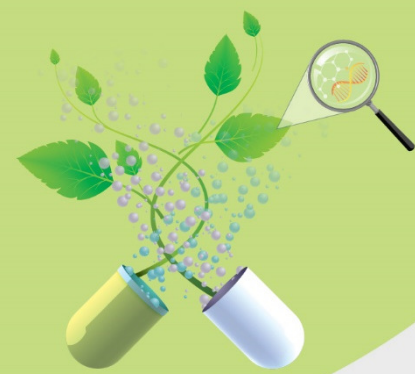




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EURO-PLANT-ACT



# MANAGEMENT OF MEDICINAL PLANTS – FROM CULTIVATION TO CONSUMER SAFETY

EURO-PLANT-ACT

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

### **1.1. Introduction**

Medicinal plants can be defined as any plant that has as constituents in one or more of its part substances that exert therapeutical potential or serve as progenitor for the production of beneficial medicines [Sofowora et al., 2013].

The therapeutical uses of medicinal plants are well-known since immemorial time and currently they represent the basis for novel drugs development [Chen et al., 2016; Jiang et al., 2022]. Furthermore, World Health Organization (WHO) declared that a very large percentage of world's population (75-80%) depends on herbal medicines for their essential healthcare needs [Marcelino et al., 2023]. In recent times, medicinal plants are commonly used as dietary supplements and consumed as foods, and also studied as greener alternatives for pests' control in agriculture, what leads to a global high demand for medicinal plant and an increased risk of extinction from overharvesting and habitat destruction [Dar et al., 2017; Chen et al., 2016; Jaing et al., 2022].

The International Union for Conservation of Nature and the World Wildlife Fund reported that globally, between 50.000 and 80.000 flowering plant species are utilized for medicinal purposes, most of the species being found in China and India [Chen et al., 2016]. Even though there are more than half a million plants in the world, many of them unexplored, and 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], a special attention should be paid to the exacerbation of an unsustainable utilization of medicinal plants from wild sources [Chen et al., 2016; Jiang et al., 2022].

The present chapter is focused on revealing the multiple roles played by medicinal plants in various sectors as agriculture, pharmaceutical field and food industry. In addition, there will also be highlighted the challenges related to medicinal plants in terms of cultivation and domestication, sustainable harvesting, bio-diversity loss, quality control, regulatory framework, and pharmacological research.



## 1.2. Medicinal plants' role in agriculture

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 the environment [Pathania 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 microbe [Ajar, 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 the environment 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].

For thousands of years, herbal medicines have served as sources of bioactive and therapeutic compounds for both industrial and agricultural applications. Several medicinal plants, formulated as extracts or essential oils, have been studied as pest control approaches, evaluating their insecticide, insect repellent or fungicide effects [Cheraghi Niroumand et al., 2016; Nxumalo et al., 2021].

Even though the synthetic pesticides continue to be the most effective strategy to control the protection and preservation of agricultural crops, their severe toxic effects, like as: (i) cancer development (e.g. 1,2-dibromoethane, ethylene dibromide, lindane, noviflumuron, spirodiclofen, ethiofencarb, methomyl, diquat, malathion, pymetrozine, propineb); (ii) reproductive toxicity/infertility (cyproconazole, flumioxazin and propiconazole); (iii) endocrine disruptive effect (vinclozolin, maneb, quizalofop-p-



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tefuryl, and zineb), and (iv) death (hydrogen cyanide), led to their total ban in organic agriculture. Another inconvenient of the overuse of synthetic pesticides is the development of resistant strains of pathogens [Nxumalo et al., 2021]

Unlike conventional pesticides, which rely on a single active ingredient, herbal pesticides contain a mix of phytochemical compounds (alkaloids, cyanogenic glycosides, phenylpropanoids, polyketides, anthocyanins, carbohydrates, amino acids, lipids, nucleic acids, terpenoids, flavonoids, phenols, saponins and tannins) that can influence both the behavior and physiology of pests, making it much harder for them to develop resistance. Thus, identifying bio-pesticides that are not only effective, but also adaptable to ecological conditions, is essential for achieving adequate pest control [Cheraghi Niroumand et al., 2016; Nxumalo et al., 2021].

Other benefits of plant-based pesticides are a reduced harmful potential, rapid biodegradation, suitability for use by small-scale farmers, and effectiveness in protecting grain from pest damage [Phokwe and Manganyi, 2023]. In this context, previous research has documented over 2.500 plant species from 235 families with demonstrated biological activity against a variety of pests. However, only a few of these have been developed for commercial use or classified as agricultural products. According to the latest studies, many herbal remedies — including plants, essential oils, and their chemical constituents — have shown inhibitory effects on insect pests [Phokwe and Manganyi, 2023].

The interest for natural pest control compounds dates since 2000 BC, the Indian book – *The Rig Veda* (4000 years old) made mention of plants with insecticidal effect. A wealth of knowledge concerning the medicinal plants effective as pest control agents is offered by Avicenna in his book known as *The Canon of Medicine*. Avicenna described 42 natural pesticides, as: black cumin (*Nigella sativa* L.), myrtle (*Myrtus communis* L.), garlic (*Allium sativum* L.), pomegranate (*Punica granatum*), euphorbia (*Euphorbia helioscopia* L.), bitter cucumber (*Citrullus colocynthis* (L.) Schrad), worm wood (*Artemisia montana* (Nakai) Pamp), olive (*Olea europaea* L.), common juniper (*Juniperus communis* L.), oleander (*Nerium oleander* L.), and others [Amrollahi-Sharifabadi et al., 2024].





Medicinal plants act as insecticides via various mechanisms of action, as follows: (i) insecticidal effect (*Allium sativum* L., *Origanum majorana*, *Ocimum basilicum*, *Nerium oleander*, *Myrtus communis*, etc); (ii) repellent effect (*Artemisia absinthium*, *Boswellia carterii*, *Myrtus communis*, *Nerium oleander*, *Ocimum basilicum*, *Origanum majorana*, *Ruta graveolens*, etc); (iii) larvicidal activity (*Allium sativum* L., *Boswellia carterii*, *Myrtus communis*, *Nerium oleander*, *Ocimum basilicum*, *Ruta graveolens*, etc); (iv) acaricidal effect (*Punica granatum*, *Allium sativum* L., etc); (v) antifeedant (*Ocimum basilicum*, *Punica granatum*, etc); (vi) developmental inhibition (*Artemisia absinthium*, *Nerium oleander*, etc), and (vii) fumigant activity (*Allium sativum* L., *Boswellia carterii*, *Ocimum basilicum*, *Origanum majorana*, etc) [Cheraghi Niroumand et al., 2016; Amrollahi-Sharifabadi et al., 2024].

A list of medicinal plants (formulated as extracts) frequently used as pesticides was published by Nxumalo et al. (2021) in an excellent review. This list includes the following plants: *Azadiracta indica*, *Bobgunnia madagascariensis*, *Lippia javanica*, *Melia azedarach*, *Solanum incanum*, and *Tephrosia vogelii* [Nxumalo et al., 2021].

Various medicinal plants proved to be also effective as fungicides protecting the fruits and crops against common fungal contamination, for example: *Acorus calamus*, *Allium cepa*, *Allium sativum*, *Aloe vera*, *Datura stramonium*, *Galenia africana*, *Moringa olifera*, *Phyllanthus niruri*, *Plumbago zeylanica*, *Ruta chalepensis*, *Thymus vulgaris* L., *Zataria multiflora*, and *Zehnerria scabra* [Nxumalo et al., 2021].

A detailed description of the medicinal plants reported in the literature as pesticides, insecticides or fungicides is presented in Table 1.1.

**Table 1.1. Medicinal plants known as natural pesticides/insecticides/fungicides** [Gahukar, 2012; Cheraghi Niroumand et al., 2016; Nxumalo et al., 2021; Phokwe and Manganyi, 2023; Amrollahi-Sharifabadi et al., 2024].

Medicinal plant	Formulation type	Pest/pathogen controlled
<i>Ailanthus excelsa</i> (Roxb.)	Leaf extract	<i>Lasiodiplodia theobromae</i> <i>Fusarium oxysporum</i>
<i>Allium cepa</i> L.	Bulb extract	<i>Alternaria alternata</i> <i>Colletotrichum gloeosporioides</i>



<b><i>Allium sativum</i> L.</b>	Bulb extract, Essential oil (bulb)	<i>Tetranychus urticae</i> <i>Fusarium oxysporum</i> <i>Alternaria alternata</i> <i>Fusarium</i> sp.	
	Garlic juice	<i>Delia radicum</i> <i>Musca domestica</i>	
	Tuber extract	<i>Anopheles stephensi</i> <i>Culex quinquefasciatus</i>	
	Fruit extract	<i>Tetranychus cinnabarinus</i>	
	Cloves extract	<i>Callosobruchus maculatus</i>	
	Essential oil (bulb)	<i>Culex pipiens</i> <i>Camptomyia corticalis</i> <i>Lycoriella ingenua</i> <i>Boophilus annulatus</i>	
	Garlic lectins	<i>Acyrtosiphon pisum</i>	
	<b><i>Artemisia absinthium</i> L.</b>	Essential oil	<i>Rhodnius prolixus</i>
		Essential oil (leaf)	<i>Ixodes Ricinus</i>
		Leaf extract	<i>Sarcoptes scabieivar. suis</i>
<b><i>Azadirachta indica</i> A. Juss.</b>	Leaf extract	<i>Hyadaphis coriandari</i> Das <i>Aspergillus flavus</i> <i>Penicillium</i> spp. <i>Mucor</i> spp. <i>Fusarium oxysporum</i> <i>Lasiodiplodia theobromae</i>	
	Seed kernel extract	<i>Aphis gossypii</i> <i>Urentius sentis</i> <i>Myzus persicae</i> <i>Schoutedenia emblica</i> <i>Cercospora rauwolfiae</i>	
	Kernel oil	<i>Hyadaphis coriandari</i> Das <i>Aphis gossypii</i> <i>Hyadaphis coriandari</i> Das <i>Coccidohystrix insolita</i> <i>Aspergillus flavus</i> <i>Penicillium</i> spp. <i>Mucor</i> spp. <i>Cercospora rauwolfiae</i>	





	Seed cake	<i>Meloidogyne</i> <i>Cercospora rauwolfiae</i> <i>Erwinia chrysanthemi</i> <i>Fusarium solani</i>	<i>incognita</i>
	Seed powder	<i>Sitophilus oryzae</i>	
<b><i>Boswellia carterii</i></b>	Essential oil of resin	<i>Lycoriella ingenua</i> <i>Sitophilus oryzae</i>	
	Essential oil	<i>Aedes aegypti</i> <i>Anopheles stephensi</i> <i>Culex quinquefasciatus</i>	
<b><i>Brassica juncea</i> (L.) Czern.</b>	Seed cake	<i>Urentius sentis</i> <i>Myzus persicae</i> <i>Meloidogyne incognita</i> <i>Erwinia chrysanthemi</i> <i>Fusarium solani</i>	
<b><i>Calotropis procera</i> (Ait)</b>	Leaf extract	<i>Fusarium oxysporum</i> <i>Lasiodiplodia theobromae</i>	
<b><i>Capsicum annuum</i> L.</b>	Essential oil (fruit)	<i>Phomopsis azadirachtae</i>	
<b><i>Catharanthus roseus</i> (L.)</b>	Leaf extract	<i>Alternaria alternata</i> <i>Fusarium oxysporum</i>	
<b><i>Chromolaena odorata</i> (L.)</b>	Leaf extract	<i>Alternaria alternata</i> <i>Alternaria longipes</i> <i>Curvularia lunata</i>	
<b><i>Crotalaria juncea</i> L.</b>	Seed cake	<i>Meloidogyne incognita</i>	
<b><i>Curcuma domestica</i> Val.</b>	Essential oil (rhizome)	<i>Phomopsis azadirachtae</i>	
<b><i>Cymbopogon citratus</i> (DC) Stapf</b>	Leaf extract	<i>Curvularia lunata</i> <i>Fusarium oxysporum</i>	
<b><i>Datura stramonium</i> L.</b>	Seed extract	<i>Fusarium oxysporum</i>	
<b><i>Diospyros lotus</i> L.</b>	Leaf extract	<i>Fusarium oxysporum</i>	
<b><i>Eucalyptus citridora</i> Hook</b>	Leaf extract	<i>Cercospora rauwolfiae</i>	
	Essential oil (leaf)	<i>Phomopsis</i>	<i>azadirachtae</i>
	Essential oil	<i>Sitophilus zeamais</i>	
<b><i>Foeniculum vulgare</i> L.</b>	Essential oil (seed)	<i>Phomopsis</i>	<i>azadirachtae</i>
	Essential oil	<i>Sitophilus zeamais.</i>	
<b><i>Mentha arvensis</i> DC</b>	Leaf extract	<i>Bombus californicus</i>	



		<i>Tetranychus macfarlanei</i>
<b><i>Myrtus communis</i></b>	Essential oil (leaf)	<i>Culex quinquefasciatus</i> <i>Aedes aegypti</i> <i>Anopheles stephensi</i> <i>Ephestia kuehniella</i> <i>Plodia interpunctella</i> <i>Acanthoscelides obtectus</i>
<b><i>Myristica fragrans Houtt.</i></b>	Essential oil (seed)	<i>Phomopsis azadirachtae</i>
<b><i>Nerium oleander</i></b>	Leaf extract	<i>Anopheles stephensi</i>
	Dust of flowers and its suspension	<i>Sitophilus zeamais</i>
	Plant (leaves, roots, and stem) extract	<i>Ceratovacuna lanigera</i>
<b><i>Ocimum basilicum</i></b>	Essential oil (aerial parts)	<i>Acyrtosiphon pisum</i> <i>Myzus persicae</i>
	Essential oil (plant)	<i>Musca domestica</i>
	Essential oil (leaf)	<i>Culex pipiens</i> <i>Lymantria dispar</i> <i>Sitophilus oryzae</i> <i>Rhyzopertha dominica</i> <i>Cryptolestes pusillus</i>
	Stem extract	<i>Culex quinquefasciatus</i>
<b><i>Ocimum gratissimum (L.)</i></b>	Leaf extract	<i>Pachliopta aristolochiae</i>
<b><i>Origanum majorana</i></b>	Essential oil (Leaf)	<i>Ephestia kuehniella</i>
	Aerial parts extract	<i>Spodoptera littoralis</i> <i>Musca domestica L</i> <i>Thrips tabaci</i>
<b><i>Punica granatum</i></b>	Fruit pericarp extract	<i>Sitophilus zeamais</i> <i>Tribolium castaneum</i>
	Leaves extract	<i>Tetranychus cinnabarinus</i>
<b><i>Ricinus communis L.</i></b>	Seed cake	<i>Meloidogyne incognita</i>
<b><i>Rosa chinensis Jacq.</i></b>	Flower extract	<i>Alternaria alternata</i>
<b><i>Ruta graveolens</i></b>	Essential oil (aerial parts)	<i>Aedes aegypti</i>
<b><i>Zingiber officinale Roscoe</i></b>	Essential oil (rhizome)	<i>Alternaria alternata</i> <i>Fusarium sp.</i>
	Plant powder	<i>Sitophilus zeamais</i>



Other medicinal plants, as nicotine, caffeine and eucalypt showed promising effects in agricultural practices. 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 has proven 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].

### **1.3. Medicinal plants' role in the pharmaceutical field**

Since ancient times up to the present day, medicinal plants represented an unconfined reservoir for the development of novel pharmaceuticals/medicines. The knowledge about the healing properties of plants dates back to the ancient cultures, as the Sumerians, the Egyptians, the Greeks and the Romans. Undoubtedly, the Traditional Chinese Medicine is the most longevive holistic system founded upon a comprehensive understanding of numerous plant, mineral, and animal substances, natural remedies to reinstate equilibrium and well-being. A significant role in the transfer of knowledge about herbal remedies was played by Ayurveda, the India's indigenous medical system, that is based on plant-derived formulations, including alkaloids and polyphenols, secondary plant metabolites [Chaachouay and Zidane, 2024].

Since the discovery of quinine from cinchona tree (*Cinchona officinalis*), a treatment for malaria, in the 17<sup>th</sup> century, significant advances were recorded in the plant-based pharmaceuticals field, findings that made considerable contributions to the antitumor and anti-infective treatments [Chaachouay and Zidane, 2024].

Natural compounds have several advantages that are primarily attributed to their molecular rigidity, which favours 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 remain the basis of many of the pharmaceutical products in use today, not only as the direct therapeutic compounds, but also as model molecules or “leads” used for drug synthesis and semisynthesis [Chaachouay and Zidane, 2024]. 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 are the source of salicylic acid [Desborough and Keeling, 2017].

In table 1.2 will be summarized a list of the most relevant plant-derived drugs that were used as treatments for different illnesses.

**Table 1.2. Relevant plant-based drugs used as therapy in different pathologies** [Chaachouay and Zidane, 2024; Desborough and Keeling, 2017; Calixto, 2019; Krishnamurti and Rao, 2016; Gao et al., 2020; Khaiwa et al., 2021]

Plant-based drug	Medicinal plant source	Year of discovery	Pharmacological effects
<b>Morphine</b>	<i>Papaver somniferum</i>	1806 – Friedrich Serturmer	analgesic
<b>Caffeine</b>	<i>Coffea arabica</i> , <i>Camelia sinensis</i>	1820 - Runge	neuroprotection
<b>Quinine</b>	<i>Cinchona officinalis L.</i>	1820 - Caventou and Pelletier	antimalarial
<b>Colchicine</b>	<i>Colchicum autumnale L.</i>	1820 – Pelletier and Caventou – first isolation 1833 – Phillip Lorenz Geiger – purified colchicine	antigout, anticancer
<b>Codeine</b>	<i>Papaver somniferum</i>	1824 – Pierre-Jean Robiquet	analgesic, antitussive
<b>Berberine</b>	<i>Coptidis rhizoma</i> <i>Berberis vulgaris L.</i> <i>Scutellaria baicalensis</i>	1830 – Buchner and Herberger	Bacillary dysentery
<b>Atropine</b>	<i>Atropa belladonna</i>	1831 - Mein	anticholinergic
<b>Papaverine</b>	<i>Papaver somniferum</i>	1848 – George Merck Fraz	anti-spasmodic



<b>Cocaine</b>	<i>Erythroxylum coca Lam.</i>	1860 - Albert Niemann	anesthetic
<b>Scopolamine</b>	<i>Datura metel L.</i>	1880 – Albert Ladenburg	sedative
<b>Ephedrine</b>	<i>Ephedra sinica Stapf</i>	1885 – Nagai – first isolation	sympathomimetic
<b>Theophylline</b>	<i>Theobroma cacao L.</i>	1888 – Albert Kossel 1895 – Fischer and Ach	diuretic
<b>Digoxin</b>	<i>Digitalis lanata</i>	1869 – Calude-Adolphe Nativelle	cardiotonic
<b>Aspirin</b>	<i>First drug obtained by synthesis</i>	1897 – Felix Hoffman	anti-inflammatory, analgesic, antithrombotic
<b>Curare</b>	<i>Chondrodendron tomentosum</i>	1943 - Winstersteiner and Dutcher	muscle relaxant
<b>Vinblastine</b>	<i>Catharanthus roseus (L.)</i>	1954 – Beer and Noble	anticancer
<b>Vincristine</b>	<i>Catharanthus roseus (L.)</i>	1961 – first isolation 1963 – FDA approval	anticancer
<b>Camptothecin</b>	<i>Camptotheca acuminata</i>	1966 – Wall and Wani	anticancer
<b>Paclitaxel/ Taxol</b>	<i>Taxus brevifolia Nutt.</i>	1971 – Wani and Wall 1993 – approved for medical use	anticancer

Other plant-based drugs that represented key pillars for the treatment of various diseases, are: convallatoxin – cardiotonic (*Convallaria majalis L.*), digitoxin – cardiotonic (*Digitalis purpurea L.*), ouabain – cardiotonic (*Strophanthus gratus (Wall. & Hook.) Baill*), glaucine – antitussive (*Glaucium flavum Crantz*), glycyrrhizin – treatment for Addison’s disease (*Glycyrrhiza glabra L.*), reserpine – antihypertensive (*Rauwolfia serpentina (L.)*), silymarin – antihepatotoxic (*Silybum marianum (L.)*), thymol – topical antifungal (*Thymus vulgaris L.*), etc [Chaachouay and Zidane, 2024].

In recent years, the pharmaceutical research shifted to the investigation of natural compounds due to their complex composition and multiple pharmacological



effects. Implementation of modern methods of analysis as high-throughput screening, computer modeling, and bioinformatics in plant-derived drugs analysis reduces considerably the duration of the discovery process and, also the associated costs [Chaachouay and Zidane, 2024].

In this context, medicinal plants will continue to be a valuable source for innovative pharmaceuticals. As proof stands the significant number of FDA approved drugs of natural origin, between 1981-2014: of the total (1562), 64 (4%) were unmodified natural products, 141 (9.1%) were botanical drug mixtures, 320 (21%) were derivatives of natural products, and 61 (4%) were synthetic drugs containing natural product pharmacophores [Calixto, 2019].

#### **1.4. Medicinal plants' role in food industry**

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].

Nowadays, the population pay more attention to what they consume and the trend of natural compounds use in drugs, dietary supplements, and, even in food ("clean label products") gains more and more ground.





Due to the multiple active compounds found in the composition of medicinal plants, their roles in food industry are variate, as preservatives, antioxidant, antimicrobials, sources of nutrients, and for changes in organoleptic characteristics. In addition, herbal products are generally recognized as safe (GRAS) and represent excellent alternatives for chemical additives [Nieto, 2020].

Rosemary (*Rosmarinus officinalis* L.), formulated as extract or essential oil showed preservative effects by exerting antioxidant and antimicrobial activity; in this light it can be incorporated into various food systems, including meats, oils, and dressings [Nieto, 2020]. In addition, rosemary extract increased the shelf-life of milk powder and of other dairy products by inhibition oxidation [Ivanišová et al., 2021].

Sage (*Salvia officinalis* L.), a plant well-known for its culinary properties, proved antibacterial and antioxidant effects, features that can be useful to increase the storage stability of frozen, vacuum-packed low-pressure mechanically separated meat from chickens [Ivanišová et al., 2021].

Lavender (*Lavandula angustifolia* L.) is frequently used in food industry (the flowers) for flavouring beverages, bakery products, ice cream, chocolated, syrup, and jellies. By adding lavender powder to a recipe of biscuits, it was augmented its stability, antioxidant, and long-term preservation, but it was also improved the content of nutrients (polyphenols) [Ivanišová et al., 2021].

Enrichment of biscuits with mint (*Mentha piperita* L.) led to improved qualities as antioxidant potential, sensory characteristics and stability [Ivanišová et al., 2021]. Addition of lemon balm (*Melissa officinalis* L.) to hamburger patties increased the antioxidant stability of the meat [Ivanišová et al., 2021].

Medicinal plants are also retrieved in the composition of functional beverages (energy drinks, sports drinks and functional and fortified drinks – dairy and plant-based beverages) to enhance their beneficial health effects and their sensory properties. The most frequently plant products used in energy drinks are caffeine and catechin (flavonoid), but other compounds as ginseng, guarana, yerba mate, ginkgo and acai are also included. Fruit-based drinks and smoothies are rich in so-called “superfruits”, like pomegranates, goji, chia, acai, and mangosteen [Maleš et al., 2022].



Apart from the effects described above, medicinal plants are added in functional foods for their pharmacological potential, as anti-inflammatory, antinociceptive, antimicrobial, antidiabetic, antioxidant, antitumoral, hepatoprotective effects, etc, the most commonly used species, are the members of Lamiaceae family. An extensive and thorough review on this subject was written by Carovic-Stanko et al. (2016) [Carovic-Stanko et al., 2016].

### **1.5. Challenges related to medicinal plants use in agriculture, pharmaceutical field and food industry**

The current overharvesting of medicinal plants for agricultural practices, in the development of novel pharmaceuticals, dietary supplements, and, in food industry requires well-established regulations to assure a sustainable production of medicinal plants for the fulfillment of future demands [Jiang et al., 2022].

In agriculture, pharmacy, and nutrition, medicinal plants have played an integral 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].

For an optimal and sustainable harvest, WHO proposed several guidelines:

- ✓ the harvest of medicinal plants should be performed during the optimal season or time period for the production of herbal products of the best quality
- ✓ the ideal harvest time (peak season and time of day) should be selected based on the quality and quantity of biologically active compounds rather than the total vegetative yield of the desired medicinal plant parts



- ✓ during harvesting, it is essential to prevent contamination by ensuring that foreign matter, weeds, or toxic plants do not mix with the harvested medicinal materials
  - ✓ the harvest should be done in appropriate conditions, avoiding dew, rain, or unusually high humidity to prevent potential damage from increased moisture, which can encourage microbial fermentation and mold growth
  - ✓ cutting tools, harvesters, and other equipment should be kept clean and properly adjusted to minimize damage and prevent contamination from soil and other materials
  - ✓ to minimize the microbial load on harvested medicinal plant materials, contact with soil should be avoided as much as possible
  - ✓ the harvested medicinal plant materials should be placed in clean baskets, dry sacks, trailers, hoppers, or other well-ventilated containers and moved to a central location for transport to the processing facility; all containers used during harvest should be kept clean and free from any contamination from previously harvested plants or other foreign materials
  - ✓ altered medicinal plant materials should be detected and removed during harvest, post-harvest inspections, and processing, to prevent microbial contamination and preserve product quality [WHO, 2003].
- *Cultivation and Domestication.* The cultivation and domestication of medicinal plants is essential to reducing 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].

Medicinal plants possess unique medicinal properties, what requires an increased care and a good management for their cultivation [Jiang et al., 2022]. In this context, WHO stated several recommendations concerning medicinal plants cultivation process:

- ✓ application of optimal conservation agriculture techniques, as “no-tillage” systems



- ✓ selection of the cultivation site considering ecological and geographical variables, previous crops that were planted and the plant protection agents used
  - ✓ climate conditions have a significant impact on the physical, chemical, and biological characteristics of plants; therefore, factors such as sunlight duration, average precipitation, and temperature—along with day and night temperature variations—should be analyzed in advance
  - ✓ the soil should have adequate levels of nutrients, organic matter, and other essential elements to support optimal growth and quality of medicinal plants. Ideal soil conditions—such as soil type, drainage, moisture retention, fertility, and pH—will depend on the specific medicinal plant species and the intended plant part for use.
  - ✓ all fertilizing agents should be used judiciously and tailored to the specific needs of the medicinal plant species and the soil's supporting capacity; these agents should be applied in a way that reduces leaching.
  - ✓ irrigation and drainage should be managed according to the specific requirements of each medicinal plant species at its various growth stages.
  - ✓ field management practices should be guided by the growth and development characteristics of each medicinal plant and the specific plant part intended for medicinal use
  - ✓ timely interventions such as topping, bud nipping, pruning, and shading can be employed to regulate plant growth and development, ultimately enhancing the quality and quantity of the produced medicinal plant material [WHO, 2003; Marcelino et al., 2023].
- *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].

The quality control of the medicinal plant products should be performed in accordance with effective Good practice guidelines, including Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), and Good Clinical Trial Practice (GCTP) by applying standardized specific methods [Efferth and Greten, 2012]. Moreover, European Medicines Agency (EMA) published a recent version (in 2022) of Quality of herbal medicinal products/traditional herbal medicinal products - Scientific guideline [EMA, 2022].

- *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.

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## **Chapter 2. Botanical characterisation and use of medicinal plants.**

### **Allelopathic and herbicidal potential of plants**

**(Balicevic R, Ravlic M)**

#### **2.1. Introduction**

Proper identification and botanical characterization represent the first and crucial step in use of any plant species. Correct identification and good knowledge on plants is essential for progress of agricultural production, discovery of new phytochemicals and pharmaceuticals, quality control of medicinal products and discovery and development of new active ingredients used as bioherbicides [Wäldchen and Mäder, 2018; Kellog et al., 2019; Šćepanović et al., 2021; Erhatic et al., 2023]. Identification tools include various methods from traditional botanic or morphological identification of a plant species, use of digital tools or more detailed methods such as plant chemical profiling and genetic methods.

Traditional identification of plants includes identification based on their morphological features using different tools. These include various plant atlases, guides and herbariums, as well as dichotomous keys which enable plant identification using taxonomic features (Figure 2.1). Dichotomous keys enable identification up to species level by dividing the groups of organisms continuously into two categories according to key characteristics. All these tools enable plant identification based on macroscopic characteristics such as fruits, flowers, and leaves, that are most often used for identification up to genera and species level [Drouet et al., 2018; Kellog et al., 2019].

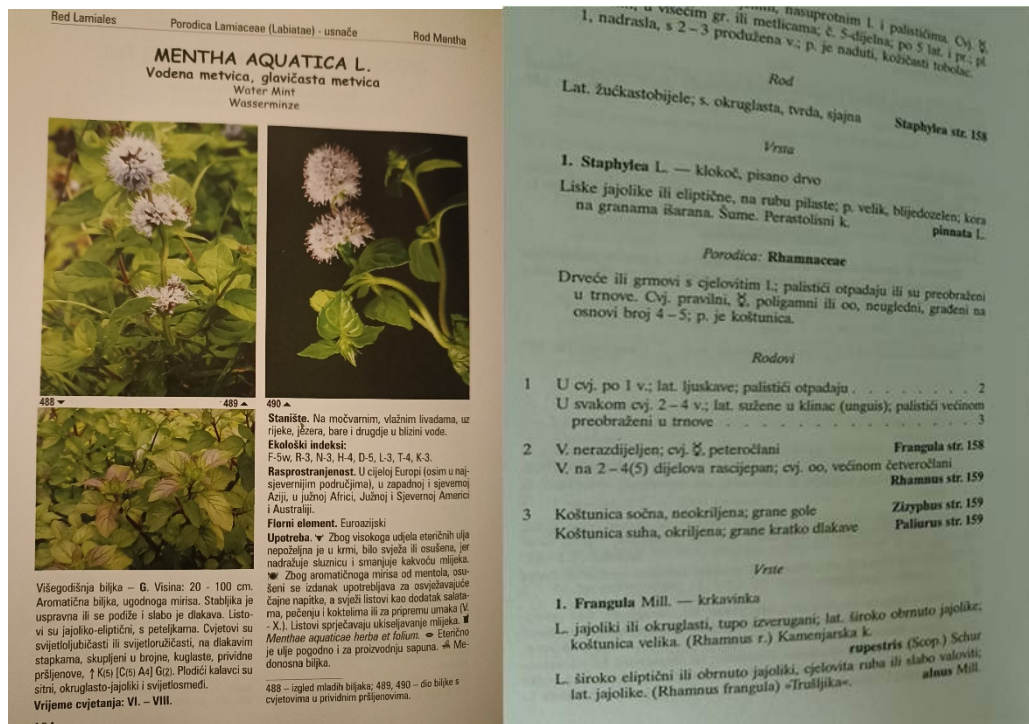


Figure 2.1. Traditional identification of plant species using atlases and dichotomous keys.

For identification both fresh and dry plant material such as herbarium specimens are used. Macroscopic identification includes observations on leaves such as leaf type (e.g. simple or compound), leaf shape (e.g., ovate, lanceolate, linear, cordate), leaf arrangement (e.g. opposite, alternate), leaf size, colour or leaf margin (e.g., entire, dentate, lobed, etc.).

Also, observations can be made based on flower (e.g. shape, colour, number of stamens etc.), shape of inflorescence (e.g. umbel, spike, raceme), type of roots (e.g., bulb etc.), as well as type, shape and size of generative organs such as fruits and seeds (Figure 2.2). Beside macroscopic, microscopic observations of anatomic elements is frequently used to confirm identification of selected plants species [Costea et al., 2019; Kellog et al., 2019].





**Figure 2.2. Fruits, seeds and leaves of plants for botanical identification.**

Plant identification can be tedious and time-consuming, requires advanced knowledge on plant morphology and anatomy, and can be a challenging process due to high morphological variability and similarity of plant species, especially ones belonging to genera with vast number of species. Therefore recently, for more rapid identification of plant species numerous digital tools are available, such as internet databases, and web and mobile applications [Wäldchen and Mäder, 2018; Hart et al., 2023]. Main advantages of this method of identification include broad availability, their user-friendly features, and speed.

Plant identification applications can be helpful to experts to additionally confirm identification of plant, or to aid in the identification of species that are outside of their area of expertise [Grgić, 2023; Hart et al., 2023]. However, the main disadvantage is the accuracy of identification up to the species level. Regardless of the fact that individual applications can identify plant species at family or genus level with 100% accuracy, the identification of the species is very often not accurate and inadequate, and requires further expert confirmation [Otter et al., 2021; Grgić, 2023]. Therefore, use of digital tools may greatly aid in botanical identification, but should not be the sole method of identification, especially for species confirmation, as misidentification can lead to use of ineffective and/or unsafe products.





Besides botanical examination, other techniques may be employed in characterization and authentication of plant materials such as DNA barcoding and characterization of chemical constituents [Kellog et al., 2019]. Important chemical constituents are identified and quantified during phytochemical screening in order to confirm and authenticate botanical materials, to differentiate chemotypes within species, as well as to detect adulterations in materials. Several methods are used such as mass spectrometry (MS), gas chromatography (GS) and high-performance liquid chromatography (HPLC) [Drouet et al., 2018; Kellog et al., 2019]. Plants can be also identified using molecular techniques such as molecular markers. Molecular markers enable for can help to differentiate two species, however the main constrain of their use is the inability to assess content of active molecules compared to phytochemical profiling and analysis [Drouet et al., 2018].

The growth of crops is inevitably accompanied by weeds, which compete with crops for light, nutrients, moisture and space causing reductions in yield quantity or their complete destruction. Weeds also reduce crop quality, harbour pests and diseases and increase production costs. Modern-day agriculture primarily relies on the use of chemical herbicides for weed management due to their high efficiency, simple application and cost-effectiveness. However, improper and excessive application of chemical herbicides may cause numerous problems, such as occurrence of weed resistant populations, herbicide residues in food chain, and adverse effects on the environment and on human and animal health [Macías et al., 2003; Singh et al., 2003].

Additionally, frequent ban on active ingredients, lack of registered plant protection products and restrictions in application of synthetic herbicides in organic agricultural systems as well as in protected areas, requires a different approach in weed management. Application of alternative measures in weed control, non-chemical and environmentally friendly which minimize and overcome the abovementioned problems, is essential. One such alternative tool for sustainable weed management is allelopathy.



**Allelopathy**, a biological phenomenon, represents both direct or indirect, harmful or beneficial effect of one plant species (donor) on the germination, establishment and growth of the other (receptor) through the production and release of chemical compounds (allelochemicals) into the environment [Rice, 1984]. Allelochemicals, which are mainly secondary plant metabolites, are present in all plants and various plant parts, in roots, rhizomes, stems, leaves, bark, flowers, fruits and seeds, in various concentrations, and are released in both natural systems and agroecosystems via root exudation, volatilization, leaching or decomposition of plant material [Weston and Duke, 2003].

Allelopathy and allelopathic interactions play an important role in agricultural systems influencing weed flora occurrence and composition as well as growth and yield of crops [Alam et al., 2001]. In agricultural systems, allelopathy can be utilized in various ways to control weeds. Allelopathic crops with substantial herbicidal effect may be implemented in organic systems where chemical weed control is not allowed or as supplementary measure in integrated weed management systems, as water extracts or essential oils, i.e. natural bioherbicides, in crop rotation, as cover crops, crop genotypes with high weed suppressive abilities, as mulches, or incorporated as residues and powders [Singh et al., 2001; Ghafari et al., 2018; Šćepanović et al., 2021]. Similarly, positive allelopathic potential could be exploited in form of biostimulators to promote crop growth, competition with weeds and crop yield [Bhadha et al., 2014; Baličević et al., 2018]. Understanding the mechanisms of allelopathic interactions and factors influencing allelopathic potential of plants can help optimize crop production and conserve biodiversity [Narwal and Tauro, 2000; Scavo et al., 2022].

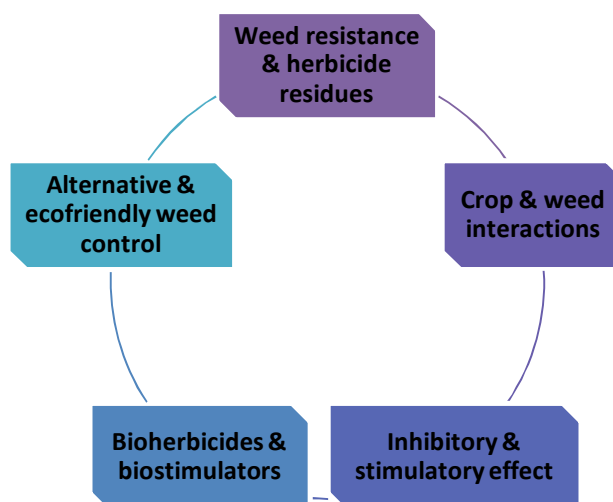


Figure 2.3. Application of allelopathy in agroecosystems.

## 2.2. Factors influencing allelopathic potential of plants

The bioactive plant secondary metabolites (allelochemicals) are present in various concentrations in all plants and plant parts [Alam et al., 2001]. Plant species, including medicinal plants, from different botanical families, both cultivated and wild, represent a great source of bioactive compounds for the development of new, safe and biodegradable bioherbicides [Bhowmik and Indjerit, 2003; Fujii et al., 2003; Amini et al., 2016]. Species belonging to Brassicaceae family, such as white mustard (*Sinapis alba*), radish (*Raphanus sativus*) and camelina (*Camellina sativa*) exerted strong herbicidal potential towards germination and growth of invasive weed species common ragweed (*Ambrosia artemisiifolia*), with 15 phenolic compounds detected in their water extracts such as phenolic aldehydes (vanillin), hydroxybenzoic acids (chlorogenic acid, vanillic acid, syringic acid) and hydroxycinnamic acids (caffeic acid, ferulic acid) [Šćepanović et al., 2021].

