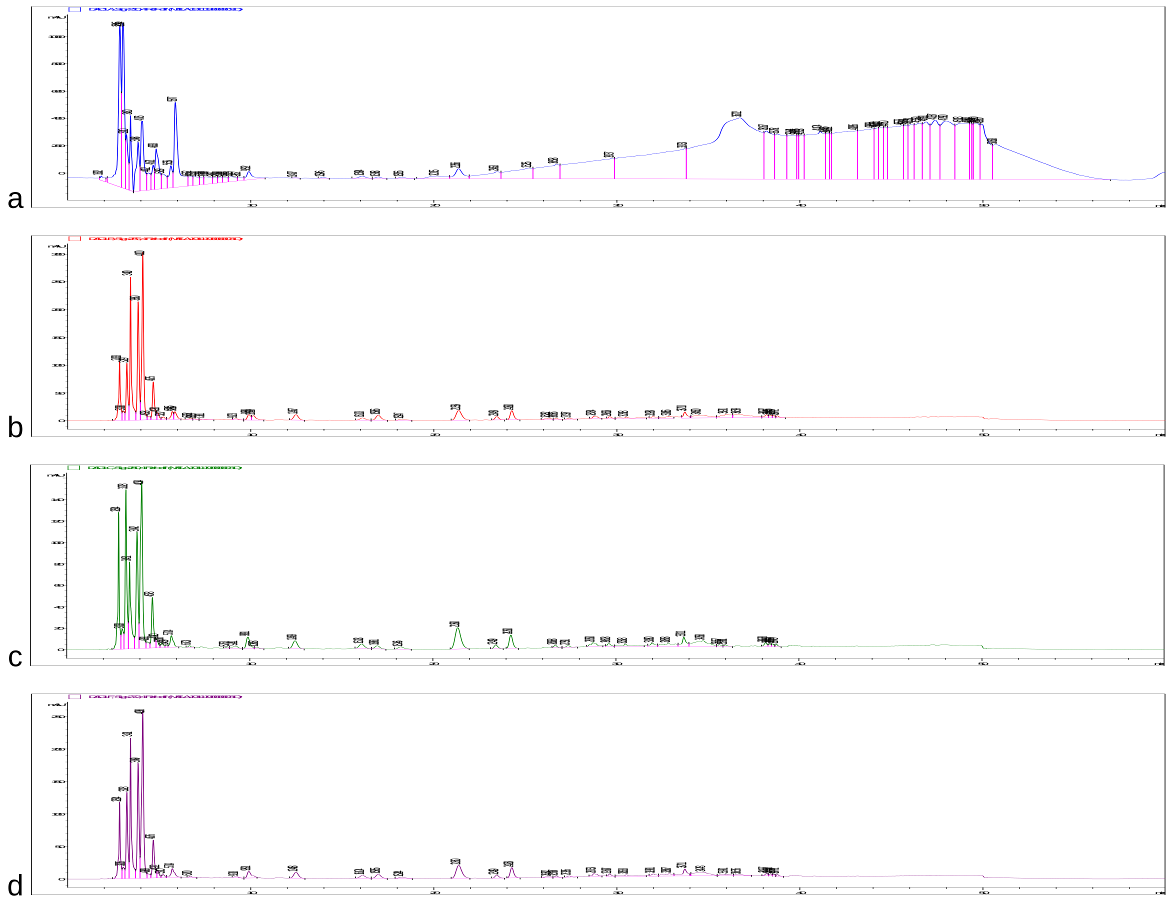


Figure 5.8. HPLC-DAD chromatograms for *M. charantia* juice samples recorded at different wavelengths λ: a-210nm, b-265 nm, c-280 nm, d- 272 nm

After updating the data from the specialized literature, no data were found to compare the results obtained in this study for the juice obtained from the four analyzed species. The richest chemical composition was identified in the juice of *M. charantia* (Figure 5.8.) and *C. metuliferus*, and the absence of compounds such as kaempferol and kaempferol-3-glucosides was found in the juice of *B. hispida*. The major constituents are epicatechin (555.22 mg/kg), catechin (201.78 mg/kg), oleanolic acid (238.10 mg/kg) and ursolic acid (195.44 mg/kg), which were identified in the juice of *M. charantia* and in *C. metuliferus* (347.67 and 191.44 mg/kg) respectively.

5.7. Separation and isolation of natural organic compounds of therapeutic interest

The separation of compounds from a mixture to obtain pure molecules has multiple applications and can be found in various fields related to organic chemistry, such as biology, pharmaceutical, food industry, etc. By separation it is possible to obtain and purify individual compounds from a complex mixture, thus allowing their analysis, characterization and further use [479].

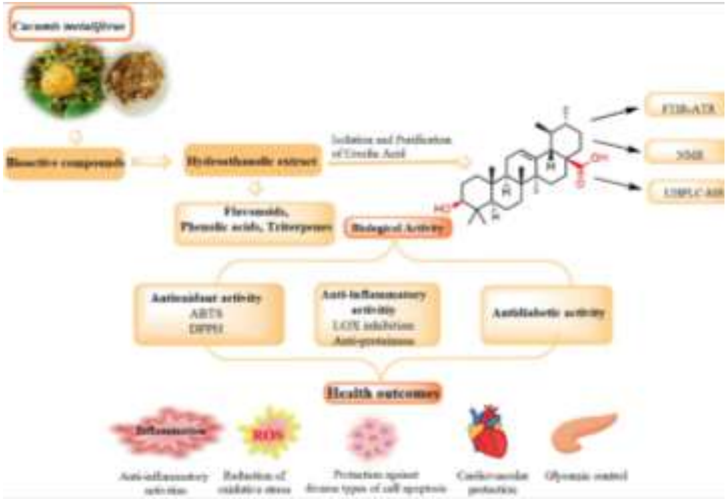


Figure 5.19. Schematic representation of the separation of ursolic acid from the hydroalcoholic extract of *C. metuliferus* and the main bioactive characteristics

The structure of the ursolic compound, the compound isolated from the extract of *C. metuliferus* was demonstrated by spectral analysis by recording the FT-IR spectra of the commercial ursolic acid (AU) and of the isolated and purified one (Figure 5.22).

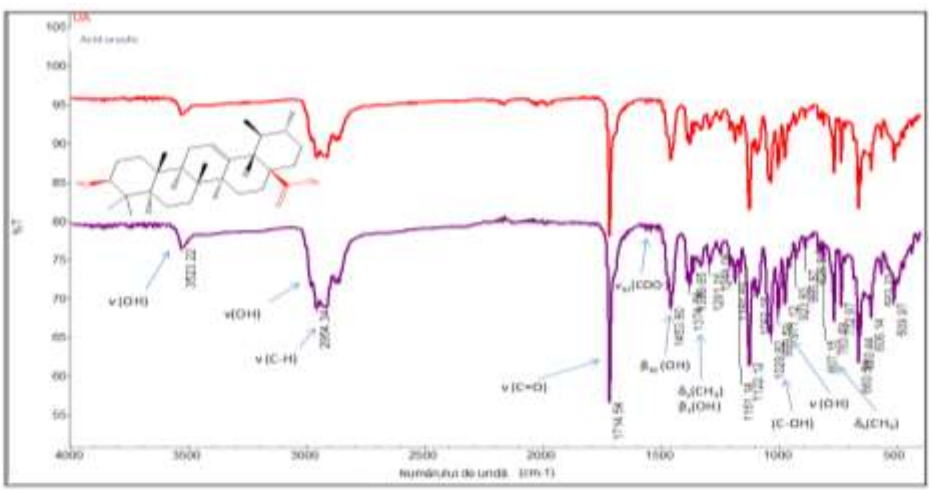


Figure 5.22. FTIR-ATR spectra of commercial ursolic acid (purple) and isolated ursolic acid (red)

The aim was to demonstrate the presence of the isolated compound, thus the interpretation and assignment of the characteristic IR bands, the vibration bands of ursolic acid and the characteristic functional groups being evident, also confirmed by the literature data of the identified acid [489,491,494].

