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EURO-PLANT-ACT



## CURRICULUM

Cooperation to implement innovative methods for the assessment of medicinal plants with central roles in pharmaceuticals, agriculture and nutrition

EURO-PLANT-ACT  
Erasmus+ Project – Partnership for Cooperation  
Project Code 2022-1-RO01-KA220-HED-000088958

Eds. Dehelean Cristina Adriana, Pinzaru Iulia Andreea



**„Victor Babeş” Publishing House**

Eftimie Murgu Square 2, room 316, 300041 Timișoara

Tel./ Fax 0256 495 210

e-mail: [evb@umft.ro](mailto:evb@umft.ro)

[www.umft.ro/editura](http://www.umft.ro/editura)

**General Director: Prof. dr. Sorin Ursoniu**

**GUIDES Collection**

**Collection Coordinator: Prof. dr. Adrian Vlad**

**Scientific Referent: Prof. dr. Daliborca Vlad**

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## CURRICULUM DESCRIPTION

**Project title:** Cooperation to Implement Innovative Methods for the Assessment of Medicinal Plants with Central Roles in Pharmaceuticals, Agriculture and Nutrition

**Project acronym:** EURO-PLANT-ACT

**Project no:** 2022-1-RO01-KA220-HED-000088958

The curriculum is addressed to specialists in fields such as agriculture, food industry, nutrition, pharmacy and is intended to provide various specialist information including the cultivation and harvesting techniques of medicinal plants, agro-biotechnological methods for the characterization of medicinal plants to promote and maintain a resource valuable ecological, methods of obtaining finished plant-based products (extracts, essential oils), but also to develop new formulas and innovative food supplements for human health in the current process of climate change and globalization.

### Professors responsible for theoretical parts:

Coordinator (UMFVBT): Dehelean Cristina Adriana, Pînzaru Iulia Andreea, Macașoi Ioana Gabriela, Șoica Codruța Marinela

Partner 1 (USVT): Alexa Ersilia, Negrea Monica, Cocan Ileana, Obistoiu Diana, Pop Georgeta

Partner 2 (UNIOS): Vrandečić Karolina, Ćosić Jasenka, Baličević Renata, Ravlić Marija

Partner 3 (UNICAL): Conforti Filomena, Statti Giancarlo

Partner 4 (ROMPAN): Voica Daniela, Avram Dana

### Professors responsible for practical parts:

Coordinator (UMFVBT): Dehelean Cristina Adriana, Pînzaru Iulia Andreea, Minda Daliana Ionela, Drăghici George Andrei

Partner 1 (USVT): Alexa Ersilia, Negrea Monica, Cocan Ileana, Obistoiu Diana, Pop Georgeta

Partner 2 (UNIOS): Vrandečić Karolina, Ćosić Jasenka, Baličević Renata, Ravlić Marija

Partner 3 (UNICAL): Conforti Filomena, Statti Giancarlo

Partner 4 (ROMPAN): Voica Daniela, Avram Dana

<b>Objectives</b>	The curriculum is proposed to establish the cognitive and professional skills necessary for the management of ecological medicinal plants with a central role in nutrition, pharmacy and agriculture. This curriculum will provide the technical and scientific knowledge regarding cultivation, selection, botanical and physico-chemical characterization of plant material, product preparation (extracts, essential oils), evaluation (pharmaco-toxicological profile) and valorization (nutrition, food industry, food supplements) them, information that will be presented to the trainers.
<b>Cognitive skills</b>	Cognitive skills necessary to deepen and use specialized scientific knowledge in the fields of agriculture, pharmacy and nutrition for the analysis and evaluation of medicinal plants with a central role in the specified fields. The ability to select technical and scientific knowledge regarding cultivation, selection, botanical and physico-chemical characterization of plant material, preparation of products (extracts, essential oils), evaluation



	(pharmaco-toxicological profile) and valorization (nutrition, food industry, food supplements).
<b>Professional skills</b>	<p>The ability to perform the botanical characterization and use of medicinal plants through the lens of analyzing the herbicidal effects of plant extracts and essential oils.</p> <p>The ability to analyze plant diseases in medicinal production and evaluate the antifungal activity of essential oils in agriculture.</p> <p>The ability to establish the agrotechnical conditions, cultivation, harvesting and storage of medicinal plants.</p> <p>The ability to analyze medicinal plants with proven efficacy against medical pathogenic bacterial strains and to evaluate the activity of medicinal plants against pathogenic bacteria prevalent in the food industry.</p> <p>Ability to know in detail the use of medicinal plants as value-added ingredients in the functional bakery and pastry, meat and dairy industries.</p> <p>The ability to analyze the pharmacological action and awareness of the health effects exerted by natural products derived from medicinal plants and to study medicinal plants and dietary reference values.</p> <p>The ability to know specific aspects related to the preparation of plant products (extracts, essential oils), phytochemical characterization and the influence of geolocation on the composition of phytocomplexes.</p> <p>Ability to analyze current safety issues of novel foods and nutrient sources (interactions between supplements/foods and drugs) and substances in dietary supplements (placed between efficacy and toxicity - plants and plant extracts).</p>
<b>Competence units</b>	<p>Knowledge of plant species and medicinal plant products (plant extracts, essential oils) with a role in agriculture, pharmacy, nutrition.</p> <p>Obtaining and characterizing medicinal plant products with a role in agriculture, pharmacy and nutrition.</p> <p>Study of functional bakery and pastry, meat and dairy products in which medicinal plants and derived products can be used as value-added ingredients.</p>
<b>Elements of innovation</b>	<p>The innovative elements are related to the interdisciplinarity and complementarity of the skills that are foreseen for implementation among students and young researchers. These elements refer to: (i) botanical characterization of medicinal plants, their use and derived products in an organic manner in agriculture based on the herbicidal, antimicrobial and antifungal effects they possess; (ii) the use of medicinal plants as value-added ingredients in the baking and pastry, meat and dairy industries; (iii) evaluation of the pharmacological action and health effects of medicinal plant products and (iv) safety analysis of the use of medicinal plants and herbal products.</p>
<b>The impact</b>	<p>The impact of this curriculum counts in the interdisciplinary, complementary and translational skills that will be acquired by students and young researchers and also in the ability to implement and use innovative techniques and methods in the management of the use of medicinal products and derived products (for example plant extracts, essential oils).</p> <p>An exhaustive knowledge in the field of medicinal plants (and their products - extracts and essential oils) with roles in pharmaceuticals, nutrition and agriculture, by combining the expertise of partners in scientific fields, such as agronomic practices (good agricultural practices in terms of cultivation,</p>



	<p>harvesting ), biological/botanical control and authentication of medicinal plants, determination of phytochemical content, biological and toxicological screening and utilization of plant products in the food industry. In addition, several strategies and methodologies to reduce the impact of climate change on medicinal plants are addressed along with conservation and sustainability measures.</p> <p>The anticipated short-term impact through the implementation of the knowledge and data presented refers to the increase in the degree of knowledge and professional training based on the development of study and experimental skills of students from all study cycles (bachelor, master and doctorate) and young researchers.</p> <p>The long-term impact is associated with the transfer of knowledge in the socio-economic environment, the development of a market segment of real interest today (medicinal plants and derived products), the analysis and safety of products based on medicinal plants or containing products derived from medicinal plants, awareness of the correct use of medicinal plants in the fields of agriculture, pharmacy and nutrition.</p> <p>Therefore, it will be created: (i) a more modern, dynamic and professional environment within the participating organizations ready to integrate new best practices and methods; (ii) a synergy with partners on the profile market; (iii) strategic planning of staff professional development in accordance with individual needs and organizational objectives; (iv) an increased capacity for professionalism needed to work at European/international level.</p> <p>Each university involved in the program intends to train qualified young people who: can develop the spirit of initiative and entrepreneurship, emphasize digital skills and the use of digital resources in learning, training. Also, to improve their professional integration skills and the creation of new enterprises (including social enterprises), to have a more active participation in society. To contribute to a better understanding and recognition of skills and qualifications in Europe and beyond.</p>
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**Activities hours - curriculum**

Total hours	Theoretical	Practical	Individual study
<b>80</b>	<b>40</b>	<b>20</b>	<b>20</b>

**Curriculum EURO-PLANT-ACT**

**THEORETICAL PART**

Chapter 1. Medicinal plants - current issues related to their role in agriculture, pharmacy and nutrition (Dehelean CA, Macașoi IG, Pînzaru IA)

Chapter 2. Botanical characterisation and use of medicinal plants (Baličević R, Ravlić M)

Chapter 3. Herbicidal effect of plant extract and essential oils (Ravlić M, Baličević R)

Chapter 4. Plant diseases in medicinal production (Ćosić J, Vrandečić K)

Chapter 5. Antifungal activity of essential oils in agriculture (Vrandečić K, Ćosić J)

Chapter 6. Agrotechnical conditions, cultivation, harvesting and storage of medicinal plants (Pop G, Obistioiu D)

Chapter 7. Medicinal plants with proven effectiveness against medical pathogenic bacterial strains (Obistioiu D, Pop G, Voica D, Avram D)