Aniya et al. (2020) screened 50 medicinal plants for their allelopathic potential against lettuce (*Lactuca sativa*) and found that star anise (*Illicium verum*, Schisandraceae) fruit, oak-leaved goosefoot (*Chenopodium glaucum*, Amaranthaceae) leaf and Chinese lantern-plant (*Physalis alkekengi*, Solanaceae) fruit were among the species with the greatest inhibitory potential on radicle and hypocotyl elongation of seedlings. Allelopathic effect of four medicinal plant species, chia (*Salvia hispanica*, Lamiaceae), black cumin (*Nigella sativa*, Ranunculaceae), wormwood

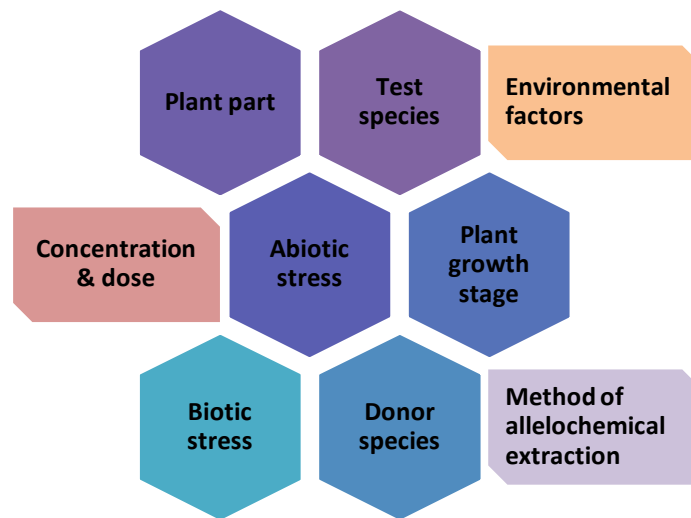


(*Artemisia absinthium*, Asteraceae), and nettle (*Urtica dioica*, Urticaceae) on the seed germination and growth characteristics of pepper (*Capsicum annuum*), spinach (*Spinacia oleracea*) and lettuce were studied by Erhatic et al. (2023).

Phytochemical composition revealed the presence of a variety of chemical compounds in various concentrations such as epicatechin, quinic acid, caffeic acid, esculetin, cinnamic acid, gallic acid and kaempferol, while nettle and wormwood had the greatest negative potential against germination of tested species.

Aromatic waters (hydrolates or hydrosols) of cypress (*Cupressus sempervirens*, Cupressaceae) and two species from Lamiaceae family, namely rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) inhibited seed germination of three lettuce varieties up to 100% compared to control in study of Politi et al. (2022). Other promising plant species with allelopathic and herbicidal effect belonging to Lamiaceae family include meadow sage (*Salvia pratensis*) [Ravlic et al., 2023a; Županić, 2023], basil (*Ocimum basilicum*), lemon balm (*Melissa officinalis*) [Petrova et al., 2015; Amini et al., 2016; Sarić-Krsmanović et al., 2019; Ravlic et al., 2022a], lavender (*Lavandula angustifolia*), peppermint (*Mentha × piperita*), apple mint (*Mentha suaveolens*), woodland sage (*Salvia nemorosa*), Russian sage (*Salvia abrotanoides*), common thyme (*Thymus vulgaris*), and oregano (*Origanum vulgare*) [Amini et al., 2016; Mirmostafae et al., 2020].

Plant species from Apiaceae family, such as parsley (*Petroselinum crispum*) [Ravlic et al., 2014], fennel (*Foeniculum vulgare*) [Ravlic et al., 2016], dill (*Anethum graveolens*), cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*) [Mirmostafae et al., 2020], and lovage (*Levisticum officinale*) [Lucić et al., 2018] are also among great candidates as potential sources of bioactive molecules with use in plant protection. Rue (*Ruta graveolens*, Rutaceae) water extracts and plant powders from aboveground biomass, fruit and leaf leachates, and essential oils obtained from leaves were also reported to have significant inhibitory potential on both crops and weeds [Makkizadeh et al., 2009, Amini et al., 2016., Ravlic et al., 2016, Mirmostafae et al., 2020].



**Figure 2.4. Factors affecting allelopathic and herbicidal potential of plants.**

Allelopathic potential of selected donor plant species depends on multiple factors. The activity is influenced by concentration or dosage, plant part, extraction method and whether plant material is fresh or dry, but also depends greatly on the target species as they differ in their sensitivity [Fujii et al., 2003; Norsworthy, 2003; Souza Filho et al., 2009; Ravlić et al., 2016; Aniya et al., 2020; Ravlić et al., 2022a].

Usually, allelopathic potential is concentration or dose dependent with higher concentrations exhibiting stronger negative effect and often completely inhibiting or delaying germination or having detrimental effects on root and shoot growth [Šćepanović et al., 2021; Erhatic et al., 2023]. Additionally, it is not uncommon for very low concentrations (e.g., 0.1 to 2%) or doses to stimulate and promote growth of test species [Baličević et al., 2018; Šćepanović et al., 2021]. Meadow sage (*S. pratensis*) water extracts in low concentrations (1% and 2,5%) significantly stimulated shoot length of lettuce by 33% and 38.9% compared to control [Županić, 2023].

Plant parts possess allelochemicals in various concentrations which is related to their different allelopathic potential [Scavo et al., 2022]. For example, Amini et al. (2016) reported that saffron crocus (*Crocus sativus*) stigma and style reduced radicle and hypocotyl of lettuce seedlings to a greater extent compared to leaf, while hyssop (*Hyssopus angustifolius*) flower promoted hypocotyl length oppose to inhibitory activity of leaf. Lavender (*L. angustifolia*) essential oil extracted from flowers showed greater decrease in seed germination and growth parameters of test species seedlings



compared to essential oil obtained from leaves [Mirmostafae et al., 2020]. Leaves are most often plant parts with the highest negative potential. According to Ravlić et al. (2019) inhibitory effect of stems of birthwort (*Aristolochia clematitis*), common evening-primrose (*Oenothera biennis*) and common poppy (*Papaver rhoeas*) was less pronounced compared to leaves.

Allelopathic potential depends on whether fresh or dry plant materials were used or when different method of allelochemicals extraction (water, ethanolic, methanolic or hydroalcoholic extracts, residues, essential oils) was employed. According to Ravlić et al. (2022a) water extracts from fresh plant material showed lower negative potential compared to water extracts from dry plant material. Dry plant material inhibited germination and growth of weed species up to 100%. Similarly, parsley (*P. crispum*) water extracts from dry biomass completely inhibited germination of hoary cress (*Lepidium draba*) contrary to extracts from fresh biomass [Ravlić et al., 2014]. Silva et al. (2014) on the other hand recorded greater phytotoxic effect of volatiles from fresh leaves compared to dry leaves of two Asteraceae shrubs on growth of lettuce and onion (*Allium cepa*) seedlings. According to Kato-Noguchi (2003), dry lemon balm (*M. officinalis*) residues inhibited germination of purple nutsedge (*Digitaria sanguinalis*) up to 30.0%, while root and shoot lengths were reduced by over 50.0%. Effect of volatile compounds from 123 medicinal and aromatic plants was reported in study of Sadeqifard et al. (2022). Results revealed that geranium (*Pelargonium graveolens*) leaves, lavender (*L. angustifolia*) flowers and horn-leaved sage (*Salvia ceratophylla*) leaves had the greatest inhibitory effect on lettuce radicle growth (100% inhibition compared to control). The greatest effect on hypocotyl growth inhibition was observed with stems of *Echinophora platyloba*, medicinal and aromatic species from Apiaceae family.

Essential oils and their components are reported to possess allelopathic and herbicidal activity. In the study of Sarić-Krsmanović et al. (2019) essential oils of basil (*O. basilicum*), sage (*S. officinalis*), thyme (*T. vulgaris*), lemon balm (*M. officinalis*) and goldenrod (*Solidago virgaurea*) were assessed for their herbicidal potential against germination and growth of velvetleaf (*Abutilon theophrasti*). The results showed that with the increase of essential oil concentration both germination and growth of





velvetleaf seedlings decreased, and the studied essential oils could be considered as potential alternative allelochemicals with herbicidal potential used in weed control as bioherbicides. Mirmostafae et al. (2020) screened inhibitory effects of 112 essential oils and their mixtures using cotton swab method. The experiment included different plant organs (root, rhizome, leaf, flower, etc.) of 97 species of aromatic and medicinal plants belonging to 16 botanical families. Oregano (*O. vulgare*) essential oil proved to be the strongest growth inhibitor, as well as several other species belonging to Lamiaceae, Geraniaceae and Apiaceae plant families. According to Souza Filho et al. (2009) hydroalcoholic extracts had higher negative potential on seed germination compared to essential oils. Similarly, differences between allelopathic effect of water extracts and plant powders are often reported (Ravlić et al., 2016, 2022a). Sekine et al. (2020) compared two methods, sandwich method and dish pack method, in order to evaluate allelopathic effect of leachates and volatile compounds of 53 spices and herbs against lettuce seedlings growth. The inhibitory activity differed among methods, with parsley (*Petroselinum sativum*) leachates, and caraway (*Carum carvi*) and dill (*A. graveolens*) volatiles being the most inhibitory.

Differences in species susceptibility towards allelopathic potential of donor species is reported in majority of studies [Baličević et al., 2018; Scavo et al., 2022; Erhatic et al., 2023] as well as different sensitivity among various genotypes of the same species [Politi et al., 2022]. Aniya et al. (2019) concluded that common bird's-foot trefoil (*Lotus corniculatus*) was the most sensitive species to shikimic acid, the main compound found in the fruits star anise (*I. verum*), compared to other test species, i.e., red clover (*Trifolium pratense*), white clover (*Trifolium repens*), alfalfa (*Medicago sativa*). *Aloe vera* (L.) Burm. F. extracts according to Baličević et al. (2018) decreased germination, root and shoot length, fresh and dry weight of barley (*Hordeum vulgare*) seedlings, while stimulating all growth parameters of oil pumpkin (*Cucurbita pepo* L. var. *oleifera*). One of the main factors which influence species sensitivity to allelopathic activity is seed size of the test species, as well as the ability of the species to metabolize allelochemicals [Vidotto et al., 2013; Bibi et al., 2023].

Phytotoxic potential of plant extracts and essential oils is affected by environmental factors, such as geographical origin, growing conditions, seasonal





variation and plant growth stage as well as abiotic and biotic environmental factors, which may increase the production of secondary metabolites in plants and enhance their inhibitory effect [Safdar et al., 2014; Khanh et al., 2018; Sarić-Krsmanović et al., 2019; Medina-Villar et al., 2020; Appiah et al., 2022; Ravlić et al., 2022a].

Phytotoxicity of plants changes seasonally in relation to seasonal fluctuations of environmental parameters [Silva et al., 2014]. Appiah et al. (2022) in their study evaluated the effect of seasonal variation on the content of carnosic acid, reported as allelochemical, and phytotoxic potential of dried rosemary (*R. officinalis*) leaves. The authors concluded that the highest inhibitory potential on lettuce was observed with samples collected from early summer (June), coinciding with the highest average concentration of carnosic acid which amounted to 15.1 mg/g dry weight, while the lowest concentration was marked in February (8.3 mg/g dry weight). Additionally, the study also reported that the concentration of carnosic acid differed in samples collected from two locations. Safdar et al. (2014) reported various inhibitory effect of extracts from parthenium weed (*Parthenium hysterophorus*) and the content of phenolic compounds in biomass collected on three locations – near the field border, near the pond and near the water channel. Similarly, species' growth stages may also influence the allelopathic performance of plants as diversity and concentration of allelochemicals vary greatly with the phenological stage of the donor plant [Ravlić et al., 2022b, Zribi et al., 2014].

Plant materials collected from vegetative, flowering and/or mature stages significantly vary in their ability to impede the germination and growth of target species. According to Ravlić et al. (2022b) water extracts from sunflower leaves collected in the flowering stage inhibited lettuce shoot length to a greater degree compared to water extracts from leaves obtained in earlier growth stage, i.e., butonisation stage. Zribi et al. (2014) tested differences in allelopathic potential of black cumin (*N. sativa*) varieties harvested at different developmental stages - vegetative (plants with 7 leaves), flowering stages (50% of flowers open) and fruiting (50% of the pods have reached a typical length). The results indicated that the Indian variety showed the highest phytotoxicity at the vegetative and Tunisian at the flowering stage. Žalac et al. (2022)



concluded that extract from older walnut tree leaves had most severe toxic effect on growth of test species.

Drought and water stress affects inhibitory capacity of plant residues and water extracts. According to Motamedi et al. (2016) safflower (*Carthamus tinctorius*) shoot residues produced under normal irrigation were less inhibitory on radish (*R. sativus*) compared to residues obtained under drought stress. Similarly, Ravlić et al. (2023b) examined effect of water stress i.e., different net irrigation water on the allelopathic potential of petunia (*Petunia hybrida*). Overall, water stress had no significant effect on the allelopathic potential of petunia, except with the highest extract concentration on the shoot length of seedlings where extracts from petunias grown at lowest net irrigation water had greater inhibitory effect compared to extracts from petunias grown at higher net irrigation water. Other agricultural practices, e.g., tillage treatments, mowing etc. may also influence allelopathic potential of plants [Biramahire et al., 2022, Ravlić et al., 2022c].

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## Chapter 3. Herbicidal effect of plant extract and essential oils (Ravlic M, Balicevic R)

### 3.1. Introduction

Allelopathic and herbicidal potential of plants can be assessed using various methods under laboratory conditions, in greenhouse using pots with soil or under field conditions. The choice of method is usually related to pathway of allelochemicals release. For example, the sandwich method is used for evaluation of leaf leachates [Amini et al., 2016; Aniya et al., 2020], while experiments with extracts simulate release of allelochemicals from plant material and soil through leaching and can be tested on both artificial media such as filter paper or in pots with soil [Šćepanović et al., 2021; Winkler et al., 2022; Ravlić et al., 2023a].

Plant powders or residues are often tested in pot experiments under laboratory conditions or in greenhouses [Ravlić, 2015; Ravlić et al., 2016]. Other methods include cotton swab method to evaluate phytotoxicity of volatile constituents of essential oils [Mirmostafae et al., 2020] and Petri dish experiments for evaluation of contact effect of essential oils [Sarić-Krsmanović et al., 2019]. Dish pack method enables assessment of volatile compounds secreted from dry plant material using multi-dishes with six wells [Kang et al., 2019; Sadeqifard et al., 2022]. Laboratory screenings may also evaluate the allelopathic potential of root exudates [Shiraishi et al., 2002; Ravlić et al., 2020; Yang and Li, 2022].

Results from plant screenings in laboratory conditions on artificial media such as filter paper or agar can be different when evaluated in soil as medium. According to Ravlić et al. (2021) emergence of redroot pigweed (*Amaranthus retroflexus*) was significantly reduced in pots with soil oppose to Petri dish bioassay in experiment with cogermination of herbs. Direct contact of test species seeds with extracts on filter paper usually causes greater detrimental effects on germination and growth of seedlings [Ravlić et al., 2014]. Beside method of release and screening method, seed density of receptor plant may also affect the degree of allelopathic potential. Lower seed density results in greater concentration of allelochemicals received per receptor plant,

therefore higher allelopathic potential is caused and vice versa when there is higher seed density the allelopathic potential is less pronounced [Aguilar-Franco et al., 2019].

### 3.2. Cultivated and wild medicinal plants with promising allelopathic and herbicidal potential

Herbicidal effect of meadow sage (*S. pratensis*) water extracts was assessed against germination and growth of two weeds species, velvetleaf (*A. theophrasti*) and common corn-cockle (*Agrostemma githago*) [Ravlić et al., 2023a]. Water extracts were prepared from dry aboveground biomass of meadow sage in five different concentrations (1%, 2.5%, 5%, 7.5% and 10%) and tested *in vitro*. Research result revealed inhibitory potential of meadow sage extracts, especially with higher concentrations (Table 3.1). Germination of common corn-cockle (*A. githago*) was significantly reduced from 7.6 % to 98.9 % compared to control.

**Table 3.1. Effect of meadow sage (*Salvia pratensis*) water extracts on seed germination and growth of weed seedlings [Ravlić et al., 2023a].**

Water extract concentration (%)	<b>Germination (%)</b>	
	<i>Abutilon theophrasti</i>	<i>Agrostemma githago</i>
Control	71.0 a	92.0 a
1 %	70.0 a	85.0 b
2.5 %	66.0 a	7.0 c
5 %	62.0 a	6.0 c
7.5 %	63.0 a	3.0 cd
10 %	64.0 a	1.0 d
Water extract concentration (%)	<b>Root length (cm)</b>	
	<i>Abutilon theophrasti</i>	<i>Agrostemma githago</i>
Control	5.35 a	6.11 a
1 %	2.81 b	6.49 a
2.5 %	1.93 c	4.09 b
5 %	1.6 c	2.5 c
7.5 %	1.32 cd	0.38 d
10 %	0.86 d	0.0 d



Water extract concentration (%)	<b>Shoot length (cm)</b>	
	<i>Abutilon theophrasti</i>	<i>Agrostemma githago</i>
Control	2.57 a	1.92 a
1 %	2.15 a	1.18 b
2.5 %	1.57 b	0.55 c
5 %	1.09 c	0.4 cd
7.5 %	1.11 c	0.23 d
10 %	1.08 c	0.0 e

Water extract concentration (%)	<b>Fresh weight (mg)</b>	
	<i>Abutilon theophrasti</i>	<i>Agrostemma githago</i>
Control	43.8 a	70.0 a
1 %	39.0 b	53.0 b
2.5 %	34.6 c	28.9 c
5 %	27.8 d	28.2 c
7.5 %	24.8 d	15.2 d
10 %	26.5 d	0.0 e

a,b,c - means followed by the same letter within column are not significantly different at P<0.05

Root and shoot length of weed seedlings was severely reduced, and even completely inhibited compared to control. Overall, greater inhibitory effect was recorded for common corn-cockle (*A. githago*) which was more sensitive compared to velvetleaf (*A. theophrasti*) (Figure 3.1).



**Figure 3.1. Effect of meadow sage (*Salvia pratensis*) water extracts on seed germination and growth of velvetleaf (*Abutilon theophrasti*): A) control and 2.5% extract, B) control and 10% extract.**

Fennel (*F. vulgare*), sage (*S. officinalis*) and rue (*R. graveolens*) water extracts were studied for their herbicidal potential against weed species hoary cress (*Cardaria draba*) by Ravlić et al. (2016). Water extracts were tested in two different concentrations with 50 and 100 g of plant biomass per liter. Results revealed that water extracts prepared from fresh and dry aboveground biomass of plants had various effect on seed germination and growth of hoary cress seedlings (Table 3.2).



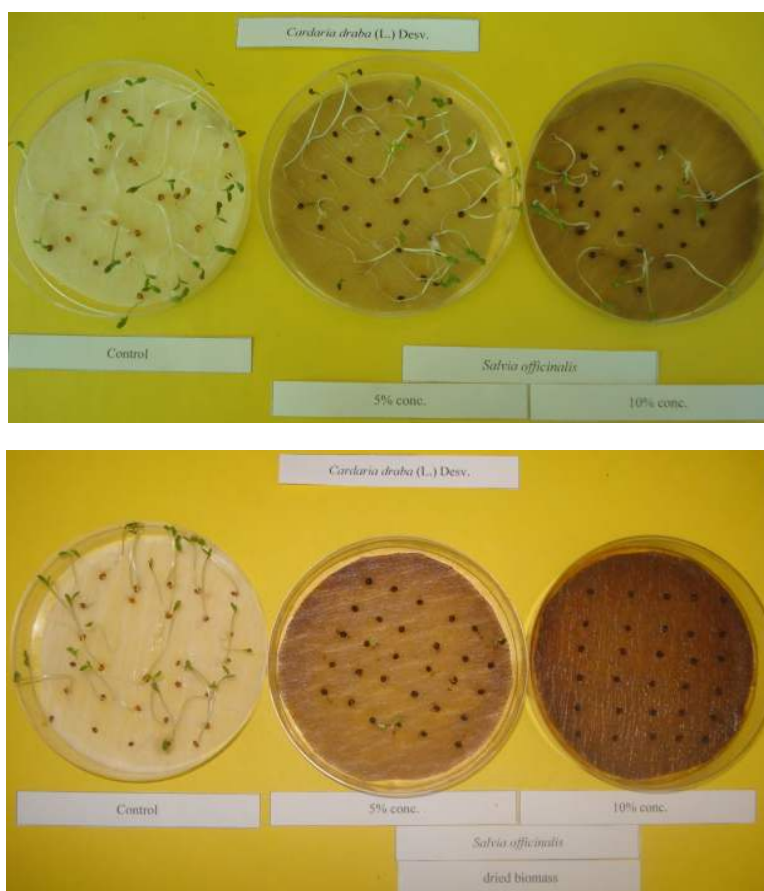
**Table 3.2. Effect of aromatic and medicinal plant water extracts on germination and seedling growth of hoary cress on filter paper in Petri dishes [Ravlić et al., 2016].**

<i>Treatments</i>	<i>Germination n (%)</i>	<i>Root length (cm)</i>	<i>Shoot length (cm)</i>	<i>Fresh weight (cm)</i>
Control	86.2 a	3.2 c	2.2 bc	0.0163 b
<b>Fresh biomass</b>				
Fenne 50 g l <sup>-1</sup>	88.7 a	2.1 d	2.6 a	0.0175 ab
100 g l <sup>-1</sup>	83.2 a	1.3 e	2.4 ab	0.0135 c
Rue 50 g l <sup>-1</sup>	62.4 bc	5.0 a	2.7 a	0.0189 a
100 g l <sup>-1</sup>	60.7 c	3.9 b	2.3 bc	0.0161 b
Sage 50 g l <sup>-1</sup>	66.9 b	3.9 b	2.3 bc	0.0157 bc
100 g l <sup>-1</sup>	65.9 bc	3.3 c	2.1 c	0.0138 c
<b>Dry biomass</b>				
Fenne 50 g l <sup>-1</sup>	1.8 f	0.1 g	0.1 e	0.0001 e
100 g l <sup>-1</sup>	0.0 f	0.0 g	0.0 e	0.0000 e
Rue 50 g l <sup>-1</sup>	12.3 e	0.2 fg	0.4 d	0.0026 d
100 g l <sup>-1</sup>	0.0 f	0.0 g	0.0 e	0.0000 e
Sage 50 g l <sup>-1</sup>	36.7 d	0.3 f	0.3 de	0.0021 de
100 g l <sup>-1</sup>	0.0 f	0.0 g	0.0 e	0.0000 e

Means followed by the same letter within the column are not significantly different at p < 0.05.

On average, extracts from fresh biomass reduced germination for up to 17%, while extracts from dry biomass for over 90%, with fennel having the highest inhibitory potential. Substantial inhibition of root length was observed with fennel extracts from fresh biomass, however rue and sage promoted root elongation. Similarly, positive effect on shoot length and fresh weight of seedling was marked for water extracts from fresh biomass. Dry biomass of all tested aromatic and medicinal plants in higher concentration completely (100%) inhibited germination and growth parameters of weed seedlings (Figure 3.2).





**Figure 3.2. Effect of *Salvia officinalis* water extracts on germination and seedling growth of hoary cress on filter paper in Petri dishes.**

The inhibitory potential of six aromatic and medicinal plant residues was tested on emergence and growth of weed species black nightshade (*Solanum nigrum*) [Ravlić, 2015, Ravlić et al., 2022a]. The effect of plant powders from dry biomass of four cultivated, basil (*O. basilicum*), chamomile (*Chamomilla recutita*), lovage (*L. officinale*), lemon balm (*M. officinalis*) and two wild, common mallow (*Malva sylvestris*) and greater celandine (*Chelidonium majus*), plant species was tested in pot experiment. Effect of dry plant powder was assessed in two rates: 10 and 20 g per kg of soil. The results showed that the greatest reduction in black nightshade emergence (63.9%) was recorded in the treatment with a higher rate of basil plant powder. Excellent inhibitory effect was recorded also with lovage and greater celandine. The reduction in root length was on average for 50%, and celandine had the greatest effect (Table 3.3).

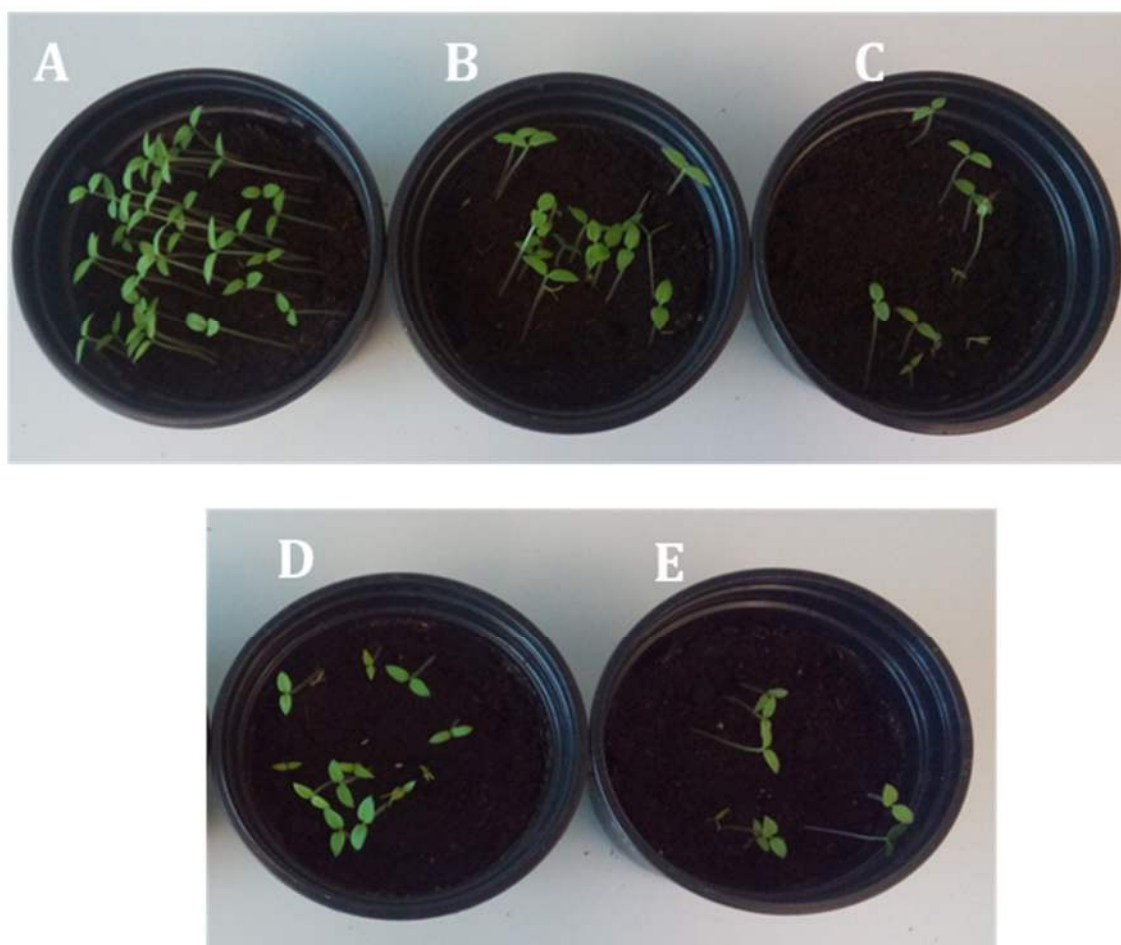


**Table 3.3. Effect of aromatic and medicinal plant powders on emergence and seedling growth of black nightshade in pot experiment [Ravlić, 2015; Ravlić et al., 2022a].**

<i>Treatment</i>	<i>g kg<sup>-1</sup></i>	<i>Emergence (%)</i>	<i>Root length (cm)</i>	<i>Shoot length (cm)</i>	<i>Fresh weight (mg)</i>
Control		61.0 <sup>a</sup>	2.2 <sup>a</sup>	4.1 <sup>a</sup>	17.7 <sup>a</sup>
Basil	10	28.5 <sup>cd</sup>	1.0 <sup>cd</sup>	2.9 <sup>d</sup>	10.3 <sup>e</sup>
	20	22.0 <sup>d</sup>	1.0 <sup>cd</sup>	2.9 <sup>d</sup>	11.7 <sup>cde</sup>
Common mallow	10	23.8 <sup>d</sup>	0.9 <sup>cd</sup>	3.2 <sup>bcd</sup>	12.6 <sup>bcd</sup>
	20	31.8 <sup>bcd</sup>	1.0 <sup>cd</sup>	3.2 <sup>bcd</sup>	12.5 <sup>cd</sup>
Chamomile	10	39.3 <sup>bc</sup>	1.1 <sup>c</sup>	3.4 <sup>bc</sup>	12.5 <sup>cd</sup>
	20	34.5 <sup>bcd</sup>	1.0 <sup>cd</sup>	3.3 <sup>bcd</sup>	13.3 <sup>bc</sup>
Lovage	10	28.5 <sup>cd</sup>	1.1 <sup>c</sup>	3.1 <sup>bcd</sup>	12.2 <sup>cde</sup>
	20	28.8 <sup>cd</sup>	0.9 <sup>cd</sup>	2.9 <sup>d</sup>	11.2 <sup>cde</sup>
Lemon balm	10	42.0 <sup>b</sup>	1.6 <sup>b</sup>	3.5 <sup>b</sup>	14.7 <sup>b</sup>
	20	37.5 <sup>bc</sup>	1.5 <sup>b</sup>	3.4 <sup>bc</sup>	14.7 <sup>b</sup>
Greater celandine	10	33.3 <sup>bcd</sup>	1.1 <sup>c</sup>	3.0 <sup>cd</sup>	12.2 <sup>cde</sup>
	20	26.8 <sup>cd</sup>	0.8 <sup>d</sup>	3.0 <sup>cd</sup>	11.0 <sup>de</sup>

Means followed by the same letter within the column are not significantly different at  $p < 0.05$ .

On the other hand, shoot length and fresh weight of black nightshade seedlings were less affected, however statistically significant reduction compared to control was recorded for all plant powders and both rates. Lovage, basil and greater celandine were the most effective and inhibited the aforementioned parameters up to 29.3% and 41.8% compared to control (Figure 3.3).



**Figure 3.3. Effect of plant powders on seedling growth of black nightshade in pots:**  
A) control; B) greater celandine 10 g kg<sup>-1</sup>; C) greater celandine 20 g kg<sup>-1</sup>; D) basil 10 g kg<sup>-1</sup>;  
E) basil 20 g kg<sup>-1</sup>.

In their study Ravlić et al. (2021) assessed seed allelopathy between herbs and weed species. The experiment included cogermination of herb and weed seeds in Petri dish experiment and evaluation on germination and seedlings growth of weed species. Results showed that seed cogermination had different effect on weed species (Table 3.4). Lovage seeds induced significant reduction in germination of redroot pigweed (*A. retroflexus*) and hoary cress (*L. draba*) for 93.6 % and 34.1%. Similarly, statistically significant inhibition in germination of black nightshade (*S. nigrum*) was recorded in treatments with lovage, basil and lemon balm. Lovage also reduced root and shoot length and fresh weight of several weed species. On average, the most susceptible weed species in the experiment were redroot pigweed and hoary cress.



**Table 3.4. Allelopathic effect of seed cogermination on germination and seedling growth of weed species in Petri dish experiment [Ravlić et al., 2021].**

Treatment	ABUTH	AMARE	CADDR	SOLNI	SORHA
	Germination (%)				
Control	55.4 a	58.3 ab	73.3 a	55.3 a	25.3 ab
Basil	58.8 a	59.2 a	70.0 a	44.3 bc	27.0 a
Chamomile	53.3 a	50.8 b	77.1 a	52.3 ab	19.3 b
Lovage	60.4 a	3.8 c	48.3 b	16.8 d	20.8 ab
Lemon balm	61.3 a	55.0 ab	67.9 a	41.5 c	20.5 ab
Root length (cm)					
Control	4.8 b	3.9 a	1.6 bc	3.1 a	4.1 a
Basil	4.8 b	3.9 a	2.2 a	3.1 a	4.1 a
Chamomile	4.8 b	3.8 a	2.0 a	2.9 a	3.6 ab
Lovage	5.6 a	1.7 b	1.2 c	1.9 b	3.9 a
Lemon balm	5.5 a	4.1 a	1.8 ab	3.2 a	3.2 b
Shoot length (cm)					
Control	4.6 bc	2.3 bc	2.1 a	1.3 a	5.9 a
Basil	4.6 bc	3.1 a	2.0 a	1.5 a	5.9 a
Chamomile	4.3 c	2.4 bc	1.9 a	1.4 a	5.5 a
Lovage	4.8 b	2.1 c	0.9 b	1.3 a	4.6 b
Lemon balm	5.1 a	2.9 ab	2.1 a	1.6 a	5.2 ab
Fresh weight (mg)					
Control	50.0 b	5.5 ab	12.9 a	8.9 a	18.9 a
Basil	54.6 a	5.9 a	11.8 b	8.1 a	18.5 a
Chamomile	43.0 c	4.7 ab	12.1 ab	7.3 ab	17.9 a
Lovage	55.0 a	2.7 c	7.4 c	6.1 b	14.7 b
Lemon balm	56.4 a	5.3 ab	12.0 ab	9.2 a	14.6 b

\* ABUTH *A. theophrasti*; AMARE *A. retroflexus*; CADDR *L. draba*; SOLNI *S. nigrum*; SORHA *S. halepense*

Results of allelopathic effect of several wild plant species on growth of tomato (*Solanum lycopersicum*) and lettuce are presented in Table 3.5. [Lišnić, 2023; Ravlić et al., 2024]. Wild species tested were Austrian yellowcress (*Rorippa austriaca* Brassicaceae), ribwort plantain (*Plantago lanceolata*, Plantaginaceae), prostrate knotweed (*Polygonum aviculare*, Polygonaceae), wild chicory (*Cichorium intybus*,



Cichoriaceae), hemp agrimony (*Eupatorium cannabinum*, Asteraceae), large yellow vetch (*Vicia grandiflora*, Fabaceae), woolly mullein (*Verbascum phlomoides*, Scrophulariaceae), common purslane (*Portulaca oleracea*, Portulacaceae) and white sweet clover (*Melilotus albus*, Fabaceae). Water extracts were prepared from dry aboveground biomass and tested in 5% concentration in Petri dish experiment. Tomato root length was significantly reduced in all treatments, except with hemp agrimony (*E. cannabinum*), while stimulatory effect was recorded in treatment with prostrate knotweed (*P. aviculare*). For lettuce, all water extracts caused significant reduction of root length, from 23.2% to 92.8% compared to control. Lettuce proved to be more sensitive as its root length was reduced, on average across all treatments, for 67.5%, while for lettuce average reduction amounted to 50.9%.

**Table 3.5. Effect of plants extracts from wild plant species on root length of tomato and lettuce** [Lišnić, 2023; Ravlić et al., 2024].

<i>Treatment</i>	<i>Root length (cm)</i>	
	<i>Tomato</i>	<i>Lettuce</i>
Control	4.37 b	2.77 a
<i>Rorippa austriaca</i>	1.50 d	0.65 e
<i>Plantago lanceolata</i>	0.93 e	0.85 de
<i>Polygonum aviculare</i>	7.11 a	2.13 b
<i>Cichorium intybus</i>	1.49 d	1.32 c
<i>Eupatorium cannabinum</i>	4.43 b	1.01 d
<i>Vicia grandiflora</i>	2.73 c	0.33 f
<i>Verbascum phlomoides</i>	0.66 ef	1.37 c
<i>Portulaca oleracea</i>	0.24 fg	0.20 f
<i>Melilotus albus</i>	0.22 g	0.25 f

Means followed by the same letter within the column are not significantly different at  $p < 0.05$ .





On average for both test species, the highest inhibitory potential was marked for common purslane (*P. oleracea*), white sweet clover (*M. albus*) and ribwort plantain (*P. lanceolata*) that reduced root length for 93.6%, 93% and 74.1% compared to control (Figure 3.4).