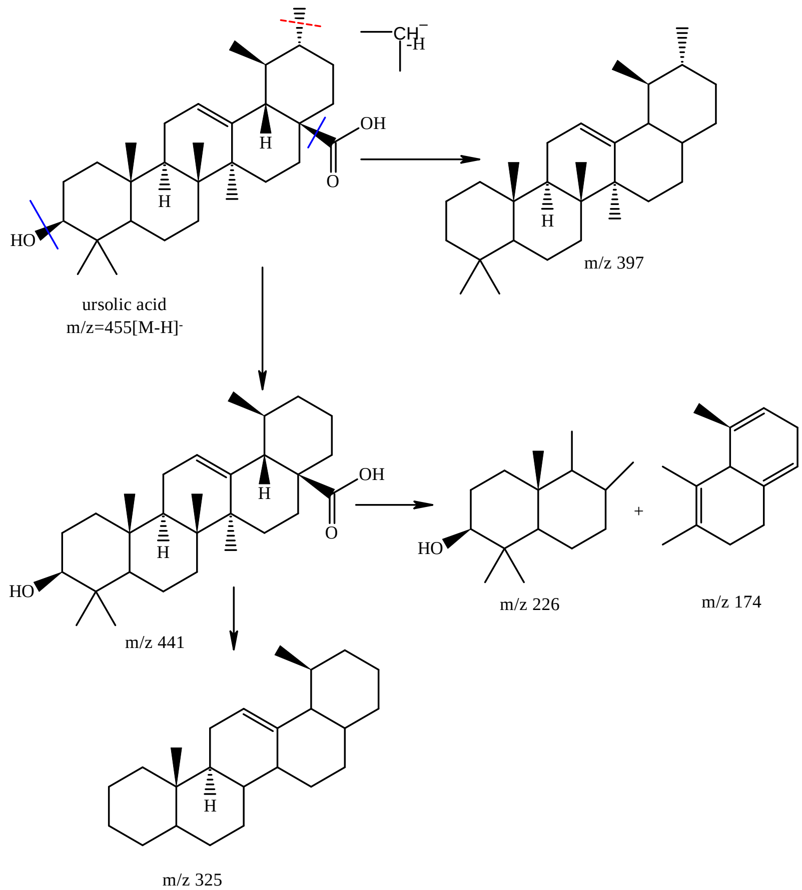


Figure 5.24. Proposed fragmentation scheme for ursolic acid

The isolated compound was further analyzed by 1H-NMR spectroscopy to further characterize the structure of the compound assumed to be ursolic acid. The obtained results were analyzed by comparing the chemical shifts of the isolated compound with data from the literature, the 1H-NMR spectrum of commercial ursolic acid.

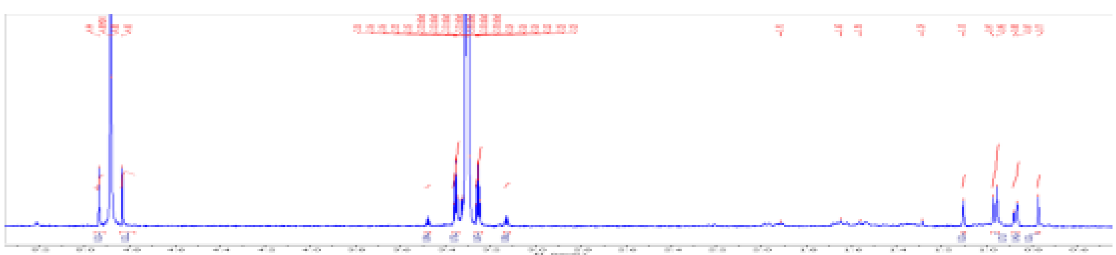


Figure 5.25. 1H NMR spectrum of ursolic acid obtained from C. metuliferus extract

The 1H-NMR spectrum, shown in Figure 5.25. of isolated AU revealed the presence in the high-field region of five methyl groups through the corresponding singlets (0.77, 0.88, 0.95, 0.97 and 1.10 ppm) and two doublet methyl groups (0.87 and 0.99 ppm), characteristic signals for the ursan-type triterpene skeleton [249].

Two singlet signals were also identified in the isolated AU at δ 1.10 ppm, characteristic for the methyl proton group of the oleanane triterpene skeleton and at δ 1.29 ppm, for the methylene protons –CH2-. The signal at 3.3 ppm in the low-field region represents H, possibly due to the hydroxyl group, and at δ =4.94 ppm, a protonic signal attributed to the olefinic proton of the triterpene.

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid): powder, white, 1H-NMR (CD3OD): δ =1.63 - 1.69 (H-1,2H, m), 1 .65 (H-2, 2H, m), 3.13 (H-3, 2H), 0.74 (H-5, 1H, d), 1.43 and 1.29 (H-6, 2H, m), 1.29 (H-7, 2H, m), 1.56 (H-9, 1H, m), 1.91 (H-11, 2H, q), 5.21 (H)-12 , 1H, t), 1.62 (H-15, 2H, m), 1.58 and 1.63 (H-16, 2H, m), 2.20 (H-18, 1H), 1.38 (H-19, 1H, m).), 1.36 (H-20, 1H, m), 1.32 (H-21, 2H, m), 1.53 (H-22, 2H, m), 0.95 (H-23, 3H, s), 0.77 (H- 24, 3H, s), 0.97 (H-25, 3H, s), 0.84 (H-26, 3H, s), 1.10 (H- 27, 3H, s), 0.87 (H-29, 3H, d), 0.95 (H-30, 3H, d).

5.8. Partial conclusions

Samples from the four species *M. charantia, C. metuliferus, B. hispida* and *T. cucumerina* were selected and analyzed for their chemical composition, using various analysis methods.

From the sample made by the spectrometric method of analysis, ICP/MS, for micro and macro elements, the presence of chemical elements with higher values in all the analyzed species such as potassium, calcium, magnesium and iron were highlighted. The comparative study between dry and freeze-dried samples demonstrated that dry plant material better preserves the presence of micro- and macroelements in the samples.

Through the HPTLC chromatographic method, the main components of the class of primary metabolites, namely carbohydrates present in the species of the Cucurbitaceae family such as maltose, arabinose, deoxyribose and others, were highlighted, data comparable to the literature, which refers to this species

HPLC/DAD analysis confirmed that the vast majority of species in the Cucurbitaceae family contain a large number of triterpenes and significant concentrations of polyphenols and flavonoids. Chemical compounds such as shikimic acid, gallic acid, quercitin, cinnamic acid, ursolic and oleanoic acid present in both hydroethaolic extracts and juice samples of cucumber species were quantified.

By using the UHPLC method, compounds specific to these plants such as cucurbitacins A, E, C, D, luteolin, guguacin, etc.

With the help of the GC/MS technique, it was possible to analyze the lipophilic part of the fruits of M. charantia, T. cucumerina and C. metuliferus, being proposed some fragmentation models of the identified compounds such as: linalool, n-heneicosan

Through successive experiments, it was possible to isolate by two different methods ursolic acid, as a primary compound in the fruits of C. metuliferus and B.hispida species. The pure compound obtained was characterized by several FT-IR, UHPLC/MS and NMR methods.