Chapter 8. The activity of medicinal plants against pathogenic bacteria prevalent in the food industry (Negrea M, Cocan I, Alexa E, Obistioiu D, Voica D, Avram D)

Chapter 9. The use of medicinal plants as value-added ingredients in the industry of functional bakery and pastry products (Alexa E, Voica D, Negrea M, Cocan I, Avram D)

Chapter 10. The use of medicinal plants as value-added ingredients in the industry of meat and dairy products (Cocan I, Negrea M, Alexa E, Obistioiu D, Voica D, Avram D)

Chapter 11. Pharmacological action and effects on health exerted by natural products derived from medicinal plants (Dehelean CA, Soica CM, Pînzaru IA)

Chapter 12. Medicinal plants and dietary reference values (Dehelean CA, Macașoi IG, Pînzaru IA)

Chapter 13. Current issues in the safety of novel foods and nutrient sources - interactions between supplements/foods and drugs (Conforti F, Statti G)

Chapter 14. Products preparation from plants (extracts, essential oils), phytochemical characterization and influence of geolocation on the composition of phytocomplexes (Conforti F, Statti G)

Chapter 15. Substances in food supplements between efficacy and toxicity - plants and plant extracts (Pînzaru IA, Macașoi IG, Dehelean CA)

Evaluation

No of hours	Observations
6	Technical and scientific part consists in 8 hours x 5 days. In total 40 hours
2	
2	
2	
2	
2	
2	
2	
2	
2	
2	
2	
2	
2	
2	



### **PRACTICAL PART**

*All the practical activities provided will be carried out in the specialized laboratories of the partner universities.*

*These activities involve the realization of innovative methods that involve:*

- (i) selection, cultivation, harvesting and characterization of organic medicinal plants,*
- (ii) evaluation of chemical composition and analysis of pharmacological properties,*
- (iii) establishing the pharmacokinetic safety profile and*
- (iv) methods of valorization of medicinal plants and products derived from them (extracts, essential oils)*

*Assessment methods: Student assessment is carried out through usual methods, namely: assessment based on the principles of double or multiple choice, assessment based on short answers, complementary or structured questions in which students intervene with solutions and/or the creation of essay topics*

*Competence certification assessment: it is carried out by using tools and methods developed in accordance with the provisions related to cognitive and professional skills, taking into account both the performance criteria and the conditions of applicability. These tools and methods are also based on the integrated assessment of different skills or various performance criteria that belong to a specific skill or different skills. The assessment emphasizes the extent to which students acquire practical skills related to the selection, cultivation, harvesting and characterization of organic medicinal plants; evaluation of chemical composition and analysis of pharmacological properties; establishing the pharmacokinetic safety profile and methods of valorization of medicinal plants and products derived from them (extracts, essential oils)*

*Study and research materials: Stimulating the participation and involvement of students and young researchers in life-long learning, during the implementation of the project but also afterwards, effective teaching-learning methods are used and adapted to the new methods that are constantly changing, used at the international level. Therefore, all programs are clearly structured, developed in four languages (English, Romanian, Italian and Croatian), related to each country within the partnership*

No of hours	Observations
	Practical part consists in 5 hours x 4 days. In total x hours
5	
5	
5	
5	





## EXTENDED CURRICULUM

### **Chapter 1. Medicinal Plants - current issues related to their role in agriculture, pharmacy, and nutrition (Dehelean CA, Macașoi IG, Pînzaru IA)**

Over the course of human history, medicinal plants have played an important role in the development of mankind, responding to basic needs such as medicine, food, fertilizers, etc. [Dar et al. 2017]. Considering the fact that there are more than half a million plants in the world, many of them unexplored, the future of medicinal plants is promising, both in the medicinal field, as well as in the nutritional and agricultural fields [Mathur and Hoskins 2017].

Over the past two decades, the agricultural field has experienced numerous major changes in terms of energy requirements and technology. Currently, the continuous growth of the population has led to a lack of food security, taking into account the limited amount of agricultural land available. It is estimated that the demand for food will increase by 70% by the year 2050. Current agricultural practices can only fulfil this need if chemical pesticides are used, which have detrimental effects on human health and on the environment [Priyanka et al. 2020]. A special focus has been placed on sustainable agriculture in recent years [Dordas 2008]. Plant microbiomes play an important role in sustainable agriculture, contributing to the growth of plants and soil fertility. The microbiome is responsible for regulating plant growth through either direct or indirect mechanisms, such as the release of growth regulators, biological nitrogen fixation, or by antagonizing pathogenic microbes [Yadav 2020]. Aside from this, natural compounds may also be used to control insect pests and weeds. Consequently, the study of plant compounds can contribute to the development of new agronomic strategies that can reduce the harm caused to human health and to the environment by using sustainable practices. Additionally, natural compounds have the advantage of requiring fewer regulatory controls for registration than synthetic compounds, which in turn reduces marketing costs [Petroski and Stanley 2009].

Nicotine belongs to the pyridine alkaloids family. It is used in agriculture as hydrochloride or sulphate salts, which are extremely effective against aphids, but are also extremely toxic to pets and people [Badhane et al. 2021]. Caffeine has been approved both as a food additive and for use in agriculture, where it proved to be useful as a poison against snails and slugs, while not causing adverse effects on human health [Hollingsworth et al. 2003]. Eucalyptol inhibited the germination of potato tubers and the growth of fungal mycelium. Additionally, it has been shown to be effective as an insecticide and in suppressing mosquito populations in northern California [Wang et al. 2014].



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Historically, medicinal plant compounds have been an important basis for the discovery of pharmaceuticals that have made significant contributions to antitumor and anti-infective treatments. Natural compounds have several advantages that are primarily attributed to their molecular rigidity, which favors protein-protein interactions as well as their ability to intervene in biological functions, which explains their effectiveness in reducing infectious diseases and cancer [Atanasov et al. 2021]. Natural products are the basis of many of the pharmaceutical products in use today. Among the most relevant examples is aspirin, the most commonly known and used drug in the world. *Salix* spp. and *Populus* spp. are the genera of plants that represent the source of aspirin [Desborough and Keeling 2017].

It is often difficult to distinguish between the medicinal and nutritional uses of plants. As a result, certain plants may only be useful from a nutritional standpoint, being used as functional or tonic foods, while other plants may be beneficial from both a nutritional and medical perspective [Jennings et al. 2015]. The World Health Organization initiated a trend toward integrative research on both food and medicine because of the importance of the connection between food and disease [WHO 2013]. The use of medicinal plants as ingredients in food gives it an added nutritional value, also known as functional food. In functional foods, a variety of substances derived from plants are present, such as alkaloids, phenols, terpenes, flavonoids, and many others. As a result, the food contains an additional nutritional value due to the presence of bioactive molecules, providing the food with an additional benefit [Mirmiran et al. 2014]. Despite having elements in common with conventional food, functional food contains additional nutritional value, which is why it may be referred to as "improved, enriched, or fortified". Bread, biscuits, and various powders or mixtures used as food supplements are examples of foods that may contain nutrient-rich food [Galanakis 2021].

In agriculture, pharmacy, and nutrition, medicinal plants have played a fundamental role in human civilization for centuries. It is important to note, however, that there are several current issues and challenges related to their use and conservation in these areas:

**Biodiversity Loss.** As a result of habitat destruction, over-harvesting, and climate change, many medicinal plants are threatened. The loss of biodiversity threatens the survival of these plants in the future [Sen and Samanta 2015].

**Sustainable Harvesting.** It's crucial to ensure a sustainable harvest of medicinal plants. Ecosystems can be disrupted, and populations can be depleted by overharvesting [Chen et al. 2016].



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*Cultivation and Domestication.* The cultivation and domestication of medicinal plants is essential to reduce the pressure on wild populations. In this way, it may be possible to ensure a consistent supply and quality of medicinal plant materials [Ramawat and Arora 2021].

*Quality Control.* In the pharmacy and herbal medicine industries, quality control is of utmost importance. In order to ensure safety and efficacy of products derived from medicinal plants, such as herbal supplements, standardization is necessary [Efferth and Greten 2012].

*Regulatory Frameworks.* There is a wide range of regulations regarding the use and sale of medicinal plants in different countries. Harmonizing these regulations and ensuring that they strike an appropriate balance between safety and accessibility are significant challenges [Thakkar et al. 2020].

*Pharmacological Research.* Validating the efficacy and safety of traditional medicinal plants remains a challenging task. There is a need for further research into the active compounds and their potential interactions with modern pharmaceuticals [Süntar 2020].

It can be concluded that medicinal plants will continue to play an important role in agriculture, pharmacy, and nutrition. In order to ensure their sustainable availability and responsible use, while respecting traditional knowledge and preserving biodiversity, it is imperative to address the current issues related to their use and conservation. To address these challenges, collaboration between government, researchers, industry stakeholders, and local communities is essential.



## **Chapter 2. Botanical characterisation and use of medicinal plants (Baličević R, Ravlić M)**

Botanical identification and use of medicinal plants chapter aims to give information on the identification and botanical characteristics of plant species as well as on the use of medicinal plants, particularly as alternative method in control of pests in agricultural production.