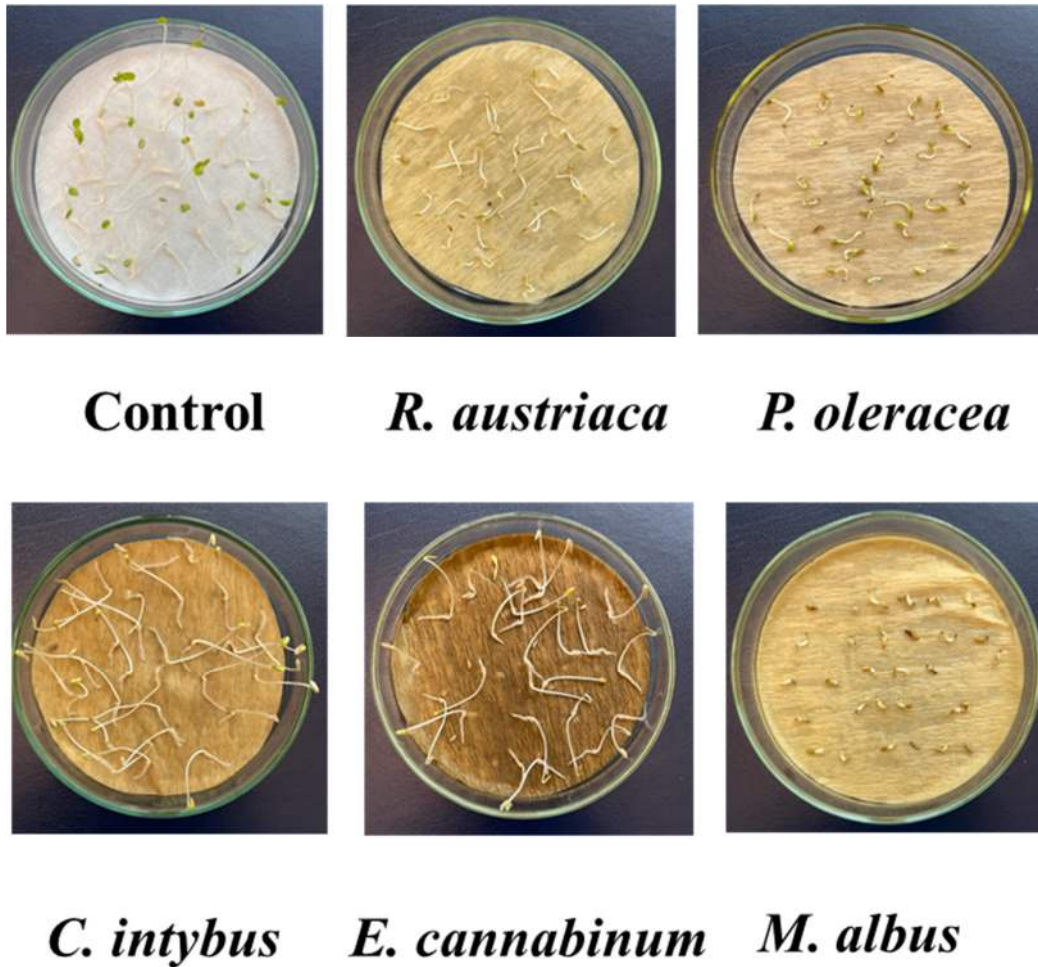


Figure 3.4. Effect of water extracts from wild plant species on growth of lettuce seedlings.

In the study of Lucić et al. (2018) allelopathic potential of lovage (*L. officinale*) was assessed. Dry aboveground biomass of lovage was used for preparation of water extracts in various concentrations (2%, 4%, 6%, 8% and 10%) which were tested in Petri dish experiment using kale and garden cress as test species. The increase in extract concentration resulted in greater negative potential on seed germination and seedlings growth of both kale and garden cress (Table 3.6). Even the lowest concentration (2%) had statistically significant inhibitory potential compared to control





treatment for all measured parameters, except for dry weight of seedlings. Garden cress proved to be more sensitive compared to kale as complete inhibitory effect was observed at 6% extract concentration as oppose to kale where complete inhibition was recorded at 8% extract concentration.

**Table 3.6. Effect of lovage (*Levisticum officinale*) extracts germination and growth of kale and garden cress seedlings [Lucić et al., 2018].**

<i>Water extract</i>	<i>Germination</i>	<i>Root length</i>	<i>Shoot length</i>	<i>Fresh weight</i>	<i>Dry weight</i>
Kale					
control	96.0 a	2.66 a	1.90 a	16.06 a	2.25 a
2%	65.3 b	0.53 b	1.45 b	10.87 b	2.34 a
4%	24.7 c	0.21 c	0.26 c	1.78 c	0.74 b
6%	16.7 d	0.16 c	0.19 cd	1.46 c	0.52 b
8%	0.0 e	0.0 d	0.0 d	0.0 c	0.0 b
10%	0.0 e	0.0 d	0.0 d	0.0 c	0.0 b
Garden cress					
control	92.0 a	3.17 a	2.01 a	12.55 a	1.83 a
2%	54.0 b	0.47 b	0.68 b	6.71 b	2.04 a
4%	8.7 c	0.20 bc	0.13 c	2.30 c	0.40 b
6%	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c
8%	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c
10%	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c

Means followed by the same letter within the column are not significantly different at  $p < 0.05$ .

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## Chapter 4. Plant diseases in medicinal production (Cosic J, Vrandecic K)

### 4.1. Introduction

Medicinal plants, like all plants, are susceptible to a variety of diseases that can affect their growth, yield, and medicinal properties [Avan, 2021]. Understanding these diseases is crucial for the cultivation and effective use of medicinal plants. Some common plant diseases that can affect medicinal plants are:

Fungal infections which are a major cause of plant diseases. Common fungal diseases include powdery mildew, root rot, and leaf spot. These can lead to wilting, yellowing of leaves, and reduced plant vigor.

Bacterial infections can cause symptoms such as wilting, leaf spots, and galls. Diseases like bacterial blight and soft rot can severely impact the health of medicinal plants.

Viruses can lead to stunted growth, mottled leaves, and reduced yields. Viral diseases are often spread by insect vectors, such as aphids.

As the demand for natural plant-based products for medicinal and health purposes continues to grow, there is a heightened focus on the quality of the raw materials sourced from these plants. Typically, the vegetative tissues and organs serve as the primary sources for these materials. However, these tissues and organs can be vulnerable to various diseases, which can lead to a decline in the quality of the economically valuable products and a potential loss of genetic diversity. Significant advancements have been achieved in identifying the organisms responsible for these diseases and understanding their pathogenic effects at the organ, cellular, and biochemical levels [Singh et al., 2016].

### 4.2. Fusarium wilt

Lavandula production show to be profitable for a small family farm, especially on the area unsuitable for other crops since it has high drought resistance and can grow in poor, rocky soils. Chemical composition of essential oils varies depending on the number of different factors: cultivars/hybrids, environmental factors, geographical



differences, agronomic factor or due to the method of oil extraction. One of the important problems in production of lavender is the occurrence of diseases, especially those caused by fungi of the genus *Fusarium* which caused disease known as Fusarium wilt [Xue-Jun et al., 2023; Özer et al., 2021; Ćosić et al., 2012].

Symptoms consisted of chlorosis and yellowing of leaves followed by wilting of leaves and branches (Figure 4.1, 4.2 and 4.3). A change in color to brown can be seen on the cross-section of the root. Brown discoloration was observed in the vascular system (Figure 4.4 and 4.5). The presence of *Fusarium sporotrichioides* Sherb was determined by morphological and molecular methods [Ćosić et al., 2012]. Isolations of the pathogen were made from the discolored tissues on potato dextrose agar (PDA). Colonies were initially white, but with age became red, and red pigments were produced in agar. Microconidia were pear shaped, oval, and fusoid, and ranged from 4.5 to 14.0 × 2.8 to 4.7 μm. Macroconidia were curved, mostly three septate, and ranged from 21.8 to 24.3 × 2.9 to 3.9 μm (Figure 4.6).

The pathogenicity test, which was performed on four-month-old lavender plants (inoculated barley and wheat grains were mixed with the substrate and placed in the root zone) showed that the obtained isolate was highly pathogenic because in sixteen days from after inoculation, 80% of the plants withered.

*Phytophthora* spp., *Armillaria* spp., *Pythium* spp. and *Fusarium* spp. are mentioned in the literature as fungal agents of lavender root diseases (soil pathogens). The types of diseases they cause are root rot and wilt. Fusarium wilt and root rot can be caused by different species such as *Fusarium oxysporum* [Özer et al., 2021], *Fusarium foetens* [Xue-Jun et al., 2023], *Fusarium sporotrichioides* [Ćosić et al., 2012]. *Fusarium oxysporum* Schlecht has been found to cause lavender wilt worldwide [Özer et al., 2021; Farr and Rossman, 2020; Garibaldi et al., 2015]. However, regardless of which *Fusarium* species it is, the symptoms of the disease and the consequences for the plants are the same.





Figure 4.1. Symptoms of Fusarium wilt of lavender.



Figure 4.2. Lavender wilt caused by *Fusarium sporotrichioides*.





Figure 4.3. Artificial infection of lavender by *F. sportotrichoides*, symptoms of wilting.



Figure 4.4. Typical symptoms of disease in vascular system.



**Figure 4.5. Brown discoloration in vascular system.**



**Figure 4.6. Macroconidia and chlamydospores of *F. sporotrichioides***

For the successful control of soil pathogens, including Fusarium species, the most important thing is to plant lavender on draining and well-drained soils to prevent the occurrence of diseases. Diseases caused by soil parasites can especially be expected in wet years, and if they occur, they are irreversible. Soil pathogens are





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present in all soils and that is why the choice of soil for planting is the most important element in preventing the occurrence of diseases.

Except lavender Fusarium wilt caused by *Fusarium oxysporum* has been also reported on cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), Japanese mint (*Mentha arvensis haplocalyx* var. *piperescens*) and basil (*Ocimum basilicum*).

### 4.3. Septoria leaf spot

Leaves with symptoms of spotting on numerous cultivars of lavender could be found, especially in wetter years [Vrandečić et al., 2014]. Initial symptoms on lower leaves included numerous, small, oval to irregular, grayish brown lesions with a slightly darker brown margin of necrotic tissue (Figure 4.7). Further development of the disease resulted in yellowing and necrosis of the infected leaves followed by premature defoliation. Similar necrotic oval-shaped lesions were observed on stems as well. The lesions contained numerous, dark, sub-globose pycnidia that were immersed in the necrotic tissue or partly erumpent (Figure 4.8).

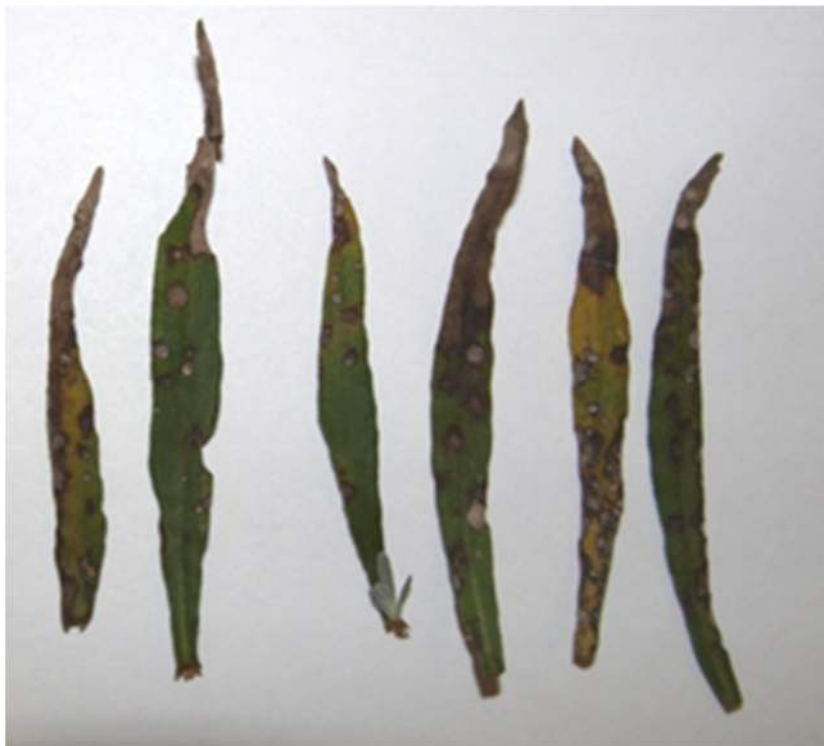
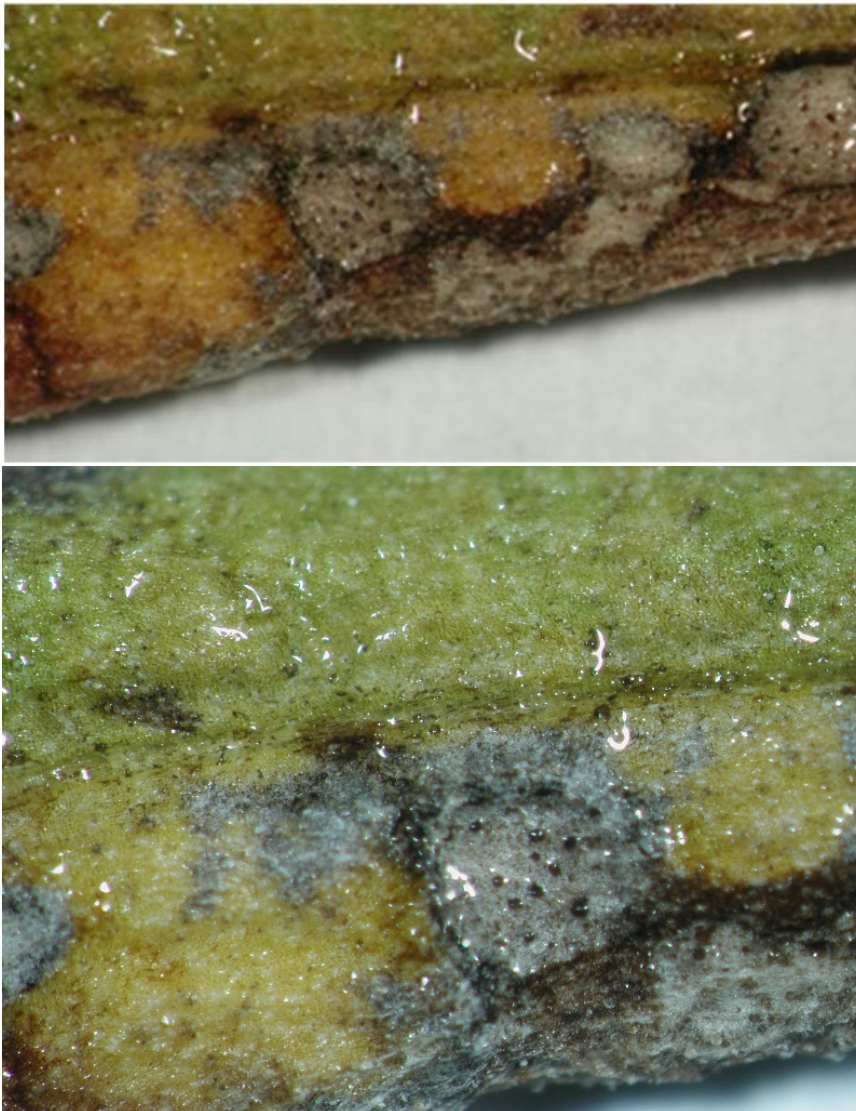


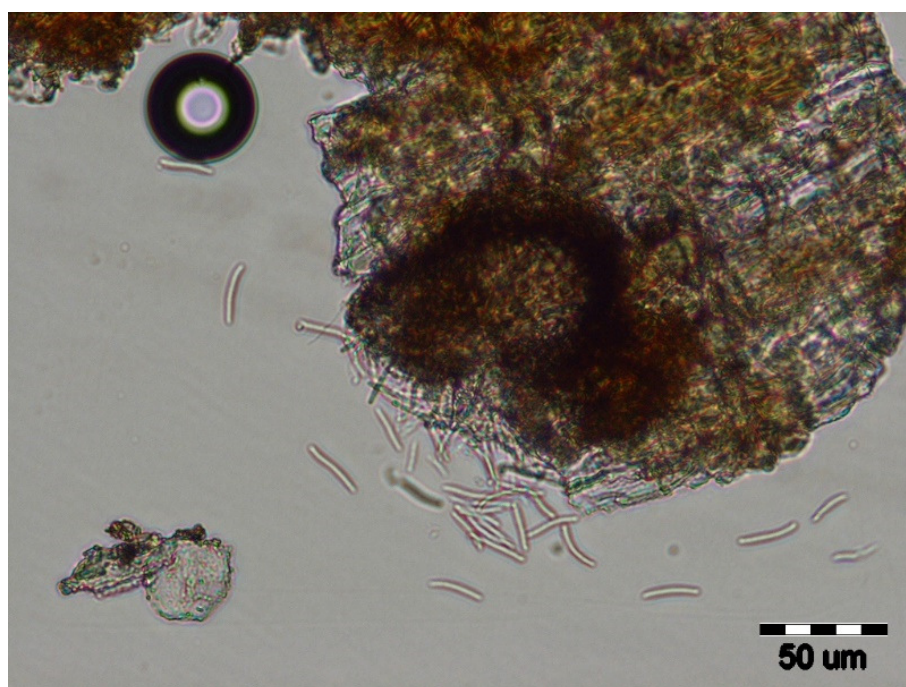
Figure 4.7. Symptoms of Septoria leaf spot on lavender.



According to Vrandečić et al. (2014) *Septoria lavandulae* on PDA forms a slow-growing, dark, circular colonies with raised center that produced pycnidia at 23°C, under 12 h of fluorescent light per day. Fungus forms numerous, dark, sub-globose pycnidia and uniform hyaline, elongate, straight or slightly curved conidia with 3 to 4 septa, with average dimensions of 17.5 to 35 × 1.5 to 2.5 μm (Figure 4.9).



**Figure 4.8. Pycnidia on lavender leaf.**



**Figure 4.9. Pycnidia and conidia of *S. lavandulae*.**

Problems can occur during wet springs and rainy summers when, according to some data, the disease reduces the quantity of flowers and oil, but also affects the quality of the obtained oil. The fungus spreads by wind-blown spores, infecting new lavender leaves if there is a sufficient wet period.

If the intensity of the infection is not strong, very often at first glance it may seem that the plants are healthy. However, if you look at the leaves more closely (especially the lower leaves in the central part of the bush), you can see small spots. Since the main protection measure is the planting of healthy planting material, care should be taken to ensure that plants with symptoms of spotting are never taken for the production of seedlings. Unfortunately, this was not taken into account in some cases on the inspected field. Cultivation of plants in sunny, airy places, planting bushes in the direction of air currents and sufficient distance between bushes will also contribute to the reduction of diseases.





#### 4.4. Alfalfa mosaic virus (AMV)

Lavender plants infected early in the growing season showed severe symptoms including stunting, bright yellow calico mosaic, yellow mottling and leaf distortion (Figure 4.10 and 4.11), while those infected at later stages of growth exhibited only mild mosaic symptoms. The disease which AMV cause on lavender is known as yellow mosaic.

The flowering of infected plants does not change in the number, size or color of flowers but plants infected with AMV have lower essential oil production quality.

AMV occurs worldwide and is one of the most important viruses infecting about 430 plant species from 51 families, and causes serious problems in some hosts. The most important hosts include alfalfa, potato, tomato, pepper, lettuce, spinach, basil, hemp, tobacco and lavender. Since numerous weed species host this virus, weeding around and within lavender fields will reduce the risk of disease. Regarding protection measures, professional literature states that the removal and burning of infected plants is urgently needed. The virus is transmitted by infected plant parts, tools or cutting machines and hands, so it is extremely important to identify the disease as soon as possible and remove the diseased plants.

Aphids transmit AMV, so insecticidal treatment of crops on which aphids usually feed (e.g. alfalfa, Prunus species) can prevent their spread to lavender. When the alfalfa crop is harvested, if aphids were present, there is a risk that they will transfer to other host plants. That is why it is recommended to leave a buffer zone of alfalfa, which is then treated with insecticides. For protection, it is important that producers of seedlings provide healthy planting material and that care is taken to ensure that there are no aphids at the place of cultivation (e.g. in the greenhouse).

Bellardi et al. (2006) found that infected *Lavandula hybrida* plants produced 8.82 ml/kg of oil, and healthy plants produced 13.8 ml/kg of oil. The same authors state that the concentration of some of the main essential oil components also changes, and because of this, the quality of the oil also decreases.





**Figure 4.10. Symptoms of Alfalfa mosaic virus on lavender.**



**Figure 4.11. Symptoms of Alfalfa mosaic virus on lavender.**





Some other important diseases of medicinal plants are summarized in Table 4.1 [Rahman Khan and Haque, 2024; McGovern, 2023; Avasthi et al., 2022; Cakir et al., 2021; Duduk et al., 2019; Pandey et al., 2019; Shi et al., 2016; Singh et al., 2016; Petrželova et al., 2015; Aktaruzzaman et al., 2015; Koike et al., 2012].

**Table 4.1. Important diseases of medicinal plants.**

<b>Medicinal plant</b>	<b>Disease and causing organism</b>
<b>Lavender</b>	Phytophthora root and crown rot ( <i>Phytophthora</i> sp.) Gray mold ( <i>Botrytis cinerea</i> )
<b>Basil</b>	Downy mildew ( <i>Peronospora belbahrii</i> ) Fusarium wilt ( <i>Fusarium oxysporum</i> f. sp. <i>basilicum</i> ) Anthracnose ( <i>Colletotrichum gleosporioides</i> and <i>C. capsica</i> ) Gray mold ( <i>Botrytis cinerea</i> ) Cercospora leaf spot ( <i>Cercospora ocimicola</i> ) Bacterial leaf spot ( <i>Pseudomonas cichorii</i> )
<b>Mentha</b>	Verticillium wilt ( <i>Verticillium alboatrum</i> var. <i>menthae</i> ) Fusarium wilt ( <i>Fusarium oxysporum</i> ) Rust ( <i>Puccinia menthae</i> ) Powdery mildew ( <i>Erysiphe cichoracearum</i> ) Rhizoctonia blight ( <i>Rhizoctonia solani</i> ) Stolon and root rot ( <i>Thielaviopsis basicola</i> ) Cercospora leaf spot ( <i>Cercospora menthicola</i> ) Corynespora leaf rot ( <i>Corynespora cassicola</i> ) Alternaria blight ( <i>Alternaria alternata</i> )
<b>Aloe vera</b>	Anthracnose ( <i>Colletotrichum gloeosporioides</i> ) Black leaf spot ( <i>Alternaria alternata</i> ) Brown leaf spot ( <i>Phoma betae</i> , <i>Phomopsis</i> sp.) Root rot ( <i>Fusarium solani</i> )
<b>Sage</b>	Powdery mildew ( <i>Erysiphe</i> sp.) Sage ( <i>Puccinia salviicola</i> ) Fusarium wilt ( <i>Fusarium oxysporum</i> ) Verticillium wilt ( <i>Verticillium dahliae</i> )
<b>Chamomile</b>	Downy mildew ( <i>Peronospora radii</i> ) Powdery mildew ( <i>Sphaerotheca macularis</i> , <i>Erysiphe cichoracearum</i> ) White rust ( <i>Albugo tragopogonis</i> )



<b>Echinacea</b>	Botrytis leaf spot and stem rot ( <i>Botrytis cinerea</i> ) Bacterial leaf spot ( <i>Pseudomonas cichorii</i> ) Cercospora leaf spot ( <i>Cercospora tabacina</i> ) Sclerotinia crown rot ( <i>Sclerotinia sclerotiorum</i> ) Sclerotium crown rot ( <i>Sclerotium rolfsii</i> ) Septoria leaf spot ( <i>Septoria lepachydis</i> ) Alternaria leaf spot ( <i>Alternaria</i> sp.)
<b>Thyme</b>	Gray mold ( <i>Botrytis cinerea</i> ) Carcoal rot ( <i>Macrophomina phaseolina</i> ) Rhizoctonia wilt ( <i>Rhizoctonia solani</i> )
<b>Rosemary</b>	Web Blight ( <i>Rhizoctonia solani</i> ) Verticillium wilt ( <i>Verticillium</i> sp.) Botrytis blight ( <i>Botrytis cinerea</i> ) Cotony soft rot ( <i>Sclerotinia sclerotiorum</i> ) Crown gall ( <i>Agrobacterium tumefaciens</i> )
<b>Fennel</b>	Fennel stem rot ( <i>Sclerotinia sclerotiorum</i> ) Leaf blight ( <i>Alternaria petroselini</i> ) Brown rot and wilt ( <i>Phytophthora megasperma</i> ) Gray mold ( <i>Botrytis cinerea</i> ) Root rot ( <i>Fusarium solani</i> ) Stem and crown rot ( <i>Fusarium avenaceum</i> ) Crown rot ( <i>Phoma glomerata</i> ) Alternaria blight ( <i>Alternaria alternata</i> )
<b>Marigold</b>	Rust ( <i>Puccinia lagenophorae</i> ) Wilt and stem rot ( <i>Phytophthora cryptogea</i> ) Powdery mildew ( <i>Leveillula taurica</i> , <i>Oidium</i> sp.) Flower bud rot ( <i>Alternaria dianthi</i> ) Damping off ( <i>Pythium</i> sp.) Leaf spot ( <i>Alternaria</i> sp., <i>Cercospora</i> sp., <i>Septoria</i> sp.)
<b>Oregano</b>	Powdery mildew ( <i>Golovinomyces biocellatus</i> ) Stem rot ( <i>Boeremia exsigua</i> var. <i>exigua</i> )



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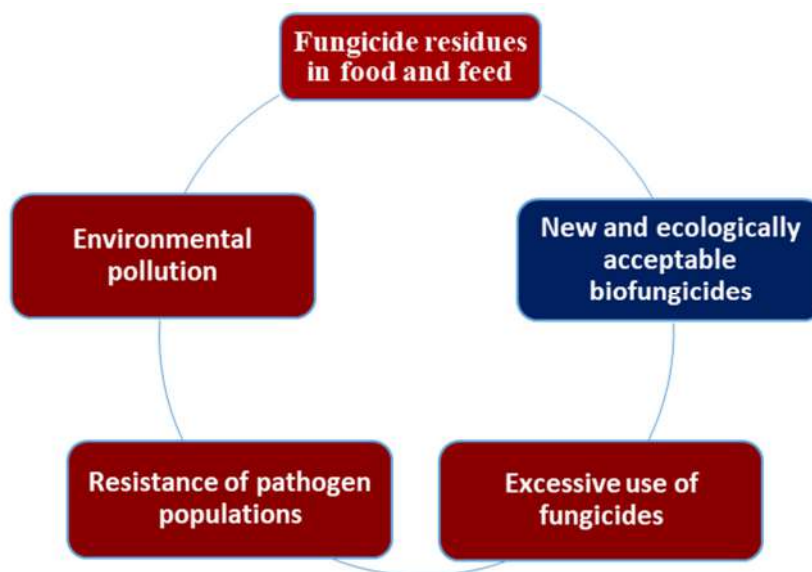
## Chapter 5. Antifungal activity of essential oils in agriculture (Vrandecic K, Cosic J)

### 5.1. Antifungal properties of essential oils against plant pathogenic fungi

Plant pathogenic fungi are among the most important pathogens of numerous crops worldwide and cause significant yield losses in many economically important crops. Phytopathogenic fungi as the causative agents of plant diseases could be airborne, soilborne and seedborne. Seedborne pathogens are a serious threat to agriculture. Seedborne fungi can colonize the seeds inducing visible symptoms and causing the economic yield and quality losses. Also, seedborne plant pathogens can remain in the seeds and inhibit germination. Subsequently, seedborne plant pathogens can be introduced into the crop on or in the seed and begin to develop at the beginning of the appearance of the young plant.

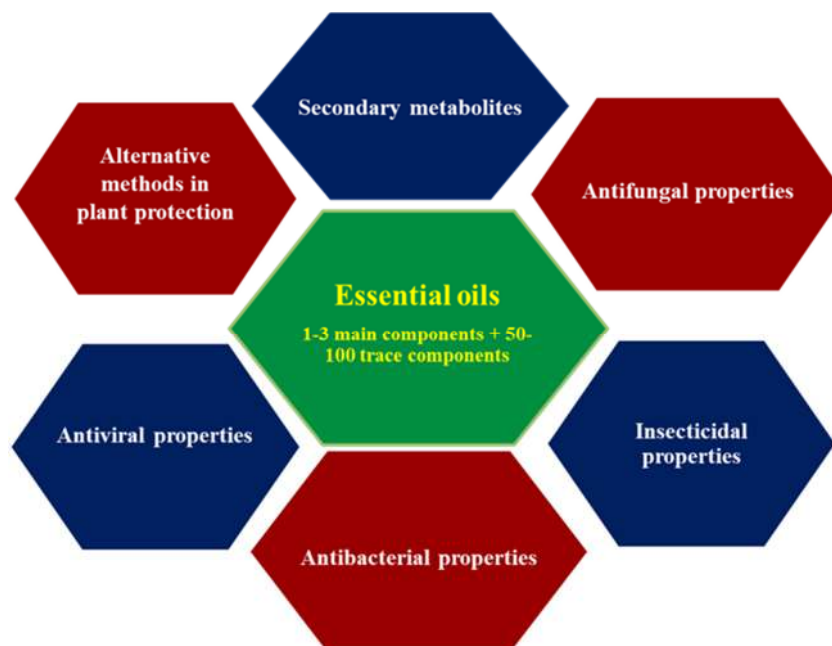
In infected seeds, there is often a change in the chemical composition and biological properties, and all of the above represents a significant threat to the yield and quality of the crop. These pathogens can be transmitted to the seedling causing its death or damaging the roots and the vascular system, with yield reduction and economic loss. Moreover, with infected seeds plant pathogens could be transmitted into areas where they were not present before. In order to avoid the spread of plant diseases through seeds, the seeds should be protected.

To reduce yield and quality losses, chemical pesticides have been used for decades to inhibit the growth of pathogenic fungi. Despite their efficacy, widespread use of synthetic pesticides is associated with significant drawbacks (Figure 5.1), including handling hazards, pesticide residues in food, feed and soil, fungal resistance to synthetic compounds, disruption of ecosystem balance and human health hazards. All of these negative impacts of pesticides have demonstrated the need for alternative non-chemical methods in plant protection.



**Figure 5.1. The drawbacks of chemical pesticides that led to alternative non-chemical methods for plants protection.**

Biological compounds (e.g. essential oils and extracts – Figure 5.2) from plants could be an important alternative that do not have hazardous effects on human health and on the environment. According to Wilkins and Board (1989) more than 1340 plants are known to be potential sources of antimicrobial compounds.



**Figure 5.2. Biological compounds with potential effects in plants protection.**



Essential oils are a complex mixture of terpenes (monoterpenes and sesquiterpenes, and their oxygenated derivatives, such as alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides), and phenolic and phenylpropanoid compounds derived from the acetate-mevalonic acid and shikimic acid pathways, respectively [Bakkali et al., 2008]. The main components make up 85% of the essential oil, while other compounds are present in small amounts or traces.

The effect of essential oils is fungistatic if complete growth inhibition occurs but can be reversed in the absence of the oil, whereas a fungicidal effect means that complete growth inhibition is irreversible even in the absence of the volatile.

The fungistatic activity of essential oils is often very clear, but, in many cases, the mode of antifungal activity is not fully 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 [Dorman and Deans, 2000]. According to Holley and Patel (2005) essential oils increase the permeability of cell membranes and decrease their function. These processes include inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions [Knobloch et al., 1988]. Essential oils inhibit fungal growth, sporulation and germ tube elongation of many plant pathogens. The antimicrobial effect of thymol and carvacrol have been reported by Sikkema et al. (1995) to cause structural and functional damage to the cytoplasmic membrane. The antimicrobial effect of eugenol is related to its ability to permeabilize the cell membrane and interact with proteins [Hyldgaard et al., 2012].

In some cases, the bioactivities of essential oils are closely related to the activity of the main components of the oils. For example, excellent antifungal activity of oils from *Origanum* and *Thymus* species is attributed to carvacrol and thymol [Begum et al., 2008; Abdolahi et al., 2010; Amini et al., 2012] while the high antifungal activity of *Syzygium aromaticum* and *Ocimum gratissimum* is attributed to eugenol [El-Zemity and Ahmed 2005; Piyo et al., 2009].

In many cases, the antimicrobial activity results from the complex interaction between the various compounds such as esters, ethers, phenols, aldehydes, alcohols, and ketones [Burt, 2004]. These interactions among compounds may lead to antagonistic, additive or synergistic effects. In agreement with the results of some



previous studies, whole essential oils have a stronger antifungal activity than their main component or a mixture of several main components. These facts lead to the assumption that the minor components are extremely important for the synergistic effect of the components. Perez-Sanchez et al. (2007) noted that fungistatic effect of *Thymus zygis* essential oils is due to the synergistic effect between minor compounds such as 3-octanol and  $\alpha$ -terpinene, rather than of the high concentration of compounds such as thymol or carvacrol.

Moreover, it is very difficult for fungi to develop resistance to a mixture of oil components with different mechanisms of antimicrobial activity [Daferera et al., 2003].

The antifungal activity of essential oils depends on applied amount of oil and the type of target pathogen. Ćosić et al. (2010) studied the effect of eleven essential oils (clove, rosemary, cinnamon leaf, sage, pine, bitter orange, mint, anise, cumin, lavender, thyme) on the mycelial growth of twelve phytopathogenic fungi (*Fusarium graminearum*, *Fusarium verticillioides*, *Fusarium subglutinans*, *Fusarium oxysporum*, *Fusarium avenaceum*, *Diaporthe helianthi*, *Diaporthe caulivora*, *Diaporthe longicolla*, *Phomopsis viticola*, *Helminthosporium sativum*, *Colletotrichum coccodes* and *Thanatephorus cucumeris*). All of these essential oils showed an inhibitory effect on some or all of the fungi studied, except for the essential oils of pine and bitter orange. Thyme, cinnamon leaf, clove and anise essential oils had the best antifungal effect.

*Rhizoctonia solani* is much more sensitive ( $EC_{50} = 0.057 - 0.486$ ) on *Thymus zygis* ssp. *sylvestris* and *Thymus zygis* ssp. *gracilis* oils than *Fusarium oxysporum* ( $EC_{50} = 0.092 - 0.630$ ) and *Colletotrichum acutatum* ( $EC_{50} = 0.110 - 0.756$ ) [Perez-Sanchez et al., 2007]. *Pimpinella anisum* essential oil also achieved various degrees of inhibition against three pathogenic fungi (Özcan and Chalchat 2006). It was the most effective against *Aspergillus parasiticus*, then followed by *Aspergillus niger* and *Alternaria alternata*.

Elshafie et al. (2015) tested the antifungal activity of *Thymus vulgaris* and *Verbena officinalis* essential oils under *in vivo* conditions in different amounts against *Monilinia laxa*, *Monilinia fructigena* and *Monilinia fructicola*. The largest applied amounts of verbena (1000 ppm) and thyme (500 ppm) essential oils significantly



reduced the diameter of lesions while smaller amounts of verbena (500 ppm) and thyme (250 ppm) essential oils obtained lower effect.

Although numerous authors claim that the antifungal activity of essential oils is satisfactory under *in vivo* conditions, there are numerous limiting factors for their use in plant protection during the growing season or during storage. The use of essential oils is limited because high amounts or concentrations are required to achieve sufficient antifungal activity. In addition, antifungal activity of essential oils depends on pH [Juven et al., 1994], temperature and water activity [Velluti et al., 2004].

In the context of the application of essential oils as a seed treatment, essential oils can be used directly to suppress the growth of phytopathogenic fungi or to control the formation and accumulation of mycotoxins. The method of application is by evaporation or direct seed treatment, and the concentrations are different and are still the subject of research. Also, to date, not enough is known about the mechanism of antifungal action of essential oils. It is possible that terpenoids and phenols, as the main components of essential oils, are responsible for the antifungal effect of essential oils. Due to their lipophilic nature and low molecular weight, terpenoids and phenols can cause structural and functional damage to pathogen cells by disrupting membrane permeability and the osmotic balance of pathogen cells [Kalagatur et al., 2015; Prakash et al., 2015; Grata, 2016].

Marín et al. (2004) examined the effect of essential oils of cinnamon, clove, oregano, lemongrass and palmarosa on the reduction of the accumulation of mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) in maize grain. Clove oil showed the best results in reducing the accumulation of both mycotoxins.

The essential oils used in the study by Perczak et al. (2019) inhibited the growth of the mycelia of *Fusarium culmorum* and *Fusarium graminearum* and reduced the concentration of mycotoxins zearalenone and trichothecene group B (DON, 3- and 15-acetyldeoxynivalenol, nivalenol and fusarenon X) in wheat seeds. The highest antifungal activity was demonstrated by cinnamon, oregano, and palmarosa essential oils. *F. culmorum* exhibited higher sensitivity to the oregano, cinnamon and verbena oils, while *F. graminearum* was more susceptible to the oregano and cinnamon oils.



Moumni et al. (2021) used seven essential oils in research in which they examined the *in vitro* influence on the growth of the mycelium of the main seedborne pathogens of cucurbits. *Cymbopogon citratus* essential oil completely inhibited mycelial growth of fungi *Stagonosporopsis cucurbitacearum* and *Alternaria alternate* at 0.6 and 0.9 mg/mL, respectively. *Lavandula dentata*, *Lavandula hybrida*, *Melaleuca alternifolia*, *Laurus nobilis*, and two *Origanum majorana* essential oils inhibited growth of *A. alternate* by 54%, 71%, 68%, 36%, 90%, and 74%, respectively.

Some essential oils may also have a stimulatory effect on the mycelial growth of some phytopathogenic fungi under *in vitro* conditions [Ćosić et al., 2010], but they may also be more effective in combination against phytopathogenic fungi than when used individually due to their synergistic effect [Nikkhah et al., 2017].

The antifungal activity of essential oils depends on the method of application. Essential oils consisting of large phenolic compounds such as thymol and eugenol have a better effect when applied directly. In contrast, essential oils with non-phenolic volatile compounds (e.g. citral and limonene) work best when the fungus is exposed to the vapors of the essential oil [Suhr and Nielsen, 2003].

Because the thymol molecule is smaller and evaporates more readily than the eugenol molecule, the compound thymol from thyme essential oil had a better effect on inhibiting growth during evaporation than eugenol from cinnamon and clove oils, even though both compounds are phenols [Suhr and Nielsen, 2003].

According to Suhr and Nielsen (2003), direct application method (contact action) of ten essential oils showed a weak inhibitory effect of all oils on various mold pathogens (*Aspergillus* sp., *Penicillium* sp.) while the oils showed a good inhibitory effect in the evaporation method.