Chapter 6. Evaluation of the biologically active properties of the plant extracts studied

6.1. Introduction

Natural products and derivatives obtained from them have been used throughout human history on a large scale, and nowadays they are increasingly found especially in the pharmaceutical market [1]. Many of these have remained neglected, showing no therapeutic or economic interest [2]. With the passage of time and the proof of the beneficial health properties of natural products, plants and plant extracts have successfully re-entered the lives of consumers.

6.4.2. Analysis of antioxidant activity by DPPH free radical inhibition method

One of the most popular spectrophotometric tests for determining the antioxidant activity of various substances and especially plant extracts is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition test which is initially colored purple, and in the presence antioxidant agents the color turns yellow. The advantages of this method are precision, low cost and ease of implementation, providing a screening of antioxidant activity [29,30] (Figure 6.9).

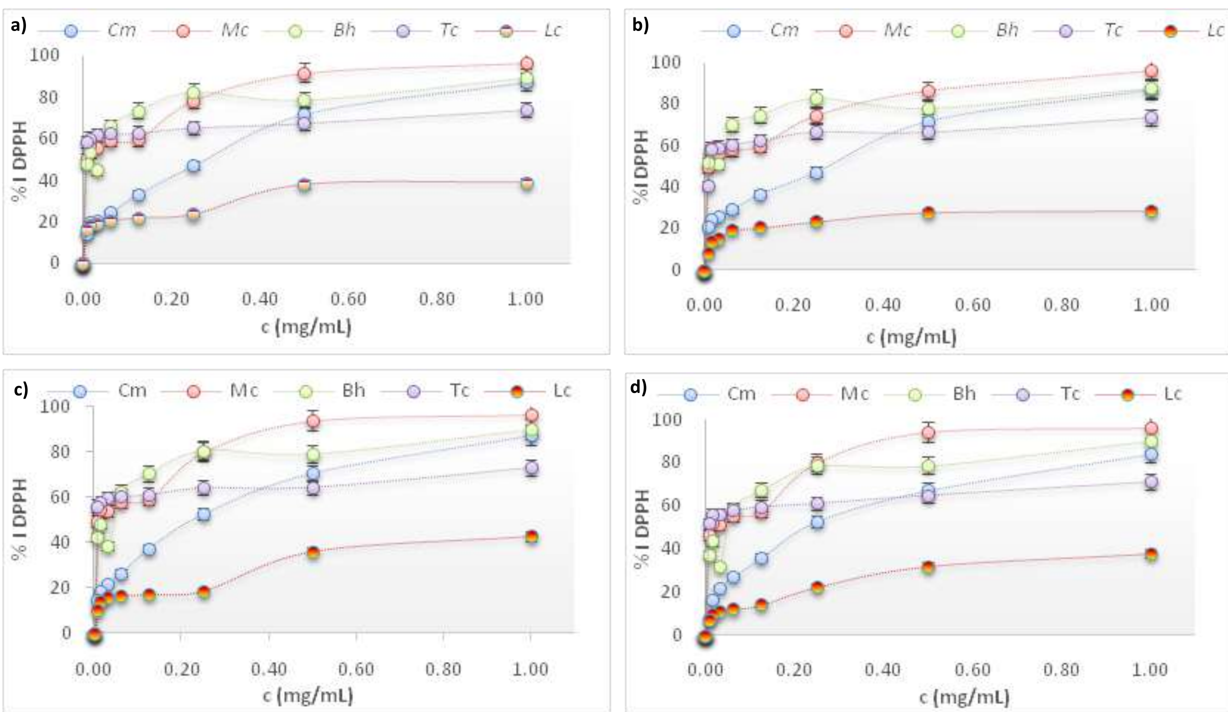


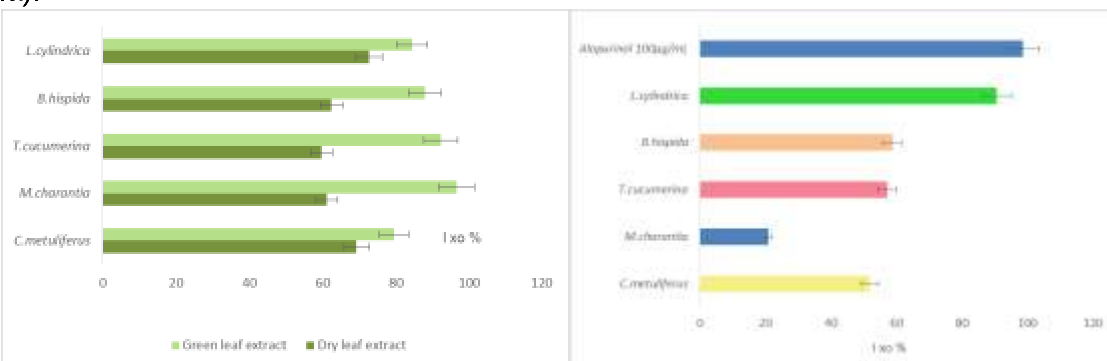
Figure 6.9. The variation of the antioxidant activity determined by the DPPH radical inhibition method during a) 15min, b) 30min, c) 45min, d) 1 hour, for the hydroalcoholic extracts from the fruits of the Cucurbitaceae family; values represent the mean of three separate experiments, error bars and standard deviation. Cm- *C. metuliferus*, Mc- *M. charantia*, Tc- *T. cucumerina*, Lc- *L. cylindrica* and Bh- *B. hispida*

6.6. Evaluation of the activity of the studied plant extracts on xanthine oxidase

Xanthine oxidase (XO) is an enzyme involved in purine metabolism, which catalyzes the oxidation of hypoxanthine and xanthine to uric acid, generating the production of reactive oxygen species (ROS).

6.6.2. Results and discussion

The evaluation of xanthine oxidase activity was carried out comparatively for the hydroethanolic extracts from fresh and dry leaves of the studied Cucurbitaceae species (Figure 6.18.a).



a) b)

Figure 6.18. Xanthine oxidase (XO) inhibition activity by the hydroethanolic extracts obtained from a) dried and fresh fruits and b) fruits of the studied Cucurbitaceae species; values represent the mean of three separate experiments, error bars and standard deviation

6.10. Partial conclusions

Following the biological studies carried out in this chapter, studies that analyzed the extracts obtained from the plant materials studied in this doctoral thesis, it can be stated that these plants have a high therapeutic potential, a potential argued from a scientific point of view but also from their use in traditional medicine, the results showing increased interest in modern alternative therapies as well.

Two methods of toxicity determination were used: MTT and the wheat germ toxicity test, and it was demonstrated that the extracts obtained from species of the Cucurbitaceae family do not show cytotoxicity. In studies to determine the viability of fibroblasts in the presence of the studied extracts, it was observed that after 24 hours of analysis cell viability increases, which means that over time a smaller number of healthy cells die, and the growth of wheat karyopses was not inhibited .

The antioxidant activity was evaluated by 6 different methods, and the results obtained by all methods were satisfactory and close to those in the specialized literature. Some of the results obtained in the methods for determining the antioxidant activity are presented for the first time for some of the analyzed plant species, therefore the data obtained were compared with other studies from the specialized literature only where possible.

The antioxidant activity determined in the case of combinations of fruit juices showed a higher value than the individual ones. It can be stated in this context that a synergy occurs in the mixtures obtained, these having an increased antioxidant effect, therefore, their consumption can prevent or reduce oxidative stress and can be exploited further in obtaining functional food products.