The use of any plants species begins with its proper identification and botanical characterization [Kellogg et al. 2019]. Identification and good knowledge on plant species is essential for improvement of agricultural productivity, discovery of new pharmaceuticals, quality control of medicinal products, and discovery and development of new bioherbicides. Plants are traditionally identified based on their morphological features using different tools. Dichotomous keys enable identification up to species level by dividing the groups of organisms continuously into two categories according to key characteristics. Plant atlases, guides and herbariums can be used. Recently, for faster identification of plant species digital tools such as internet databases and mobile applications are available. Plant identification can be time-consuming and a challenging process due to the high morphological variability and similarity of species belonging to genera with large number of species [Wäldchen et al. 2018].

Cultivated and wild medicinal plants have wide variety of application and use in both traditional and modern medicine, as a source of income, and in agricultural production [Bolouri et al. 2022]. In plant protection, medicinal plants are used as sustainable tool in integrated pest management systems as effective alternatives to synthetic pesticides due to the wide variety of biologically active compounds they possess. Plant extracts and essential oils derived from medicinal plants show herbicidal, antifungal, antibacterial, antiviral, nematocidal and insecticidal activity [Chen et al. 2016]. Plant based pesticides can be applied to plants as synthetic pesticides without adverse effects on environment, non-target organisms, and on plants, human and animal health. They can act as insect repellents and attractants, or antifeedants, inhibit oviposition and have ovicidal and larvicidal effects [Souto et al. 2021]. Essential oils extracted from many plants possess antifungal properties and are effective against plant pathogens [Abd et al. 2021]. Medicinal plants with allelopathic potential are also used for weed control in different ways e.g., in crop rotation, as cover crops or catch crops. Intercropping of plants with allelopathic potential suppresses weed growth by releasing allelochemicals and through weed-crop competition. Surface mulches with plant residues, incorporation of plant biomass and application of plant extracts prepared with water or methanol in various concentration reduces weed germination and growth. Plant species with



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positive allelopathic effect can be utilized as biostimulators for promotion of crop growth [Aniya et al. 2020].

In summary, the meticulous botanical profiling of medicinal plants is indispensable for accurately recognizing and employing them. These plants boast a storied tradition in traditional medicine and remain subjects of ongoing scientific exploration and bioprospecting. Consequently, the meticulous record-keeping, preservation, and sustainable oversight of these invaluable resources are imperative to ensure their enduring accessibility for healthcare and various other purposes.



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### **Chapter 3. Herbicidal effect of plant extract and essential oils (Ravlić M, Baličević R)**

The herbicidal impact of plant extracts and essential oils pertains to the application of naturally occurring compounds sourced from plants to manage or hinder the proliferation of undesired weeds, vegetation, or pests in agricultural and horticultural environments. This method is a component of the wider domain of natural or organic pest control. Herbicidal effect of plant extract and essential oils aims to present the research on plant extracts and essential oils obtained from medicinal plants and their potential use in agriculture as a tool in controlling weed germination and growth. Modern agriculture relies primarily on the application of synthetic herbicides as simple, cost-effective and highly efficient method of weed control [Jouini et al. 2020]. However, excessive and improper use of synthetic herbicides may lead to occurrence of weed resistant populations, herbicide residues in food, negative impact on the environment and on human and animal health. Similarly, restrictions in synthetic herbicide application, ban on active ingredients and public demand for food produced in organic systems calls for implementations of new methods in weed control [Curl et al. 2020]

Medicinal plants, both cultivated and wild, represent a great source of bioactive compounds. These compounds are sources of potential bioherbicides, and the majority of them are biodegradable under environmental factors and therefore their use is considered as sustainable and environmentally friendly tool in integrated pest management. The bioactive plant secondary metabolites (allelochemicals) are present in various concentrations in plants and plant parts (roots, leaves, stems, seeds etc.) and include phenolic compounds, alkaloids and terpenoids. Application of plant extracts and essential oils results in various effects on the targeted weeds species [De Mastro et al. 2021]. They may inhibit or decrease weed germination, result in loss of seed vigour, delay germination, reduce root and shoot length of seedlings, induce root necrosis and reduce accumulation of seedling biomass [Liu et al. 2015]. Herbicidal potential of plant extracts and essential oils depends on multiple factors. Plant species from different plant families and genus differ notably in their effect, as well as particular species population. Geographical origin, agricultural practices, as well as seasonal variation and plant growth stage have influence on the herbicidal efficacy [Kumar et al. 2021]. Environmental factors such as microclimatic location, light and temperature intensity, water and nutrient availability and other abiotic and biotic factors may increase the production of secondary metabolites in plants and enhance their herbicidal potential [Roberts et al. 2022]. The activity is influenced also by concentration, extraction method (cold/hot extraction, water extracts, methanolic extracts, ethanolic extracts, essential oils), and whether plant material is



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fresh or dry [Hasan et al. 2021]. Herbicidal potential also depends greatly on the target weed species as they differ in their sensitivity. Similarly, it is important for the plant extracts and essential oils not to have adverse effects on the crop when they are applied. Plant extracts and essential oils may be applied on weed seeds or seedlings alone or in combination with lower doses of synthetic herbicides [Zhang et al. 2022].

Screening plant species under laboratory conditions is the first step in evaluating the herbicidal potential, both on artificial medium and in the soil, followed by glasshouse experiments. The trials should consider different factors, such as plant species, extraction method, concentration, application method and target species in order to fully evaluate efficacy of plant extracts and essential oils [Schein et al. 2022].

In summary, utilizing plant extracts and essential oils for herbicidal purposes presents a promising and eco-friendly strategy for weed and pest control in agriculture. Despite their numerous benefits, it is vital to take into account their varying effectiveness, limited residual effects, and the potential for non-selectivity when integrating them into pest management plans. Achieving successful outcomes hinges on precise application and dosage.



## Chapter 4. Plant diseases in medicinal production (Ćosić J, Vrandečić K)

Plant diseases in medicinal cultivation carry substantial ramifications for the quality, yield, and safety of medicinal plant species. Pathogenic infections can exert pronounced influence on the quantitative and qualitative aspects of bioactive constituents within these plants, consequently exerting cascading effects on their therapeutic efficacy and safety profile within the domain of herbal medicine. The main contents of the part related to plant diseases in medicinal plant production is to present the research in the area of plant pathogens in medicinal plants focused on studying the diseases caused by various pathogens, that can affect the health and productivity of these valuable plants [Sofowora et al. 2013]. Among all biotic disease agents, fungal pathogens are the most important. According to the host range, plant pathogens are divided into polyphagous, oligophagous and monophagous. For a disease to occur, three conditions must be met: (i) the pathogen needs to be present on or in the plant; (ii) the environmental conditions need to be suitable for the pathogen and (iii) the plant must be susceptible to the disease [Pandit et al. 2022].

Understanding plant-pathogen interactions and developing effective management strategies are crucial for maintaining the quantity and quality of medicinal plants.

Identification and characterization of plant pathogens is the first step in their management. On plants, they can cause various symptoms which are, in some cases, very typical for some diseases and on the basis of these symptoms we can determine the causative agent of the disease. Much more often, the symptoms are not specific, and then we do additional analyses in order to determine the cause of the disease. Pathogen biology and infection mechanisms are part of studying the biology and infection mechanisms of plant pathogens helping to understand how they interact with medicinal plants and cause diseases and yield losses [Venbrux et al. 2023].

This knowledge aids in identifying critical points of vulnerability and developing targeted control measures.

Disease management strategies: Developing effective disease management strategies is crucial for preventing and controlling plant diseases in medicinal crops. Researchers explore various approaches, including cultural practices, chemical control, biological control, and the use of resistant cultivars or genetic engineering techniques to mitigate disease outbreaks [Scortichini 2022].

The basic advantages of chemical protection of plants are contained in the quick, cheap and effective suppression of plant pathogens, but these advantages are reduced by the negative





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indirect impact on human health, environmental pollution and ecological balance [Marchand 2023].

Integrated disease management means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of plant pathogens and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimize risks to human health and the environment. Integrated disease management approaches involve combining multiple strategies to control plant diseases. Researchers investigate the effectiveness of integrated pest management (IPM) practices in order to minimize disease incidence [Razdan and Sabitha 2009].

In summary, plant diseases within the context of medicinal plant production present notable impediments to the integrity, productivity, and safety of medicinal flora and herbs. These pathogenic affections hold the capacity to perturb the composition of bioactive constituents, crop output, and general vitality of these botanical specimens. The imperative adoption of sustainable and ecologically harmonious disease management approaches assumes paramount significance in sustaining the uninterrupted supply of superior-grade medicinal plants for employment in herbal medicine and allied spheres. Furthermore, the enforcement of rigorous quality assurance measures and safety assessments assumes critical relevance in safeguarding consumer well-being within the herbal medicine sector.