## 5.2. Possibilities of use of essential oils against phytopathogenic fungi

In research on influence of twelve essential oils on the growth of the mycelium of the phytopathogenic fungus *Rhizoctonia solani* (on three temperatures 15, 20, and 30°C and quantity 25 µL) complete inhibition of mycelial growth of the *R. solani* fungus was determined when anise, tea tree and thyme oils were applied (for all temperatures)



and regardless of the days of incubation [Ereš, 2022]. Sweet orange oil had the weakest antifungal effect, followed by clove, pine, cypress and eucalyptus oils.

The effect of thyme and anise oil compared to the control at 15 °C is shown in the Figure 5.3 and the comparison of essential oils effects on the mycelial growth by temperature on the eighth day after inoculation has shown in the Table 5.1.

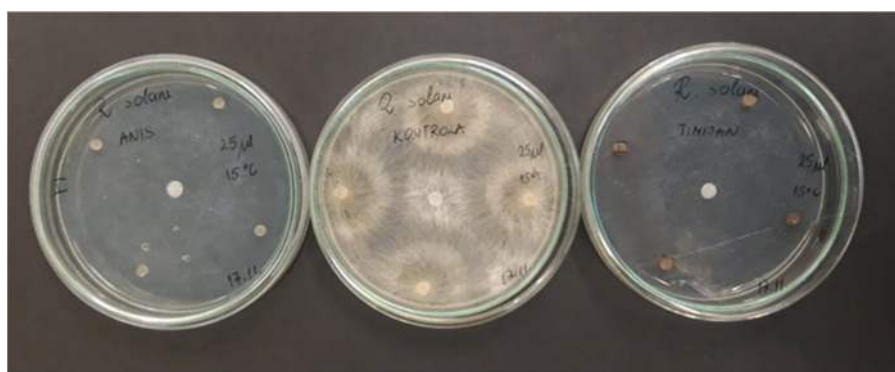


Figure 5.3. Influence of essential oil of anise and thyme compared to the control (4th day after inoculation at 15 °C)

Table 5.1. Comparison of essential oils influence on *R. solani* micelia growth depending on temperature on the eighth day.

OIL	15 °C	20 °C	30 °C	LSD 0.05 0.01
ANISE	30	30	30	0.00 0.00
PINE	0	0	3.33	6.99 10.09
CINNAMON BARK	30	18.33	30	18.50 28.02
CITRONELLA	30	20.58	30	9.46 14.33
TEA TREE	30	30	29.25	1.04 1.58
CYPRESS	0	0	13.33	8.57 12.98



EUCALYPTUS	17.92	0	2.08	18.38 27.84
CLOVE	0	0	0	0.00 0.00
LAVENDER	30	0	27	3.33 5.04
ORANGE SWEET	0	0	0	0.00 0.00
ROSEMARY	4.83	0	30	9.66 14.63
THYME	30	30	30	0.00 0.00

Different essential oils, including anise, thyme, cumin, peppermint, lavender, sage, lemon balm, rosemary, myrtle, cinnamon leaf, basil, white pine, eucalyptus, cedar, bergamot, mandarin, cypress, patchouli, ginger, bitter orange, sandalwood, and camphor, have varying effects on the growth of the grey mould fungus *Botrytis cinerea*. The oils were applied in three different amounts (3, 5, and 7  $\mu$ l), and measurements were taken on the third and ninth day.

According to Table 5.2, all oils, except for those of bitter orange, sandalwood, and camphor, exhibited some level of antifungal activity. Thyme and anise oil demonstrated the strongest antifungal activity compared to the water control. Conversely, the oils of bitter orange, sandalwood, and camphor were found to stimulate the growth of the *B. cinerea* fungus [Grgić et al., 2016].

**Table 5.2. Inhibition zone (mm) in first measurement, 3 days after inoculation.**

Oil	Amount of oil		
	3 $\mu$ l	5 $\mu$ l	7 $\mu$ l
<i>Carum carvi</i>	30,87 $\pm$ 0,31 a <sup>AB</sup>	32,63 $\pm$ 0,13 a <sup>A</sup>	32,75 $\pm$ 0,14 a <sup>A</sup>
<i>Cedrus atlantica</i>	10,63 $\pm$ 4,01 e <sup>F</sup>	13,38 $\pm$ 1,07 d <sup>DE</sup>	15,25 $\pm$ 1,45 fg <sup>EF</sup>
<i>Cinnamomum camphora</i>	0,38 $\pm$ 0,24 g <sup>I</sup>	4,75 $\pm$ 0,95 f <sup>F</sup>	3,88 $\pm$ 1,07 jk <sup>HI</sup>
<i>Cinnamomum verum</i>	17,00 $\pm$ 0,29 d <sup>D</sup>	18,88 $\pm$ 0,24 c <sup>C</sup>	18,75 $\pm$ 1,01 de <sup>D</sup>
<i>Citrus aurantium</i>	4,13 $\pm$ 0,43 f <sup>HI</sup>	2,88 $\pm$ 0,13 f <sup>F</sup>	2,63 $\pm$ 0,24 k <sup>I</sup>
<i>Citrus bergamia</i>	10,25 $\pm$ 1,03 e <sup>F</sup>	18,00 $\pm$ 1,06 c <sup>C</sup>	18,13 $\pm$ 1,60 de <sup>DE</sup>



<i>Citrus reticulata</i>	9,63 ± 0,47 e <sup>F</sup>	10,00 ± 0,61 e <sup>E</sup>	7,25 ± 1,20 i <sup>G</sup>
<i>Cupressus sempervirens</i>	9,63 ± 0,66 e <sup>F</sup>	13,00 ± 2,33 d <sup>DE</sup>	16,88 ± 0,63 efg <sup>DE</sup>
<i>Eucalyptus globulus</i>	11,13 ± 1,07 e <sup>EF</sup>	9,88 ± 1,57 e <sup>E</sup>	15,00 ± 1,24 g <sup>EF</sup>
Kontrola / Control	4,38 ± 0,24 f <sup>HI</sup>	4,63 ± 0,63 f <sup>F</sup>	4,63 ± 0,55 jk <sup>GHI</sup>
<i>Lavandula officinalis</i>	27,63 ± 0,72 b <sup>BC</sup>	30,63 ± 0,97 a <sup>AB</sup>	28,63 ± 0,55 c <sup>BC</sup>
<i>Melissa officinalis</i>	24,00 ± 0,89 c <sup>C</sup>	31,88 ± 0,13 a <sup>A</sup>	31,88 ± 0,47 ab <sup>AB</sup>
<i>Mentha x piperita</i>	30,50 ± 1,37 ab <sup>AB</sup>	32,63 ± 0,13 a <sup>A</sup>	28,25 ± 1,36 c <sup>C</sup>
<i>Myrtus communis</i>	17,75 ± 1,05 d <sup>D</sup>	18,13 ± 2,56 c <sup>C</sup>	17,25 ± 1,20 efg <sup>DE</sup>
<i>Ocimum basilicum</i>	16,63 ± 1,01 d <sup>D</sup>	27,00 ± 0,87 b <sup>B</sup>	30,13 ± 0,66 bc <sup>ABC</sup>
<i>Pimpinella anisum</i>	32,50 ± 0,00 a <sup>A</sup>	32,13 ± 0,55 a <sup>A</sup>	32,88 ± 0,24 a <sup>A</sup>
<i>Pinus sylvestris</i>	15,13 ± 0,63 d <sup>DE</sup>	16,50 ± 1,02 c <sup>CD</sup>	20,13 ± 0,83 d <sup>D</sup>
<i>Pogostemon patchouli</i>	9,38 ± 0,68 e <sup>FG</sup>	11,38 ± 0,83 d <sup>DE</sup>	12,13 ± 0,47 h <sup>F</sup>
<i>Rosmarinus officinalis</i>	17,88 ± 0,66 d <sup>D</sup>	17,88 ± 1,52 c <sup>C</sup>	17,63 ± 0,60 ef <sup>DE</sup>
<i>Salvia officinalis</i>	24,38 ± 0,31 c <sup>C</sup>	27,13 ± 0,38 b <sup>B</sup>	28,88 ± 0,90 c <sup>BC</sup>
<i>Santalum album</i>	2,63 ± 0,97 fg <sup>HI</sup>	3,13 ± 0,43 f <sup>F</sup>	2,50 ± 0,29 k <sup>I</sup>
<i>Thymus vulgaris</i>	31,25 ± 0,43 a <sup>AB</sup>	32,63 ± 0,13 a <sup>A</sup>	31,88 ± 0,24 ab <sup>AB</sup>
<i>Zingiber officinale</i>	5,50 ± 0,41 f <sup>GH</sup>	5,25 ± 0,60 f <sup>F</sup>	6,13 ± 0,55 ij <sup>GH</sup>

For the average values (comparison by columns) marked with the same lowercase letter, no statistically significant differences were found at the significance level of  $P < 0.05$ , and for the average values marked with a capital letter, no statistically significant differences were determined at the significance level of  $P < 0.01$  LSD test.

Table 5.3. Inhibition zone (mm) in second measurement 9 days after inoculation

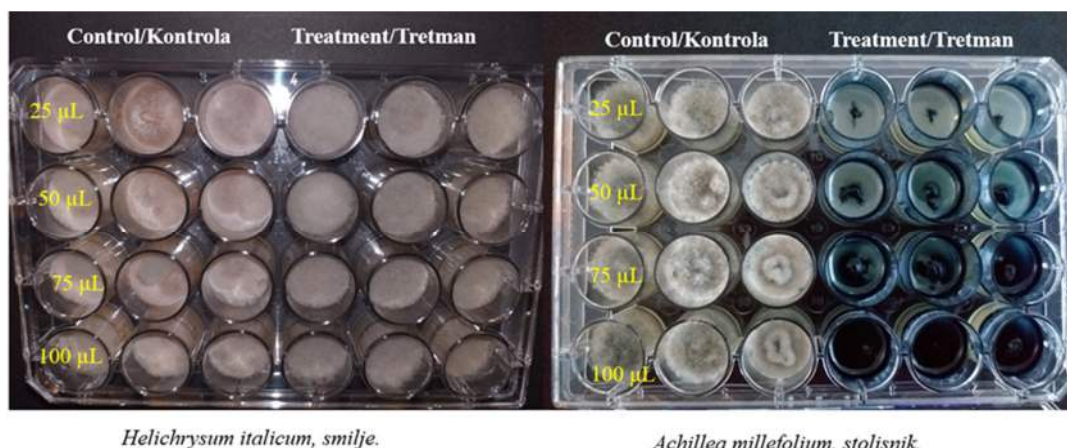
Oil	Amount of oil		
	3 µl	5 µl	7 µl
<i>Carum carvi</i>	0,00 ± 0,00c <sup>B</sup>	0,00 ± 0,00d <sup>D</sup>	6,13 ± 0,24e <sup>E</sup>
<i>Cinnamomum verum</i>	0,00 ± 0,00c <sup>B</sup>	1,13 ± 0,66d <sup>D</sup>	0,50 ± 0,50g <sup>F</sup>
<i>Lavandula officinalis</i>	11,75 ± 0,52b <sup>A</sup>	25,50 ± 3,51b <sup>B</sup>	21,50 ± 1,70c <sup>C</sup>
<i>Mentha x piperita</i>	13,63 ± 0,52ab <sup>A</sup>	16,38 ± 1,97c <sup>C</sup>	13,75 ± 1,33d <sup>D</sup>
<i>Pimpinella anisum</i>	0,00 ± 0,00 c <sup>B</sup>	0,00 ± 0,00d <sup>D</sup>	27,13 ± 0,13b <sup>B</sup>
<i>Rosmarinus officinalis</i>	1,38 ± 0,94c <sup>B</sup>	2,63 ± 1,01d <sup>D</sup>	3,38 ± 1,30f <sup>EF</sup>
<i>Thymus vulgaris</i>	18,75 ± 5,39a <sup>A</sup>	32,63 ± 0,13a <sup>A</sup>	31,88 ± 0,24a <sup>A</sup>
<i>Zingiber officinale</i>	1,25 ± 0,72c <sup>B</sup>	1,13 ± 0,72d <sup>D</sup>	2,00 ± 0,61fg <sup>F</sup>



For the average values (comparison by columns) marked with the same lowercase letter, no statistically significant differences were found at the significance level of  $P < 0.05$ , and for the average values marked with a capital letter, no statistically significant differences were determined at the significance level of  $P < 0.01$  LSD test.

In a study conducted by Jelenić et al. (2020), the researchers examined the impact of nine essential oils derived from native Croatian plants on the suppression of *Botrytis cinerea* mycelial growth. The essential oils investigated were *Mentha x piperita*, *Salvia officinalis*, *Rosmarinus officinalis*, *Lavandula hybrida*, *Origanum compactum*, *Thymus vulgaris*, *Hiperici oleum*, *Achillea millefolium*, and *Helichrysum italicum*. The results indicated that the oils of *Thymus vulgaris* and *Mentha x piperita* exhibited a positive influence on mycelial growth suppression in both the volatile and macrodilution methods. Furthermore, these oils demonstrated a fungistatic effect even after 96 hours at all tested concentrations.

On the other hand, *Achillea millefolium* and *Hiperici oleum* did not show suppression effects in the volatile method, but did exhibit suppression of mycelial growth in the macrodilution method (Figure 5.4 and Table 5.4). The essential oil of *Helichrysum italicum* actually stimulated the growth of *B. cinerea* mycelium in both methods. These findings suggest that essential oils could serve as a biocontrol agent for grapevine gray mold control, potentially replacing traditional pesticides. Further research is needed to investigate the effectiveness of essential oils in *Botrytis control* under both in vitro and in vivo conditions.



*Helichrysum italicum*, smilje.

*Achillea millefolium*, stolisnik.

**Figure 5.4. Microdilution method – Helichrysum and Achillea.**



**Table 5.4. Influence of essential oils on *B. cinerea* mycelium growth – macrodilution method after 96 h (0 = supression of growth, 1 = 25% of mycelium growth, 2 = 50% of mycelium growth, 3 = 75% of mycelium growth, 4 = 100 % of mycelium growth).**

Essential oil/concentration	25 $\mu$ L	50 $\mu$ L	75 $\mu$ L	100 $\mu$ L
H. oleum	1	1	0	0
A. millefolium	0	1	0	0
H. italicum	4	4	4	4
L. hybrida	0	0	0	0
S. officinalis	0	0	0	0
M. piperita	0	0	0	0
T. vulgaris	0	0	0	0
R. officinalis	3	3	2	0
O. compactum	3	3	1	0

Nine essential oils (anise, pine, cinnamon bark, citronella, tea tree, cypress, eucalyptus, clove, lavender) were tested for in vitro antifungal activity on two soilborne phytopathogenic fungi *Globisporangium ultimum* and *Globisporangium irregular* [Petrić et al., 2021]. Essential oils were applied in three amounts (5, 15 and 30  $\mu$ L). The zone of inhibition was measured on the fourth and eighth day after the inoculation.

The effectiveness of the essential oils varied depending on the amount applied and the specific fungi species. After eight days, clove, anise, cinnamon bark, and citronella oils displayed the strongest antifungal activity against *G. ultimum* (Table 5.5), regardless of the amount applied. However, for *G. irregulare*, clove oil exhibited the most potent antifungal activity across all amounts tested. On the other hand, pine and cypress oils demonstrated the weakest antifungal activity against both pathogens, regardless of the amount applied. These results highlight the significance of considering the specific essential oil, the amount used, and the targeted fungi species when utilizing essential oils for their antifungal properties.



**Table 5.5. The effect of essential oils on mycelial growth of *Globisporangium ultimum* (inhibition zone in mm).**

Oil	5 $\mu$ L		15 $\mu$ L		30 $\mu$ L	
	4. day	8. day	4. day	8. day	4. day	8. day
Lavender	0	0	30.0	24.0	23.8	18.7
Citronella	13.0	0	25.0	23.0	30.0	30.0
Chajevac	0	0	30.0	18.3	22.4	22.4
Cinnamon bark	14.3	0	16.7	15.0	17.6	15.0
Eucalyptus	0	0	0	0	20.7	20.6
Pine	0	0	0	0	0	0
Anise	10.3	0	30.0	18.3	19.4	14.7
Cypress	0	0	0	0	0	0
Clove	16.7	15.0	21.0	18.0	23.4	16.4
Control	0	0	0	0	0	0
LSD 0.05	0.26	0.11	0.31	1.17	1.38	1.49

An in vitro study was conducted to investigate the impact of different volumes of twelve essential oils on the growth of economically significant phytopathogenic fungi, *Fusarium oxysporum* and *Botrytis cinerea* [Palfi et al., 2019]. The antifungal activity of the essential oils was compared to commercial fungicide.

The results showed that the effectiveness of the essential oils in inhibiting fungal growth decreased over time and varied depending on the specific phytopathogenic fungus and the volume of essential oil applied. Among the essential oils tested, thyme oil exhibited the most potent antifungal effect on both fungi, with the lowest IC<sub>50</sub> values. On the other hand, the essential oils of eucalyptus and lemon had the weakest antifungal effect, with the highest IC<sub>50</sub> values.

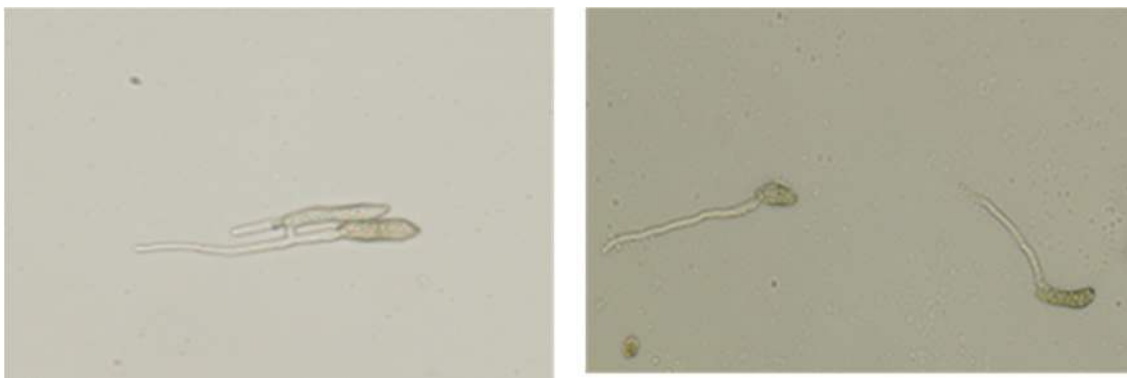
The certain essential oils, when applied at specific volumes, demonstrated comparable or even better inhibitory effects on mycelial growth compared to the tested fungicides.

As part of research about the possibility of controlling *Passalora fulva* with essential oils, Novak (2012) investigated the effectiveness of the following oils: aniseed, rosemary, cloves, cinnamon leaf, medicinal sage and thyme. Figures 5.5 and 5.6 show conidia germination recorded on the control and in thyme and cinnamon oil.

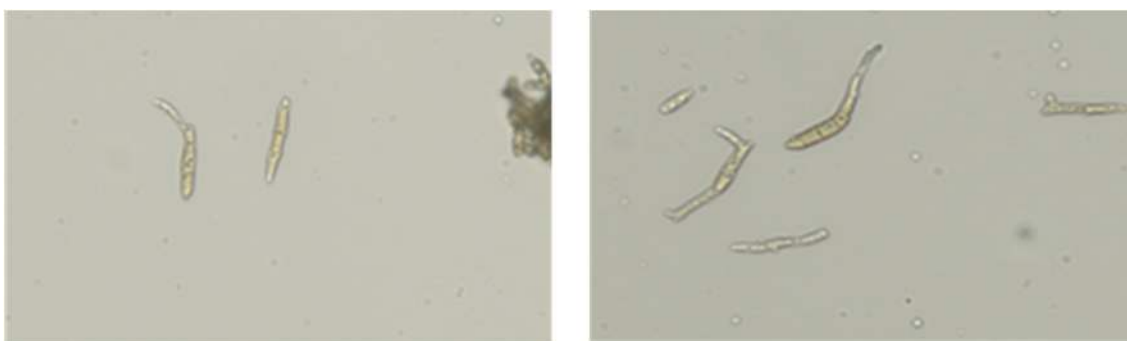




It is evident from the pictures that significantly longer germ tubes were recorded in the control than in the oils.



**Figure 5.5. Germination of conidia in the control (photo courtesy of A. Novak).**



**Figure 5.6. Germination of conidia in thyme and cinnamon oil (photo courtesy of A. Novak)**

Tables 5.6 and 5.7 show the results of statistical data obtained based on the length of germ tubes measured after 7 days for each oil at a concentration of 1%. It is evident from the data that all oils give better results compared to the control. The best results were recorded when using clove oil. In the case of other oils, there were no significant statistical differences between them regardless of oil concentration.



**Table 5.6. The mean values of germ tube length in oils at a concentration of 1% (10 µl/1 ml) measured after 7 days [Novak, 2012].**

Oil	Mean value
anise	2,201 c
rosemary	3,9603 c
clove	1,1826 c
cinnamon	5,372 b
medicinal sage	1,446 c
thyme	1,609 c
Control	20,927 a

**Table 5.7. Analysis of variance for the length of germ tubes measured after 7 days between different oils at concentration 1% (10 µl/1 ml) [Novak, 2012].**

Sources of variability	n-1	SS	s <sup>2</sup>	F <sub>exp.</sub>
Total	209	17877,61		
Between the oils	203	8846,912	43,58085	34,53*
Oils	6	9030,703	1505,117	

Some other important findings of in vitro antifungal activity of essential oils are summarized in Table 5.8.

**Table 5.8. Antifungal effect of some essential oils on phytopathogenic fungi.**

Tested plants	Types of phytopathogenic fungi	References
<b><i>Cestrum nocturnum</i></b> (night-booming jasmine)	Botrytis cinerea, Colletotrichum capsici, Fusarium oxysporum, F. solani, Phytophthora capsici, Sclerotinia sclerotiorum	Al-Reza et al. (2010)
<b><i>Cinnamomum verum</i></b> ( <i>C. zeylanicum</i> ) (Ceylon cinnamon tree)	Alternaria alternata, Alternaria sp., Aspergillus niger, Aspergillus sp., Botrytis cinerea, Colletotrichum coccodes, C. gloeosporioides, Diaporthe helianthi, D. phaseolorum var. caulivora, Fusarium avenaceum, F. oxysporum, F. fujikuroi, Fusarium oxysporum f.sp. ciceri, F. oxysporum f.sp. lycopersici, F. oxysporum f.sp. melonis, F. oxysporum f.sp. fragariae, F. proliferatum, F. verticillioides, F. culmorum, Helminthosporium sativum, Passalora fulva, Penicillium sp., Phomopsis longicolla, Phomopsis viticola, Rhizopus sp., R. stolonifer	Behtoei (2012), Gupta et al. (2008), Siripornvisal et al. (2009), Sukatta et al. (2008), Velluti et al. (2003), Ćosić et al. (2010), Novak (2012), Roselló et al. (2015), Park et al. (2017)
<b><i>Malaleuca alternifolia</i></b> (tea tree)	Botrytis cinerea	Li et al. (2017), Shao et al. (2013)
<b><i>Origanum compactum</i>, <i>Origanum vulgare</i></b> (oregano)	Botrytis cinerea, Giberella fujikuroi, Fusarium culmorum, F. graminearum	Adebayo et al. (2013), Rosello et al. (2015) Zhao et al. (2021) Harčárová et al. (2021)



<b><i>Foeniculum vulgare</i></b> (fennel)	Botrytis cinerea, F. oxysporum f.sp. fragariae, Macrophomina phaseolina, Sclerotinia sclerotiorum, Verticillium fungicola var. fungicola	Soylu et al. (2007), Tanović et al (2009), Khaledi et al. (2014), Park et al. (2017), Pedrotti et al (2017)
<b><i>Thymus vulgaris</i></b> (thyme)	Alternaria alternata, Aspergillus niger, A. parasiticus, A. flavus, Botrytis cinerea, Diplodia sp., Cryphonectria parasitica, Colletotrichum coccodes, Diaporthe helianthi, D. phaseolorum var. caulivora, Fusarium avenaceum, F. graminearum, F. oxysporum, F. subglutinans, F. verticillioides, Helminthosporium sativum, Macrophomina phaseolina, Passalora fulva, Phomopsis longicolla, P. viticola, Phytophthora cactorum, Rhizoctonia solani, Pythium aphanidermatum, Rhizopus stolonifer, Sclerotinia sclerotiorum, Monilinia laxa, M. fructigena, M. fructicola	El-Zemity and Ahmed (2005), Kim et al. (2008), Ćosić et al. (2010), Moghtader (2012), Abdolahi et al. (2010), Abdollahi et al. (2011), Amini et al. (2012), Novak (2012), Harčárová et al. (2021), Elshafie et al. (2015)
<b><i>Pinus sylvestris</i></b> (scot pine)	Colletotrichum coccodes, Helminthosporium sativum	Ćosić et al. (2010)
<b><i>Ocimum basilicum</i></b> (basil)	Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Botrytis cinerea, Fusarium proliferatum, F. verticillioides, F. graminearum, Pyricularia grisea	Piyo et al. (2009), Abdolahi et al. (2010) Harčárová et al. (2021)
<b><i>Salvia officinalis</i></b> (sage)	Cladobotryum sp., Colletotrichum coccodes, Fusarium verticillioides, Helminthosporium sativum, Mycogone perniciosa, Passalora fulva, Verticillium fungicola. var. fungicola, Phomopsis viticola	Tanović et al. (2009), Ćosić et al. (2010), Novak (2012)
<b><i>Carum carvi</i></b> (caraway)	Macrophomina phaseolina, Curvularia lunata, Fusarium equiseti, Alternaria alternata, Botryodiplodia theobromae, Colletotrichum corchori	Begum et al. (2008)
<b><i>Illicium verum</i></b> (star anise)	Aspergillus fumigatus, Aspergillus niger	Alhajj et al. (2019)
<b><i>Mentha x piperita</i></b> (peppermint)	Botrytis cinerea, Verticillium dahliae	Tančinova et al. (2022), Luković et al. (2019)
<b><i>Lavandula angustifolia</i></b> (lavender)	Botrytis cinerea	Tančinova et al. (2022)
<b><i>Cymbopogon flexuosus</i></b> (lemongrass)	Botrytis cinerea	Tančinova et al. (2022)
<b><i>Eugenia caryophyllus</i></b> (clove)	Fusarium equiseti, F. culmorum, F. poae, F. avenaceum, F. oxysporum f. sp. lycopersici	Grzanka et al. (2021), Sharma et al. (2017)
<b><i>Sesamum indicum</i></b> (sesame)	Botrytis cinerea	Samara et al. (2021)



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

### 6.1. Introduction

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: biological, ecological, technological and socio-economic [Civitaresse et al., 2023].

Cultivation technologies for medicinal and aromatic plants are determined by the species and type of culture (annual, biennial and perennial), by the harvested plant organ (leaves, flowers, roots, etc.) and by the cultivation area (for species with ecological plasticity big) [Pecingină, 2020].

By zoning the crops is meant the establishment of their favourability zones, based on the confrontation of the natural conditions of the respective area with the biological requirements of the species to be cultivated.

Optimization of plant growth and development conditions is done according to the biological requirements for temperature, water, light and soil.

### 6.2. Elements of technology for cultivating medicinal and aromatic plants

In Romania, the production of medicinal and aromatic plants is regulated by Law No. 491/2003 regarding Medicinal and Aromatic Plants, as well as beekeeping products. The plant material can be obtained from two sources: by cultivating medicinal and aromatic plants, and by harvesting these species from the wild.

The quality of the cultivated medicinal and aromatic plant production is determined by the following factors: biological factors (biological certification and the quality value of the seed and planting material); ecological factors (soil and climate); and technological factors (crop rotation and placement, fertilization, soil works, sowing or planting, maintenance works, and harvesting) [Muntean et al., 2016].



*Biological factors.* The active ingredient content of medicinal and aromatic plants is influenced by: the hereditary production; quality requirements of the cultivar; 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].

*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].

The main technological steps in cultivating medicinal and aromatic plants include:

- **Crop rotation**
- **Fertilization**
- **Soil works**
- **Biological material (seeds and planting material)**
- **Maintenance works (weed, disease, and pest control, and irrigation)**
- **Harvesting and conditioning**

To develop the technology for cultivating medicinal and aromatic plants, we rely on the knowledge of agrotechnical and phytosanitary measures to ensure efficient and high-quality economic production.



### ***Crop rotation***

An essential role in the correct zoning of medicinal plants is the establishment of crops in the rotation and their rotation, which represents the order (succession) of cultivating plants over time on the same land area. Observing crop rotation is very important to interrupt the biological cycle and spread of pests and diseases. It considers the vegetation cycle duration (annual, biennial, and perennial crops), the sowing or planting period (spring, summer, or autumn), and the organ or part of the crop used.

The preceding plant must meet certain conditions from a rotation perspective, such as:

- Medicinal and aromatic plants with small seeds and slow growth in the early vegetation stages should be cultivated after plants that leave the land very clean of weeds.
- Contain optimal amounts of nutrients.
- Free the land early for good soil preparation until sowing or planting, while perennial plants are cultivated outside the rotation.

Species from the same botanical family as preceding plants are not recommended due to common diseases and pests.

### ***Fertilization***

The growth and development of plants are influenced by fertilizer administration. Providing nutrients is necessary depending on the growth stages of the plants to stimulate balanced vegetative mass growth with the content of active principles. In rational fertilization, we consider: the existing nutrient reserves in the soil and ensuring the optimal nutrient ratio based on the specific consumption of each species. Specific nutrient consumption studies show that species cultivated for herba or folia require more nitrogen, those for flores et fructus require more phosphorus, and those for radix et rhizoma require more potassium. Research results highlight the contribution of microelements regarding increased plant mass production and the quality of active principles.



### ***Soil Works***

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 for which plants have certain requirements: species with low and very low seed set are sown shallow, and a favourable ratio between the water-air regime and an adequate supply of nutrients must be ensured in the soil [Liu et al., 2021].

Soil works include operations executed with various machines and equipment on the soil, aimed at loosening, breaking down, levelling the soil, incorporating fertilizers and amendments, and combating weeds, diseases, and pests in medicinal and aromatic plant crops through preventive methods. These works are carried out differently for each species.

A significant contribution of soil works is that sowing or planting will be done under proper conditions, and the plants will have optimal growth and development conditions, resulting in a good and high-quality harvest.

### ***Biological material (seeds and planting material)***

It must meet standards regarding germination, purity, botanical composition, and sanitary status. It should come from the previous harvest, as the germination ability is quickly lost. The seed quantity is determined based on crop density, thousand seed weight (TSW), purity, and seed germination. For propagation by seedlings or cuttings, quality indices must be met, ensuring they are healthy, respecting specific distances, densities, and planting depths for the crop. Irrigation immediately after transplanting is essential for successful plant survival.

Water influences the quantity and quality of plant production, in the sense that absolutely all vital biochemical and physiological processes consumed in the plant

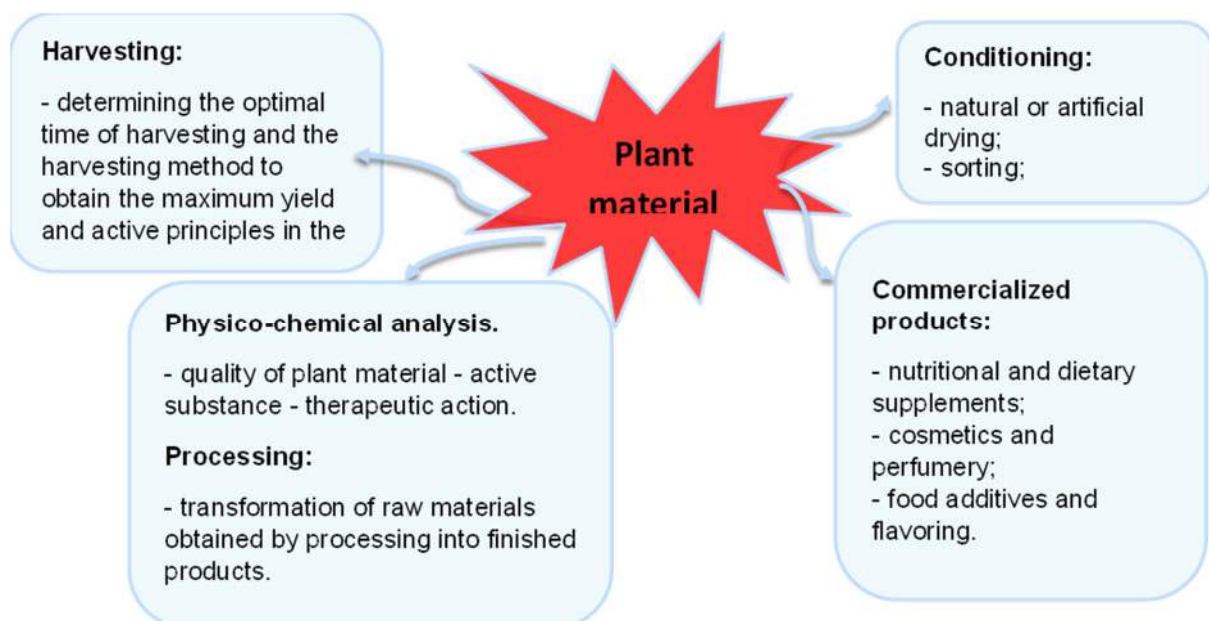


body take place in the presence of water. The importance of water is found in: (i) it forms the soil solution; (ii) it transports mineral and synthetic substances into the plant; (iii) due to its components - oxygen and hydrogen - it participates in equal proportions with carbon dioxide in the chlorophyll assimilation process (synthesis of organic matter); (iv) is the medium of oxidation and reduction reactions; (v) facilitates absorption and circulation through vessels; (vi) maintains cellular tension; (vii) releases or absorbs energy and regulates tissue temperature through transpiration and evaporation [Herzog et al., 2021].

### **Maintenance Works**

Weed control is achieved through a series of measures integrating preventive, curative, and biological measures.

In the Figure 6.1, the steps are presented to ensure the quality of plant material and maintain active principles to ensure high-quality raw material. For the therapeutic use of plant material, two stages are important: the optimal harvesting moment and the harvesting method, ensuring the content of active principles in the parts of the plant to be utilized.



**Figure 6.1. Flow of operations in the process of obtaining plant material.**





The optimal harvesting moment must be chosen to ensure the plant material contains the maximum amount of active principles; rules are very strict, possibly expressed in terms of calendar, seasons, and even month, vegetation phenophase, or even the specific time of day for harvesting. Meteorological conditions, which affect the initiation of the harvesting operation, are decisive for the harvesting moment. Generally, medicinal and aromatic plants should be harvested in dry weather, in the morning after the dew has lifted, or in the afternoon until sunset. Medicinal plants containing volatile oils should be harvested particularly in the morning before sunrise.

The harvesting method refers to the harvesting action itself, which can be mechanical or manual, aiming to preserve the maximum active principles in the collected material. Additionally, for the protection of medicinal and aromatic plants and ensuring species perpetuation, specific important rules must be observed for perennial species harvesting for subsequent years. Harvesting is done differently depending on the species, the part of the plant used, and the season.

### **6.3. Harvest, yield and processing**

Harvesting medicinal and aromatic crops is a critical step in the cultivation process to ensure the plants are at their peak of potency and that essential oils and medicinal compounds are preserved. The timing and techniques for harvesting can vary depending on the plant species and the part of the plant being collected (e.g., leaves, flowers, roots, seeds).

The harvest timing can vary by species and by the plant part to be harvested. Generally, herbs are harvested when they are in full bloom but before they start to set seed, as this is when essential oil content is often highest. Sharp tools like pruners and shears are used to minimize damage during harvest. Harvesting is usually done during dry conditions as wet plants can mould or rot during drying process.

Processing medicinal and aromatic crops is a critical step to extract, preserve, and prepare the valuable compounds within these plants for various uses, including herbal remedies, essential oils, teas, culinary applications, and more. The specific processing methods can vary widely based on the plant species and the desired end product [Vasanthkumar et al., 2023].



After processing, the dried or processed materials should be stored in airtight containers, away from direct light, moisture, and temperature fluctuations.

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 adept 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 labeling, are imperative for the sustenance of medicinal plant quality, thereby catering to a spectrum of applications, encompassing herbal medicine and pharmaceuticals.

Recent advancements in medicinal and aromatic crops are transforming the way we view and use these plants. These innovations include sustainable cultivation, genetic improvement, and research into their medicinal properties. As the world prioritizes sustainability and health, these crops are poised to play a pivotal role in various industries, including agriculture and medicine.

The future holds great promise for these versatile and valuable plants, with precision breeding and genomic research opening up new possibilities in healthcare, agriculture, and the culinary world.

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## **Chapter 7. Medicinal plants with proven effectiveness against medical pathogenic bacterial strains (Obistoiu 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].

In the near future, it will have been a hundred years since Alexander Fleming discovered penicillin. Since that time, antibiotics have demonstrated incalculable value in terms of both mental and material benefits, playing a pivotal role in saving countless lives. However, the advent of the antibiotic era was accompanied by the emergence of a new threat, namely antimicrobial resistance. This has currently reached a point where the successful completion of the centenary of the antibiotic era is being limited [Rahman and Sarker, 2020].

The current role of scientists around the world is to address the challenge of discovering new sources of effective antimicrobial drugs or to design and synthesize



them. Throughout history, medicinal plants have been a valuable source of molecules with therapeutic potential. Folk medicine in various civilizations has historically been based on natural products, and currently, medicinal plants remain an important source for identifying novel drug leads [Atanasov et al., 2015].