The most pronounced activity, evaluated by the molybdenum reduction method, was demonstrated by extracts from the fruits of C. metuliferus and B. hispida, which can be useful in obtaining plant-based treatments against oxidative stress and its subsequent effects generated in the body ..

Through the method of inhibiting the DPPH radical, all analyzed samples showed a strong antioxidant effect that is directly proportional to the concentration of the extracts, both from the leaves and from the fruits of the analyzed plant species (family Cucurbitaceae and Fabaceae).

The evaluation of the antioxidant potential by the ABTS radical inhibition method performed comparatively for two types of hydroethanolic and methanolic extracts showed varied values. Thus, the hydroethanolic samples from the fruits of L.cylindrica and T. cucumerina showed an increased inhibitory effect at low concentrations, compared to the other species, while the methanolic extracts were advantageous for all representatives, standing out bitter cucumber and kiwano. In the case of extracts, the one from the leaves of the species M. charantia was noted, being a potential source rich in antioxidants for obtaining teas and other food or pharmaceutical products.

The results obtained by the FRAP method for the samples from the fruits and leaves of the species studied in this thesis did not vary significantly, being within an IC50 range between 34.63 µM Eq Fe (II)/g and 45.37 µM Eq Fe (II) /g.

Extracts from the fruits of winter cucumber and snake cucumber showed the highest capacity to chelate metal ions (Fe^{2+}), and in the case of leaf extracts, those of the plant species L.cylindrica and C. metuliferus. Therefore, in the case of excess iron in the body, the analyzed plant species are important sources of useful compounds that can prevent the toxic effects on organs, cells or the triggering of lipid peroxidation that occur due to the accumulation of iron.

The anti-inflammatory activity of the plant samples was carried out by several methods, and the results varied for the plant species analyzed. M. charantia and C.sieberiana showed the highest potential in fighting inflammation. In the lipoxygenase inhibition tests, the extracts from the leaves of L. cylindrica proved to be more active, and in the albumin denaturation test, the extracts from the fruits of T. cucumerina.

It has been experimentally proven that fresh plant material has a greater potential to inhibit xanthine oxidase compared to dry, comparing extracts obtained from fresh and dried leaves of species of the Cucurbitaceae family in the study on xanthine oxidase activity.

Analysis of antidiabetic properties for plant materials by inhibiting the activity of two e representative enzymes, highlighted the species M. charantia and C. metuliferus that had greater capacity to inhibit α-amylase and β-glucoxidase.

The preliminary study on some gram-positive and gram-negative bacteria highlighted the antibacterial potential of the plant materials belonging to the species L. cylindrica, the other extracts from the analyzed plant species being less active against the tested strains of microorganisms.

Chapter 7. Encapsulation of some hydroethanolic extracts obtained from the studied plant species

Microencapsulation is a process by which an active material or principle, which can be an ingredient, a bioactive substance or a mixture, is encapsulated in a protective matrix in the form of microcapsules [558,559]. Studies on this topic are relatively new, interest grew only in the 7th and 8th decades of the 20th century. The first application of microcapsules was in the manufacture of carbonless paper in the stationery industry [560]. Today, due to the fact that microcapsules can be obtained with different properties, with specific characteristics, with a special appearance, color or taste and with a varied range of principles, they have applications in various fields such as the pharmaceutical, cosmetic, food, textile, agricultural or of new materials [561–563].

7.4. Characterization of microcapsules based on plant extracts

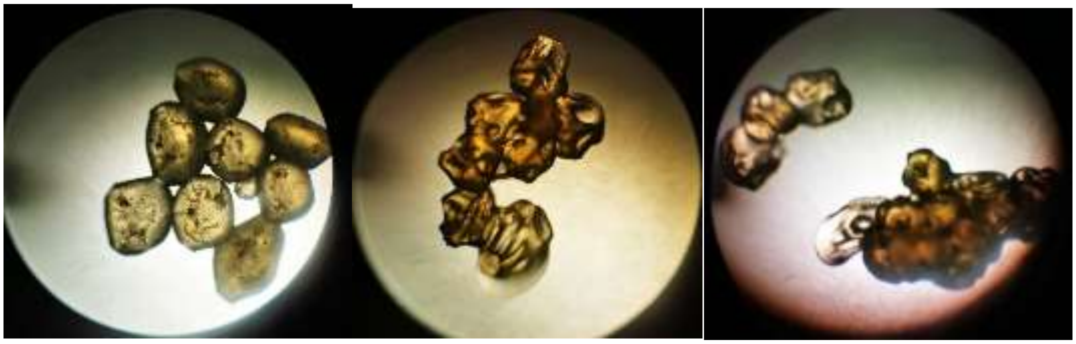
After obtaining the microcapsules, they can be characterized with the help of several techniques that can provide various information about the product obtained, such as shape, size, structure, size distribution, chemical composition, properties related to release, etc. Thus, an accessible method is optical microscopy (OM), which allows direct visualization of microcapsules and provides information on shape, size and surface appearance

A more advanced technique is scanning electron microscopy (SEM) which uses an electron beam to obtain high-resolution images of microcapsules. In this way, details of the shape, surface and structure of the capsules can be obtained and the morphology evaluated. SEM analysis is generally coupled with Energy Dispersive X-ray Spectroscopy (SEDX) for compositional analysis of samples.

Fourier transform infrared (FTIR) spectroscopy can be used to obtain absorption spectra that provide information on the chemical composition of the microcapsules. Thermal analysis such as thermogravimetric (TGA) and differential scanning (DSC) provides information on the thermal stability, moisture content and thermal behavior of microcapsules [573,574].

7.4.2. Results and discussion

From the images recorded under the optical microscope, it can be seen that microcapsules of different shapes and colors were obtained that do not resemble each other, although extracts with the same type of solvent, ethanol 70%, were used (Figure 7.3). The microcapsules of *B. hispida* and *T. cucumerina* indicate a more intense green color, the other species have microspheres colored in yellow-orange to brown, while in the control sample, only with alginate are slightly transparent white microspheres. The shape of these microsystems varies greatly, being very irregular in the case of the sample containing *L.cylindrica* extract, and those containing *C. metuliferus* and *M. charantia* extracts are similar, and those containing *B.hispida* extract are much rounder and without deformations.



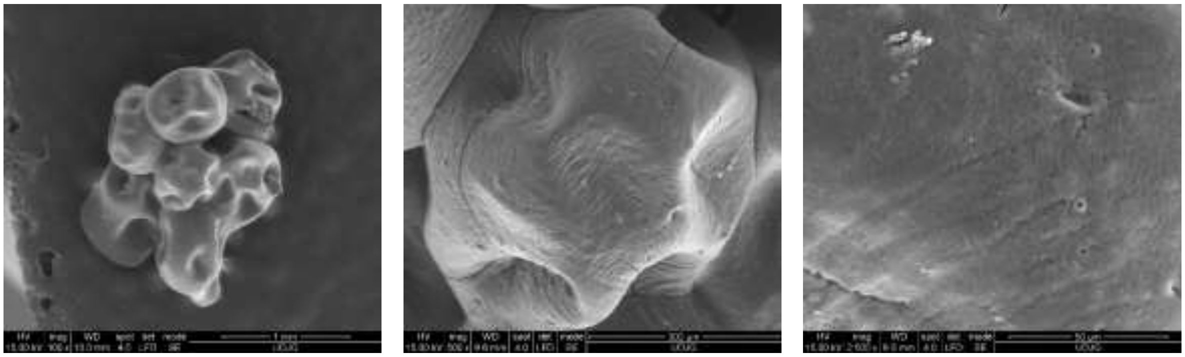
a. b. c.

d. e. f.