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## Chapter 5. Antifungal activity of essential oils in agriculture (Vrandečić K, Ćosić J)

Antifungal activity of essential oils in agriculture is the part of course which aims to give information about essential oils and their potential antimicrobial properties as natural alternatives for controlling plant pathogens. Essential oils may not completely replace traditional fungicides, but they can serve as valuable tools in integrated pest management strategies [Nazzaro et al. 2017]. Biological compounds extracted from plants could be one of the most important alternatives which do not have any hazardous effects on human health and environment. The history of using plant-based compounds such as essential oils and extracts against phytopathogens almost returns to the times when the plant diseases were ascribed. It is very well known for a long time that essential oils have antifungal, antibacterial and antiviral activity. Many essential oils exhibit antifungal properties and can be effective against a range of plant pathogens. They can inhibit the growth and reproduction of fungi responsible for diseases such as grey mold, damping-off, and root rot. Many research are ongoing, and most of them are in vitro. Essential oils can be applied directly to plant tissues or used as foliar sprays to control pathogens. Antifungal effects of the essential oils depend on the application method. They can be volatilized and used as fumigants to control airborne pathogens. Larger phenolic components (thymol, eugenol) have the best effect if are applied directly to medium, whereas smaller components (citral, allyl isothiocyanate) are most efficient when are used as volatiles [Montenegro et al. 2020]

Essential oils often contain more than 50 components, of which 1 to 3 are the main components representing 90 % or more of the whole volume. All the other components are often represented by less than 1%. Their biological activity depends on chemical composition, which is determined by the plant species, applied concentration, geographical origin, environmental conditions and agronomic practice. Also, the antifungal effectiveness of essential oils is affected by the sensitivity of targeted species [Moumni et al. 2021].

Expression of fungistatic or fungicidal effect of essential oils is often very clear, but, in many cases, the mode of antifungal action is not completely understood. The mode of action of essential oils depends on the type of target organisms and is mainly related to their cell wall structure and outer membrane arrangement. They increase cell membranes permeability and reduce their function, inhibit fungal growth, sporulation and germ tube elongation of many plant pathogens. In large number of cases the antimicrobial activity results from the complex interaction between the different compounds like esters, ethers, phenols, aldehydes, alcohols and ketones. In some cases, the bioactivities of essential oils are closely related with the



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activity of the main components of the oils. On the other hand, results of many previous researches stated that whole essential oils have higher antifungal effects than their major component or mixture of several major components. These facts lead to the assumption that the minor components are extremely important for the component's synergistic activity. Also, it is very difficult for the fungi to develop resistance to a mixture of oil components with different mechanisms of antimicrobial activity [Basak and Guha 2018].

It is important to consider the specific essential oil, concentration, and target pathogen when using essential oils as plant protectants. It is essential to conduct small-scale in vitro and in vivo trials to determine the efficacy and potential phytotoxic effects of essential oils on the target pathogens [Beressa et al. 2020].

In summary, the utilization of essential oils for their antifungal properties in agricultural settings presents a prospective, ecologically sustainable approach to mitigate the deleterious effects of fungal diseases on crop cultivation. Essential oils serve as valuable components within integrated pest management paradigms, thereby safeguarding agricultural productivity while mitigating collateral ecological impact. It is imperative to underscore that the efficacy of essential oils necessitates an adept understanding of application methodologies and the nuanced dynamics of particular crops and associated fungal diseases.



## Chapter 6. Agrotechnical conditions, cultivation, harvesting and storage of medicinal plants (Pop G, Obistioiu D)

Agrotechnical prerequisites, cultivation methodologies, harvesting techniques, and storage procedures constitute integral components of medicinal plant production. The cultivation and enduring safeguarding of medicinal plant specimens are contingent upon the amalgamation of diverse factors and methodologies. The production of plant raw material is conditioned, both quantitatively and qualitatively, by a series of factors including biological, ecological, technological and socio-economic [Civitarese et al. 2023]

*Biological factors.* The active ingredient content of medicinal and aromatic plants is influenced by the hereditary production, the quality requirements of the cultivar, and the cultural value of the seed material.

*Ecological factors.* In addition to a diverse and rich flora, our country has a wide variety of climate and soil conditions. The productivity of cultivated medicinal and aromatic plants and the quality of their production is conditioned by biological factors (biological and cultural value of the planting material); ecological factors (soil climate, orography); ecological zoning of the plants and by technological factors (rotation, fertilization, soil tillage, sowing or planting, soil work, harvesting and conditioning of production) [Liu et al. 2015].

*Temperature.* During ontogeny, the main biological and physiological phenomena (water and nutrient uptake, their rate of movement, chemical reactions, plant growth and development) take place under optimal conditions at a certain temperature - the 'harmonic optimum' - which is differentiated according to species [Wróbel et al. 2020].

*Humidity.* Water influences the quantity and quality of plant production, in the sense that absolutely all vital biochemical and physiological processes consumed in the plant body take place in the presence of water. The importance of water is found in: (i) the formation of soil solution; (ii) the transport of mineral and synthetic substances into the plant; (iii) the participation in equal proportions with carbon dioxide in the chlorophyll assimilation process (synthesis of organic matter) due to its components - oxygen and hydrogen; (iv) the oxidation and reduction reactions as reaction medium; (v) easing the absorption and circulation through vessels; (vi) the maintenance of cellular tension; (vii) the release or absorption of energy and regulation of tissue temperature through transpiration and evaporation [Herzog 2021].

*Light.* Light plays a special role in plant life. Through light, the Sun's energy is integrated into the plant as potential energy. Light energy is absorbed by chlorophyll, which through the process of photosynthesis converts carbon dioxide taken from the leaves into monosaccharides [Kubica et al. 2020].



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*Soil.* Soil is important because of its characteristics: texture, structure, soil solution, soil reaction and buffering capacity.

Soil texture, i.e. the grain size composition of the soil, influences the development and absorption capacity of the root system, water circulation, nutrient ion retention, cation exchange capacity, microbiological activity, etc.

Soil structure is a very important factor in soil fertility, influencing gas exchange, thermal regime, water circulation and the way elementary particles are grouped into structural aggregates. Soil structure and texture are those insufficiencies considered imperative requirements for plants' development: species with low and very low seed set are sown shallow, and a favorable ratio between the water-air regime and an adequate supply of nutrients must be ensured in the soil [Liu et al. 2021].

In summary, the prosperous cultivation of medicinal plants necessitates meticulous deliberation of agrotechnical parameters, encompassing soil attributes, climatic conditions, and solar exposure. The implementation of appropriate cultivation techniques, encompassing judicious seed selection, organic methodologies, and adopt pest control strategies, stands as pivotal determinants of plant vitality. Timely harvest, accompanied by prudent drying and processing techniques, serves to perpetuate the medicinal attributes. Furthermore, diligent storage practices, in conjunction with precise labelling, are imperative for the sustenance of medicinal plant quality, thereby catering to a spectrum of applications, encompassing herbal medicine and pharmaceuticals.



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## **Chapter 7. Medicinal plants with proven effectiveness against medical pathogenic bacterial strains (Obistioiu D, Pop G, Voica D, Avram D)**

Throughout history, medicinal flora has been employed for an extensive period to alleviate diverse medical maladies, with a subset of these botanical species demonstrating empirically verified efficacy against pathogenic bacterial strains. The utilization of medicinal plants possessing antibacterial attributes has acquired pertinence in light of the escalating challenge posed by antibiotic resistance, precipitating an exploration of alternative therapeutic modalities. Plants and other natural sources can provide many structurally diverse and complex compounds. Plant extracts and essential oils with antifungal, antibacterial, and antiviral properties have been analyzed worldwide as potential sources of new antimicrobial compounds, food preservatives, and alternative treatments for infectious diseases. Antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticidal properties have been attributed to essential oils. Essential oils (EO) can therefore be a potent tool for combating resistant microorganisms [Chouhan et al. 2017; Duque-Soto et al. 2023]. Although pioneering works have elucidated several components' mechanisms of action in the past, detailed knowledge of the mechanisms of action of the vast majority of compounds is still lacking [Chouhan et al. 2017].

EO (volatile oils) are aromatic, oily liquids extracted from plants (leaves, buds, fruits, flowers, herbs, branches, bark, wood, roots, and seeds [El et al. 2016, Safaei-Ghomi and Ahd 2010]. In recent years, the interest has increased in researching and developing novel antimicrobial agents derived from diverse sources to combat microbial resistance. Adding essential oils to antibiotics may reduce the antimicrobial minimum inhibitory concentration (MIC), with the greatest effect observed with aminoglycosides such as amikacin [Chouhan et al. 2017, Basavegowda and Baek 2022].

The composition, functional groups of the active components, and their synergistic interactions determine the antimicrobial activity. The antimicrobial mechanism of action differs dependent on the type of natural product or microorganism strain. It is well known that Gram-positive bacteria are more susceptible to the activity of natural plant products than Gram-negative bacteria. This is because Gram-negative bacteria have a rigid and more complex outer membrane, rich in lipopolysaccharides (LPS), thereby limiting the diffusion of hydrophobic compounds. In contrast, Gram-positive bacteria are surrounded by a thick wall of peptidoglycan that is not dense enough to resist to the small molecules of antimicrobials, thereby facilitating their access through the cell membrane. Due to the lipophilic extremities of



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lipoteichoic acid in the cell membrane, Gram-positive bacteria can also facilitate the penetration of hydrophobic compounds of EO [Chouhan et al. 2017, Balouiri et al. 2016].