Medicinal plants provide an almost boundless supply of bioactive compounds, and their application as antimicrobial agents has been utilized in various ways. Natural antimicrobial agents can work independently or alongside antibiotics to boost effectiveness against a broad spectrum of microbes [Fazly Bazzaz et al., 2016].

The antimicrobial compounds derived from medicinal plants have the potential to inhibit the growth of bacteria, fungi, viruses, and protozoa through mechanisms that differ from those of currently used antimicrobials. This may confer significant clinical value in the treatment of resistant microbial strains. Some of these active compounds demonstrate both intrinsic antibacterial activity and antibiotic resistance-modifying activities. Others, while not effective as standalone antibiotics, can help overcome antibiotic resistance when combined with antibiotics. The chemical complexity of these compounds suggests a promising therapeutic potential, as they may exhibit fewer side effects and a reduced likelihood of developing resistance compared to synthetic drugs [Ruddaraju et al., 2020].

EO (volatile oils) are aromatic, oily liquids extracted from plants (leaves, buds, fruits, flowers, herbs, branches, bark, wood, roots, and seeds [El Kollu 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 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].

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].

The *Lamiaceae*, or mint family, encompasses 236 genera and over 7,000 species, making it the largest family within the order *Lamiales*. This family is almost globally distributed, with many species cultivated for their aromatic leaves and attractive flowers. *Lamiaceae* is particularly valued by humans for herbs that provide flavor, fragrance, or medicinal benefits. Most family members are perennial or annual herbs with square stems, though some are woody shrubs or subshrubs. Typically, the leaves are simple, oppositely arranged, fragrant, and contain volatile oils. The flowers generally form in clusters, featuring two-lipped, open-mouthed, tubular corollas (fused petals) and five-lobed, bell-shaped calyxes (fused sepals). The fruit is usually a dry nutlet [<https://www.britannica.com/plant/Lamiaceae>, accessed on 2024].



### ***Origanum vulgare (oregano)***

It is an aromatic plant widespread in Asia, Europe, and North Africa. Traditionally, oregano is used to treat respiratory issues, digestive disorders, dermatological conditions, and various other inflammatory and infectious ailments. Oregano essential oils have shown MICs (Minimum Inhibitory Concentrations) ranging from 0.03 to 100 µg/mL against *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Acinetobacter baumannii* [Thielmann et al., 2019; Lu et al., 2018].

### ***Thymus vulgaris (thyme)***

Thyme is an aromatic shrub native to the Mediterranean region. Its aerial parts are traditionally used as an antihelmintic, antispasmodic, and carminative in digestive disorders, as well as for respiratory issues such as cough, bronchitis, laryngitis, and sore throat. Thyme essential oils have shown minimum inhibitory concentrations (MICs) ranging from 512 to 1024 µg/mL against *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. The main constituents of thyme essential oil are monoterpenic compounds such as carvacrol, thymol, γ-terpinene, and p-cymene, which act synergistically to enhance its antimicrobial efficacy [Antih et al., 2021].

### ***Rosmarinus officinalis (rosemary)***

Rosemary is a shrub native to the Mediterranean region, cultivated as an aromatic ornamental plant. Rosemary leaves have many traditional uses based on their antibacterial, carminative, antispasmodic, and choloretic actions. It has also been reported to treat urinary tract infections, leishmaniasis, as well as other microbial infections and inflammations. The essential oil, obtained from the aerial parts, showed MICs ranging from 0.3 to 1.72 µg/mL for *Bacillus cereus*, *B. subtilis*, *Bacillus pumilis*, *Salmonella poona*, *S. aureus*, and *E. coli*, and from 7.03 to 450 µg/mL for *L. monocytogenes*, *P. aeruginosa* [Santos et al., 2017; Hussain et al., 2010]. Limonene, camphor, eucalyptol, α-pinene, Z-linalool oxide, and borneol are among the major constituents of rosemary essential oil, compounds responsible for its antibacterial



activity. Ethanol extracts of rosemary have also demonstrated MICs ranging from 4.10 to 8.10  $\mu\text{g/mL}$  against *Staphylococcus aureus*, *E. coli* and *Salmonella sp* [Manilal et al., 2021].

### ***Mentha x piperita* (peppermint)**

It is a hybrid of *Mentha spicata* L. (spearmint) and *Mentha aquatica* L. (watermint). *M. x piperita* is native to the Mediterranean region and is cultivated worldwide. Traditionally, it has been used to treat a wide range of ailments, including skin irritation, sunburn, sore throat, fever, muscle pain, nasal congestion, indigestion, and infectious diseases. Essential oils derived from peppermint have shown antimicrobial, antimalarial, and anti-giardial activities *in vitro*. They exhibited minimum inhibitory concentrations (MICs) ranging from 0.5 to 8  $\mu\text{g/mL}$  against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *P. aeruginosa*, *E. coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* [Abolfazl et al., 2021]. Monoterpenes such as menthol and menthone have been reported to be responsible for the antibacterial activity. An *in vivo* study demonstrated that peppermint essential oil ointments improved the healing process in a wound model infected with *S. aureus* and *P. aeruginosa* [Modarresi et al., 2019].

### ***Salvia officinalis* L. (sage)**

Sage is an aromatic plant native to the Mediterranean and Middle Eastern regions but has been naturalized worldwide. In traditional medicine, *S. officinalis* is used to treat various disorders, including ulcers, gout, rheumatism, inflammation, dizziness, tremors, paralysis, diarrhea, and hyperglycemia. Sage essential oils have shown minimum inhibitory concentrations (MICs) ranging from 7.08 to 450  $\mu\text{g/mL}$  against *Y. lipolytica*, *L. monocytogenes*, and *P. aeruginosa*. Ethanol extracts exhibited MICs of 62.5  $\mu\text{g/mL}$  against *Streptococcus pyogenes* [Wijesundara and Rupasinghe, 2019]. Z-linalool oxide, limonene, camphor,  $\alpha$ -pinene, and borneol are the main constituents responsible for the essential oil's antibacterial activity, while rosmarinic acid, quercetin, ellagic acid, ursolic acid, epigallocatechin gallate, and chlorogenic acid might be involved in the antibacterial activity of the alcoholic extract.



### ***Ocimum basilicum (basil)***

This annual plant, native to Africa and tropical Asia, is cultivated worldwide. Traditionally, the plant is used to treat headaches, coughs, diarrhea, warts, and digestive disorders. The essential oil obtained from the aerial parts has shown MIC values ranging from 0.023 to 0.047 mg/mL against *Vibrio spp.* [Snoussi et al., 2016], while its ethanolic extract has shown MIC values ranging from 0.06 to 2.2 mg/mL against *B. subtilis*, *S. aureus* and *E. coli* [Adigüzel et al., 2005]. The major constituents of basil essential oil are linalool and estragole. Other compounds detected in relatively low concentrations include eucalyptol, methyl cinnamate, menthone, 1,8-cineole, eugenol, borneol, camphor, and germacrene. The main constituent, linalool, is believed to be largely responsible for the antibacterial activity of the essential oil. Seasonal variations in its concentration may lead to reduced antibacterial effects during the summer [Snoussi et al., 2016].

### ***Matricaria chamomilla (chamomile)***

It is a plant native to southern and eastern Europe. Traditionally, it is used to treat cough, menstrual and gastrointestinal pain, rheumatism, eczema, skin irritations, gingivitis, and eye inflammations. Ethanolic extract from chamomile has shown MICs ranging from 9.75 to 156.25 µg/mL against *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis* and *P. vulgaris* [Cvetanović et al., 2019]. α-bisabolol might be involved in the observed antibacterial activity. In a mouse model, topical formulations with *M. chamomilla* demonstrated faster wound healing than corticosteroids applied to tongue ulcers [Martins et al., 2009]. In clinical studies, a wild chamomile mouthwash administered to a patient with methotrexate-induced oral mucositis successfully treated the patient within 4 weeks [Mazokopakis et al., 2005], and the efficacy of *M. chamomilla* on oral mucositis induced by anticancer therapy was confirmed in a clinical study evaluating 98 cases of head and neck cancers. The results suggested that treating mucositis was accelerated by oral rinsing with chamomile preparations [Petronilho et al., 2012].

Plants and their natural products offer a promising source of antibacterial agents, and further investigation into this area represents a productive avenue of

research. One of the future challenges for phytochemicals is the development of effective methods and forms of administration that can deliver the active substance, the antimicrobial compound, to the target site in systemic infections.

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)**

Food safety is a worldwide issue with major public health consequences. Poor food handling practices can lead to the presence of numerous pathogenic organisms. Antimicrobial agents play a vital role in controlling these microbes and safeguarding food safety and human health. The increasing preference for natural, safe, and sustainable food preservation techniques has spurred research into the use of plant-based antimicrobials as substitutes for synthetic preservatives. The food industry is now investigating innovative strategies that integrate different physical methods with various natural antimicrobials [Bouarab Chibane et al., 2019].

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].

Plant-based antimicrobials form the primary category of natural preservatives, consisting of secondary metabolites that target microbial cells. Various plant parts, including seeds, fruits, peels, leaves, and roots, are abundant in these antimicrobials. These include phenolic compounds (such as simple phenols, phenolic acids,



anthocyanins, flavonoids, and quinones), tannins, essential oils, terpenoids, glucosinolate derivatives, alkaloids, and thiols [McClements et al., 2021].

Most plant extracts are generally recognized as safe (GRAS) and have been granted qualified presumption of safety (QPS) status in the U.S. and EU [Saeed et al., 2019].

Plant-based antimicrobial extracts or compounds, such as moso bamboo extract (Takeguard®) or a blend of various natural antimicrobial extracts (Biovia™ YM10) including green tea extract, have been suggested as alternatives to chemical preservatives [Bouarab Chibane et al., 2019]. Moreover, the European Food Safety Authority (EFSA) has approved rosemary (*Rosmarinus officinalis* L.) extract, which possesses antimicrobial properties, as a food additive (E 392) [EFSA ANS Panel 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]. Due to their potential as natural food preservatives, flavouring 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 [Lodhia, 2009].

Although plant-derived antimicrobials exhibit intrinsic antimicrobial properties, their utilization within the food industry is constrained by several substantial challenges. These challenges encompass issues such as chemical instability, difficulties in effectively dispersing these agents within food matrices, a restricted array of commercially available formulations, and the potential for imparting undesirable flavour profiles.



The stability of plant-based antimicrobials is often compromised under conditions prevalent in food processing or storage. Their antimicrobial efficacy is influenced by a variety of factors, including fluctuations in pH levels, temperature variations, and the concentration of the antimicrobials. Furthermore, the direct incorporation of plant extracts into food products frequently results in alterations to sensory attributes, such as flavour and texture, which may be considered undesirable. Additionally, the bioavailability of these plant-derived compounds and their potential to enhance food safety can be negatively impacted by interactions with macronutrients and other food components. Consequently, several stabilization techniques have been proposed to improve the overall stability of these antimicrobials, facilitate their release during storage, and mitigate any adverse effects on the sensory qualities of food products. Among these techniques are nano-emulsions (i), encapsulation methods (ii) and the integration of antimicrobials into active packaging systems (iii).

The application of these stabilization strategies can significantly enhance the stability of bioactive compounds, augment their antimicrobial effectiveness, and achieve controlled release during food storage [McClements et al., 2021; Castro-Rosas et al., 2017; Pinto et al., 2021].

Encapsulating plant antimicrobials in edible colloidal delivery systems offers a promising way to boost their effectiveness and minimize adverse interactions with food components.

(i) The most widely used delivery system for these antimicrobials is *oil-in-water nano-emulsions*, which consist of lipid nanoparticles dispersed in water. These nano-emulsions can be created from food-grade ingredients like plant-based emulsifiers and various stabilizers using standard processing techniques, such as mixing, sonication, and homogenization [McClements et al., 2021].

Nano-emulsions of essential oils derived from lemongrass, clove, thyme created through microfluidization of the primary emulsion, reduced *E. coli*. Incorporating alginate into the aqueous phase facilitates the application of these nano-emulsions in the coating materials for fruits and vegetables [Salvia-Trujillo et al., 2015].

An antibacterial nano-emulsion was created from anise seed extract using the ultrasound emulsification technique. This nano-emulsion effectively inhibited the



growth of *E. coli* and *Sal. typhimurium*, unlike the bulk extract, which did not impact these bacteria [Ghazy et al., 2021].

The use of nano-emulsions, such as those containing plant essential oils, to manage pathogens in plant-based foods has shown effectiveness. For instance, a nano-emulsion of oregano oil at a concentration of 0.1% successfully reduced the levels of *L. monocytogenes*, *Sal. typhimurium*, and *E. coli* on lettuce [Bhargava et al., 2015].

(ii) *Spray-drying and encapsulation* are frequently employed methods to enhance the stability and effectiveness of plant-derived antimicrobials in food products. Spray-drying involves atomizing a liquid solution or suspension into a stream of hot air, which quickly evaporates the droplets and produces a dry powder. This dry powder form of plant antimicrobials is more stable and easier to manage compared to its liquid counterpart.

Encapsulation, on the other hand, involves enclosing a natural antimicrobial within a protective matrix, such as a polymer or lipid, to boost its stability and functionality.

In the research conducted by Chen et al., eugenol and thymol were co-encapsulated into zein-casein nano-capsules using spray-drying. The powders produced exhibited favourable properties including good water hydration, stability during storage, controlled release over 24 hours, and effective bactericidal and bacteriostatic activities against *E. coli* and *L. monocytogenes* in milk whey, respectively [Chen et al., 2015]. Thyme essential oil, when encapsulated by spray-drying with casein and maltodextrin as wall materials, demonstrated antibacterial effects against thermotolerant coliforms and *E. coli* in meat burgers [Radünz et al., 2020].

(iii) *Active packaging* entails the intentional addition of specific substances to either the packaging material or the package's headspace to improve the functionality of the packaging system. This type of packaging can help maintain food quality and prolong the product's shelf life by enabling direct interaction between the food and bioactive agents purposefully included in the packaging. Antimicrobial packaging is a specific form of active packaging where its effectiveness relies heavily on the rate at



which the biologically active molecules, embedded within the polymer matrix, migrate [Arruda et al., 2022].

The most commonly utilized biopolymers for incorporating plant antimicrobials are chitosan, starch, carrageenan, cellulose, and alginate.

In relation to active chitosan films, incorporating 1% apple peel polyphenols into chitosan films improved their antibacterial effectiveness against *B. cereus*, *E. coli*, *Sal. typhimurium*, and *S. aureus* [Riaz et al., 2018]. Additionally, coating fresh cucumbers with chitosan infused with oregano essential oil reduced the number of total mesophilic bacteria as well as total yeasts and molds during storage at 10 °C for 15 days [Gutiérrez-Pacheco et al., 2020]. Furthermore, a bio-composite film made from cassava starch and whey protein, which was loaded with rambutan peel extract and clove oil, slightly inhibited *B. cereus*, *E. coli*, and *S. aureus* in vitro, and also decreased the total viable count in salami stored for 10 days [Chollakup et al., 2020].

Plant antimicrobials have garnered significant interest as viable alternatives to synthetic preservatives in the food industry, providing advantages like improved safety, longer shelf life, and higher consumer acceptance. 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. To optimize their use and boost their effectiveness, it's essential to gain a deeper understanding of how these plant antimicrobials and their combinations work, particularly regarding their impacts at molecular and cellular levels on target microorganisms.

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## **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 herbs have been a part of human life from birth to death. They frequently appear on our tables in various forms of food, are utilized for technical and bioenergetic purposes, and play a significant role in medicine, pharmacy, and the food industry. Over the past decade, the use of herbs and spices has grown. These plants often grow wild without chemical additives, and some studies have indicated that they have higher nutritional value compared to many other common food plants. Medicinal herbs are becoming increasingly important due to their potential health benefits, attributed to their nutritional content, including vitamins, phenolics, anthocyanins, flavonoids, and tannins. These raw materials are considered promising and advantageous both economically and ecologically for the food industry.

Medicinal plants are used for designing functional foods that are defined as "similar in appearance to a conventional food", and are demonstrated to have physiological benefits or to reduce the risk of chronic disease beyond basic nutritional functions [Devaraj and Mahalingam, 2021]. They are also used to improve the physical appearance, taste and food additive in bakery products.

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].

### **9.1. Medicinal plants used to improve the taste, colour 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 colouring agents in bakery and pastry in order to enhance the colour of foodstuffs or to make it look tastier and more attractive to the consumer [Arraiza and de Pedro, 2009].

Salihi et al. explored the impact of substituting wheat flour with plant extracts like blueberry and cranberry (at 3%, 6%, and 9% concentrations) and compared the resulting biscuits to control samples in terms of physicochemical and colour parameters. The findings indicated that higher concentrations of plant extracts in the biscuits significantly enhanced sensory attributes such as odour and taste. The study concluded that incorporating plant extracts into biscuits improved their physicochemical and sensory properties [Salihi et al., 2023].

Wang et al. [Wang et al., 2012] utilized ultra-fine grinding technology to create hawthorn bread, incorporating 3% hawthorn powder, 0.6% salt, 18% sugar, and 0.5% bread amendment in the formula to enhance its health benefits and unique flavour. Adding hawthorn to whole wheat flour bread supports the normal functioning of the digestive and circulatory systems and has an anti-hyperglycemic effect. This affordable ingredient is suitable for consumption by individuals with type 2 diabetes [Borczak et al., 2016].

Balestra et al. [Balestra et al., 2011] developed a functional bread by incorporating ginger, achieving satisfactory physicochemical and sensory properties. Their research indicated that up to 3% ginger powder could be added to the bread without negatively affecting its rheological properties.

Kaushal et al. also explored the functional and sensory characteristics of cookies enhanced with ginger powder. The functional and organoleptic qualities of



cookies containing 12% ginger powder were significantly better compared to the control samples [Kaushal et al., 2019].

## 9.2. 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 behaviour of bread gluten caused by polyphenols have been reported [Czajkowska–González et al., 2021].

The objective of the study conducted by Bourekoua and colleagues was to assess the impact of enriching gluten-free bread with acerola fruit powder on its physical, sensorial, and antioxidant properties. All quantities of acerola fruit powder tested demonstrated an improvement in textural parameters, as evidenced by a decrease in firmness and chewiness and an increase in springiness. Moreover, the incorporation of acerola fruit powder into enriched breads resulted in enhanced antioxidant properties [Bourekoua et al., 2021].

Another medicinal plant used is *Moringa oleifera*, which is recognized as an excellent source of phytochemicals, with potential applications in functional and medicinal food preparations due to its nutritional and medicinal properties; many authors have experimented with incorporating it mainly in biscuits, cakes, brownies, and sandwiches.

According to Ogunsina et al. adding *Moringa oleifera* seed flour influences the sensory characteristics of various breads and biscuits; however, these changes are not significant when using a ratio of 90% flour to 10% *Moringa oleifera* for bread and 80% flour to 20% *Moringa oleifera* for biscuits. Additionally, while the taste reflected the typical flavour of *Moringa oleifera* seeds, it remained acceptable in bread. The





nutritional profile of both products improved, with increased levels of protein, iron, and calcium [Ogunsina et al., 2011].

In their study, Agba et al. investigated the potential of incorporating decolorized *Moringa oleifera* leaf powder into cookies as a means of addressing consumer demand for healthier food options. They sought to address the challenge of low acceptability associated with the green colour of the powder. The findings indicated that neither the decolourization nor the addition level (2.5 or 7.5%) exerted a notable impact on water activity or flour functionality. However, minor variations in cookie colour were discernible. The *Moringa*-enriched cookies demonstrated an enhanced spread ratio and elevated protein, phenolic content, antioxidant activity, and *in vitro* protein digestibility in comparison to the control cookies [Agba et al., 2024].

EI-Gammal et al. incorporated *Moringa oleifera* at varying concentrations (5%, 10%, 15%, and 20%). The findings indicated that *Moringa oleifera* leaf powder exhibited elevated levels of protein and crude fibers, along with essential minerals such as calcium, magnesium, phosphorus, and iron. The addition of *Moringa oleifera* to the preparation of wholemeal sliced bread resulted in a notable increase in protein content. Furthermore, the intake of magnesium, calcium, and iron increased in comparison to the control [EI-Gammal et al., 2016].

Devisetti et al. evaluated the effect of *Moringa oleifera* leaf flour in sandwiches, reaching similar conclusions. The protein content in puffed sandwiches increased, reaching 21.6 g in 100 g of product. Additionally, the dietary fiber content was presented at 14.8 g per 100 g of product, and there was also a reduction in fat content of 3.7 g per 100 g of product, which was accompanied by a high presence of phenolic compounds and flavonoids. With regard to the sensory characteristics of the sandwiches, an acceptable result was obtained in terms of texture [Devisetti et al., 2015].



### 9.3. 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.

There has been a swift shift back to natural products due to the negative impact of artificial or harmful foods on human health. Consequently, the bakery industry has seen significant innovations and developments over the years. A key focus in the industry now is the incorporation of natural products into foods. Medicinal plants are being used to enhance the physical appearance, taste, and as food additives in bakery products, as well as for creating functional foods.

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.

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## **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 meat and dairy industry faces major challenges in extending shelf life, maintaining sensory quality and ensuring food safety. In this context, the application of medicinal plants has become a promising solution due to their antimicrobial and antioxidant properties. This chapter provides an extensive survey on the use of medicinal plants as value-added ingredients in the meat and dairy industry, and gives an overview of the benefits these plants bring in the context of preservation, nutritional enhancement and food safety. With their strong bioactivity, herbal compounds have been recognized as effective natural additives that contribute to extending the shelf-life of food products, improving flavour and optimizing nutritional value.

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 industrialised 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].

Medicinal plants contain a variety of bioactive compounds such as polyphenols, flavonoids, tannins, alkaloids and essential oils that can prevent spoilage and improve the quality of meat products. These compounds provide antioxidant and antimicrobial properties that are extremely valuable in the food industry. Their use in meat and dairy



products improves food safety and reduces the need for synthetic chemical preservatives. 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].

TG Dikme (2023) emphasized that medicinal and aromatic plant extracts have been integrated into traditional meat, dairy and bakery products, thus adding significant value [Dikme, 2023]. For example, rosemary and thyme compounds are widely used in meat and dairy to prevent oxidation and microbiological spoilage. A study conducted by B Kaptan and GT Sivri (2018) showed that herbal essential oils are effective in protecting dairy products against pathogenic microorganisms and extending shelf life [Kaptan and Sivri, 2018].

In another study, Nieto (2020) discussed the importance of thyme, one of the most widely used herbs in the food industry, due to its antioxidant and antimicrobial properties, being used in meat, dairy and fish to extend shelf life [Nieto, 2020].

The benefits of using medicinal plants in the food industry are not limited to shelf-life extension or flavour enhancement. These plants also bring significant health benefits to consumers. Grigoriadou et al. (2023) emphasized that Mediterranean herbs, such as oregano, rosemary and thyme, are natural sources of antioxidants that protect the body against oxidative stress, thereby reducing the risk of chronic diseases [Grigoriadou et al., 2023].

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 colour 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].



Another study by Iriondo-DeHond et al. (2018) looked at the use of herbal by-products in the food industry and showed that they can provide health benefits, including improving digestion and reducing inflammation, due to their high fiber and phenolic compounds content [Iriondo-DeHond et al., 2018].

### **10.1. Use of medicinal plants in the meat industry**

In the meat industry, herbs are used to reduce lipid oxidation and inhibit the growth of harmful bacteria such as *Salmonella* and *Listeria monocytogenes*. DA Delesa (2018) demonstrated that the addition of herbal extracts such as oregano and rosemary to meat products significantly reduced lipid degradation, thus helping to maintain the sensory quality and safety of the products [Delesa, 2018]. Also, Alirezalu et al. (2020) highlighted that several Mediterranean medicinal plants such as rosemary, have the ability to preserve meat, extending shelf life and improving flavour without affecting textural properties [Alirezalu et al., 2020].

Another important aspect of the application of medicinal plants in the meat industry is their effect on the nutritional quality of the products. The study conducted by Singh et al. (2015) on the use of *Moringa oleifera* in meat products showed that this plant not only improves the oxidative stability of meat, but also provides a natural source of essential micronutrients such as vitamin C and beta-carotene, making the products more nutritionally attractive [Singh et al., 2015]. Rosemary and thyme, in particular, are known for their intense flavours, which are appreciated in products such as sausages, minced meat and other processed meats. Grigoriadou et al. (2023) pointed out that these plants are used to both improve flavour and reduce the amount of salt and other synthetic additives in meat, providing a healthier and more appealing product for consumers [Grigoriadou et al., 2023].

One of the meat industry's biggest challenges is extending shelf life without compromising product quality. By adding herbal extracts, meat producers can reduce the need for synthetic preservatives. Nieto (2020) showed that thyme essential oil was highly effective in preventing the growth of harmful bacteria and extending the shelf life of fresh and processed meats [Nieto, 2020].



Likewise, Pérez-Alvarez et al. (2019) emphasized that the addition of plant extracts, such as oregano, can increase the shelf life of meat products without affecting sensory quality. This is crucial in the context of modern industry, where demands for more natural and chemical additive-free foods are increasing [Pérez-Alvarez et al., 2019].

Food safety is another important aspect of using medicinal plants in the meat industry. Puvača et al. (2020) showed that medicinal plants contribute to reducing the bacterial load in meat products by providing natural protection against bacterial contamination [Puvača et al., 2020]. Essential oils from thyme, oregano and rosemary have demonstrated the ability to inhibit the growth of pathogenic bacteria without affecting product quality.

Also, Iriondo-DeHond et al. (2018) showed that the addition of herbal extracts to meat products can reduce the risk of food contamination and extend shelf life, while providing health benefits to consumers due to their antioxidant and antimicrobial properties [Iriondo-DeHond et al., 2018].

### **10.1.1. Application of medicinal plants in different meat products**

#### *a) Sausages and processed meat products*

Sausages and other processed meat products are highly susceptible to lipid oxidation and bacterial contamination. The addition of herbal extracts helps prevent these problems. Pérez-Alvarez et al. (2019) showed that sausages treated with oregano extracts had an extended shelf life and improved flavour compared to sausages that did not contain natural additives [Pérez-Alvarez et al., 2019].

#### *b) Cured and smoked meat*

Matured and smoked meat is another area where herbs are used to improve both sensory quality and microbiological stability. Herbs such as thyme and rosemary are commonly used in cured meat preparations to add distinct flavours and inhibit the growth of harmful microorganisms. Grigoriadou et al. (2023) demonstrated that the addition of thyme to matured meat significantly improved microbiological stability by preventing the growth of pathogenic bacteria [Grigoriadou et al., 2023].



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### *c) Meat marinated and ready to cook*

Herbs are also often used in meat marinades to improve flavour and extend shelf life. Marinades based on rosemary, oregano and thyme not only add attractive flavours, but also help protect meat from oxidation during the cooking process. Puvača et al. (2020) have shown that these herbs are highly effective in maintaining the freshness and quality of meat prepared for cooking [Puvača et al., 2020].

## **10.2. Use of medicinal plants in the dairy industry**

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 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 a study conducted by Puvača et al. (2020), researchers showed that the use of herbs such as thyme and basil in cheeses improved microbiological quality, contributing to shelf-life extension and improved flavour [Puvača et al., 2020]. El-Sayed and Youssef (2019) showed that medicinal plants can improve the oxidative stability of dairy products, especially those with high fat content, such as cheeses and butter [El-Sayed and Youssef, 2019]. Herbs such as rosemary and sage are used to prevent rancidity and fat oxidation, thus helping to extend shelf life and maintain the sensory quality of products.



Another example is the use of rosemary and sage in yoghurts and cheeses, which Kaptan and Sivri (2018) demonstrated to improve microbiological stability and extend shelf life without compromising the natural flavour of the product [Kaptan and Sivri, 2018].

One of the biggest challenges in the dairy industry is extending the shelf life of perishable products such as yogurt, cheese and milk without using synthetic preservatives. Herbs offer a natural solution to this problem. Dikme (2023) has shown that essential oils from plants such as thyme, basil and oregano have the ability to inhibit the growth of harmful microorganisms such as *Listeria monocytogenes* and *Escherichia coli*, commonly found in dairy products, thus extending the shelf life of dairy products [Dikme, 2023]. These bacteria are responsible for many foodborne diseases and spoilage of dairy products.

In another study, Puvača et al. (2020) demonstrated that extracts of oregano and thyme significantly extended the shelf life of yogurt and cheeses by preventing bacterial contamination and mould formation [Puvača et al., 2020]. This is crucial for perishable products such as yoghurt, which require optimal preservation to safely reach the final consumers. One of the biggest challenges in the dairy industry is to extend the shelf life of perishable products such as yoghurt, cheese and milk without using synthetic preservatives. Herbs offer a natural solution to this problem.

Besides preservation, herbs play an important role in improving the sensory qualities of dairy products. Herbs add natural flavours and can reduce the need for artificial additives such as synthetic flavours or flavour enhancers. Nieto (2020) pointed out that thyme and mint are commonly used in dairy products to improve flavour and provide a freshness sensation, especially in products such as yogurt and cheese [Nieto, 2020]. In the same study Nieto (2020) emphasized that the use of plant extracts such as rosemary not only improves the stability of dairy products, but also provides a natural preservation solution that meets consumer demand for healthier and less processed foods [Nieto, 2020].

Pérez-Alvarez et al. (2019) explored the use of herbal extracts in yogurts and other fermented dairy products, highlighting that these natural flavors not only enhance the sensory experience, but also contribute to a longer-lasting sensation of freshness





[Pérez-Alvarez et al., 2019]. In particular, herbs such as mint and basil are added to yogurts to bring a touch of freshness, and are especially preferred in light dairy products and for healthy diets.

Also, in ripened cheeses, herbs not only improve taste but also help prevent the growth of harmful microorganisms. Kaptan and Sivri (2018) demonstrated that rosemary and thyme essential oils added to cheeses improved not only taste but also microbiological stability, preventing the formation of bacteria and moulds that could compromise product quality [Kaptan & Sivri, 2018].

Another major advantage of using medicinal plants in the dairy industry is their potential to enrich products with functional properties. Medicinal plants are rich in antioxidants, fiber and other bioactive substances, which can contribute to improving consumer health. By adding herbal extracts, dairy products become an important source of phenolic compounds and flavonoids, which have been associated with reduced risk of chronic diseases such as cardiovascular disease and type 2 diabetes.

Iriondo-DeHond et al. (2018) discussed the importance of functional dairy products incorporating herbs to provide additional health benefits. They pointed out that antioxidants in herbs such as rosemary and oregano help to neutralize free radicals in the body, thereby preventing cell damage and improving overall health [Iriondo-DeHond et al., 2018].

In addition, many herbs have beneficial digestive properties. For example, peppermint is known for its soothing effects on the digestive tract and is used in dairy products for people suffering from digestive problems. Grigoriadou et al. (2023) highlighted the benefits of herbs for digestive health and how they can be integrated into functional dairy products such as probiotic yogurts [Grigoriadou et al., 2023].

### **10.2.1. Application of medicinal plants in different dairy products**

#### *a) Yoghurts and fermented products*

Yogurts and other fermented dairy products are one area in which herbs are widely used because of their ability to improve flavor and contribute to digestive health. El-Sayed and Youssef (2019) pointed out that the addition of thyme and oregano to yogurts not only improves flavor, but also contributes to the growth of probiotic bacteria,



improving gut health and microbiota balance [El-Sayed and Youssef, 2019]. In addition, these herbs have antibacterial properties that help prevent contamination with pathogenic bacteria.

*b) Cheeses*

Cheeses are another important application area for herbs. Herbal extracts, such as rosemary and basil, are used to improve taste and prevent fat oxidation, which can lead to rancidity. Puvača et al. (2020) demonstrated that the addition of herbal extracts to ripened cheeses helps to prevent the growth of harmful bacteria and moulds, contributing to shelf-life extension [Puvača et al., 2020].

*c) Butter and other high-fat dairy products*

High-fat dairy products such as butter are prone to lipid oxidation, which can affect both taste and quality. The use of herbs with antioxidant properties, such as rosemary and oregano, helps to prevent oxidation and maintain the taste and texture of butter for longer. Nieto (2020) pointed out that essential oils from these herbs are often used in butter to prevent rancidity and to provide a fresh and natural taste [Nieto, 2020].

### **10.3. Perspectives and challenges in the use of medicinal plants**

Although medicinal plants offer numerous benefits, there are also challenges in standardizing their use in industry. Variability in phytochemical composition depending on species, season and extraction methods may influence their efficacy in food products. Paswan et al. (2021) discussed the need for further research on the standardization of herbal extracts for their effective integration into food production chains [Paswan et al., 2021].

In conclusion, medicinal plants bring significant value in the food industry, especially in meat and dairy products, where they contribute to shelf-life extension, improved nutritional value, and protection against oxidation and microbiological degradation. Their use as natural value-added ingredients offers a viable alternative to synthetic chemical additives, thus contributing to a healthier and safer diet.



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## Chapter 11. Pharmacological action and effects on health exerted by natural products derived from medicinal plants

(Dehelean CA, Șoica CM, Pînzaru IA)

### 11.1. Introduction

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. Plants contain a variety of bioactive compounds which can have diverse effects on the human body: minerals and vitamins that are necessary components of a healthy human diet, as well as numerous primary and secondary metabolites that influence human nutrition and health.

Secondary metabolites are not essential in plants, but these compounds exhibit biological activity that makes them very useful as ingredients for formulating traditional and modern medicines. Of particular importance are the bioactive components consisting of phytochemical substances such as polyphenols, carotenoids, terpenes, alkaloids, coumarins and essential oils (Figure 11.1) [Samtiya et al., 2021].

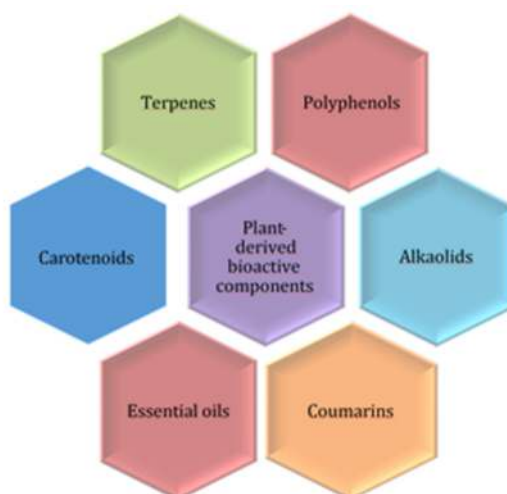


Figure 11.1. Plant-derived bioactive compounds.

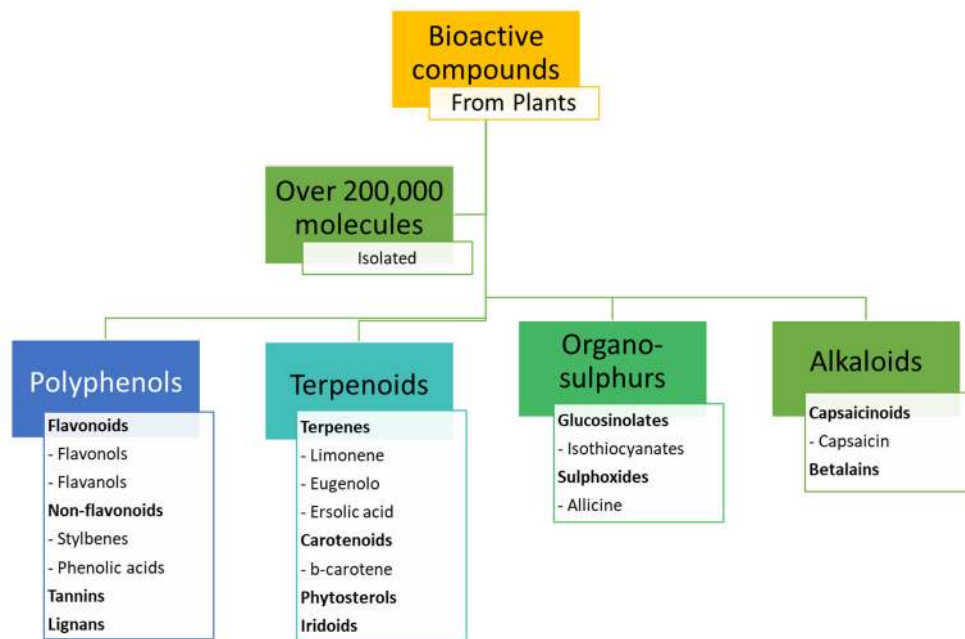


Figure 11.2. Bioactive compounds present in fruits and vegetables [Desjardins, 2014].

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].