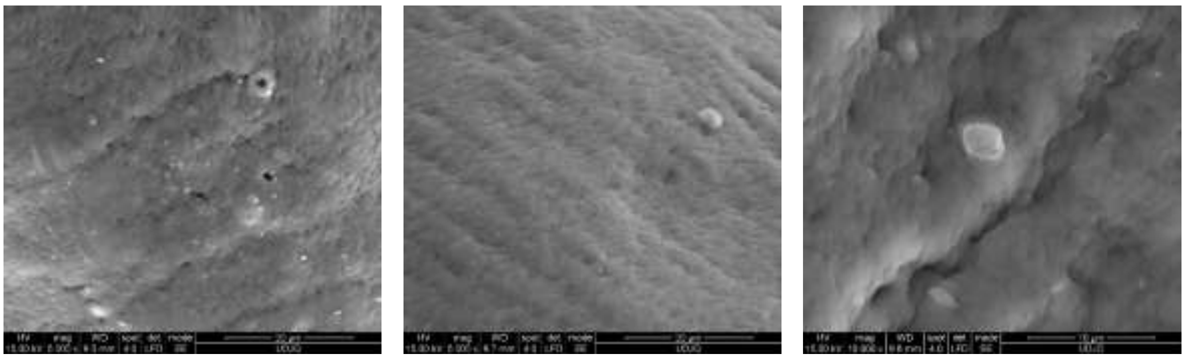
Figure 7.3. Images recorded with the optical microscope (OM) for the microcapsules containing the extracts of a. *B.hispida*; b. *C. metuliferus*; c. *M. charantia*; d. *T. cucumerina*; e. *L. cylindrica*; f. positive control

The SEM images (Figure 7.4) show the microcapsules obtained from the extracts of each analyzed species, images taken at different sizes. Thus, different irregular shapes can be observed in the case of the species C. metuliferus, T. cucumerina and L.cylindrica; and spherical for the samples of B.hispida and M. charantia, the emerging beam can only contain a local information (a 'pixel') from the image.

For microcapsules with C. metuliferus extract (Figure 7.4), several well-defined microspheres can be observed, with irregularities on the surface (Figure 7.4.a), with dimensions around 300 µm (Figure 7.4.b). At 5000X magnification, the surface of the microcapsule wall is observed with a regular appearance (eg Figure 7.4.d), in well-ordered polymer chains in rows, evident at 10000X magnification (Figure 7.4.e). Through EDX analysis, the composition of the chemical elements, in order, carbon, oxygen, chlorine, calcium, or nitrogen, present in microcapsules, in different percentages, at 5000X and 10000X magnification (Figure 7.5.e) were highlighted.



a) b) c)



d) e) f)

Figure 7.4. SEM images of microcapsules with C. metuliferus extract: a) 100X (1mm); b) 500X (300 µm); c) 2500X (50 µm); d) and e) 5000X (20 µm); f) 10000X (10 µm)

7.5. Partial conclusions

Microcapsules were obtained containing hydroethanolic extracts from the fruits of plant species with therapeutic properties adapted to growth and cultivation in Romania: M. charantia, C. metuliferus, B. hispida, T. cucumerina and L. cylindrica.

Microcapsules were obtained by external ionotropic gelation with alginate in the presence of calcium chloride solution.

The best encapsulation yield was obtained from the extracts obtained from the species C. metuliferus, 92.24% and T. Cucumerina, 94%, and the degree of encapsulation was 91.05% and 91.22%, respectively .

Through optical microscopy, images were obtained regarding the appearance of each type of microcapsule made, these indicating the appearance of round or slightly elongated microspheres, with some irregularities on the surface, depending on the species used in the process of obtaining the microcapsules.

With the help of scanning electron microscopy (SEM) images, images were obtained that confirm the appearance of microspheres, with more information regarding the morphology of the surfaces of the obtained microcapsules, but also the appearance of the microcapsule wall.

The EDX compositional analysis, coupled with the SEM technique, of the analyzed images provided valuable information about the presence of chemical elements in the microcapsules, such as carbon, oxygen, chlorine, boron, potassium, calcium and nitrogen, elements specific to the compounds in the plant products and the calcium alginate used when obtaining microcapsules.

Chapter 8. General conclusions and originality of the research activity

8.1. General conclusions and research originality

At the beginning of the research of this doctoral thesis, many more activities and research directions were proposed, but as is known, in research the unpredictable is always present, so it was possible to partially achieve the initial research directions and reorient to others, until the "finished" product was obtained, i.e. this doctoral study.

In the doctoral work entitled "*Study of chemical compounds from plant species of therapeutic interest*" research was carried out on five species adapted to growth and cultivation in Romania through collaboration with SCDL Buzău and the Buzău Genetic Resources Bank, as well as an African plant, all of therapeutic interest. The study was oriented towards several main directions of analysis of plant materials such as: identification, separation, isolation and characterization of biologically active compounds, evaluation of therapeutic potential and obtaining and characterization of a compound of importance for the medical/food field.

The interest in the study of the species *M.charantia, L.cylindrica* and *B.hispida* is due to the popularity of these species worldwide, being a current research topic and analyzed from interdisciplinary perspectives, while the species *C.siberiana, C.metuliferus* and *T. cucumerina* are less studied species, but which in recent years have proven interesting, both from the point of view of their chemical composition and their therapeutic potential. Thus, although the plants analyzed in this thesis seem different, they have many aspects in common.

The original scientific contribution made as a result of the studies and researches is presented synthetically in the following conclusions.

For the first time, the varieties *M.charantia* Rodeo, *L.cylindrica* Elida, *B.hispida* Zefir, *C.metuliferus* Tempus and *T.cucumerina* Felix, obtained through collaboration with the Bank of Plant Genetic Resources for Vegetable, Floriculture, Aromatic and Medicinal Plants, were analyzed. BRGVEG Buzău and the Research-Development Station for Vegetable Cultivation, SCDL Buzău, Romania.

Studies were carried out in collaboration with colleagues from Burkina Fasso, Africa on various anatomical parts of the plant C. sieberiana collected from the Hauts-Bassins region, from the forest of Dienderesso, Burkina Faso.

The plant species selected in the study from the families Cucurbitaceae and Fabaceae, show a valuable therapeutic potential, being demonstrated through extensive use in holistic medicine, as well as through laboratory studies and analyses, proving to have antibacterial, antioxidant, anti-inflammatory and antidiabetic properties. *M. charantia*, the most studied of the selected species, is particularly known for its antidiabetic properties. Trichosanthes cucumerina stands out for its benefits in the gastrointestinal tract, *B. hispida* for its antinociceptive action, *L. cylindrica* for its bronchodilator and hepatoprotective properties, and *C. metuliferus* for its antimalarial action. The species *C. sieberiana* stands out for its valuable therapeutic properties, being used in traditional African medicine.

The characterization of plant cells and tissues from the leaves and fruits of the studied species was carried out using CLSM microscopy.

Various conventional extraction methods have been used, such as decoction, maceration and Soxhlet extraction using different solvents, but also non-conventional ones such as ultrasound-assisted sequential extraction.

Sequential extraction assisted by ultrasound using solvents with different polarities, proved to be the best method for separating and isolating the compounds of interest for in vitro studies, extraction that allowed obtaining TEC values (total extractable components) within a range of 44.3 - 61.5%.

Primary and secondary metabolites from the classes of saponins, terpenoids and compound alkaloids that have a valuable therapeutic potential were highlighted in the analyzed plant species

Juices from the species *M.charantia* Rodeo, *C.metuliferus* Tempus, *B.hispida* Zefir and *T.cucumerina* Felix were obtained and analyzed for the first time, proving to be of great interest to the scientific world considering the number of citations of the results obtained .