Several studies have demonstrated that bioactive molecules can attach to the cell surface and traverse the cell membrane's phospholipid barrier. Their accumulation disrupts the structural integrity of the cell membrane, which can be detrimental, altering cell metabolism and causing cell mortality [Basavegowda and Baek 2022]. The interaction between antimicrobials in a mixture can have three distinct outcomes: synergistic, additive, or antagonistic [Chouhan et al. 2017, Yang et al. 2022, Zhang et al. 2017].

High morbidity and mortality rates are observable evidence of the alarming multi-resistance increase of pathogens. It is one of the greatest obstacles clinicians and researchers confront. The existing ineffective medical treatments have prompted the search for new and effective medicines to address this issue. Due to the rise of antibiotic-resistant bacteria and the dearth of novel antibiotics on the market, it is necessary to develop alternative strategies for treating infections caused by the action of different drug-resistant bacteria. Among the proposed strategies are the creation of antibiotic alternatives and the discovery or development of adjuvants. Combining antibiotics with non-antibiotic medications is one possibility. Antibiotics may also be combined with adjuvants or antimicrobial agents selected from the naturally occurring bioactive compounds reservoir [Balouiri et al. 2016].

It is imperative to recognize that the efficacy of medicinal plants can exhibit variability contingent upon factors including the modality of plant preparation, concentration, and the particular bacterial strain under consideration. Furthermore, notwithstanding the demonstrated antibacterial attributes of these botanical agents, they may not invariably serve as a singular substitute for conventional antibiotic agents. Frequently, they are employed in tandem with complementary medical interventions or as prophylactic measures aimed at bolstering general health.

Prior to the utilization of medicinal plants for therapeutic purposes, it is incumbent to seek the counsel of a healthcare practitioner to ascertain the secure and efficacious application, particularly in scenarios involving severe bacterial infections.



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## **Chapter 8. The activity of medicinal plants against pathogenic bacteria prevalent in the food industry (Negrea M, Cocan I, Alexa E, Obistioiu D, Voica D, Avram D)**

The assessment of medicinal plants' efficacy against prevalent pathogenic bacteria within the food industry has emerged as a progressively significant domain in the realms of food safety and public health. The ubiquity of pathogenic bacteria within the food production milieu underscores the imperative of investigating alternative, naturally-derived approaches to mitigate these microbial agents, which have the potential to incite foodborne maladies and epidemic outbreaks. The prevention of food spoilage and the emergence of pathogens that cause food poisoning is usually achieved through the use of chemical additives that have a number of negative effects, including: the human health hazards of chemical compounds, the occurrence of chemical residues in the food and feed chains and acquisition of microbial resistance to the chemicals used.

As a result of these worries, it's more important than ever to find a natural, healthy, and safe alternative to preservatives. For some time, plant extracts have been used to prevent food poisoning and preserve food [Mostafa et al. 2018].

Some of the challenges facing bread manufacturers include extending shelf life by reducing rancidity and decreasing microbial spoilage, as these changes lead to spoilage of bread and other bakery products. To overcome these difficulties and increase shelf life, commercially available antioxidants and chemical preservatives such mold inhibitors are used. Bread may be utilized as a functional food to efficiently boost the intake of herbs that promote human health and prevent disease as it is one of the most significant and widely consumed foods worldwide [Ibrahim et al. 2015].

According to the "back to nature" movement, using natural herbs and medicinal plants in meals is seen as an alternative to using synthetic chemicals [Nieto 2020].

Medical herbs have been used for thousands of years in cuisine and are inexpensive, readily available, and healthy. Additionally, because they contain advantageous phytochemicals, they are utilized in several medicinal formulations to both cure and prevent ailments. Additionally, herbs are utilized in the food sector as natural antioxidants to prevent oxidation of lipids, enhance the nutritional value of food, and provide taste to a variety of drinks [Lourenço et al. 2019].

Since plants contain a variety of vital antifungal compounds, such as phenolic compounds, glucosinolates, cyanogenic glycosides, oxylipins, and alkaloids, plant extracts have been thoroughly studied as bio-preservatives in bakery products [Axel et al. 2017].





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Due to their potential as natural food preservatives, flavoring agents, and decontaminating agents, plant essential oils are attracting a lot of interest in the food industry since they are also Generally Recognized as Safe – GRAS [Colombo et al. 2020]

Numerous studies have been done to determine whether essential oils can prolong the shelf life of bread. As a result, essential oils have antifungal properties. Thyme, cinnamon, and clove oils were known to inhibit spoilage fungus, whereas orange, sage, and rosemary oils had only a negligible effect, according to studies carried out previously [Liu et al. 2017]. Researchers have reported that cinnamon, clove and cardamom oil were found to suppress growth of microorganisms in cookies [Sulieman et al. 2023].

Hyssop is a significant medicinal herb that is used to tea blends to have antifungal, antispasmodic, and cough-relieving benefits. Its essential oil is utilized in the food industry and is high in pinocamphone, -pinene, myrtenol, linalool, methyl eugenol, and limonene [Hatipoğlu et al. 2013].

According to the studies conducted by Gavahian et al., a variety of essential oils, such as thyme, cinnamon, oregano, and lemongrass, can stop the development of dangerous germs in bread items, extending their shelf lives and improving their safety [Gavahian 2020]. The potential of *Thymus vulgaris* essential oil was previously investigated against *Aspergillus*, *Penicillium*, *Ulocladium*, *Cladosporium*, *Trichoderma*, *Rhizopus*, *Chaetomium* and *Aspergillus niger*, showing antifungal activity [Khalili et al. 2015].

Other studies highlighted that palmarosa oil, with specific rose fragrance, appears to be a good candidate to be used as an antibacterial agent against *Bacillus subtilis* in the bakery industry [Santamarta et al. 2021].

In summary, the utilization of medicinal plants substantiated for their antibacterial attributes in the food sector exhibits potential in augmenting food safety and diminishing the prevalence of foodborne pathogenic microorganisms. Ongoing research in this realm persists in scrutinizing the efficacy of distinct plant-derived extracts and essential oils against pathogenic bacteria within diverse food matrices. As consumer preferences trend toward enhanced food safety and a predilection for natural solutions, the integration of medicinal plants as natural food preservatives may foreseeably witness burgeoning adoption within the food industry.



## **Chapter 9. The use of medicinal plants as value-added ingredients in the industry of functional bakery and pastry products (Alexa E, Voica D, Negrea M, Cocan I, Avram D)**

Medicinal plants can be added as such or in the form of extracts, essential oils in bakery products with the following purpose: i) to improve the sensory properties of the products; ii) for an antioxidant role through the inclusion of polyphenolic active principles, and iii) for an antimicrobial role due to the biologically active antifungal and antibacterial compounds found in medicinal plants [Milla et al. 2021].

### *Medicinal plants used to improve the taste, color and aroma of bakery products*

Medicinal plants as: dill, parsley for leaves, sage, basil, thyme, chervil, watercress, coriander, cumin, anise, and others are used in the bakery and pastry for the purpose of seasoning the products. Medicinal plants added in different forms of dough preparation improve the sensory properties, having positive or negative effects on its rheological properties. A previous study showed that the quality parameters of bread (H/D report, volume, porosity) obtained by addition of 5% cumin as infusion are superior compared to the control [Sayed et al. 2018]. On the contrary, other studies have reported that the addition of aromatic plants leads to the worsening of the rheological properties of the dough as: gumminess, strength, adhesiveness, elasticity, chewiness, among others, this effect being due to the polyphenolic compounds present in plants' composition that exert antioxidant activity [Czajkowska–González et al. 2021]. Some active principles from medicinal plants as curcumin are used as coloring agents in bakery and pastry in order to enhance the color of foodstuffs or to make it look tastier and more attractive to the consumer [Arraiza and de Petro 2009].

### *Medicinal plants as antioxidants agents in bakery products*

An antioxidant effect obtained after the enrichment of wheat bread with extracts of *Camellia sinensis*, *Asparagus racemosus* and *Curcuma longa* was reported by Pop et al. They highlighted that the addition of 5% extracts increased the antioxidant capacity of the bread without altering the sensory properties [Pop et al. 2016]. Antioxidant properties of green tea powder substituting some flour in sponge cakes was also reported [Ma and Ryu 2018].

Even if the beneficial effects regarding the increase in the antioxidant capacity of bakery products by the addition of medicinal plant extracts are evident, studies regarding the changes in the behavior of bread gluten caused by polyphenols have been reported [Czajkowska–González et al. 2021].



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### *Medicinal plants as antimicrobial agents in bakery products*

Several types of essential oils, especially those belonging to Lamiaceae family and Umbelliferae, are mentioned as antimicrobial agents in the bakery industry, resulting in a product with extended shelf life and enhanced safety [Gavahian et al. 2020]. Sitara et al. evaluated the essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula asafoetida*) against fungi seeds as: *F. oxysporum*, *F. moniliforme*, *F. nivale*, *F. semitectum*. All extracted oils showed fungicidal activity [Sitara et al. 2008].

Due to its chemical composition, *Origanum vulgare* helps to extend the shelf life and the nutritional qualities of many products, such as bread and bakery products, cereals [Chis et al. 2017]. The oregano plant is rich in fiber, antioxidant activity, phenolic content and can be used up to 2% in bread to improve nutritional and sensory qualities, specific volume and shelf life, also having an inhibitory action on molds [Muresan et al. 2012]. Further studies are necessary for the development of common strategies for the control and prevention of fungal and mycotoxin development in bakery and pastry products.