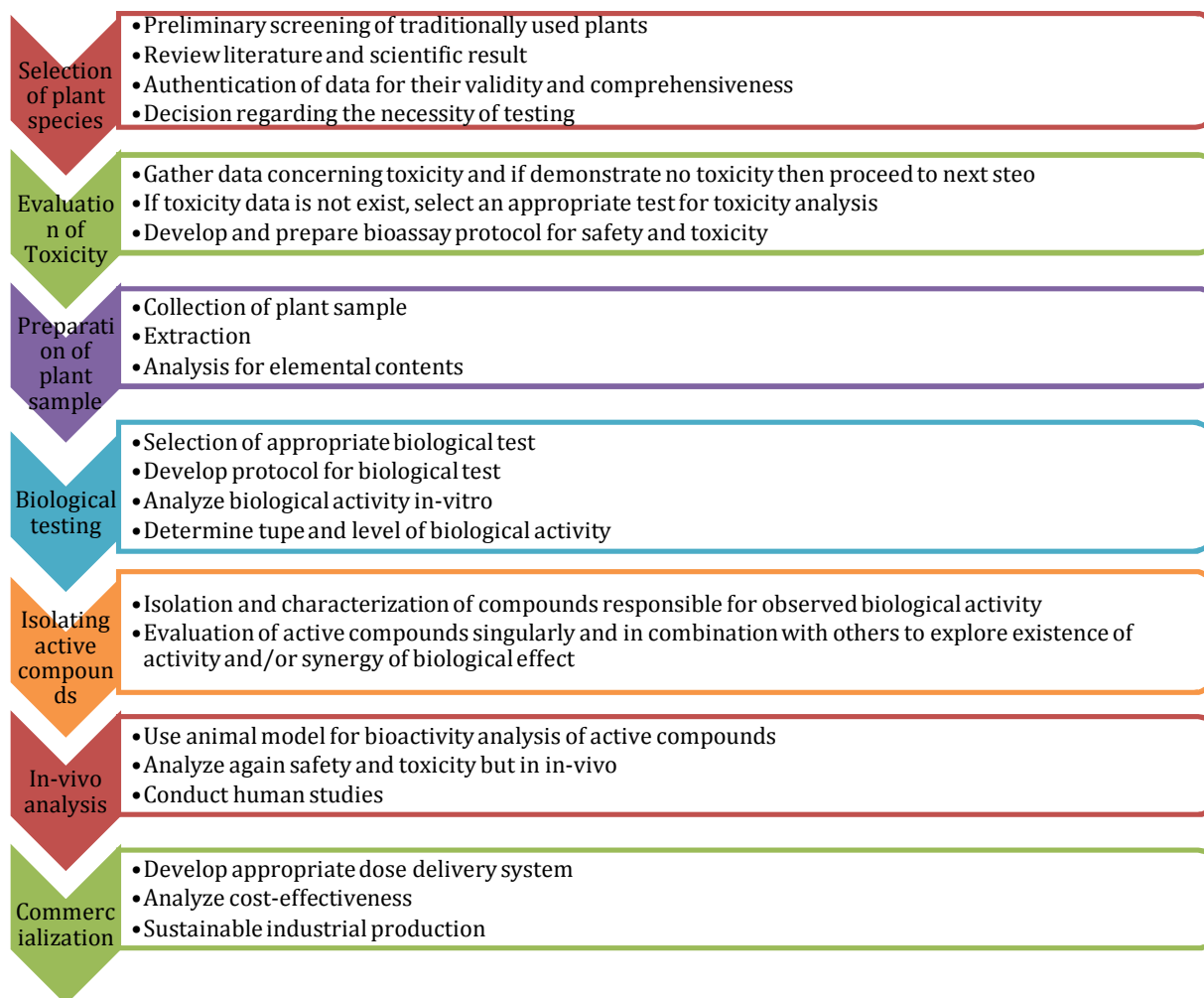
Morphine was the first plant compound isolated and used in human medicine. It came from the *Papaver somniferum* species, and it marked the beginning of the age of drug discovery in 1803 [Krishnamurti, 2016]. Since then, over 70,000 plant species have been studied and used in traditional medicine due 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 use of metabolomic studies is also used to identify new biological targets, elucidate mechanisms of action, and 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 (Figure 11.3).



**Figure 11.3. The flow chart of medicinal plant study [Azmir, 2013].**

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



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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 thalylglucerase alpha, 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.

### **11.2. Plant-derived bioactive compounds as antioxidants**

Phenolics are considered the key phytochemical compounds that can help to maintain better human health due to their proven strong antioxidative activity, which can help to reduce the risk of certain chronic diseases: heart disorders, arthritis, neurodegenerative diseases, cancer, arteriosclerosis. Antioxidants are compounds that scavenge free radicals in the human system. While the human body has a natural antioxidant defence system that keeps free radicles in check. Natural antioxidants found in food, particularly fruits, vegetables, and other plants, plays important role in disease prevention and in the situation of an excess of free radicals. Among phenolic compounds, flavonoids are the most abundant. They consist of anthocyanins, anthocyanidins, flavonols, flavones, and flavanones that have been shown to possess antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties [Jideani et al., 2021] (Figure 11.4).

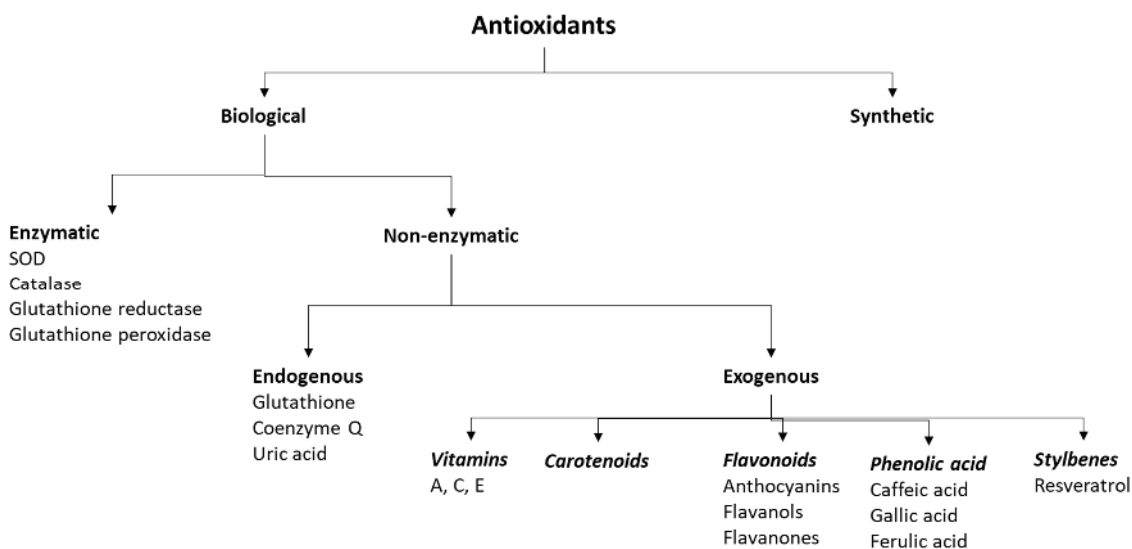


Figure 11.4. Classification of antioxidants [Kotha et al., 2022].

A particular class of plant-derived bioactive molecules are essential oils that are a mixture of chemical compounds having less molecular weight, such as terpenoids, carbonyl compounds, alcohols, aliphatic compounds, and polyphenols. They are extracted from various medicinal plants in the pharmacological industry due to their antioxidant, antifungal, antimicrobial and antiviral properties. The essential oils were taken out from different plant parts with leaves, fruits, flowers, bark, and root by using steam distillation, solvent extraction, and hydrodistillation. Several in vitro, in vivo, and clinical trials have shown the safety and effectiveness of antioxidant essential oils (EOs) in oral health. The products derived from different medicinal plants such as *Azadirachta indica*, *Thymus vulgaris*, *Asparagus racemosus*, *Juglans regia*, and *Ocimum sanctum* possess different types of phytochemicals and some used in pharmaceuticals [Kumar et al., 2021].

### 11.3. Plant-derived bioactive compounds as anti-inflammatory

Inflammation is a molecular biological response of living organisms to a series of exogenous stressors: chemical, mechanical, and infectious microorganisms. The effect of inflammation is the release of inflammatory cytokines and non-cytokines mediators such as reactive oxygen species (ROS) and nitric oxide (NO) that can end up in various chronic diseases, including cardiovascular diseases, diabetes, dementia,



and cancer. Several treatment strategies are in the medical trials to prevent these chronic inflammation stages. Among those strategies, herbs and spices-derived organic phytochemicals have long been used as an essential therapeutic tool due to their reputed medicinal effects on anti-inflammation.

In recent decades, hundreds of research and review articles have been published regarding the anti-inflammatory activities of plants (Table 11.1).

**Table 11.1. Anti-inflammatory activity of some medicinal plants [Nunes et al., 2020].**

Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
01	<i>Acacia catechu</i>	Mimosaceae	Bark, wood, flowering tops, gum.	Tannin, gum, catechuic acid
02	<i>Azadirachta indica</i>	Meliaceae	Leaf, root, oil, seed, gum, fruit, flower.	Margosine, bitter oil, azadirachtin.
03	<i>Caesalpinia crista</i>	Caesalpinaceae	Seeds, root, leaf, root bark.	Oleic, linoleic, palmitic, stearic acid, phytosterols.
04	<i>Cassia angustifolia</i>	Caesalpinaceae	Pods, dried leaves.	Emodin, eatharitin, mucilage, senna-picrin, opleanic acid.
05	<i>Coriandrum sativum</i>	Umbelliferaeapiaceae	Leaf, bark, flower	Tannin, cathartin, malic acid, cathartin, albuminoids.
06	<i>Cuscuta reflexa</i>	Convolvulaceae	Plant, seed, fruit, stem.	Cuscutine, flavonoid, glucoside, bergenin, coumarin.
07	<i>Enicostema littorale</i>	Gentianaceae	Whole plant.	Alkaloids, gentiocrucine



Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
08	<i>Erythrina variegata</i>	Papilionaceae	Leaves, bark, roots, flower.	2-Hydroxygenistein, genistein.
09	<i>Euphorbia hirta</i>	Euphorbiaceae	Plant, roots, leaves	Ascorbic acid, $\beta$ -amyrin, choline, inositol, linoleic acid, $\beta$ -sitosterol.
10	<i>Euphorbia tirucalli</i>	Euphorbiaceae	Root, plant (milk, juice).	$\beta$ -sitosterol, ellagic acid, citric acid, malic acid, eupholglucose.
11	<i>Fagonia cretica</i>	Zygophyllaceae	Leaves, twigs, bark.	Betulin
12	<i>Ficus benghalensis</i>	Moraceae	Aerial roots, bark, seeds, leaves, buds, fruits, latex.	Skin, fruits contain 10% tannin.
13	<i>Ficus carica</i>	Moraceae	Fruit, root.	Alkaloids, ascorbic acid, caffeic acid, niacin, linoleic acid, lutein, $\beta$ -carotene, pantothenic acid, $\beta$ -amyrin.
14	<i>Ficus religiosa</i>	Moraceae	Bark, leaves, fruits, tender shoots, seeds.	The bark contains tannins, rubber, wax.
15	<i>Foeniculum vulgare</i>	Apiaceae	Fruit, root, seeds, leaves.	Ascorbic acid, estragole, coumaric acid, caffeic acid, $\alpha$ -terpinene, scoparone, scopoletin, cynarin, D-limonene, $\alpha$ -phellandrene.
16	<i>Gentiana kuroo</i>	Gentianaceae	Rhizomes (roots)	Gentiopicroine, gentianic acid



Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
17	<i>Gloriosa superba</i>	Liliaceae	Rhizome, tuber, leaves, flower	Choline, colchicine, stigmasterol, salicylic acid, 2-methylcolchicine.
18	<i>Glycyrrhiza glabra</i>	Papilionaceae	Roots, leaves.	Genistein, eugenol, bergapten, glycyrrhizin, acetophenone, estragole, camphor, ascorbic acid, apigenin, anethole.
19	<i>Gmelina arborea</i> Roxb	Verbenaceae	Whole plant.	Betulin
20	<i>Grewia asiatica</i>	Tiliaceae	Leaves, roots, fruits, bark.	Betulin
21	<i>Hibiscus rosa-Sinensis</i>	Malvaceae	Buds, roots, leaves, flower	Quercetin, ascorbic acid.
22	<i>Hygrophila auriculata</i>	Acanthaceae	Roots, leaves, seeds.	Oleic and linoleic acids in seed oil, palmitic acid, stearic acid.
23	<i>Manihot esculenta</i>	Euphorbiaceae	Tuberous roots.	Ascorbic acid, palmitic acid, lauric acid, stearic acid, oleic acid.
24	<i>Martynia annua</i>	Pedaliaceae	Fruits, leaves.	Pelargonidin-3,5-diglucoside, cyanidin-3-galactoside, semi-drying oil.
25	<i>Momordica charantia</i>	Cucurbitaceae	Whole plant	5-Hydroxytryptamine, alkaloids, ascorbic acid, $\beta$ -carotene, cholesterol, lutein, diosgenin, lanosterol, lycopene, momordicin,





Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
				charantin niacin, momordicoside.
26	<i>Moringa oleifera</i>	Moringaceae	Roots, bark, leaves, seeds.	Choline, moringinine, myristic, ascorbic acid, $\beta$ -carotene, niacin, oleic acid, spirochin, stearic acid, tocopherol, vanillin.
27	<i>Nelumbo nucifera</i>	Nymphaeaceae	Whole plant.	Anonaine, ascorbic acid, $\beta$ -carotene, copper, erucic acid, glutathione, hyperoside, myristic acid, nuciferine, oxoushinsunine, rutin, stearic acid, trigonelline, kaempferol, D-catechin.
28	<i>Nicotiana tobacum</i>	Solanaceae	Leaves.	1,8-Cineole, 4-vinylguaiacol, acetaldehyde, acetophenone, alkaloids, anabasine, nicotinic acid, nicotine, scopoletin, quercitrin, sorbitol, tocopherol stigmasterol, trigonelline.
29	<i>Nigella sativa</i>	Ranunculaceae	Seeds.	$\alpha$ -spinasterol, ascorbic acid, $\beta$ -sitosterol, carvone, D-limonene, linoleic acid, myristic acid, methionine, nigellone, stearic acid, stigmasterol, tannin, thymoquinone, hederagenin.



Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
30	<i>Ocimum basilicum</i>	Lamiaceae	Whole plant	Acetic acid, ascorbic acid, aspartic acid, apigenin, arginine.
31	<i>Plumbago zeylanica</i>	Plumbaginaceae	Root, leaves, root, bark.	Plumbagin, droserone, 3-chloroplumbagin, chitranone, zeylinone, elliptone, isozeylinone.
32	<i>Portulaca oleraceae</i>	Portulacaceae	Stem, leaves, seeds.	Oleracins I and II, acylated betacyanins, carbohydrate, galacturonic acid, mucilage.
33	<i>Pterocarpus marsupium</i>	Fabaceae	leaves, flower, gum Heartwood,	Alkaloids, gum, essential oil, semi-drying fixed oil.
34	<i>Solanum melongena</i>	Solanaceae	Roots, leaves, tender fruits.	Ascorbic acid, alanine, arginine, caffeic acid.
35	<i>Solanum nigrum</i>	Solanaceae	Whole plant.	Solenin, solasodine.
36	<i>Stereopermum suaveolens</i>	Bignoniaceae	Roots, flower	Mucilage, albumin, sugar, wax, lapachol, dehydrotectol, $\beta$ -sitosterol, <i>n</i> -triacontanol.
37	<i>Tephrosia purpurea</i>	Fabaceae	Whole plant	Tephrosin, betulinic acid, lupeol, rutin.
38	<i>Terminalia chebula</i>	Combretaceae	Mature, immature fruits.	Ascorbic acid, gallic acid, ellagic acid, chebulic acid.



Number	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
39	<i>Thespesia populnea</i>	Malvaceae	Whole plant	Gossypol, herbacetin, kaempferol.
40	<i>Thespesia populneoides</i>	Malvaceae	Whole plant	Populneol, gossypol, kaempferol, quercetin-5-glucoside, calycopterin, kaempferol-5-glucoside, kaempferol-3-glucoside.
41	<i>Tinospora cordifolia</i>	Menispermaceae	Stem	Alkaloids, starch.
42	<i>Vernonia cinerea</i>	Asteraceae	Whole plant	Linoleic acid, lupeol, vernolic acid.

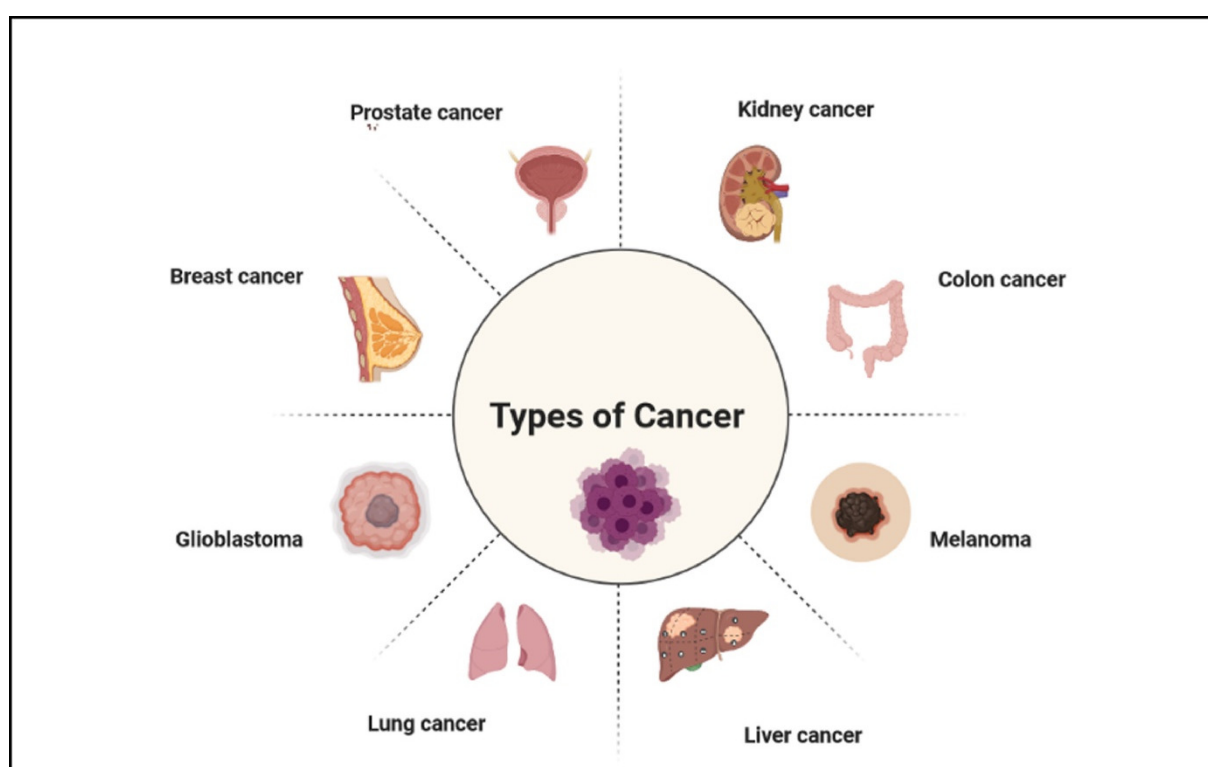
Polyphenolic compounds such as tannins, lignans, coumarins, saponins, and especially the flavonoids have drawn significant attention due to their modulation effects on inflammasomes. The flavonoids represent a group of vegetal pigments with extensive distribution in nature, being available in fruits, seeds, flowers, and barks. Flavonoids have an anti-inflammatory capacity since they inhibit the production of inflammatory mediators by modulating the arachidonic acid pathway, inhibiting several enzymes such as ATPase, prostaglandin, cyclooxygenase, lipoxygenase, NADH oxidase, protein kinase, hydrolases, peroxidases, metallopeptidases, tyrosinases, and phospholipases. Thus, flavonoids have been the target of increasing interest as a potential therapeutic drug in inhibiting or even decreasing inflammatory activity.

#### 11.4. Plant-derived bioactive compounds as anticancer

Cancer is a disorder that rigorously affects the human population worldwide. It is an extreme metabolic disorder that has seen significant advancement in treatment plans and preventative remedies. It is also called neoplastic disease, characterized by the uncontrolled proliferation followed by the constant multiplication of human cells. Medicinal plants, through the diversity of their chemical constituents, have the potential



to regulate cancer stem cell pathways. The most well-known plant-derived anticancer compounds of medical importance include those especially good at attacking the cytoskeleton system of cell microtubules which include the Vinca alkaloids, and taxanes, e.g., docetaxel (Taxotere), paclitaxel (Taxol) and others. It is extremely important to know about the different types of cancers along with targets responsible for them to find a therapeutic option for their treatment (Figure 11.5).



**Figure 11.5. Types of cancers [Roy et al., 2022].**

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.



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## Chapter 12. Medicinal plants and dietary reference values

(Dehelean CA, Șoica CM, Pînzaru IA)

Dietary reference values and medicinal plants are two distinct but interconnected aspects of health and nutrition. Dietary reference values are established to guide individuals in maintaining a balanced and healthy diet, providing benchmarks for the optimal intake of nutrients necessary for the proper functioning of the body. On the other hand, herbs can help achieve these dietary goals, bringing additional health benefits.

Globally, nutrition plays an essential role in preventing mortality and improving quality of life [English et al., 2021]. Because of this, dietary intervention requires significant effort on the part of both health care providers and patients. It has been found that, many times, nutritional counselling of patients is treated superficially, with most of the focus being on pharmacological interventions [Hever, 2017].

The main benefits of the plant-based diet have also been highlighted in relation to the scientific progress in the field. Whole-food diets, which include vegetables, fruits, legumes, whole grains, nuts, seeds, herbs and spices, are recognized for their ability to prevent chronic diseases such as cardiovascular disease and cancer. Several associations dedicated to the prevention of these diseases, as well as the United States Department of Agriculture, have emphasized the importance of ensuring an adequate intake of fiber, minerals and vitamins, recommending that half of the plate should consist of vegetables and fruits [McGuire, 2016; USDA, 2020].

In addition, there is a growing interest in the use of medicinal plants as an integral part of a healthy lifestyle. Herbs such as ginger, turmeric, echinacea and many others are traditionally used for their therapeutic and preventive properties. These plants contain bioactive compounds that can provide antioxidant, anti-inflammatory and antimicrobial effects, contributing to overall health and disease prevention [Pan et al., 2013].

In the current context, there is a growing interest in integrative research that combines traditional knowledge of medicinal plants with modern scientific research



methods. Thus, more solid evidence can be obtained regarding the efficacy and safety of using these plants for therapeutic purposes. As more and more studies highlight the benefits and mechanisms of action of medicinal plants, they are becoming more integrated into treatment protocols and dietary recommendations [Tapsell et al., 2006].

In conclusion, a holistic approach that includes both dietary reference values and the use of medicinal plants can lead to improved health and chronic disease prevention, providing a solid foundation for a healthy and balanced lifestyle.

### **12.1. Plant macronutrients**

The ideal ratio of macronutrients in food is still a hotly 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 lactation [Clemente-Suárez et al., 2021]. There are a number of optimal sources of carbohydrates, including vegetables, fruits and grains. It is common to label certain plant-based foods 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 replacing rice with 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 way [Alcorta et al., 2021].

Carbohydrates are essential macronutrients that provide energy to the body, being particularly important for the optimal functioning of the brain and nervous system [Slavin, 2013]. Complex carbohydrate sources such as whole grains, vegetables and legumes are preferred over simple carbohydrates found in refined sugars and processed foods because of their health benefits. They release energy gradually, keeping blood sugar levels stable and providing long-term satiety [Mann and Truswell, 2012].



In addition, dietary fibers, an important category of carbohydrates, play a crucial role in digestive health. Fibers help regulate intestinal transit, preventing constipation and reducing the risk of digestive conditions such as diverticulosis and colon cancer [Anderson et al., 2009]. Fibers also contribute to lowering blood cholesterol levels and weight control, promoting cardiovascular health [Soliman, 2019].

Consuming a variety of carbohydrate sources, including vegetables, fruits, whole grains and legumes, ensures an adequate intake of vitamins, minerals and phytonutrients, essential for maintaining general health [Bach-Faig et al., 2011]. Adapting carbohydrate intake to individual needs and lifestyle can contribute to a balanced diet and prevent chronic diseases, while providing the energy needed for daily activities [Marriott et al., 2010].

### *Proteins*

The recommended protein intake is 0.8 g/kg/day for adults. Several recent studies recommend an increase in this intake to 1.2 g/kg/day for people over 65 years of age [Lonnie et al., 2018]. Although food marketing has largely focused on animal proteins, all essential amino acids are synthesized by bacteria or plants, so they can be easily obtained from plant products [Hertzler et al., 2020]. Plant-based foods rich in protein include nuts, legumes, seeds, soybeans, and whole grains. Despite the fact that these plants tend to contain a lower amount of essential amino acids than animal products, some studies suggest that this difference can be beneficial [Gorissen et al., 2018].

For example, plant foods are often accompanied by fibers, vitamins, and phytonutrients that may contribute to better overall health and reduced risk of chronic diseases [Satija et al., 2016]. These aspects emphasize the importance of a balanced and varied diet that includes both plant and animal proteins, depending on individual needs and lifestyle.

Furthermore, the combination of plant protein sources can compensate for individual deficiencies in essential amino acids. For example, the combined consumption of legumes and whole grains such as rice and beans can provide a complete profile of essential amino acids. This method is known as "protein



supplementation" and is essential for vegans and vegetarians to ensure adequate protein intake [Young and Pellett, 1994].

For athletes and people with an intense level of physical activity, protein requirements may be higher than what is usually recommended. They could benefit from up to 1.6-2.2 g/kg/day to support muscle recovery and performance [Phillips and Van Loon, 2011]. Protein supplements such as whey, pea, or hemp protein powders can be helpful in meeting these increased needs.

It is also important to consider the quality of protein consumed. High-quality proteins are those that contain all the essential amino acids in appropriate proportions for the body's needs. High-quality protein sources include eggs, dairy products, lean meats, and certain plant proteins such as soy and quinoa [Hoffman and Falvo, 2004].

Therefore, an adequate and quality protein intake, adapted to individual needs and dietary preferences, is crucial for maintaining health and optimal functioning of the body throughout life [Brouns et al., 2003].

### *Dietary fatty acids*

Fatty acids have a wider recommended intake range than other macronutrients, ranging from 20% to 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. Omega-3 fatty acids are mainly found in flax seeds, chia seeds, hemp seeds, soybean seeds, walnuts and wheat germ. Omega 3 derived from plants has many advantages over marine products because it does not contain heavy metals such as mercury, lead or other industrial pollutants [Liu et al., 2022].

Omega-6 fatty acid, on the other hand, is found in most plants and is an essential fatty acid. For this reason, certain modern diets tend to be excessive in omega-6 fats by eating foods rich in these fats but low in omega-3 fats. Inflammatory and chronic diseases were more likely to develop as a result of this increased omega-6/omega-3 ratio [Nur Mahendra et al., 2023].

To balance this ratio and to reduce the risk associated with excessive omega-6 intake, it is recommended to increase the consumption of foods rich in omega-3 and to reduce the sources rich in omega-6, such as refined vegetable oils. An effective way



to improve this balance is to include fatty fish in your diet, such as salmon, mackerel and sardines, which are excellent sources of long-chain omega-3 fatty acids such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). These forms of omega-3 are directly usable by the body and have beneficial effects on cardiovascular health and brain function [Calder et al., 2020].

In addition, omega-3 supplements based on fish oil or krill oil may be considered to ensure adequate intake, especially for people who do not consume enough fish. It is important to monitor the quality of these supplements to avoid contaminants and ensure efficacy [Innes and Calder, 2020].

Attention to sources of omega-6 is also recommended. For example, sunflower, corn and soybean oils, commonly used in processed products, are high in omega-6 and should be consumed in moderation. Replacing them with oils higher in omega-3 or oils with a more balanced ratio, such as olive oil, can help maintain a healthy balance of these essential fatty acids [Simopoulos, 2016].

Therefore, maintaining a healthy omega-6/omega-3 ratio is essential for preventing inflammation and chronic diseases, and a balanced diet rich in natural sources of omega-3 and moderate in omega-6 can provide numerous health benefits [Simopoulos, 2002].

## 12.2. Plant micronutrients

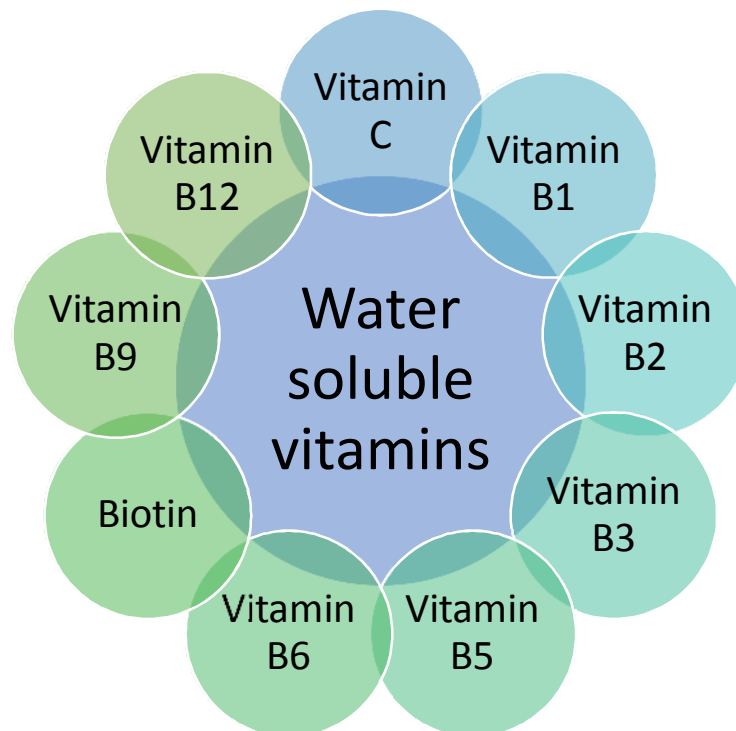
It is important to note that although studies have focused on the macronutrient content of plants, more recent studies have highlighted the beneficial role of other micronutrients in plant-based products. A healthy diet should contain a variety of micronutrients such as vitamins, minerals and phytonutrients [Assunção et al., 2022].

### *Vitamins*

Vitamins and minerals are defined “essential” because they cannot be synthesized in human organism and therefore must be obtained by the diet. They are required by in varying amounts throughout life to coordinate various physiological functions to maintain health. Vitamins are organic compounds that can be divided into two categories, based on their relative solubility in water and oils:



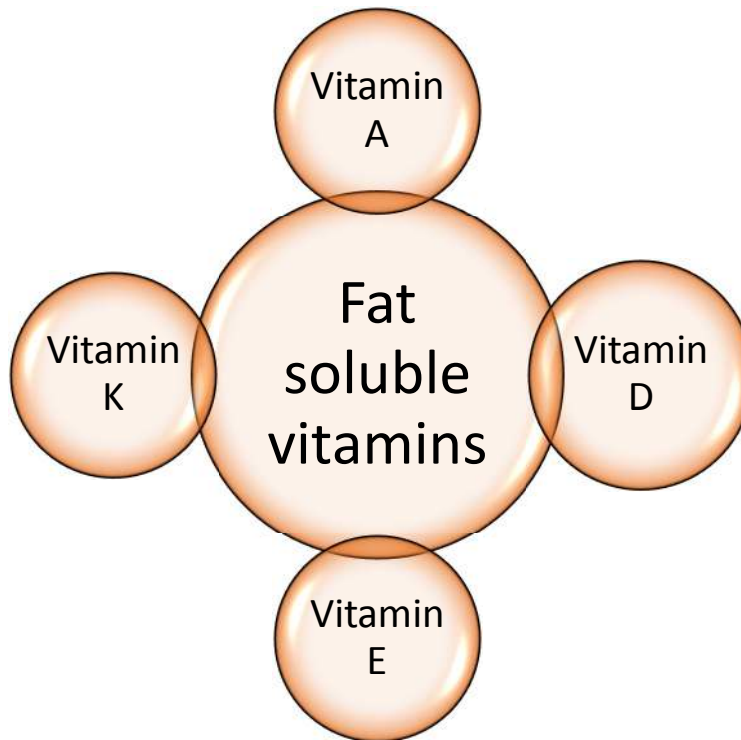
- water-soluble vitamins: they are soluble in water but not in fats;
- fat-soluble vitamins: they are soluble in fats (and organic solvents) but not in water.



**Figure 12.1. Water soluble vitamins.**

Vitamins are essential micronutrients for maintaining health and optimal functioning of the body. Water-soluble vitamins, such as vitamin C and B-complex vitamins, are water-soluble and must be consumed daily because the body cannot store them in large quantities. Vitamin C is a powerful antioxidant, supporting the immune system and skin health, while B vitamins are crucial for energy metabolism and nerve health [Mahan and Raymond, 2016]. On the other hand, fat-soluble vitamins, such as vitamins A, D, E, and K, dissolve in fat and are stored in the body.





**Figure 12.2. Fat soluble vitamins.**

Vitamin A is essential for vision and immunity, vitamin D for bone health, vitamin E as an antioxidant, and vitamin K for blood clotting and bone health [Gropper et al., 2018]. A balanced and varied diet, rich in plant sources, can ensure the necessary intake of vitamins for optimal health.

The vitamins are obtained from the food, but some are acquired through the microorganisms in the gut flora that make biotin and vitamin K. Deficiencies of vitamins as well as excess intake can produce disorders and chronic or acute toxicity. Vegetables and fruits are a rich source of micronutrients. Vitamin deficiencies can lead to various health problems. For example, a lack of vitamin C can cause scurvy, a condition characterized by weakness, anemia, and skin problems [Carr and Maggini, 2017].

Deficiencies of B vitamins can lead to various conditions such as beriberi (vitamin B1 deficiency), pellagra (vitamin B3 deficiency), and megaloblastic anemia (vitamin B12 deficiency) [Kennedy, 2016]. Vitamin D is essential for calcium absorption, and its deficiency can lead to rickets in children and osteomalacia in adults



[Holick, 2007]. Vitamin A is vital for visual health, and deficiency can lead to xerophthalmia and night blindness [Sommer, 2008]. Vitamin K deficiencies can cause blood clotting problems, which can lead to excessive bleeding [Shearer, 2009].

In addition to preventing deficiencies, vitamins also play preventive and therapeutic roles. For example, it has been shown that vitamin E can help protect cells from oxidative damage, and vitamin D supplementation can improve bone health and reduce the risk of osteoporosis [Traber and Stevens, 2011]. Vitamin C not only supports the immune system, but also promotes wound healing and collagen synthesis [Carr and Maggini, 2017]. The B vitamin complex is essential for the functioning of the nervous system and for energy production, having important roles in maintaining mental health and preventing depression and anxiety [Kennedy, 2016].

Natural sources of vitamins are varied and can be found in many foods. Fresh fruits and vegetables are excellent sources of water-soluble vitamins, while nuts, seeds and vegetable oils are rich in fat-soluble vitamins [Gropper et al., 2018]. In addition, animal products such as liver, fatty fish and dairy products are good sources of vitamins A and D [Mahan and Raymond, 2016].

To ensure adequate vitamin intake, it is important to eat a varied diet that includes a wide range of foods. Vitamin supplements may be necessary in certain circumstances, such as in the case of a diagnosed deficiency, certain medical conditions, or during periods of growth and development [Traber and Stevens, 2011]. However, it is essential to consult a healthcare professional before starting any supplementation regimen to avoid hypervitaminosis or unwanted interactions with other medications [Kennedy, 2016].

Additionally, for individuals with special needs or specific health conditions, minerals and trace elements supplementation may be necessary under the supervision of a healthcare professional. For example, pregnant women, the elderly and athletes may have increased nutritional requirements and may benefit from appropriate supplementation to ensure sufficient intake of essential nutrients.



Water soluble vitamins – key points:

- ❖ *Vitamin C (ascorbic acid)*: powerful antioxidant, supports the immune system, skin health and iron absorption. Sources: citrus fruits, strawberries, bell peppers.
- ❖ *B vitamins (B1, B2, B3, B5, B6, B7, B9, B12)*: essential for energy metabolism, nerve health and blood cell production. Sources: whole grains, legumes, nuts, seeds, green leafy vegetables.

Fat soluble vitamins – key points:

- ❖ *Vitamin A*: essential for vision, immune system and skin health. Sources: carrots, sweet potatoes, spinach.
- ❖ *Vitamin D*: helps absorb calcium and phosphorus, essential for bone health. Sources: exposure to the sun (in the case of plants, indirectly through the diet of animals that consume them).
- ❖ *Vitamin E*: antioxidant, protects cells from damage. Sources: Nuts, seeds, spinach.
- ❖ *Vitamin K*: important for blood clotting and bone health. Sources: green leafy vegetables (kale, spinach), broccoli, Brussels sprouts.

*Minerals*

Minerals and trace elements are essential for maintaining the health and proper functioning of the body. Minerals such as calcium, iron, magnesium, potassium and zinc play crucial roles in processes such as bone health, oxygen transport, muscle and nerve function, water balance regulation and immunity. Plant sources of these minerals include green leafy vegetables, legumes, nuts and seeds.

Calcium is fundamental for building and maintaining strong bones and teeth, preventing conditions such as osteoporosis. Rich sources of calcium include broccoli, almonds and fortified tofu [Weaver, 2014]. Iron is essential for the production of hemoglobin and the transport of oxygen in the blood. Iron deficiency can lead to anemia, manifested by fatigue and weakness. Legumes, spinach and pumpkin seeds are excellent sources of plant iron [Abbaspour et al., 2014].



Magnesium contributes to normal muscle and nerve function, protein synthesis and blood sugar control. Rich sources of magnesium are pumpkin seeds, almonds and spinach [Rosanoff et al., 2012]. Potassium is crucial for maintaining water balance and the proper functioning of cells and nerves. Bananas, potatoes and avocados are excellent sources of potassium [He and MacGregor, 2008]. Zinc plays an essential role in the functioning of the immune system and in the wound healing process. Nuts, sesame seeds and chickpeas are rich sources of zinc [Prasad, 2013].

Trace elements such as copper, manganese, selenium and iodine are required in smaller amounts but are vital for energy production, carbohydrate and lipid metabolism, cell protection and thyroid function. Copper is essential for red blood cell formation and immune system function, and as rich sources are nuts, seeds, and dark chocolate [Harvey et al., 2003]. Manganese plays a crucial role in carbohydrate and lipid metabolism, being present in whole grains, nuts and tea [Aschner and Aschner, 2005].