For the first time, juices and extracts from various anatomical parts of some species of the Cucurbitaceae and Fabaceae families were studied comparatively by quantifying different primary and secondary metabolites such as saccharides, polyphenols, flavonoids, carotenoids, triterpenoids, vitamin C.

The highest concentration of total phenolic compounds was highlighted in the fruits of the species *C. metuliferus* (77.04 ± 0.45 mg EqAG/g SU) and in the leaves of *T. cucumerina* (58.55 ± 0.55 mg EqAG /g SU).

The highest content of β-carotene (103.65 ± 0.70 μg EqβC/g SU) was found in M. charantia, followed by C. metuliferus, the species *T. cucumerina* having the lowest concentration of β-carotene (3.75 ± 0.21 mg EqβC/g SU).

Vitamin C proved to be abundant in the leaves of the species studied, with the best values being highlighted for the extracts of *T. cucumerina* (19.65±0.01 mgAA/10 g SU) and *C. metuliferus* (16.86 ± 0 .06 mgAA/10 g SU).

The fruits of the studied species have a higher concentration of saccharides with values between 20 and 40 μg EqG/mL compared to the leaves of the same species, but with a lower content than other species of the Cucurbitaceae family presented in the literature.

A comparative analysis of the micro- and macroelements from the naturally dried and lyophilized plant material obtained from the fruits of the studied species was carried out and it was demonstrated that all the species have an increased potassium content, and the naturally dried samples are richer in elements, such as phosphorus, potassium , calcium, magnesium, sulfur, sodium, iron, manganese, boron, nickel, copper, selenium, molybdenum, cobalt, zinc, barium, chromium and aluminum.

Through the ICP/MS method, the presence of chemical elements with high values, such as potassium, calcium, magnesium and iron, was highlighted in all the analyzed species. The comparative study between dry and freeze-dried samples demonstrated that dry plant material better preserves the presence of micro- and macroelements in the samples.

Through the HPTLC chromatographic method, the main components of the class of saccharides, primary metabolites, present in the species of the Cucurbitaceae family, such as maltose, arabinose, deoxyribose, were highlighted.

By HPLC/DAD chromatography it was confirmed that the vast majority of species in the Cucurbitaceae family contain a large number of triterpenoids and significant concentrations of polyphenols and flavonoids. Chemical compounds such as shikimic acid, gallic acid, quercitin, cinnamic acid, ursolic and oleanoic acid present in both hydroethaolic extracts and juice samples of cucumber species were quantified.

Thus, in the juice of *M.charantia* and in that of *B.hispida*, gallic acid was identified in the highest concentration (3386.78 ± 1.60 mg/kg and 525.24 ± 0.80 mg/kg respectively), in that of *C.*

metuliferus catechin 928.74 ± 0.97 mg/kg, and in T.cucumerina the oleanic acid (352.79 ± 7.97 mg/kg).

By using the UHPLC/MS method of the extracts from the fruits and leaves of the species of the Cucurbitaceae family, compounds specific to these plants were highlighted, such as cucurbitacins A, E, C, D, luteolin, guguacin, etc.

The lipophilic part of the fruits of *M. charantia, T. cucumerina* and *C. metuliferus* was analyzed by GC/MS chromatography, the structure being demonstrated by the fragmentation mechanisms proposed for identified compounds such as: linalool, n-heneicosan, ocimene, 2, 6-tritertbutyl-phenol.

Separation and isolation by two different methods of ursolic acid as a primary compound in the fruits of *C. metuliferus* and *B.hispida* species. The pure compound obtained was characterized by FT-IR, UHPLC/MS and NMR.

Different biologically active properties of the studied species were studied by evaluating cytotoxicity, antioxidant and anti-inflammatory activity.

The studies carried out to prove the antioxidant properties of the obtained extracts were carried out by 6 different methods, the DPPH and ABTS free radical inhibition methods, the FRAP method and the evaluation of the chelation capacity of metal ions, for some of the analyzed plant species being carried out for the first time given in this thesis

The antioxidant activity determined in the case of combinations of fruit juices showed a higher value than the individual ones. The antioxidant activity by inhibiting the DPPH radical of the juice combinations highlighted the synergistic effect of the *M.charantia* species on the other species, as well as the chelation activity of the metal ions. The same synergistic effect was also proven in the case of the combination of kiwano and snake cucumber.

It can be stated in this context that there is a synergy in the obtained mixtures having an increased antioxidant effect, therefore, their consumption can prevent or reduce oxidative stress and they can be exploited further in obtaining functional food products.

The most pronounced activity, evaluated by the molybdenum reduction method, was demonstrated by extracts from the fruits of *C. metuliferus* and *B. hispida*, which can be useful in obtaining plant-based treatments against oxidative stress and its subsequent effects generated in the body.Through the method of inhibiting the DPPH radical, all analyzed samples showed a strong antioxidant effect that is directly proportional to the concentration of the extracts, both from the leaves and from the fruits of the analyzed plant species (family Cucurbitaceae and Fabaceae).

The evaluation of the antioxidant potential by the ABTS radical inhibition method performed comparatively for two types of hydroethanolic and methanolic extracts showed varied values. Thus, the hydroethanolic samples from the fruits of *L. cylindrica* and *T. cucumerina* showed an increased inhibitory effect at low concentrations, compared to the other species, while the methanolic extracts were advantageous for all representatives, standing out bitter cucumber and kiwano. In the case of extracts, the one from the leaves of the species *M. charantia* was noted, being a potential source rich in antioxidants for obtaining teas and other food or pharmaceutical products.

The results obtained by the FRAP method for the samples from the fruits and leaves of the species studied in this thesis did not vary significantly, being within an IC50 range between 34.63 µM Eq Fe (II)/g and 45.37 µM Eq Fe (II) /g.

The highest chelation capacity of metal ions (Fe^{2+}) was demonstrated by the extracts from the fruits of the winter cucumber and the snake cucumber, and in the case of the extracts from the leaves of the species r of *L.cylindrica* and *C. metuliferus* plants. Therefore, in the case of excess iron in the body, the analyzed plant species are important sources of useful compounds that can prevent the toxic effects on organs, cells or the triggering of lipid peroxidation that occur due to the accumulation of iron.

Two methods of toxicity determination were used: MTT and the wheat germ toxicity test, and it was demonstrated that the extracts obtained from species of the Cucurbitaceae family do not show cytotoxicity.

In studies to determine the viability of fibroblasts in the presence of the studied extracts, it was observed that after the 24 hours of analysis the cell viability increases, which means that a smaller number of healthy cells die over time.

The anti-inflammatory activity of the plant samples was carried out by several methods, and the results varied for the plant species analyzed. *M. charantia* and *C.sieberiana* showed the highest potential in fighting inflammation. In the lipoxygenase inhibition tests, the extracts from the leaves of *L. cylindrica* proved to be more active, and in the albumin denaturation test, the extracts from the fruits of *T. cucumerina*.

It has been experimentally proven that fresh plant material has a greater potential to inhibit xanthine oxidase compared to dry.

The analysis of the antidiabetic properties of plant materials by inhibiting the activity of two representative enzymes, highlighted the species *M. charantia* and *C. metuliferus* that had a greater capacity to inhibit α-amylase and β-glucooxidase.

The preliminary study on gram-positive and gram-negative bacteria highlighted the antibacterial potential of the *L. cylindrica* species, the other extracts from the analyzed plant species being less active against the tested strains of microorganisms.