In summary, the incorporation of medicinal plants as value-added constituents in the bakery and pastry sector offers a prospect for the development of functional foods that amalgamate gustatory attributes with potential health-enhancing properties. This aligns with the escalating consumer inclination toward products endowed with wellness-promoting attributes, rendering the inclusion of these botanical components a distinctive marketing feature for manufacturers. This diversification also augments the spectrum of more health-conscious and nutritionally enriched bakery and pastry commodities available to consumers. The achievement of this endeavor hinges upon meticulous formulation, stringent quality assurance measures, and unambiguous information dissemination to consumers.



## **Chapter 10. The use of medicinal plants as value-added ingredients in the industry of meat and dairy products (Cocan I, Negrea M, Alexa E, Obistioiu D, Voica D, Avram D)**

The incorporation of medicinal plants as value-added constituents in the meat and dairy sector signifies an evolving phenomenon that unites gastronomic ingenuity with prospective health-promoting attributes. This approach encompasses the inclusion of a diverse range of medicinal plants into meat and dairy products, culminating in the provision of functional foods to consumers. These products not only cater to sensory preferences but also offer the potential for health-enhancing attributes. Medicinal plants are also used in food with the aim of bringing functional value to the food product in which they are added for health promotion, as in recent times cardiovascular or gastrointestinal diseases, hypertension, diabetes and cancer are on the rise in industrialized and well-developed countries. Researchers are therefore looking for ways to prevent these diseases or alleviate their consequences by producing healthier or functional foods. Thus, the use of medicinal plants with beneficial effects on health is known from traditional medicine. At the same time, the use of medicinal plants also aims to reduce fat or salt content [Krickmeier et al. 2019].

The addition of natural antioxidants and antimicrobials to meat and meat products is one of the important strategies in the development of healthier and novel meat products. In this regard, several studies using herbs, spices, fruits and vegetable extracts have shown that the addition of these extracts to raw and cooked meat products reduced lipid oxidation, improved colour stability and total antioxidant capacities, which are important characteristics for shelf-stable meat products [Hygreeva et al. 2014].

The main active components/phytochemicals responsible for the antioxidant activity of plant derivatives are polyphenols, flavonoids, phenolic diterpenes and tannins [Zhang et al. 2010]. An important factor to be considered when using herbs as antioxidants is the minimum effective concentration, as most of them, due to their high antioxidant content, can imprint very intense color and taste [Oswell et al. 2018].

Some spices with lower antioxidant potential require a higher dose of use. This is the case for cumin and cardamom, with the lowest dose found for cooked beef (1%), as determined by Qureshi et al [Qureshi et al. 2023].

Milk and dairy products are one of the most common foods in the diets of all population groups and are consumed as such, and represent a suitable medium for the growth of undesirable microorganisms. Some spoilage microorganisms can adversely affect visual appearance and



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commercial value, whereas others are pathogens that affect product safety. Recent studies have recorded the efficacy of natural plant compounds when introduced directly into milk or into cheese by immersion or spraying [Clarke et al. 2019; Ritota and Manzi 2020].

Herbs as additive substitutes are widely used in the dairy industry. Due to their rich content of vitamins, minerals and other biologically active substances they have beneficial effects on digestion, activity and emotional state of the cardiovascular system [Ogneva 2015; Stanislav et al. 2019].

In addition, herbs give dairy products a pronounced taste and smell specific to plants, as well as an attractive appearance. Biologically active substances from plant materials, including medicinal plants, represent a promising direction in the production of medicinal, preventive and functional animal products [Stanislav et al. 2019].

In summary, the integration of medicinal plants as supplementary constituents within the meat and dairy sector presents an avenue for the development of functional foods that harmonize sensory attributes with potential health-enhancing characteristics. In response to the escalating consumer inclination toward products conferring potential wellness benefits, the inclusion of these botanical elements establishes a distinctive marketing advantage for producers. The efficacy of this endeavor hinges on meticulous formulation, rigorous quality assurance, and forthright communication with consumers, congruent with the surging consumer demand for more health-conscious and nutritionally enriched meat and dairy selections.



## **Chapter 11. Pharmacological action and effects on health exerted by natural products derived from medicinal plants (Dehelean CA, Soica CM, Pînzaru IA)**

The pharmacological action of natural products derived from medicinal plants and their effects on health are of considerable interest to pharmacology, medicine, and natural health practitioners. The natural products contain a variety of bioactive compounds which can have diverse effects within the human body. Throughout the history of medicine, plants have played a crucial role due to their remarkable therapeutic properties. Even today, new bioactive molecules are being discovered through the exploration of plants. Today, more than half of the drugs used for the treatment and prevention of various diseases originate from plants. In addition, traditional medicine is the primary method of treatment for most diseases throughout the world [Gad et al. 2013].

Morphine was the first plant compound isolated and used in human medicine. It was obtained from the *Papaver somniferum* species, and it marked the beginning of the age of drug discovery in 1803 [Krishnamurti and Rao 2016]. Since then, over 70,000 plant species have been studied and used in traditional medicine due to their remarkable biological properties. More recently, the number of herbal medicines discovered has increased due to scientific advances in fields such as genomics and proteomics. The metabolomic studies are also used to identify new biological targets, to elucidate mechanisms of action, and to maintain evidence of the benefit of drugs and therapeutic effects that have been developed [Nasim et al. 2022]. Research in the medical field is primarily focused on discovering the most promising compound that will be effective in treating a multitude of pathologies, including cancer, cardiovascular disease, and neurodegenerative disorders [Thomford et al. 2018]. In order to obtain a medicine, the first steps include the isolation and purification of the compounds from their natural sources. Next, the compounds are evaluated pharmacologically and toxicologically to select those with the best results with targeted effects. It is becoming increasingly prevalent for scientists to derivatize compounds of vegetable origin to make them more effective at interacting with biological targets. As a final step, toxicology, pharmacokinetics and pharmacodynamics tests are carried out, and if the results are positive, the compounds of vegetable origin can be considered potential therapeutic candidates [Dzobo 2022]

From the standpoint of therapeutic use, plants provide several methods of administration. The most common method of treatment is the use of home remedies, such as herbal teas. There are also certain plant extracts that can be used in various pharmaceutical forms, such as



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tinctures and powders, in crude form or in standardized fractions. The packaging of bioactive compounds in the form of medicines is also possible [Nasim et al. 2022].

Plants produce signaling molecules such as cytokinin, auxin, and salicylic acid, as well as secondary metabolites such as alkaloids, polyphenols, and terpenoids, which play an integral role in plant physiological processes. The release of these molecules is especially important in stressful conditions in order to protect the plant. Traditional medicine relies heavily on these compounds due to their small molecule sizes and diverse mechanisms of action [Lepri et al. 2023].

Biotechnological progress has also resulted in the development of therapeutic proteins from vegetables. Herbal medicines can be used to treat a wide variety of conditions, including cancer, HIV, cardiovascular diseases, and diabetes. The remedies are known as biological products from plants and have the advantage of making therapeutic proteins more easily than methods based on animal cell cultures or microbial fermentation. In addition, they are characterized by a lower risk of microbial contamination, which makes them a competent platform and one of the fastest growing product classes in the pharmaceutical industry. Many drugs used in the modern world are based on proteins derived from plants [Chen 2016]. For example, carrots produce taliglucerase alfa, a substance that is used to treat Gaucher's disease. Also, influenza vaccines are undergoing clinical trials, and vaccines against COVID-19 based on virus-like particles represent an important biopharmaceutical candidate [Rosales-Mendoza 2020]

Natural products have attracted the attention of the pharmaceutical industry, resulting in an increased interest in plant-based medicines. Natural medicines have a number of advantages over synthetic medicines, including lower risks, increased therapeutic efficiency, and easier metabolism and absorption. Moreover, the purification and standardization processes of a single compound are more convenient, facilitating its use in modern drug delivery systems.

To conclude, natural products derived from medicinal plants exert a wide range of pharmacological actions. Various aspects of health can be benefitted from their therapeutic potential, ranging from the control of inflammation to the protection against infections and oxidative stress. In both traditional and modern medicine, these natural products have played an important role in the development of pharmaceutical drugs. To ensure safety and efficacy, their use should be guided by scientific evidence and medical expertise.



## Chapter 12. Medicinal plants and dietary reference values (Dehelean CA, Soica CM, Pînzaru IA)

Dietary reference values and medicinal plants are two distinct aspects of health and nutrition. As part of the dietary guidelines, dietary reference values are established in order to guide individuals in maintaining a balanced and healthy diet, and medicinal plants can contribute to the achievement of these dietary goals. At the global level, nutrition plays a critical role in preventing mortality [English et al. 2021]. In light of this, dietary intervention requires significant effort both on the part of healthcare providers as well as on the part of patients. It has been found that counselling patients from a nutritional standpoint is often treated superficially, with the majority of the focus being placed on pharmacological interventions [Hever and Cronise 2017]. The main benefits of the plant-based diet have also been emphasized in conjunction with the scientific progress in the field. The definition of whole food is the association of vegetables, fruits, legumes, whole grains, nuts, seeds, herbs, and spices. Several associations dedicated to the prevention of cardiovascular diseases and cancer, as well as the United States Department of Agriculture, have emphasized the importance of providing an adequate intake of fiber, minerals, and vitamins by ensuring that half of the plate consists of vegetables and fruits [McGuire 2016]. The ideal ratio of macronutrients in food is still an intensely debated topic. There is ample evidence that has highlighted the health benefits of a plant-based diet [Yokose et al. 2021]. The most important macronutrients that can be found in the plant kingdom include carbohydrates, proteins, and fatty acids.