Selenium is a powerful antioxidant that protects cells from oxidative damage and supports thyroid function. Sources of selenium include Brazil nuts, sunflower seeds, and mushrooms [Rayman, 2000]. Iodine is essential for the synthesis of thyroid hormones, which regulate metabolism. Rich sources of iodine include seaweed, fish and dairy products [Zimmermann and Boelaert, 2015].

A varied and balanced diet can ensure adequate intake of minerals and trace elements for optimal health. Regular consumption of foods rich in these essential nutrients can help prevent deficiencies and support the proper functioning of the body. It is important to pay attention to dietary diversity and include a wide range of vegetables, fruits, whole grains, nuts and seeds in your daily diet.

*Minerals – key points:*

- ❖ *Calcium*: essential for healthy bones and teeth, blood clotting and normal muscle and nerve function. Sources: green leafy vegetables (e.g. spinach, kale), broccoli.
- ❖ *Iron*: required for the production of hemoglobin and the transport of oxygen in the blood. Sources: legumes (beans, lentils), chia seeds, quinoa.



- ❖ *Magnesium*: important for muscle and nerve function, blood sugar regulation and protein synthesis. Sources: nuts, seeds, spinach.
- ❖ *Potassium*: regulates water balance and blood pressure. Sources: bananas, potatoes, avocados.
- ❖ *Zinc*: contributes to the immune system and wound healing. Sources: pumpkin seeds, cashews, chickpeas.
- ❖ *Phosphorus*: essential for building bones and teeth and for energy production. Sources: fish, chicken, lentils.
- ❖ *Selenium*: it has antioxidant properties and supports the functioning of the thyroid gland. Sources: Brazil nuts, sunflower seeds, mushrooms.

Trace elements – key points:

- ❖ *Copper*: needed for energy production and connective tissue formation. Sources: mushrooms, nuts, seeds.
- ❖ *Manganese*: involved in carbohydrate and lipid metabolism. Sources: nuts, green leafy vegetables, pineapple.
- ❖ *Selenium*: antioxidant that protects cells from damage. Sources: Brazil nuts, sunflower seeds, mushrooms.
- ❖ *Iodine*: essential for thyroid function. Sources: seaweed, blueberries, beans.

Plant-based foods are rich sources of vitamins and essential micronutrients. Below are foods that contain some vitamins and minerals (Figure 12.3).



Nutrient	PRI/AI	Content (per 100 g)	Bioavailability (%)
•(Pro)-vitamin A	•650 µg/d	<ul style="list-style-type: none"> <li>•Carrot 694 µg</li> <li>•Kale 335 µg</li> <li>•Mango 26 µg</li> <li>•Orange 8 µg</li> </ul>	•0-36%
•Folate	•330 µg/d	<ul style="list-style-type: none"> <li>•Spinach 300 µg</li> <li>•Broccoli 230 µg</li> </ul>	•60-98%
•Vitamin C	•95 mg/d	<ul style="list-style-type: none"> <li>•Kale 100 mg</li> <li>•Broccoli 47 mg</li> <li>•Orange 51 mg</li> <li>•Kiwi 79 mg</li> </ul>	•89-90%
•Vitamin K	•70 µg/d	<ul style="list-style-type: none"> <li>•Kale 623 µg</li> <li>•Spinach 394 µg</li> <li>•Kiwi 79 µg</li> </ul>	•5%
•Potassium	•3500 mg/d	<ul style="list-style-type: none"> <li>•Spinach 539 mg</li> <li>•Kale 400 mg</li> <li>•Banana 374 mg</li> <li>•Kiwi 312 mg</li> </ul>	•60-85%
•Calcium	•950 mg/d	<ul style="list-style-type: none"> <li>•Kale 180 mg</li> <li>•Spinach 105 mg</li> <li>•Kiwi 30 mg</li> <li>•Orange 23 mg</li> </ul>	•20-40%

Figure 12.3. Vitamins and minerals found in different fruits and vegetables.

### *Phytonutrients*

Phytonutrients, also known as phytochemicals, are bioactive compounds present in plants that provide significant health benefits. They are not considered essential nutrients, but they help prevent disease and maintain health. Phytonutrients have antioxidant, anti-inflammatory and anti-carcinogenic properties, among others.

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, 2022].

## 1. Flavonoids

Flavonoids are an important class of phytonutrients present in plants, recognized for their multiple benefits on human health. These bioactive compounds are found in various fruits, vegetables, teas and other plant-based foods, giving them not only their vibrant colors but also antioxidant, anti-inflammatory and antiviral properties [Manach et al., 2004]. Through these properties, flavonoids contribute to protecting cells against oxidative stress, reducing inflammation and supporting the immune system [Panche et al., 2016].

Studies have shown that regular consumption of flavonoids can reduce the risk of chronic diseases such as cardiovascular disease, diabetes and certain types of cancer [Hollman et al., 1999; Arts and Hollman, 2005]. Therefore, including flavonoid-rich foods in the daily diet can bring significant benefits to overall health and long-term well-being [Bondonno et al., 2019].

### *Types of flavonoids and their sources:*

- *Quercetin*: helps reduce inflammation and may have antiviral effects. Sources: onions, apples, berries.
- *Catechins*: powerful antioxidants that protect cells from damage. Sources: green tea, dark chocolate, apples.
- *Anthocyanins*: contribute to heart health and have antioxidant properties. Sources: blueberries, blackberries, eggplant.
- *Luteolin*: it has anti-inflammatory and antioxidant effects, helping to protect cells. Sources: parsley, green pepper, chamomile.
- *Flavonols*: benefits for cardiovascular health and reducing cancer risk. Sources: tea, onion, broccoli.
- *Isoflavones*: contribute to hormonal balance and bone health. Sources: soy, tofu, chickpeas.



## 2. Carotenoids

Carotenoids are a class of essential phytonutrients present in many plants and plant-based foods. These bioactive compounds are natural pigments that impart vibrant colours from yellow and orange to deep red to fruits and vegetables [Fraser and Bramley, 2004]. Carotenoids are known for their multiple health benefits, including antioxidant properties and important roles in maintaining healthy eyes, skin, and the immune system [Krinsky and Johnson, 2005]. Eating foods rich in carotenoids is associated with reduced risk of chronic diseases such as cardiovascular disease and certain types of cancer [van Poppel et al., 1993; Rao and Rao, 2007]. Because of these benefits, including a variety of colourful fruits and vegetables in your daily diet can significantly contribute to overall health and well-being [Johnson, 2002].

- *Beta-carotene*: precursor of vitamin A, essential for vision and immunity.

Sources: carrots, sweet potatoes, pumpkin.

- *Lutein and zeaxanthin*: protect the eyes against macular degeneration.

Sources: spinach, kale, corn.

## 3. Glucosinolates

Glucosinolates are natural compounds found in various cruciferous vegetables known for their beneficial health properties. These phytonutrients play an essential role in protecting the body against various diseases, including cancer. Glucosinolates contribute to the detoxification of the body and have anti-inflammatory, antioxidant and anti-carcinogenic effects [Traka and Mithen, 2009]. A regular consumption of glucosinolate-rich vegetables such as broccoli, cabbage, cauliflower, and Brussels sprouts may help maintain health and prevent chronic diseases [Higdon et al., 2007].

Studies have shown that glucosinolates and their hydrolysis products, such as sulforaphane, can induce phase II enzymes of detoxification, which help neutralize potential carcinogens and eliminate them from the body [Zhang et al., 1992]. Also, these compounds can inhibit inflammation by modulating inflammatory pathways and reducing the production of pro-inflammatory cytokines [Herr and Büchler, 2010].

Glucosinolates and their derived isothiocyanates have been intensively studied for their anticancer effects. It has been found that they can inhibit the growth of tumor



cells and induce apoptosis in various types of cancer cells [Clarke et al., 2008]. For example, consumption of cruciferous vegetables has been associated with a reduced risk of colorectal, lung and prostate cancer [Verhoeven et al., 1996].

In addition, the antioxidant effects of glucosinolates contribute to the protection of cells against oxidative stress, a major factor in the development of chronic diseases and aging [Fahey et al., 2001]. Therefore, including cruciferous vegetables in your daily diet not only provides nutritional benefits, but also offers long-term protection against a variety of ailments.

- *Sulforaphane*: contributes to the detoxification of the body and has anti-cancer properties. Sources: broccoli, Brussels sprouts, cauliflower.
- *Glucoraphanin*: precursor of sulforaphane, with antioxidant and anti-inflammatory effects. Sources: broccoli, cauliflower, kale.
- *Glucobrassicin*: converts to indole-3-carbinol, which supports hormonal health and has anti-cancer properties. Sources: cabbage, cauliflower, kale.
- *Glucoiberine*: contributes to the protection against cardiovascular diseases and supports liver function. Sources: broccoli, Brussels sprouts, kale.

#### 4. Phytosterols

Phytosterols are bioactive compounds found in plants that structurally resemble cholesterol and are known for their cardiovascular health benefits. These nutrients can help reduce the level of LDL cholesterol ("bad" cholesterol) in the blood, thus contributing to the prevention of cardiovascular disease [Katan et al., 2003]. Phytosterols also have anti-inflammatory and antioxidant properties, supporting overall body health [Awad and Fink, 2000]. The main sources of phytosterols include nuts, seeds, legumes and vegetable oils [Jones & AbuMweis, 2009]. Regular consumption of foods rich in phytosterols can have a positive impact on the health of the heart and circulatory system [Plat and Mensink, 2005].

Studies have shown that phytosterols reduce the intestinal absorption of cholesterol, thereby causing a decrease in serum LDL cholesterol levels. A meta-analysis conducted by Katan et al. (2003) showed that a daily intake of 2-3 grams of



phytosterols can reduce LDL cholesterol levels by about 10%. This reduction is significant and may contribute to lowering the risk of cardiovascular disease.

Phytosterols have also been associated with anti-inflammatory properties. Awad and Fink (2000) demonstrated that these compounds can reduce inflammatory markers in various experimental models, suggesting a potential benefit in the prevention and management of chronic inflammatory diseases.

In addition, phytosterols exert antioxidant effects, protecting cells against oxidative stress. Oxidative stress is a major factor in the pathogenesis of chronic diseases, including cardiovascular disease and cancer [Moghadasian, 2000]. Therefore, eating foods rich in phytosterols not only helps control cholesterol levels, but also protects against oxidative and inflammatory damage.

Dietary sources of phytosterols are diverse and include nuts (almonds, pistachios), seeds (sunflower, pumpkin), legumes (beans, peas) and vegetable oils (olive oil, canola oil) [Jones and AbuMweis, 2009]. Including these foods in your daily diet can be an effective strategy for improving cardiovascular health and reducing the risk of chronic diseases.

- *Beta-sitosterol*: helps lower cholesterol levels and supports cardiovascular health. Sources: Nuts, seeds, legumes.
- *Campesterol*: contributes to the maintenance of cardiovascular health and has anti-inflammatory effects. Sources: vegetable oils, avocados, almonds.
- *Stigmasterol*: supports joint health and may have anti-cancer effects. Sources: soy, beans, peanuts.
- *Brassicasterol*: has antioxidant properties and can help maintain skin health. Sources: mustard seeds, cabbage, kale.

## 5. Polyphenols

Polyphenols are a diverse group of natural compounds found in plants, recognized for their powerful antioxidant and anti-inflammatory properties. These phytonutrients play a crucial role in protecting cells against oxidative stress and chronic inflammation, thus contributing to the prevention of cardiovascular disease, cancer and other degenerative conditions [Scalbert et al., 2005]. Polyphenols are found in many



foods, including fruits, vegetables, tea, coffee and red wine. Regular consumption of polyphenol-rich foods can support heart, brain, and immune system health, promoting longevity and overall well-being [Williamson, 2017].

Studies have shown that polyphenols exert beneficial effects through various mechanisms, including neutralizing free radicals, reducing inflammation, and modulating the expression of genes involved in antioxidant and anti-inflammatory processes [Pandey and Rizvi, 2009]. For example, flavonoids, a subgroup of polyphenols, have demonstrated the ability to improve endothelial function and reduce blood pressure, thereby contributing to the prevention of cardiovascular disease [Hollman and Katan, 1999].

Resveratrol, a polyphenol found in red wine, has been associated with anti-cancer and protective effects on the cardiovascular system. Resveratrol can induce cancer cell apoptosis and inhibit cell proliferation, thereby providing protection against tumor development [Baur and Sinclair, 2006].

In addition, green tea polyphenols such as epigallocatechin gallate (EGCG) have been studied for their neuroprotective effects. These substances can reduce the risk of neurodegenerative diseases such as Alzheimer's and Parkinson's by reducing oxidative stress and inflammation in the brain [Mandel et al., 2011].

Food sources of polyphenols are varied and include berries, citrus fruits, apples, onions, spinach, dark chocolate and various types of tea [Manach et al., 2004]. Including these foods in your daily diet can help maintain health and prevent chronic disease.

- *Resveratrol*: has anti-inflammatory and antioxidant properties, beneficial for heart health. Sources: red grapes, red wine, blueberries.

- *Curcumin*: anti-inflammatory and powerful antioxidant. Sources: turmeric.

- *Quercetin*: contributes to reducing inflammation and has antiviral effects. Sources: apples, onions, green tea.

- *Epicatechin*: beneficial for cardiovascular health and brain function. Sources: dark chocolate, green tea, apples.

- *Ellagic acid*: has anticarcinogenic properties and supports skin health. Sources: pomegranate, strawberries, walnuts.



## 6. Terpenoids

Terpenoids, also known as isoprenoids, are a large class of organic compounds found in many plants and certain animals. These aromatic molecules are responsible for the characteristic odors and flavors of many plants and have a wide range of health benefits. Terpenoids have anti-inflammatory, antioxidant, antimicrobial and anticarcinogenic properties, contributing to the body's protection against various diseases and infections [Thoppil and Bishayee, 2011]. Common sources of terpenoids include citrus fruits, peppermint, eucalyptus, oregano, and lavender. The consumption and use of terpenoids can support overall health and well-being, providing both internal and external benefits [Gershenzon and Dudareva, 2007].

Terpenoids are involved in various biological and therapeutic processes. For example, limonene, a major terpenoid found in citrus peel, is known for its anticancer properties and ability to modulate enzymes involved in body detoxification [Crowell, 1999]. Carvacrol, present in oregano, has demonstrated strong antimicrobial effects against a wide range of bacteria and fungi [Burt, 2004].

Another important terpenoid is menthol, found in peppermint, which has analgesic and anti-inflammatory properties and is commonly used in personal care products and topical medications to relieve pain and inflammation [Eccles, 1994]. Eucalyptol, the main component in eucalyptus oil, is known for its antioxidant effects and ability to improve respiratory function [Juergens et al., 2003].

Terpenoids also play a crucial role in the protection of plants against pathogens and herbivores, contributing to their natural defenses [Gershenzon and Dudareva, 2007]. These compounds are widely used in aromatherapy and the pharmaceutical industry for their therapeutic benefits.

- *Limonene*: it has anti-inflammatory and anticarcinogenic effects. Sources: citrus fruits (lemon peel, orange).

- *Menthol*: used for its calming and anti-inflammatory effect. Sources: mint, eucalyptus.

- *Carvacrol*: has antimicrobial and anti-inflammatory properties. Sources: oregano, thyme.





- *Linalool*: known for its sedative and anxiolytic effects. Sources: lavender, coriander.

- *Beta-caryophyllene*: helps reduce inflammation and supports nervous system health. Sources: black pepper, cloves.

## 7. Other phytonutrients

- *Allicin*: has antibacterial and antiviral properties. Sources: garlic, onion.

- *Capsaicin*: may help reduce pain and has anti-inflammatory properties. Sources: hot peppers.

- *Apigenin*: contributes to reducing inflammation and may have anti-cancer effects. Sources: parsley, chamomile, celery.

- *Lutein*: beneficial for eye health and reduces the risk of macular degeneration. Sources: spinach, kale, egg yolk.

- *Anthocyanins*: powerful antioxidants that support cardiovascular and immune health. Sources: blueberries, blackberries, black cherries.

### 12.3. Health benefits of phytonutrients

Phytonutrients play a crucial role in the prevention and management of various chronic diseases. For example, carotenoids and polyphenols are powerful antioxidants that protect cells against oxidative stress and DNA damage, thereby reducing the risk of cancer and cardiovascular disease [Liu, 2004]. Flavonoids in green tea and red wine have been associated with improved heart health by reducing inflammation and improving endothelial function [Arts and Hollman, 2005].

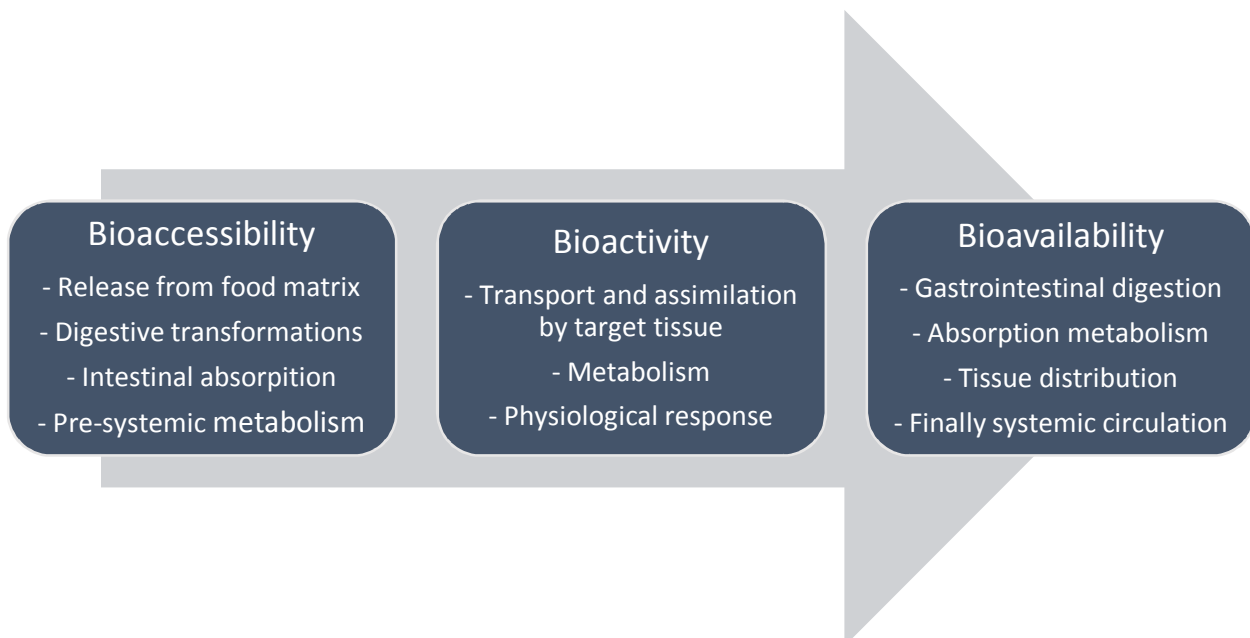
Also, glucosinolates in cruciferous vegetables can activate detoxification enzymes in the liver, contributing to the elimination of carcinogenic substances from the body [Higdon et al., 2007]. Phytosterols in nuts and seeds can reduce LDL cholesterol levels, thereby improving the lipid profile and reducing the risk of atherosclerosis [Plat and Mensink, 2005].

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.



Legumes and beans can be considered both as vegetables and as an alternative to meat thanks to a comparable nutritional profile, rich in proteins, iron and zinc. *Phaseolus vulgaris* (beans) are a low glycaemic source of complex carbohydrates, vitamins, minerals, protein, fiber, and phytochemical compounds with a various of bioactive properties. Their dietary inclusion is beneficial to improving disease conditions such as cardiovascular disease and type-2 diabetes, gut microbial diversity, colon health, and chronic low-grade inflammation [Mullins, 2021]. Anthocyanins, mainly delphinidin, petunidin, and malvidin, are some of the most abundant phytochemicals found in beans and have been shown to improve glycemic control and reduce the risk for cardiovascular disease.

Several key factors influence the bioavailability of phytonutrients in the human metabolism system. During digestion, phytonutrients undergo enzymatic breakdown in the gastrointestinal tract before being absorbed through the intestinal wall and entering the bloodstream. However, digestion efficiency can vary depending on the specific phytonutrient and an individual's digestive enzymes [Siddiqui, 2023].



**Figure 12.4. The processes suffered by phytonutrients in the human body.**

The term 'Bioactivity' includes all the physiological effects it generates [Takur, 2020].



#### 12.4. Integrating phytonutrients into the diet

To benefit from the protective effects of phytonutrients, it is essential to eat a varied and balanced diet rich in fruits, vegetables, nuts and seeds. Regular consumption of phytonutrient-rich foods not only provides protection against chronic disease, but also supports overall health by improving immune function, reducing inflammation, and protecting against oxidative stress [Williamson, 2017].

Supplementation with phytonutrients can also be considered, but it is preferable to obtain these compounds from whole foods, as interactions between different phytonutrients and other bioactive compounds in food can amplify the beneficial effects.

Among phytonutrients, polyphenols are a class of naturally occurring compounds that have attracted attention. A number of beneficial biological actions are associated with it, such as antioxidant properties and the ability to regulate cell function [Zhang et al., 2022]. A number of other compounds such as flavonoids, stilbenes and curcuminoids play an important role in the prevention of 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 diseases [Monjotin, 2022].

These phytonutrients contribute to overall health by protecting the body against free radicals, reducing inflammation and supporting the immune system. Eating a variety of fruits, vegetables, nuts and seeds ensures an adequate intake of phytonutrients, thus promoting a balanced diet and multiple health benefits.

##### *Stilbenes*

Stilbenes are an important class of organic compounds found both in nature and in various industrial applications. They are known for their structure based on two benzene nuclei linked by an ethylene bridge (C=C), which gives them unique physical and chemical properties [Hecht, 2001]. Stilbenes are of major interest in organic chemistry due to their ability to undergo different photochemical reactions and form derivatives with diverse applications [Brimioulle et al., 2015].



In nature, stilbenes are found in plants, where they play essential roles in defence mechanisms against pathogens. A notable example is resveratrol, a naturally occurring stilbene present in grapes and other fruits, which has attracted the attention of researchers for its potential benefits to human health, including antioxidant and anti-inflammatory properties [Baur and Sinclair, 2006]. Resveratrol has been extensively studied for its protective effects against cardiovascular disease and its ability to prevent certain types of cancer [Afaq and Mukhtar, 2003].

In addition to their presence in nature, stilbenes have extensive applicability in industry. They are used as precursors in the synthesis of polymers, dyes, and optoelectronic materials [Ravindranath et al., 2002]. Also, due to their fluorescent properties and ability to form liquid crystals, stilbenes are used in the development of materials for displays and other advanced technologies [Miller et al., 1992].

### *Curcuminoids*

Curcuminoids are a class of polyphenolic compounds found mainly in the plant *Curcuma longa*, known as turmeric. These compounds are responsible for the vibrant yellow color of turmeric and have attracted the attention of researchers due to their potential health benefits [Aggarwal et al., 2007].

Curcuminoids are known for their antioxidant, anti-inflammatory and anticancer properties, being intensively studied for their applicability in modern medicine [Goel et al., 2008]. The best-known curcuminoid is curcumin, which has shown promising effects in numerous preclinical and clinical studies. In addition to curcumin, turmeric also contains demethoxycurcumin and bisdemethoxycurcumin, which contribute to the overall biological activity of turmeric extract [Anand et al., 2007].

These substances have been traditionally used in Ayurvedic and Chinese medicine to treat various conditions, including inflammation, pain, and digestive disorders [Gupta et al., 2013]. Currently, research is focused on elucidating the molecular mechanisms by which curcuminoids exert their therapeutic effects and on developing formulations that improve their bioavailability [Anand et al., 2007].

The food and nutritional supplement industry also capitalizes on curcuminoids for their natural colouring and preservative properties, providing a healthy alternative



to synthetic additives [Priyadarsini, 2014]. Thus, curcuminoids continue to be the subject of intense and innovative research, having the potential to significantly contribute to the improvement of human health and the development of added value products [Lao et al., 2006].

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. Studies show that plant-based diets help reduce inflammation, improve immune function, and manage body weight. They can also help prevent type 2 diabetes and lower blood pressure, thanks to the high content of fibers, vitamins, minerals and antioxidants present in plant foods.

In addition to individual health benefits, plant-based diets can have a positive impact on the environment. Intensive agriculture for meat and dairy production contributes significantly to greenhouse gas emissions, deforestation and excessive water consumption. Reducing the consumption of animal products and increasing the consumption of plant-based foods can help reduce the global ecological footprint, contributing to a more sustainable environment.

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 fibers. Nevertheless, their use should be guided by scientific evidence, and individuals should be aware of potential interactions and dosages in order to ensure their safety and effectiveness. It is essential that people interested in including herbs in their diet consult a health professional for appropriate and personalized recommendations. In addition, educating consumers about safe and ethical sources of medicinal plants is crucial to prevent contamination and misuse. Thus, an informed and integrated approach can maximize the benefits of a plant-based diet and the use of medicinal plants, contributing to overall improved health and well-being.



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## **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 with 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: 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 place 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



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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].

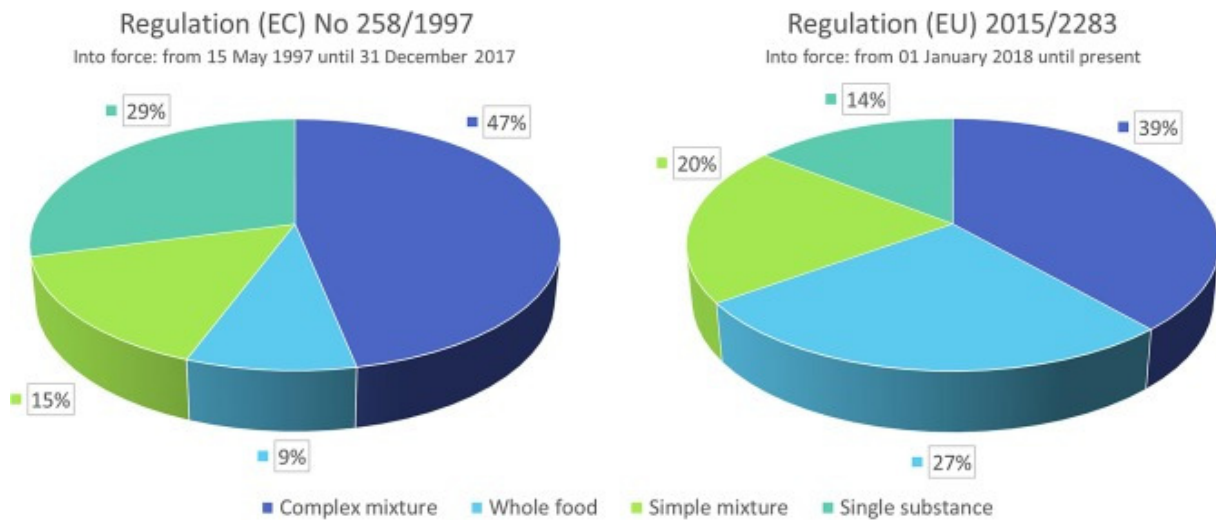
*Yarrowia lipolytica* yeast biomass is a large source of protein, exogenous amino acids, essential trace minerals and lipid compounds, mainly unsaturated fatty acids, as well as a source of B vitamins [Jach and Malm, 2022]. Of the 26 extracts approved as novel foods by the European Union, 23 have been 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, 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.

The European Food Safety Authority (EFSA) have the role to identify and characterize any hazards linked to the consumption of novel foods, and assessing the risk associated to their consumption under the proposed conditions of use. EFSA became operational in 2003 and in 2018, with the implementation of Regulation (EU) 2015/2283 (repealing and replacing Regulation No 258/97), it became the sole EU entity responsible for carrying out such risk assessments. All novel foods received by EFSA have been clustered by their source, in order to draw further attention to the highly heterogeneous profile of these regulated products.

Novel foods have been grouped into single substances, simple mixtures, complex mixtures, and whole foods, as per the respective definitions provided by EFSA's novel food Guidance document [EFSA NDA Panel, 2016a]. In the study of Ververis and co-workers [Ververis et al., 2020] an in-depth analysis of the experience gained by EFSA in the risk assessment of novel foods and of the reasoning behind the most frequent scientific requests by EFSA to applicants is made. The study analysed all novel food applications received by EFSA from January 2003 till the end of 2019. The results showed that the number of novel food applications received by EFSA



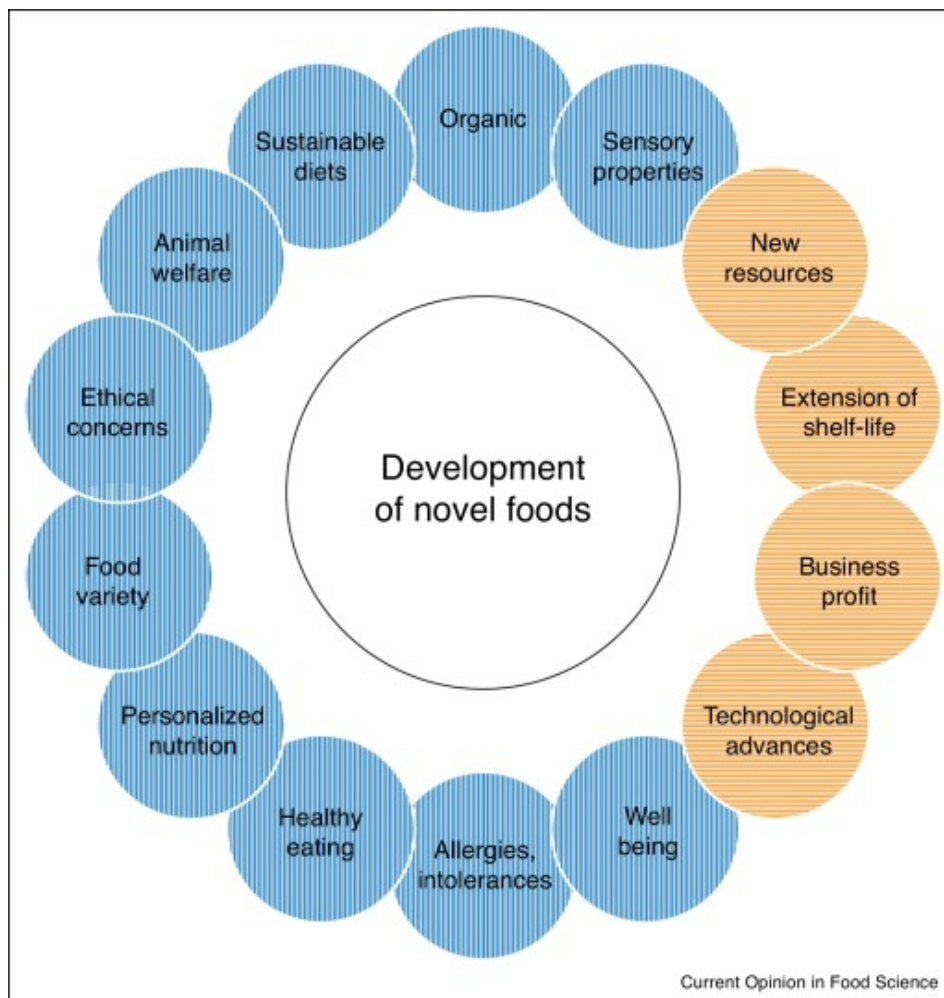
between 2003 and 2017 was low (an average of 5 applications per year) while in 2018, with the implementation of the new regulation, it peaked to 40 in 2018 and 39 in 2019.



**Figure 13.1. The type of novel foods that entered EFSA’s risk assessment (% of the novel food applications) [Ververis et al., 2020].**

Complex mixtures represent a considerable proportion of the novel foods assessed by EFSA. The proportion of novel whole foods has increased since the implementation of the new regulation, whereas the proportion of novel single substances has decreased.

The motives for the development of novel foods are different from the perspective of the consumers and the industry. Two interrelated trends have dominated the scientific research about novel foods in Western countries: i) the impact of food production on the environment, climate change, and animal welfare has encouraged people to avoid eating meat, and meat alternatives and replacements are made from plant-based alternatives, insects, and artificial meat; ii) the awareness of the connection between food and health has created a market for products with health-enhancing properties.



**Figure 13.2. Motives for the development of novel foods from the perspective of the consumers (in blue) and the industry (in yellow) [Tourila and Hartmann, 2020].**

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 Glycoprotein P. 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].

A key prerequisite for successful therapy is the efficacy and safety of the drugs used. These parameters are tested in large clinical trials before new drugs are approved. But, it has to be taken into account that several factors may alter the drugs' characteristics when used in the general population like mutations in the drug target, leading to changes in protein expression or resistance mechanisms.

Wiesner and co-workers studied the levothyroxine interactions with food and dietary supplements [Wiesner et al., 2021]. Levothyroxine (l-thyroxine, l-T<sub>4</sub>) is a drug of choice for treating congenital and primary hypothyroidism. The results proved that l-T<sub>4</sub> ingestion in the morning and at bedtime are equally effective, and also that the co-administration of l-T<sub>4</sub> with food depends on the drug formulation. Coffee, soy products,





fiber, calcium or iron supplements, and enteral nutrition resulted in decreased I-T4 absorption.

In the study of Duda-Chodak and Tarko the possible side effects of polyphenols and their interaction with medicines and food supplements were evidenced [Duda-Chodak and Tarko, 2023]. Polyphenols are a large group of compounds that comprise phenolic acids, flavonoids, lignans, stilbenes, etc. Their activity depends on both their bioaccessibility (the amount of an ingested nutrient that is available for absorption in the gut after digestion) and their bioavailability (the fraction of an ingested nutrient that reaches the systemic circulation and the specific sites where it can exert its biological action).

The main factors which affect the bioavailability/bioaccessibility of polyphenols are food matrix, food processing, and digestive enzymes. Moreover, some polyphenolic compounds can exert their biological activity only after the biotransformation by intestinal microbiota. Polyphenols, due to their antioxidant properties and the ability to quench free radicals and reactive oxygen species, exert a beneficial effect on human health, and they are believed to slow down the aging process as well as to be useful in the prevention of the development of many diseases. For this reason, diets which are rich in polyphenol compounds, as well as dietary supplements containing them, have become popular. However, it should not be forgotten that the consumption of polyphenolic compounds, especially in large amounts in a purified form (supplements instead of fruits and vegetables), can cause side effects or even have a negative impact on our health.

Polyphenols are able to chelate the ions of transition metals (e.g., Fe) in the intestine so that it cannot be absorbed, leading to the development of anaemia. Among potent inhibitors of iron absorption are various teas which are rich in catechins. Flavonoids are able to form complexes with proteins and this leads to the inhibition of specific enzymes like digestive enzymes. In some diseases may be beneficial, e.g. in the treatment of diabetes but in healthy individuals, any disorders in the activity of digestive enzymes are unfavourable, as they cause unpleasant symptoms in the digestive system, as well as the impaired assimilation of some nutrients.



Hesperetin, luteolin, quercetin, catechin and rutin are flavonoids that produce an inhibition of  $\alpha$ -amylase. Tea polyphenols, extracts of raw African pear, herbal extracts containing rosmarinic acid and extracts from *Rubus corchorifolius* inhibit  $\alpha$ -glucosidase and  $\alpha$ -amylase. Epigallocatechin-3-gallate (EGCG) inhibit lactase. Polyphenols can alter drug absorption, distribution, and metabolism. When the metabolism of a drug is limited, its concentration in the blood or tissues increases, causing various effects which are sometimes very dangerous. In addition, polyphenols can also affect drug transport through their interaction with the drug transporters, e.g., P-glycoprotein. This means that there is a considerable risk for an adverse impact of the drug–polyphenol interactions, especially for drugs with a narrow therapeutic index such as warfarin, cyclosporine A, and digoxin.

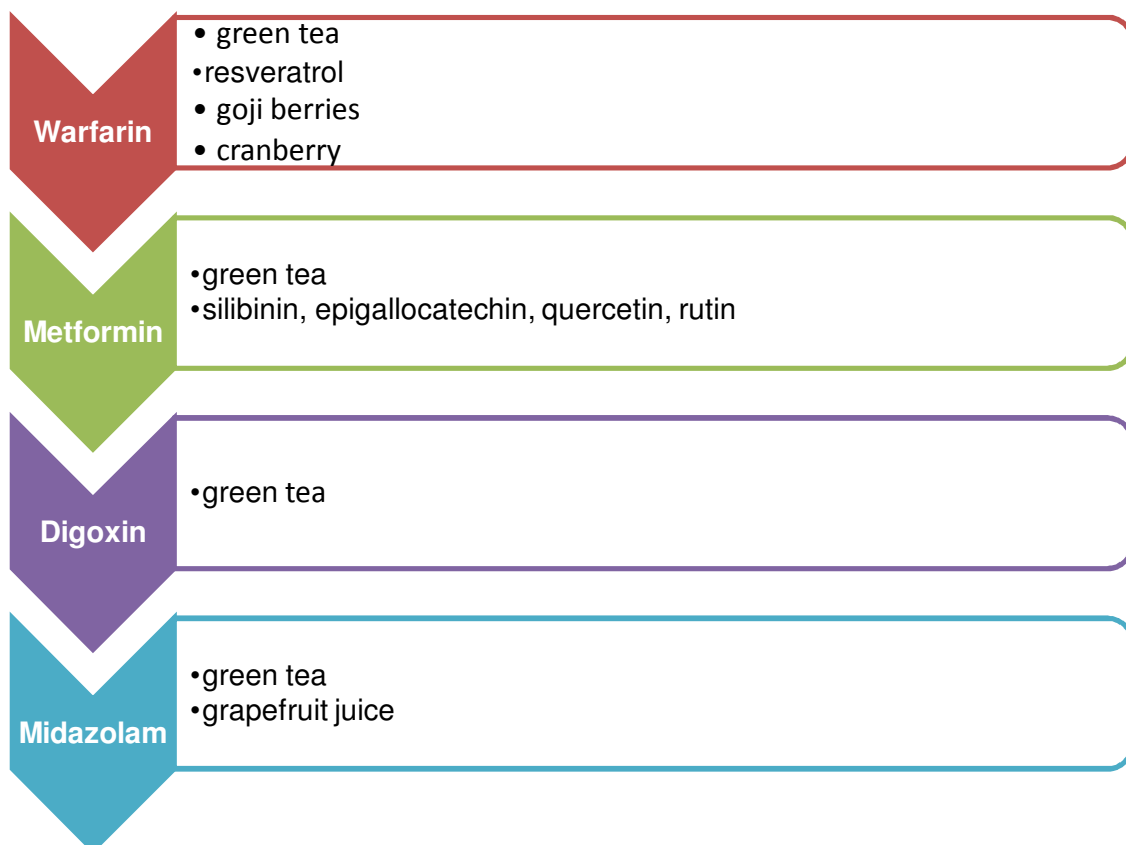


Figure 13.3. Polyphenol-drug interaction.