Microcapsules were obtained containing hydroethanolic extracts from the fruits of plant species with therapeutic properties adapted to growth and cultivation in Romania: *M.charantia, C.metuliferus, B.hispida, T. cucumerina* and *L.cylindrica*.

Microcapsules were obtained by external ionotropic gelation with alginate in the presence of calcium chloride solution.

The best encapsulation yield was obtained from the extracts obtained from the species *C. metuliferus*, 92.24% and *T. cucumerina,* 94%, and the degree of encapsulation was 91.05% and 91.22%, respectively .

Through optical microscopy, images were obtained regarding the appearance of each type of microcapsule made, these indicating the appearance of round or slightly elongated microspheres, with some irregularities on the surface, depending on the species used in the process of obtaining the microcapsules.

By scanning electron microscopy (SEM), images were obtained that confirm the appearance of microspheres, with more information on the morphology of the surfaces of the microcapsules obtained, but also on the appearance of the microcapsules wall.

The EDX compositional analysis, coupled with the SEM technique, of the analyzed images provided valuable information about the presence of chemical elements in the microcapsules, such as carbon, oxygen, chlorine, boron, potassium, calcium and nitrogen, elements specific to the compounds in the plant products and the calcium alginate used when obtaining microcapsules.

8.2. Future research directions

The results obtained in the studies of this PhD study, which focused on six plant species*: M. charantia, C. metuliferus, B. hispida, T. cucumerina, L. cylindrica* and *C. siberiana*, generated new research perspectives which propose the detailed exploitation of some directions that have not been exploited to the maximum.

Thus, further research will have as major research directions the isolation and separation of new molecules, specific to the analyzed plant species and their analysis in detail, through in vitro, in vivo and in silico studies.

Obtaining by various separation techniques new pure compounds from the species studied, especially the continuation of the study on the species *C. metuliferus*.

Carrying out in silico studies of compounds isolated from the species *C. metuliferus and B. hispida* with the aim of highlighting certain properties such as anti-inflammatory and antidiabetic and with the idea of elucidating their mechanism of action.

Carrying out in vitro and in vivo studies for the isolated molecules but also for the most biologically active extracts to highlight the cicatrizing activity, with the aim of obtaining a product with this action.

More in-depth physico-chemical characterization of the microcapsules encapsulating compounds from these plants and in vitro evaluation of antioxidant, anti-inflammatory and antimicrobial potential; but also the release of compounds of therapeutic interest through the in vitro digestion method.

Obtaining new microcapsules by different techniques, using other materials for encapsulation with the aim of selecting the optimal method of obtaining microcapsules, which would be safer and with increased therapeutic potential and more economically advantageous.

Carrying out studies to identify the antiviral and antitumor properties of fruit and leaf extracts of the species studied in this study.

The realization of collaborations and new projects with interdisciplinary connection that will aim to promote the studied species on the Romanian market, this being possible by disseminating the research results from the thesis and bringing to the attention of the scientific community but also the general public, i.e. the consumer, the phytochemical composition and properties biologically active of these species with the aim of promoting and valorizing these species from a therapeutic and food point of view.

The capitalization of the results and the business plan made during the doctoral studies within the project "Academic excellence and entrepreneurial values - scholarship system to ensure opportunities for training and development of the entrepreneurial skills of doctoral students and post-doctoral students" - ANTREPRENORDOC, Contract no. 36355/23.05.2019 POCU/380/6/13 - SMIS code: 123847 based on the studies in the doctoral thesis.

Chapter 9. Dissemination of results

9.1. List of original publications

List of original and collaborative publications (Cumulative Impact Factor= 38.675)

1. Anna Cazanevscaia Busuioc, Andreea-Veronica Dediu Botezatu, Bianca Furdui, Costel Vinatoru, Filippo Maggi, Giovanni Caprioli, Rodica-Mihaela Dinica; "Comparative Study of the Chemical Compositions and Antioxidant Activities of Fresh Juices from Romanian Cucurbitaceae Varieties" Molecules 2020, 25, doi:10.3390/molecules25225468, F.I.=4,927; (37 citations).
2. Eliasse Zongo, Anna Busuioc*, Roland Nâg-Tiero Meda, Andreea Veronica Botezatu, Maria Daniela Mihaila, Ana-Maria Mocanu, Sorin Marius Avramescu, Benjamin Kouliga Koama, Sami Eric Kam, Hadidiatou Belem, Franck Le Sage Somda, Clarisse Ouedraogo , Georges Anicet Ouedraogo, Rodica Mihaela Dinica "Exploration of the Antioxidant and Anti-Inflammatory Potential of *Cassia Sieberiana* DC and *Piliostigma Thonningii (Schumach.*) Milne-Redh, Traditionally Used in the Treatment of Hepatitis in the Hauts-Bassins Region of Burkina Faso" Pharmaceuticals 2023, 16, 133, doi:10.3390/ph16010133, F.I.=5.215 (2 citations).
3. Anna Cazanevscaia Busuioc, Giorgiana Valentina Costea, Andreea Veronica Dediu Botezatu, Bianca Furdui,* and Rodica Mihaela Dinica*; "*Cucumis metuliferus* L. Fruits Extract with Antioxidant, Anti-Inflammatory, and Antidiabetic Properties as Source of Ursolic Acid" Separations 2023, 10, 274. https://doi.org/10.3390/separations10050274, F.I.=3,344.
4. Bălănescu Fănică, Andreea Veronica Botezatu, Fernanda Marques, Anna Busuioc, Olivian Marincaş, Costel Vînătoru, Geta Cârâc, Bianca Furdui, and Rodica Mihaela Dinica "Bridging the Chemical Profile and Biological Activities of a New Variety of *Agastache Foeniculum* (Pursh) Kuntze Extracts and Essential Oil", Int. J. Mol. Sci. 2023, 24, 828, doi:10.3390/ijms24010828, F.I.=6,208 (1 citation).
5. Balanescu Fanica, Anna Cazanevscaia Busuioc, Andreea Veronica Dediu Botezatu,Steluta Gosav, Sorin Marius Avramescu, Bianca Furdui, and Rodica Mihaela Dinica, "Comparative Study of Natural Antioxidants from *Glycine Max, Anethum Graveolens* and *Pimpinella Anisum* Seed and Sprout Extracts Obtained by Ultrasound-Assisted Extraction", Separations 2022, 9, 152, doi:10.3390/separations9060152, F.I.=3.344; (8 citations).
6. Dinica Rodica-Mihaela, Sandu Cristina, Botezatu Dediu Andreea Veronica, Basil Anna Cazanevscaia, Balanescu Fanica, Ionica Mihaila Maria Daniela, Dumitru Caterina Nela,Furdui Bianca, Iancu Alina Viorica-" Allantoin from Valiable Romanian Animal and Plant Sources with Promising Anti-inflammatory Activity as a Nutricosmetic Ingredient", Sustainability, 2021, Volume 13, Issue 18, Article Number 10170, DOI 10.3390/su131810170, F.I.= 3,889; (12 citations).
7. Kevine Kamga Silihe, Stephane Zingue, Marius Trésor Kemegne Sipping, Anna Busuioc Cazanevscaia, Andreea Veronica Dediu Botezatu, Dieudonne Njamen, Rodica Mihaela Dinica;" The Antioxidant Potential of *Ficus Umbellata Vahl* (Moraceae) That Accelerates In Vitro and the In Vivo Anti-Inflammatory Protective Effects” Appl. Sci. 2022, 12, 9070. https://doi.org/10.3390/app12189070, F.I. =2.838.