**Carbohydrates.** The recommended daily intake of carbohydrates under normal conditions is approximately 130 grams, excluding pregnancy and breastfeeding [Clemente-Suárez et al. 2021]. There are a number of optimal sources of carbohydrates, including vegetables, fruits, and cereals. It is common to label certain products of vegetable origin as primary sources of carbohydrates, such as whole tubers and potatoes. The protein content of these products is satisfactory despite the fact that they are considered high in energy, but low in protein. By substituting rice for chicken, for example, the nitrogen balance is maintained. These results demonstrate that vegetable-based food sources can meet nutritional requirements in a healthy and balanced manner [Alcorta et al. 2021].

**Protein.** The recommended consumption of protein is 0.8 g/kg/day for adults. Several recent studies recommend an increase in this intake to 1.2 g/kg/day for individuals over the age of 65 [Lonnie et al. 2018]. Although food marketing has largely focused on proteins of animal origin, all essential amino acids are synthesized by bacteria or plants, so they can easily be obtained from vegetable products [Hertzler et al. 2020]. Plant-based foods rich in protein include nuts,





legumes, seeds, soybeans, and whole grains. In spite of the fact that these plants tend to contain a smaller amount of essential amino acids than animal products, some studies suggest that this difference can be beneficial [Gorissen et al. 2018].

Dietary fatty acids. Fatty acids have a wider recommended intake range than other macronutrients, ranging between 20% and 35% of total calories for adults over 19 years of age [Poli et al. 2023]. There are only two essential fatty acids in the diet: omega-3 and omega-6. The omega-3 fatty acids are primarily found in flax seeds, chia seeds, hemp seeds, soy seeds, walnuts, and wheat germ. Omega 3 derived from plants has many advantages over marine products because it contains no heavy metals such as mercury, lead, or other industrial pollutants [Liu et al. 2022]. The omega-6 fatty acid, on the other hand, is found in most plants and is an essential fatty acid. Due to this, certain modern diets tend to be excessive in omega-6 fats by consuming foods high in this fat, but low in omega-3 fats. Inflammatory and chronic diseases were more likely to develop as a result of this increase in omega-6/omega-3 ratio [Nur Mahendra et al. 2023].

It is important to note that although studies have focused on the macronutrient content of plants, more recent studies have highlighted the beneficial role of other micronutrients in plant-based products. A healthy diet must contain a variety of micronutrients, such as vitamins, minerals, and phytonutrients [Assunção et al. 2022]. Among the phytonutrients, polyphenols are a class of compounds of natural origin that have attracted attention. A series of beneficial biological actions are associated with it, such as antioxidant properties and the ability to regulate the function of cells [Zhang et al. 2022]. A number of other compounds, such as flavonoids, stilbenes, and curcuminoids, play an important role in preventing cardiovascular diseases, neurodegenerative diseases, and cancers. In addition, these micronutrients are often enzyme cofactors and have pleiotropic and synergistic effects, decreasing the risk of chronic disease [Monjotin et al. 2022].

A plant-based diet has numerous health benefits, with scientific evidence showing that regular consumption of vegetables, fruits, whole grains, nuts and seeds can significantly reduce the risk of chronic diseases, such as cardiovascular disease.

In conclusion, medicinal plants and dietary reference values are closely related in the context of promoting nutrition and health. A well-rounded and balanced diet can benefit from the consumption of medicinal plants, as they contain essential nutrients, antioxidants, and dietary fiber. Nevertheless, their use should be guided by scientific evidence, and individuals should be aware of potential interactions and dosages to ensure their safety and effectiveness.



### **Chapter 13. Current Issues in the Safety of Novel Foods and Nutrient Sources - interactions between supplements/foods and drugs (Conforti F, Statti G)**

Recently, there has been considerable attention paid to the safety of novel foods and nutrients, particularly in the context of interactions between supplements/foods and medications. Novel foods are foods or ingredients that are "new" compared to those traditionally understood. They are defined as foods that have not been consumed to any significant extent in the European Union prior to May 15, 1997, when the first novel foods regulation came into force. They can be new foods, foods produced using new technologies and production processes, or foods that are traditionally consumed outside the EU [Grimsby 2020]. Food or food ingredients covered by this regulation must not: (i) present a risk to the consumer; (ii) mislead the consumer; (iii) differ from the other foods or food ingredients for whose substitution they are intended, to such an extent that their normal consumption would be nutritionally disadvantageous to the consumer [Fortin 2022].

The concept of "novel food" is not a recent one. New types of foods, ingredients, or food production methods have always come to Europe from all over the world, for example: corn, potatoes, and tomatoes from America have been imported to Europe since the 15th century, as have rice and pasta that were imported from Asia, or coffee from East Africa to the more recent Chia seeds and quinoa. Until a few decades ago, new foods on the market were mainly represented by concentrated extracts of natural active ingredients of different origins (phytosterols, lycopene, omega-3-rich oils), in recent years the focus is gradually shifting to the use of specific sources to obtain nutritionally sound foods, formulated even without resorting to traditionally used raw materials and ingredients [Siegrist and Hartmann 2020]. In fact, current research focuses on different categories of food sources and production processes. Novel foods can be alternative sources of protein, carbohydrates or food supplements. Underutilized legume crops, edible fungi, terrestrial and aquatic plants and microalgae and insect are important sources of protein with less impact on the environment [Quintieri et al. 2023]. Insect farming for example has lower greenhouse gas emissions and can therefore be a viable alternative to animal protein sources [Van Huis and Oonincx 2017]. *Yarrowia lipolytica* yeast biomass is a large source of protein, exogenous amino acids, essential trace minerals and lipid compounds, mainly unsaturated fatty acids, as well as a source of B vitamins [Jach and Malm 2022]. Of the 26 extracts approved as novel foods by the European Union, 23 have been approved for use in food supplements (FS). These include: fungal origin extracts, animal extracts, algae extracts and plant extracts [Ververis et al. 2020]. Utilizing plant-derived substances is a common practice in the production of food supplements,



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and notably, those derived from botanical products and plant extracts have witnessed substantial growth. This rapid expansion has prompted extensive scientific research aimed at examining the potential advantages and drawbacks linked to their consumption.

Drug interactions are a major concern for both pharmaceutical companies and regulatory agencies. The European Medicines Agency developed and subsequently updated special guidelines in 2013, known as the "Guideline on the Investigation of Drug Interactions," which outline a comprehensive approach to assessing a drug's interaction potential. Drug-drug, drug-food, and drug-classical supplement interaction studies are conducted for each drug under development.

The Food and Drug Administration (FDA) does not require manufacturers of dietary supplements to demonstrate their safety and efficacy, although such supplements must still submit a safety record. Manufacturers and distributors of supplements are required to report any serious side effects to the FDA through the "MedWatch" system, a program dedicated to reporting the safety of medical products. Clinical studies on supplements, given their increasing growth, have increased significantly in recent years. For information on studies currently underway, the NIH National Center for Complementary and Integrative Health (NCCIH) can be consulted [Iwatsubo 2020]. Drugs, food, and supplements can interfere with each other in both kinetics and dynamics. In pharmacodynamic interactions, a substance (drug, food or supplements) modifies tissue sensitivity to other substances, exerting the same effect (agonist) or blocking the effect (antagonist).

These effects habitually occur at the receptor level but can also occur at the intracellular level. In pharmacokinetic interactions, the administration of substances may alter the absorption, distribution, metabolism, or excretion of another substance. Thus, the amount and persistence of the drug where the receptor is expressed is altered. During the absorption phase, co-administration of drugs or herbal products may reduce or increase the absorption of one or both administered substances by acting, for example, on gastric pH or by interacting with intestinal P-glycoprotein. During the distribution phase they may interfere with binding to plasma proteins while in the metabolism phase they may act as enzyme inducers reducing the effectiveness of a substance. Finally, in the elimination phase they can increase or inhibit renal excretion, leading to reduced efficacy or the appearance of toxic effects [Sprouse and Van Breemen 2019].

In conclusion, it is clear that the safety of novel foods and nutrient sources is a complex issue, particularly in terms of interactions with medications. It is essential to gain a better understanding of these interactions in order to optimize healthcare outcomes. It is the



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responsibility of healthcare providers, governmental bodies, and patients to address these challenges, ensuring that dietary habits and supplement use are aligned with medication regimens to maximize safety and efficacy. Individualized approaches to healthcare may provide more tailored solutions for individuals, taking into account their unique responses.