8. Sara El Moujahed, Rodica Mihaela Dinica, Hicham Abou Oualid, Mihaela Cudalbeanu, Andreea-Veronica Botezatu-Dediu, Anna Cazanevscaia Busuioc, Fouad Ouazzani Chahdi, Youssef Kandri Rodi, Faouzi Errachidi "Sustainable biomass as green and efficient crosslinkers of collagen: Case of by-products from six pomegranate varieties with global commercial interest in Morocco" Journal of Environmental Management, Volume 335, 1 June 2023, 117613, https://doi.org/10.1016/j.jenvman.2023.117613, F.I. =8.91

9.2. Participation in projects, summer schools and conferences

Projects:

- Directory: PN-III-P1-1.1-MC-2019-1767 – Mobility projects for researchers, UEFISCDI, 2019;
- Mobility within the EXPERT project at the University of Bucharest, Faculty of Chemistry, Department of Organic Chemistry, February 2020;
- Participant in the project "PROACTIVE HEALTH WITHOUT BORDERS, Joint Operational Program Romania-Republic of Moldova 2014-2020 - ENI -2nd Call for proposals", 2021 GALAțI;
- Participant in the Academic Excellence and Entrepreneurial Values project - scholarship system to ensure opportunities for training and development of entrepreneurial skills of doctoral students and post-doctoral students - ANTREPRENORDOC, Contract no. 36355/23.05.2019 POCU/380/6/13 - SMIS code: 123847- 2022, GALAțI.

Prizes:

- Third Prize - within the 2018 SCDS-UDJG Doctoral School Conference, Galati,
- Third Prize - oral presentation at the 2019 SCDS-UDJG Doctoral School Conference, Galati,
- III Prize - poster at the 2019 SCDS-UDJG Doctoral School Conference, Galati,
- III Prize - within the SCDS-UDJG Doctoral School Conference 2020, Galati
- Premiul Award-The most Active Team of Students în cadrul International Summer School „Food Safety and Healthy Living” FSHL-2020;
- Premiul I- în cadrul Conferinței Școlii Doctorale SCDS-UDJG 2021;
- Premiul II- în cadrul Conferinței Școlii Doctorale SCDS-UDJG 2021;
- Premiul I- în cadrul Conferinței Școlii Doctorale SCDS-UDJG 2022;
- Premiul I- în cadrul Conferinței Școlii Doctorale SCDS-UDJG 2023;
- Mențiune- în cadrul Conferinței Școlii Doctorale SCDS-UDJG 2023;

Premierea rezultatelorcetării UEFISCDI :

- PN-III-P1-1.1- PRECISI-2021- 56862

Conference

• International Conference "10ème édition du Colloque Franco-Roumain de Chimie Appliquée -COFrRoCA June, 2018";

• Scientific Conference of SCDS-UDJG Doctoral Schools, 6th June 2018 Edition, GALAțI;

• GALMED National Congress with International Participation, VIIIth Edition March 2018, GALAțI;

• IasiCHEM 2018 Conference, Chemistry Faculty Days "Alexandru Ioan Cuza University" Iași, 2018;

• International Conference "Conférence Internationale des Sciences de la VIE et des Technologies pour le BIEN-ÊTRE, LIFE 2018", Cluj - Napoca, Romania

• Scientific Conference of SCDS-UDJG Doctoral Schools, 7th Edition 2019, GALAțI;

• IUPAC International Chemistry Congress, Paris, 2019;

• Conference "Utilizzo dei prodotti di scarto della canapa industriale come fonte di biopesticidi", University of Camerino (UNICAM), Italy 2019;

• Scientific Conference of SCDS-UDJG Doctoral Schools, 8th Edition 2020, GALAțI;

• Conference "Food safety - a shared responsibility" July 2020;

• Advances in Food Chemistry Conference, AdFoodChem, April 2021;

• International Conference "INTELLIGENT VALORISATION OF AGRO-INDUSTRIAL WASTES", October 2021 Chisinau;

• "Young Researchers' International Conference on Chemistry and Chemical Engineering-YRICCCE III", Cluj-Napoca 2021;

• Scientific Conference of SCDS-UDJG Doctoral Schools, IXth Edition 2021, GALAțI;

• EuroAliment 2021 conference, GALAțI;

• 17th International Conference "Students for Students", 2021;

• International Conference "European Integration - Realities and Perspectives", 16th Edition, 2021, GALAțI;

• "11th International Conference The Danube - Axis of European Identity" 11th edition, June, 2021 Galați;

• "International Conference On Contemporary Scientific and Technological Aspects Towards An Entrepreneurial Approach" 1st EDITION, 2022, GALAțI;

• EUROINVENT, 14th edition, 2022, Iasi;

• Scientific Conference of SCDS-UDJG Doctoral Schools, 10th Edition 2022, GALAțI;

• National Chemistry Conference XXXVIth edition, Călimănesti-Căciulata, 2022;

• SCDS-UDJG Doctoral Schools Scientific Conference, XIth Edition 2023, GALAțI.

Summer schools:

• Participation in the International Summer School "Food Safety and Healthy Living" FSHL-2020 July 2020;

• Participation in the International Summer School "CHEMISTRY for Everyday Life" Camerino, Italy, September 2020

Courses:

- English for Scientific and Engineering Purposes, 2018
- Academic writing (academic writing) in the technical field and fundamental sciences. Ethics in scientific research and management of research projects" 2018
- Advanced methods of experimental data processing and analysis" 2018

- Instrumental separation and identification techniques and data analysis (chromatography, NMR, MS, IR, electroanalysis, multivariate analysis)" 2019
    - "Working practices regarding the creation and publication of scientific works II - In the technical field", Galați, May 2019
    - "Editing and publishing scientific articles" DFCTT, Galați, June 2019
    - "From B cells to yeast-diverse approaches to rapid discovery of COVID-19 therapeutic antibodies" August 2020
    - "Disease and the Hippo Pathway: Cellular and Molecular Mechanisms" July 2020
    - "Sewage Screening as an Early Outbreak Alert Tool and SARS-Covid-2 Fate in waters "July 9, 2020
    - "Catalysts / Recent Advances in Biocatalysis for Biomanufacturing" June 2020
    - "Nutrients/ Diet and CKD: Old and New Concepts" July 2020
    - "Strategies to maximize LC-MS/MS uptime and efficiency", September 2020
    - "Modernizing the analyzes of naturally occurring toxins in food and water" September 2020
    - "Dioxin Session 1 | Moving away from magnetic sectors for legacy POP's analyses" September 2020
    - "Dioxin Session 2 | Advances in instrumentation for emerging POP's analyses" September 22, 2020
    - "Innovation Lifecycle Insights" December 2020
    - "Functional ingredients, obtaining and characterization" 2020;
    - "Web of Science for beginners" January 2021
    - "Funding data in Web of Science" January 2021
    - "Choosing a different Carrier Gas in GC" March 2021
    - "Improve your researcher profile" March 2021
    - "Basic of LPGC/Vacuum GC for fastest methods using the MS detector" April 2021
    - "Basics in liquid chromatography" April 2021
    - "Faster method development and optimization in (U)HPLC) by choosing a suitable column type" May 2021
    - "HILIC-Basic and new methods and applications for the analysis of polar compounds" May 2021
    - "Build a Rock-Solid Analytical Foundation with Restek CRMs" June 2021
    - "Our favorite productivity tools for staying ahead in LC-MS/MS food testing workflows" June 2021.

Contests:

• FameLab Romania national final - Grand Final 2019, organized by the British Council;
• Grand Prize - BluAct competition in Galati, Romania 2020.