## **Chapter 14. Products preparation from plants (extracts, essential oils), phytochemical characterization and influence of geolocation on the composition of phytocomplexes (Conforti F, Statti G)**

Plant-based industries are dependent on the preparation of products from plants, including extracts and essential oils, as well as the phytochemical characterization of these products. As a result of the geographic origin of plants, phytocomplexes (the complex mixture of phytochemicals in plants) are significantly influenced by their geolocation. The preparations that can be obtained from the plants are numerous. Extracts are preparations that can be liquid, semisolid, or solid in consistency, obtained using various extraction techniques. Extracts are preparations derived from the complete or partial evaporation of solutions obtained by exhaustion of dried plant drugs, using suitable solvents [Azwanida 2015]. Using maceration or percolation processes, the extracts obtained can be classified as follows: (i) fluid extracts: these extracts contain the same amount of active ingredient found in the starting plant drug; (ii) soft extracts: during the concentration process, a honey-like consistency is achieved and they are 2 to 6 times more concentrated than fluid extracts; (iii) dry extracts: these are solid powders obtained by complete evaporation of the solvent used for extraction [Abubakar and Haque 2020].

Other preparations obtained through maceration or percolation are the tinctures. These are liquid solutions obtained by processing plant drugs with an appropriate solvent. Commonly, a hydroalcoholic solution (a mixture of water and alcohol) is used, the alcohol content of which is chosen according to the solubility of the active ingredients to be extracted. The main distinction between extracts and tinctures lies in the fact that, in the former, an evaporation process is carried out to increase the concentration of the active ingredients in the preparation. In contrast, tinctures can also be obtained by simply diluting the corresponding fluid extract. Another substance extracted plant materials is oleoresin, which contains a mixture of essential oils (volatile aromatic components) and resins (non-volatile components). This extraction is usually done by solvent or high-pressure extraction processes [Hudz et al. 2020]. Finally, we have essential oils, which can be obtained from plant material that undergoes steam distillation, hydrodistillation or cold pressing, depending on the plant and the type of oil to be obtained. They consist of a complex mixture of chemical compounds, including terpenes, aldehydes, ketones, alcohols and others, which give them both their distinctive aroma and potential therapeutic properties [Aziz et al. 2018].



Chromatographic techniques, which rely on interactions with a stationary phase (solid or liquid) and a mobile phase (liquid or gas), are commonly used to separate, identify and quantify phytocomplexes [Coskun 2016], as follows:

- 1) Column chromatography: is a separation technique based on the differential distribution of the components of a mixture between a mobile phase (solvent) and a stationary phase (column packed with solid material or gel). It is commonly used for purification and separation of mixtures of organic compounds [Revathy et al. 2011].
- 2) Gas Chromatography (GC): in this case, the mobile phase is a gas, and the stationary phase is a coating or column packed with solid material. Samples are vaporized and injected into the column for separation. It is a widely used technique for analyzing mixtures of volatile and thermally stable compounds [McNair et al. 2019].
- 3) High-Performance Liquid Chromatography (HPLC): the mobile phase is a liquid pumped through a column filled with small stationary particles. Separation is based on chemical and physical interactions between the mixture components and the stationary phase [Rahimi et al. 2020].
- 4) High-Performance Thin-Layer Chromatography (HPTLC): is a variant of thin-layer chromatography (TLC) that uses highly uniform thin layers of stationary material (silica) on a glass or aluminum plate as the stationary phase and a liquid mobile phase for separation. Unlike TLC the seeding is not done manually but with a special machine, the run is done in a chamber (Automatic Development Chamber) at controlled temperature, humidity and saturation. Visualization is done using a specific instrument (Visualizer) that allows image acquisition on a computer [Ramu and Chittela 2018].

The phytochemical composition of a natural extract can vary greatly depending on the plant of origin, method of extraction, growing conditions, and the part of the plant used. The active ingredient content of a plant is influenced by several environmental factors, such as climatic conditions, altitude, latitude and soil composition, as well as biotic factors. Light and temperature are critical for plant photosynthesis, which in turn affects the production of secondary compounds. Temperature can also affect the rate of enzymatic reactions. Altitude and latitude have a significant impact on the chemical composition of medicinal plants, with variations often noted between plants growing in mountainous areas and those growing in lowlands [Altemimi et al. 2017]. Biotic factors, such as interactions between different plant species or the presence of nearby organisms, can influence the production of secondary metabolites in plants. Mutual interaction between plants and other organisms can lead to changes in phytochemical composition. In summary, the active ingredient content of plants is



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influenced by a complex array of environmental and biotic factors, making it important to consider these influences in phytochemical analysis and production of medicinal plants [Shan et al. 2023].

As a result, the preparation of products from plants, including extracts and essential oils, as well as the phytochemical characterization of these products are vital for a variety of industries. Geographic location plays a significant role in determining the composition of phytocomplexes, as well as the quality, authenticity, and therapeutic properties of plant-based products. It is crucial to understand these geographical influences in order to standardize, ensure quality control, and develop plant-based products that are safe and effective.



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## **Chapter 15. Substances in food supplements between efficacy and toxicity - plants and plant extracts (Pînzaru IA, Macașoi IG, Dehelean CA)**

Supplements are products that are consumed in addition to the usual diet in order to provide additional nutrients that are beneficial to the health and well-being of the body. In this regard, dietary supplements serve the purpose of supplementing the diet, containing ingredients such as vitamins, minerals, amino acids, herbs, or botanicals, and can be administered in the form of lozenges, capsules, or various liquid pharmaceutical forms [Wierzejska 2021].

Traditional medicine is based on plants that are a main and important source of many drugs used today, such as aspirin and morphine. Additionally, numerous products of vegetable origin are used in the manufacture of food supplements. The popularity of plant-based products has increased recently, a trend that can be explained by the long history of their use. According to a survey conducted between 2003 and 2006, approximately 20% of adults regularly consume herbal supplements [Bailey et al. 2011]. It is important to note that one of the most significant problems associated with this high intake of food supplements is that most patients do not report to their doctors, and many supplements can interfere with medical treatment. These supplements have gained popularity due to their perceived natural and holistic qualities, offering a wide array of health benefits. However, it's essential to understand that just because something is natural doesn't necessarily mean it's safe, and the efficacy of these substances can be closely tied to their potential for toxicity [Ronis et al. 2018].

Regarding the effectiveness of the substances found in food supplements, special attention must be paid to the following topics: nutrient density, traditional medicine, synergy and bioavailability and adaptogenic properties.

**Nutrient Density.** Many plant-based food supplements, such as those containing vitamins, minerals, and antioxidants, can provide essential nutrients that are often lacking in the standard diet. These nutrients are crucial for various bodily functions, including energy production, immune support, and overall health [Drewnowski and Fulgoni 2014].

**Traditional Medicine:** Many plant extracts have a long history of use in traditional medicine systems around the world. For instance, herbs like ginseng, echinacea, and turmeric have been used for centuries for their potential health benefits, including immune support and anti-inflammatory properties [Boy et al. 2018].

**Synergy and Bioavailability:** Some plant compounds are believed to work in synergy with other components in their natural form, increasing their bioavailability and potential health benefits.





This is often referred to as the "entourage effect," where multiple compounds in a plant work together for a more significant impact [Nair and Augustine 2018].

**Adaptogenic Properties:** Certain plant extracts, like adaptogens (e.g., ashwagandha and rhodiola), have been associated with stress reduction, improved stamina, and enhanced mental clarity, offering a potential natural solution for the demands of modern life [Todorova et al. 2021].

At the opposite pole is the toxicity of the substances used in food supplements, based on which are found mainly: dosage and concentration, allergenic reactions, interactions, purity and contamination and lack of regulation.

**Dosage and Concentration.** Natural substances in plant extracts can be potent, and when consumed in high concentrations, they may lead to toxicity. Overdosing on some plant-based supplements can result in adverse effects, including digestive issues, headaches, or more severe health concerns [Brima 2017].

**Allergenic Reactions.** Individuals can be allergic to plant compounds, even if they are considered generally safe. Allergic reactions can range from mild skin irritations to severe anaphylactic responses [Shahali and Dadar 2018].

**Interactions.** Plant-based supplements may interact with medications or other supplements, leading to unexpected side effects or diminished effectiveness. It is crucial to consult a healthcare professional before adding new supplements to your regimen, especially if you're taking prescription medications [Sprouse and Van Breemen 2016].

**Purity and Contamination.** The source and quality of plant-based supplements are critical. Poorly sourced or contaminated products can introduce toxins or harmful substances into the body, leading to adverse effects [Ratajczak et al. 2020].

**Lack of Regulation.** The dietary supplement industry is not as tightly regulated as pharmaceuticals, leading to variations in quality and safety among different products. It's important to choose reputable brands that follow good manufacturing practices [Dwyer et al. 2018].

In conclusion, substances in food supplements derived from plants and plant extracts can offer a range of health benefits, but their efficacy and safety depend on various factors, including dosage, purity, individual tolerance, and potential interactions with other substances. It's crucial for individuals to approach these supplements with caution, do their research, consult with healthcare professionals, and pay close attention to product quality and sourcing to ensure they receive the potential benefits while avoiding toxicity and adverse effects.



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