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

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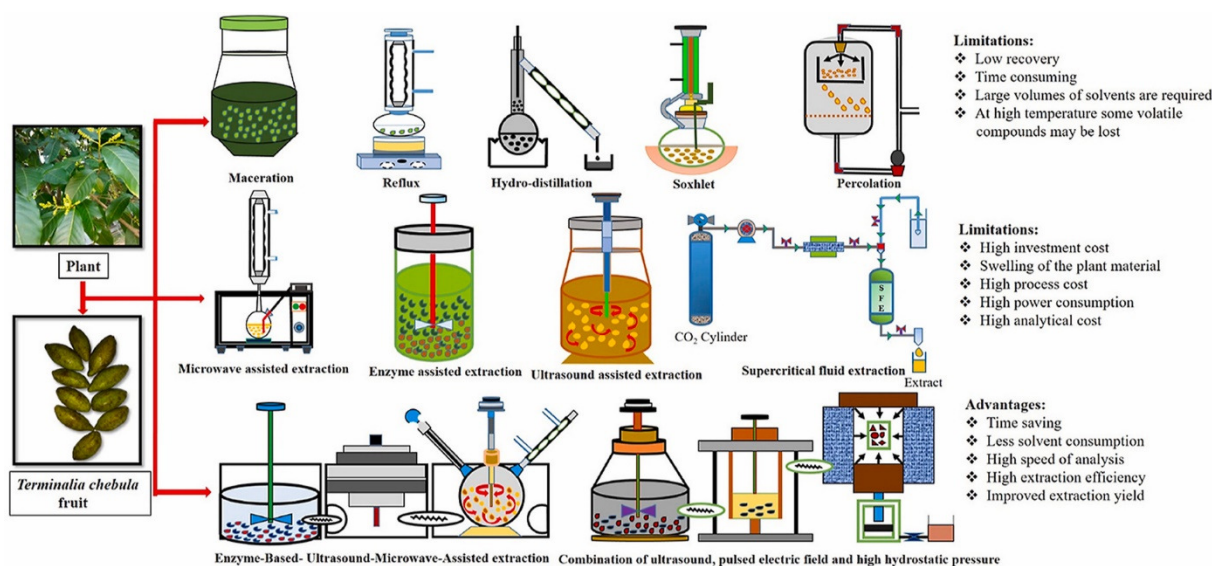
## **Chapter 14. Products preparation from plants (extracts, essential oils), phytochemical characterization and influence of geolocation on the composition of phytocomplexes (Conforti F, Statti G)**

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. Many environmental elements can affect the quality, growth, and distribution of medicinal plants. For some plants, for example liquorice, cultivation is often unproductive. The chemical profile of plants is known to reflect the resilience of plants within the same species due to different climatic conditions, date of collections, allelopathy and many other environmental conditions. For example, *H. officinalis* subsp. *aristatus* collected in different areas characterized by different environmental and climatic conditions and in different years, revealed important differences in essential oil composition [Guerrini et al., 2021].

The concept of preparation of medicinal plant involves the proper and timely collection of the plant, authentication by an expert, adequate drying, and grinding. This is followed by extraction, fractionation, and isolation of the bioactive compound where applicable. 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 (maceration, infusion, decoction, percolation, digestion and Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extraction) (Figure 14.1).

Extracts are preparations derived from the complete or partial evaporation of solutions obtained by exhaustion of dried plant drugs, using suitable solvents [Azwanida, 2015].



**Figure 14.1. Techniques for extraction of bioactive compounds [Jha, 2022].**

The choice of an appropriate extraction method depends on the nature of the plant material, solvent used, pH of the solvent, temperature, and solvent to sample ratio. It also depends on the intended use of the final products. The solvent used for the extraction of medicinal plants is also known as the *menstruum*. The choice of solvent depends on the type of plant, part of plant to be extracted and nature of the bioactive compounds.

Using maceration or percolation processes, extracts can be obtained that 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 2020].

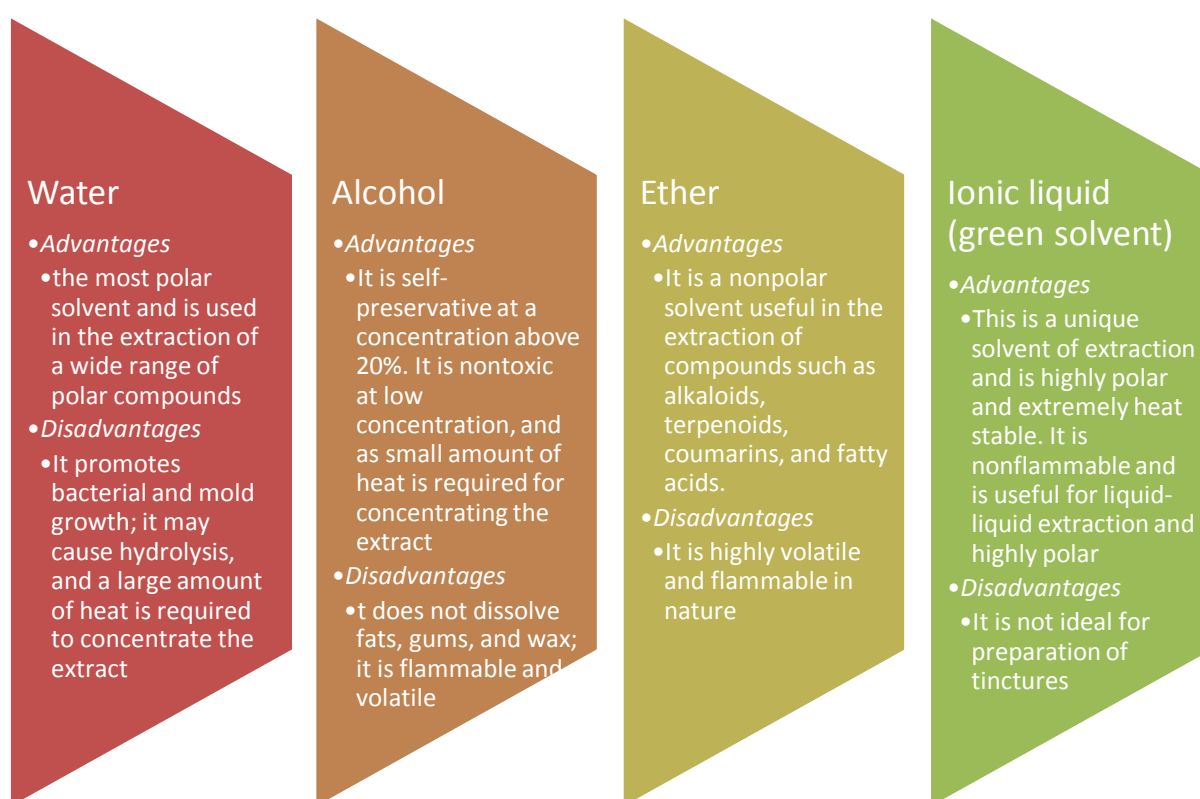
Other preparation obtained through maceration or percolation are the tinctures. These are liquid solutions obtained by processing plant drugs with an appropriate solvent. Commonly, a hydroalcoholic solution (a mixture of water and alcohol) is used, the alcohol content of which is chosen according to 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].



**Figure 14.2. Properties of solvent of extractions.**

One of the advanced extraction procedures in preparation of medicinal plants is Microwave-assisted extraction which uses mechanism of dipole rotation and ionic transfer by displacement of charged ions present in the solvent and drug material. Microwave-assisted extraction has special advantages such as minimizing solvent and time of extraction but this method is suitable only for specific phenolic compounds while some compounds like tannins and anthocyanins may be degraded because of high temperature involved.

Ultrasound-assisted extraction is another advanced extraction procedure. This method involves application of sound energy to disrupt plant cell wall and increase the drug surface area for solvent penetration with realising of secondary metabolites. The advantages of this process are: it is applicable to small sample; it reduces the time of extraction and amount of solvent used, and maximizes the yield but this method is difficult to be reproduced; also, high amount of energy applied may degrade the phytochemical by producing free radical [Abubakar and Haque, 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].

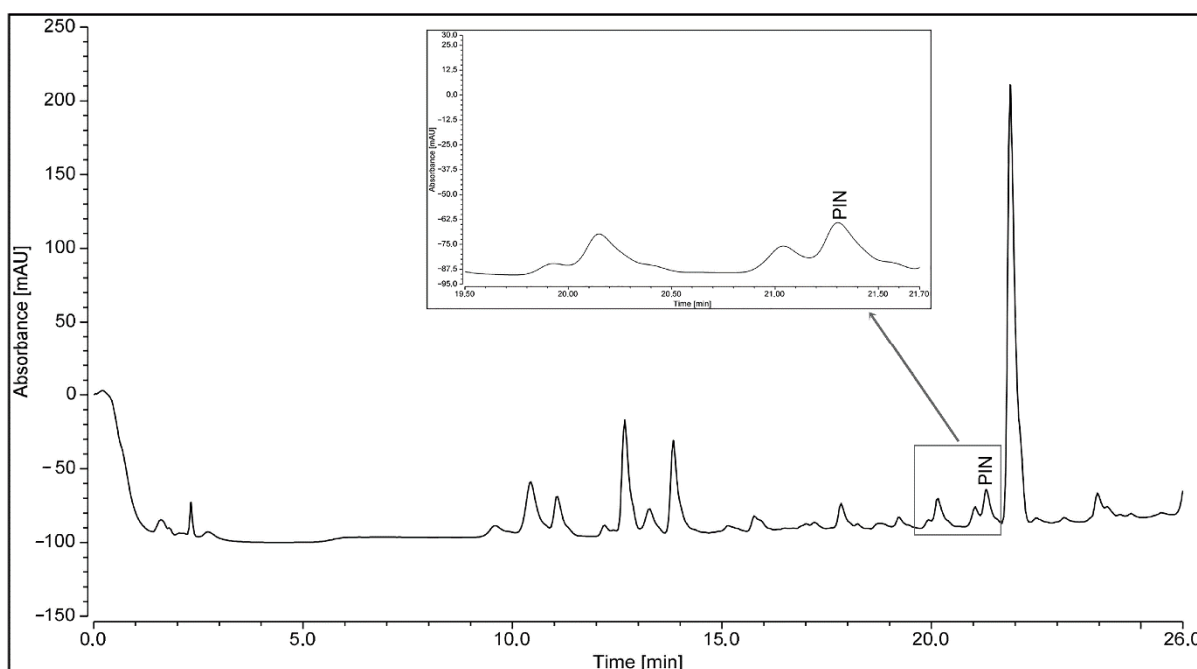
In order to deal with the problems related to the herbal extract chemical complexity, the researchers adopted the herbal fingerprinting approach. Since the entire pattern of compounds (phytocomplex) characterizes the chemical composition of the herbal drug, the chromatographic fingerprint represents a comprehensive qualitative methodology, in which the entire chromatograms were evaluated during data analysis to discriminate among different genotypes.

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]. There are many reports about fingerprint techniques to address the identity and quality of botanicals, which are mainly chromatographic analysis, including high performance liquid chromatography (HPLC), gas chromatography (GC), ultra performance liquid chromatography (UPLC), and capillary electrophoresis (CE). Spectroscopy methods are also applied to gain fingerprints [Donno et al., 2016].

- 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]. Identification of pinosylvin in *Pinus nigra* subsp. *laricio*, a naturally occurring stilbenoid suppressing LPS-induced expression of pro-inflammatory cytokines and mediators and inhibiting the JAK/STAT signaling pathway are shown below [Perri et al., 2023].

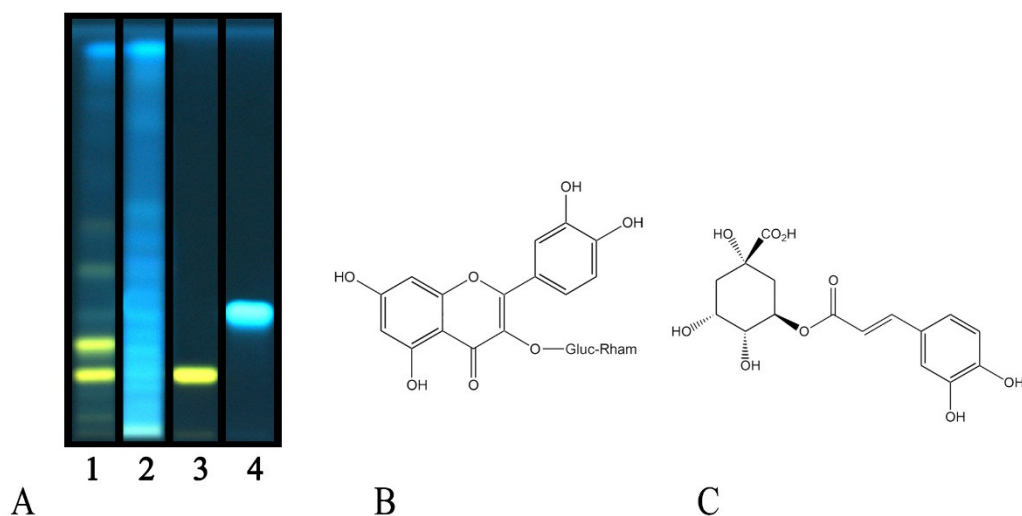


**Figure 14.3. HPLC chromatogram of *Pinus nigra* subsp. *laricio* knotwood extract: pinosylvin (PIN) identification.**

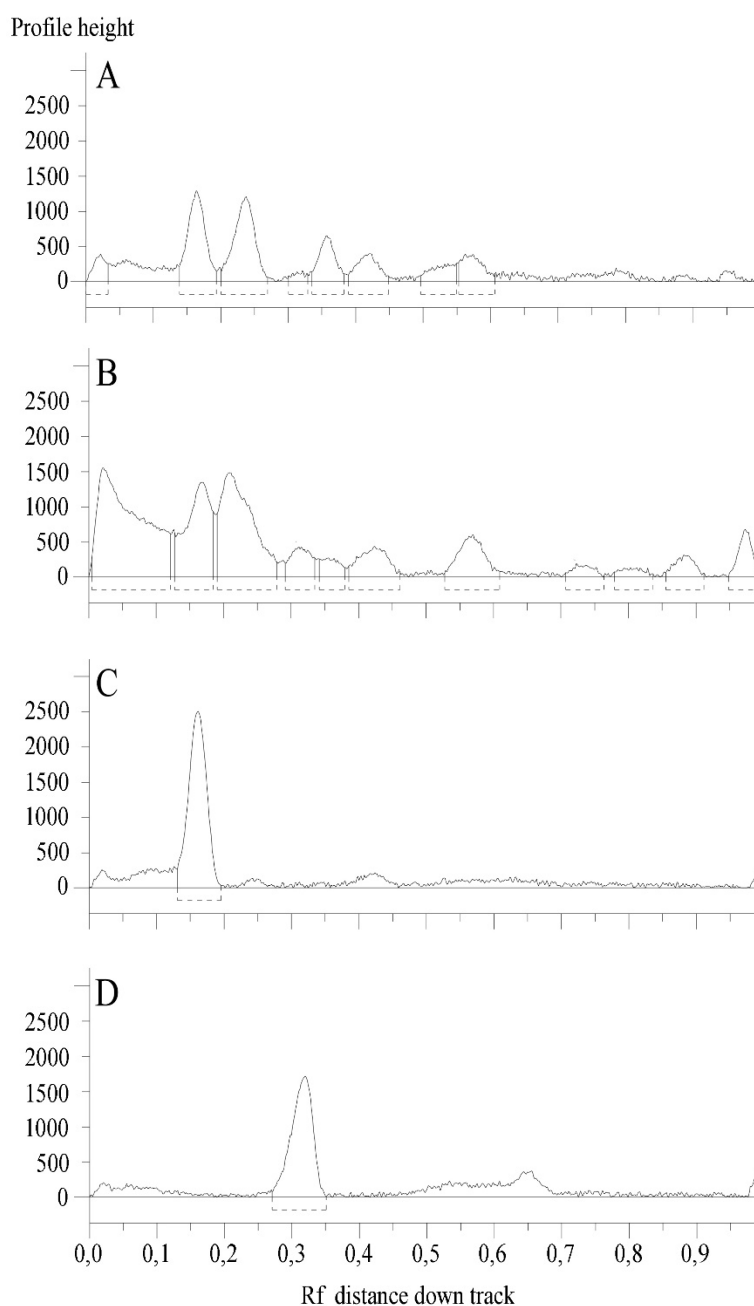


- 4) High-Performance Thin-Layer Chromatography (HPTLC - High-Performance Thin-Layer Chromatography): is a variant of thin-layer chromatography (TLC) that uses highly uniform thin layers of stationary material (silica) on a glass or aluminum plate as the stationary phase and a liquid mobile phase for 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].

Below is an example of HPTLC analysis [Amodeo et al., 2019].



**Figure 14.4. (A) HPTLC analysis of the ethyl acetate fractions of *Chenopodium album* L. (Amaranthaceae) and *Sisymbrium officinale* (L.) Scop. (Brassicaceae). Tracks: 1, *C. album* L.; 2, *S. officinale* (L.) Scop.; 3, rutin; 4, chlorogenic acid. (B) rutin. (C) chlorogenic acid.**



**Figure 14.5. HPTLC chromatograms of analyzed samples and standards. A, *C. album* L.; B, *S. officinale* (L.) Scop.; C, rutin (Rf = 0.16).; D, chlorogenic acid (Rf = 0.32).**

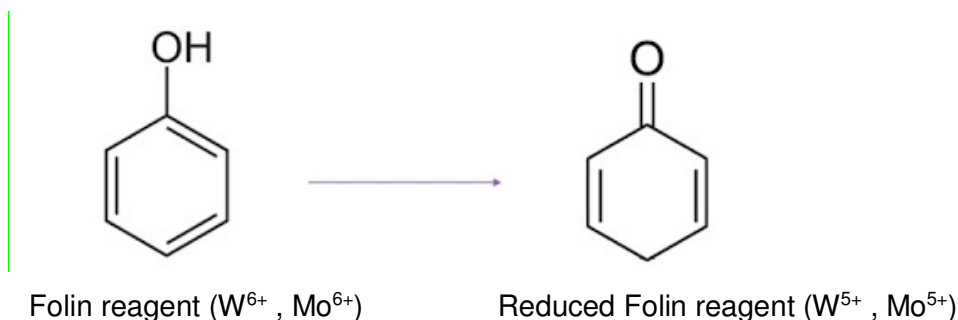
The total polyphenol content could be determined by the Folin-Ciocalteu method, using chlorogenic acid as a standard. The Folin-Ciocalteu reagent is composed of a mixture of two acids: phosphomolybdic ( $H_3PMo_{12}O_{40}$ ) and



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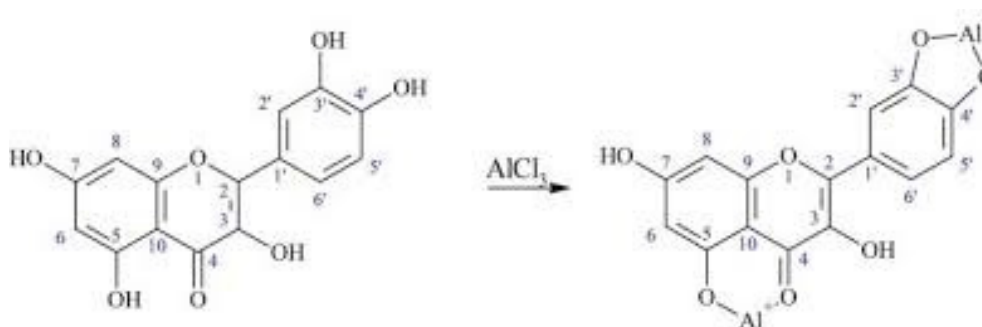


phosphotungstic ( $\text{H}_3\text{PW}_{12}\text{O}_{40}$ ). In basic conditions, the chemical reaction leads to the reduction of this reagent and, macroscopically, an intense blue color can be observed which indicates the quantity of polyphenols present in the sample.



**Figure 14.6. Principle of Folin-Ciocalteu method.**

The total flavonoid content could be evaluated with a colorimetric assay based on the formation of a flavonoid-aluminum complex with maximum absorption at 430 nm.



**Figure 14.7. Reaction of flavonoid-Aluminium Chloride ( $\text{AlCl}_3$ ).**

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 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.

In verification of quality, purity and integrity of the botanicals, special techniques and strategies are applied due to the complex nature of the components of the herbals (Figure 14.3). Thermal stability of the samples, determination of the mass and enthalpy variation, high sensitivity, reproducibility and rapid response to the variation of results are the qualities of the thermal techniques like thermogravimetric analysis and differential thermal analysis. In thermal analysis, time and temperature functions are used as defining parameters.

The temperature ranges from 25 to 1000 °C are used in the thermal analytical procedures. Mass Signals are obtained during the heating processes of the analysis which reveal about the mass loss (mass lost in the thermal degradation process) in different defining steps (endothermic and exothermic). To establish the pharmacopeia standards of the medicinal herb formulations HPTLC fingerprinting profile was used, phytochemical components of the formulations can be disclosed and efficacy, safety, and quality can be assured [Balekundri and Mannur, 2020].

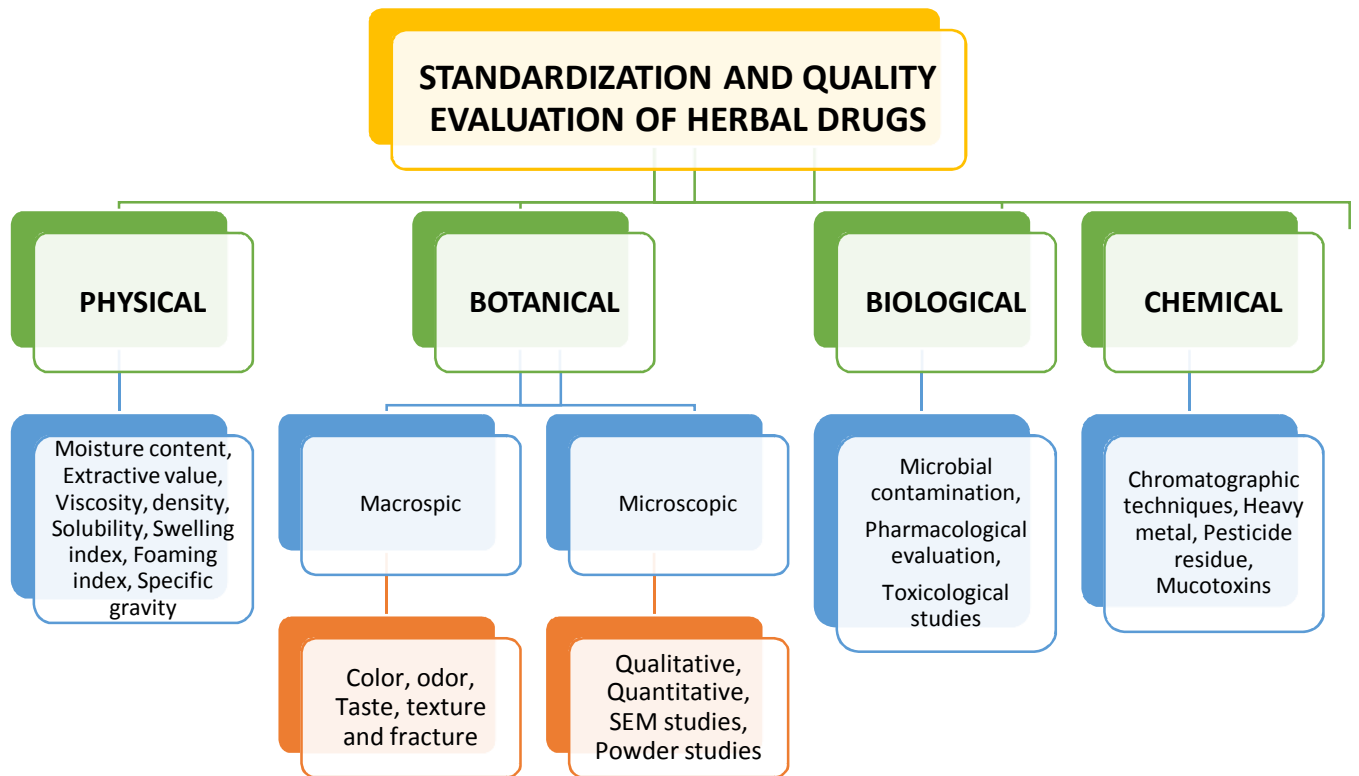


Figure 14.8. Methods of herbal standardization.

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

### **15.1. Introduction**

Supplements are products that are consumed in addition to the regular diet to provide additional nutrients that are beneficial to the body's health and well-being. In this regard, food supplements are intended to supplement the diet, containing ingredients such as vitamins, minerals, amino acids, herbs or botanical substances and can be administered in the form of pills, capsules or different liquid pharmaceutical forms [Wierzejska, 2021].

Traditional medicine is based on plants that are a primary and important source of many drugs in use today, the most common examples being aspirin and morphine. In addition, numerous plant-based products are used in the manufacture of dietary supplements. The popularity of herbal products has increased recently, a trend that can be explained by the long history of their use. According to surveys, approximately 20% of adults regularly consume herbal supplements [Bailey et al., 2011; Wierzejska, 2021].

It is important to note that one of the most significant problems associated with this high intake of dietary supplements is that most people do not report to their doctors, and many supplements can interfere with their medical treatment. These supplements have gained popularity due to their perceived natural and holistic qualities, offering a wide range of health benefits. However, it is essential to understand that just because something is natural does not necessarily mean it is safe, and the effectiveness of these substances can be closely related to their potential for toxicity [Ronis et al., 2018; Bailey et al., 2011].

In addition, the chemical complexity of plants may contribute to their beneficial effects, but also to their potential risk to induce adverse reactions. The active substances in plants can vary significantly depending on the plant species, the part used (leaves, root, flowers), the processing method and the growing conditions. This



makes standardization and quality control of herbal supplements essential to ensure their safety and effectiveness. It is also important to consider possible interactions between supplements and prescribed medications, as they may negatively influence medical treatments or cause unanticipated side effects [Ronis et al., 2018; Bailey et al., 2011].

On the other hand, the perceived benefits of herbal supplements, such as boosting the immune system, improving digestion, reducing inflammation, and fighting stress, contribute to their continued growth in popularity. It is essential that we promote consumer education about the responsible use of dietary supplements and encourage consultation with health professionals before starting any new supplement regimen. Only in this way can the benefits of these products be maximized while minimizing the potential risks associated with their inappropriate use [Wierzejska, 2021; Bailey et al., 2011; Ronis et al., 2018].

## 15.2. Plant-derived substances used in dietary supplements

Plant-derived substances used in dietary supplements include a wide range of compounds obtained from different parts of plants, such as leaves, flowers, roots and seeds. These substances are often valued for their beneficial health properties. Some of the most common plant-derived substances used in dietary supplements are shown in Table 15.1.

**Table 15.1. Plant-derived substances used in food supplements and their beneficial properties.**

CATEGORY	EXAMPLES	PROPERTIES	SOURCES
<b>POLYPHENOLS</b>	Flavonoids, Resveratrol, Ellagic Acid	Antioxidant, anti-inflammatory	Fruits, vegetables, green tea, red wine
<b>ALKALOIDS</b>	Caffeine, Theobromine, Morphine	Strong pharmacological activity	Coffee, tea, cocoa, opiate poppy
<b>TERPENOIDS</b>	Carotenoids (beta-carotene), Phytosterols	Eye health, cholesterol reduction	Mint, basil, thyme





<b>GLYCOSIDES</b>	Cardiac glycosides (digitalis), Saponins (yucca, quillaja)	Various medicinal properties	Digitalis, yucca, quillaja
<b>TANNINS</b>	Tannins	Antioxidants, digestive health	Tea, wine
<b>PHYTONUTRIENTS</b>	Lycopene, Sulforaphane, Anthocyanins	Beneficial effects on health	Tomatoes, broccoli, berries
<b>ESSENTIAL FATTY ACIDS</b>	Omega-3, Omega-6	Crucial for the proper functioning of the body	Vegetable oils, flaxseeds, nuts, fatty fish
<b>POLYSACCHARIDES</b>	Beta-glucans	Stimulation of the immune system	Mushrooms, oats
<b>LIGNANS</b>	Lignans	Antioxidant, phytoestrogenic	Flax seeds, sesame, whole grains
<b>ESSENTIAL OILS</b>	Lavender, Eucalyptus, Tea Tree	Aromatherapy, various health benefits	Lavender, Eucalyptus, Tea Tree

### 15.3. Effectiveness of plant-derived substances

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 herbal dietary 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 body functions, including energy production, immune support, and overall health [Drewnowski et al., 2014; Nicklas et al., 2014; Fekete et al., 2023]. For example, vitamin C from acerola extracts or vitamin E from wheat germ oil are recognized for their role in cell protection and support of immune functions. Vitamin C is essential for collagen synthesis and immune system function, while vitamin E acts as a powerful antioxidant that protects cells from oxidative stress [Lehoczki et al., 2023; Fekete et al., 2023].



Minerals such as magnesium in spirulina supplements are essential for the proper functioning of the nervous and muscular systems. Magnesium contributes to over 300 biochemical reactions in the body, including the regulation of muscle tone and nerve impulses [Ward, 2014].

Multivitamin and mineral supplements have also been associated with various health benefits, including reduced cancer risk and improved general health. Studies have shown that certain combinations of vitamins and minerals can reduce cancer incidence and overall mortality, particularly in nutritionally deficient populations [Nicklas et al., 2014; Ward, 2014].

However, it is important to note that the effects of supplements may vary depending on the specific dose and combination of nutrients, as well as the health status of the individual. Therefore, it is recommended to consult a specialist before starting any supplementation regimen [Lehoczki et al., 2023; Drewnowski et al., 2014; Nicklas et al., 2014; Fekete et al., 2023].

### *Traditional Medicine*

Many plant extracts have a long history of use in traditional medicine systems around the world. For example, herbs such as ginseng, echinacea, and turmeric have been used for centuries for their potential health benefits, including immune support and anti-inflammatory properties [Boy, 2018; Wang et al., 2018; Singh et al., 2017; Nicklas et al., 2014; Fekete et al., 2023].

In traditional Chinese medicine, ginseng is used to improve energy and vitality. It is recognized for its ability to increase resistance to stress and to improve physical and mental performance [Wang et al., 2018]. In addition, ginseng is considered an adaptogen, helping the body to adapt to various types of stress and to enhance the overall functioning of the body [Kiefer & Pantuso, 2003; Fekete et al., 2023].

Echinacea is popular in traditional North American medicine for the prevention and treatment of colds. Studies have shown that echinacea extracts can boost the immune system and reduce the duration of cold symptoms. One study found that taking echinacea can reduce the severity and duration of respiratory symptoms [Barrett, 2003; Hudson, 2011; Fekete et al., 2023].



Turmeric, known for its active curcuminoids, is used in Ayurvedic medicine to reduce inflammation and to improve joint health. Curcumin, the main active compound in turmeric, has strong anti-inflammatory and antioxidant properties [Singh et al., 2017; Aggarwal et al., 2007; Nicklas et al., 2014]. Research has shown that curcumin can inhibit inflammatory molecules in the body, providing protection against chronic inflammatory conditions [Gupta et al., 2013; Fekete et al., 2023].

Turmeric, in addition to its uses in Ayurvedic medicine, is also used in other cultures to treat digestive ailments and for its antioxidant properties [Singh et al., 2017; Aggarwal et al., 2007]. Curcumin in turmeric has been extensively studied for its potential in the prevention and treatment of chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease [Gupta et al., 2013; Fekete et al., 2023].

Ginseng is used in various traditional preparations to treat fatigue, improve cognitive function and boost the immune system. Ginseng extracts have been studied for their potential effects in improving cognitive function and physical endurance [Wang et al., 2018; Reay et al., 2005; Fekete et al., 2023]. In traditional North American medicine, echinacea was used not only for colds, but also for various infections and inflammations, having an important place in traditional herbal medicine [Barrett, 2003; Fekete et al., 2023].

### *Synergy and Bioavailability*

Some plant compounds are thought to work synergistically 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, 2018; Singh et al., 2017; Bertuccioli et al., 2019; Nicklas et al., 2014; Fekete et al., 2023].

For example, resveratrol in grapes may be more effective when consumed with other antioxidants present in the fruit. Studies have shown that antioxidants in grapes, such as quercetin and catechins, can work synergistically to improve the bioavailability of resveratrol and potentiate its beneficial effects on cardiovascular health [Nair, 2018; Baur and Sinclair, 2006; Nicklas et al., 2014; Fekete et al., 2023].



Curcumin in turmeric has increased the bioavailability when combined with piperine in black pepper. Piperine, an active compound in black pepper, can increase the absorption of curcumin by up to 2000%, facilitating its passage through the intestinal barrier and reducing hepatic metabolism [Shoba et al., 1998; Gupta et al., 2013; Nicklas et al., 2014; Fekete et al., 2023]. This combination is often used in supplements to maximize the anti-inflammatory and antioxidant benefits of turmeric [Bertuccioli et al., 2019; Fekete et al., 2023].

Thus, synergy and bioavailability play a critical role in the effectiveness of herbal supplements, ensuring that nutrients are absorbed and used efficiently by the body. This concept is crucial in developing supplements that maximize health benefits by using natural compounds in their optimal form.

#### *Adaptogenic properties*

Certain plant extracts, such as adaptogens (eg, ashwagandha and rhodiola), have been associated with reduced stress, improved endurance, and improved mental clarity, offering a potential natural solution to the demands of modern life [Todorova, 2021; Panossian and Wikman, 2010; Chandrasekhar et al., 2012; Nicklas et al., 2014; Fekete et al., 2023].

Adaptogens help the body adapt to stress and maintain homeostasis. These plant compounds work by modulating the stress response, balancing biological processes and improving the body's ability to cope with physical, chemical and biological stress. This means that adaptogens can help reduce the negative effects of prolonged stress and prevent conditions associated with chronic stress, such as fatigue, anxiety and sleep disorders [Panossian and Wikman, 2010; Todorova, 2021; Nicklas et al., 2014; Fekete et al., 2023].

Ashwagandha (*Withania somnifera*) is known for its ability to reduce levels of the stress hormone cortisol and to improve sleep quality. Studies have shown that ashwagandha can significantly reduce stress and anxiety levels, contributing to more restful sleep and overall well-being. A randomized, double-blind, placebo-controlled trial showed that a high-concentration of ashwagandha extract significantly reduced stress and anxiety scores compared to placebo [Chandrasekhar et al., 2012; Fekete



et al., 2023]. In addition, ashwagandha has been associated with improved physical endurance and cognitive performance, making it useful in managing the demands of modern life [Auddy et al., 2008; Fekete et al., 2023].

Rhodiola (*Rhodiola rosea*) is valued for its ability to improve mental and physical performance under stress. Studies suggest that rhodiola can increase work capacity, reduces mental fatigue, and improves alertness and mental clarity. One study demonstrated that rhodiola can significantly reduce mental fatigue and improve performance in cognitive tasks, contributing to better stress management [Olsson et al., 2009; Fekete et al., 2023]. Rhodiola works by influencing neurotransmitter systems and the hormonal response to stress, helping to maintain homeostasis and the body's adaptation to stressful conditions [Panossian and Wikman, 2010; Fekete et al., 2023].

In addition to ashwagandha and rhodiola, there are other adaptogens that provide similar benefits. For example, eleuthero (*Eleutherococcus senticosus*), also known as Siberian ginseng, is used to improve energy and stamina and to reduce fatigue [Panossian et al., 2008; Fekete et al., 2023]. Schisandra (*Schisandra chinensis*) is another adaptogen renowned for its properties to improve physical and mental performance and to protect against oxidative stress [Winston and Maimes, 2007; Fekete et al., 2023].

Adaptogens are an important category of natural supplements that can help manage stress and improve overall health, providing a holistic solution to the demands of modern life.

#### *Additional benefits*

In addition to adaptogens, herbal supplements can provide specific benefits for different body systems. For example, ginkgo biloba leaf extract is known to improve blood circulation and support cognitive function. Studies have shown that ginkgo biloba can improve cerebral blood flow and have beneficial effects on memory and cognitive function, being useful in treating conditions such as dementia and Alzheimer's disease [Weinmann et al., 2010; Gschwind et al., 2017; Nicklas et al., 2014; Fekete et al., 2023].



Milk thistle seeds (*Silybum marianum*) are used to support liver function and detoxification. Silymarin, the main active compound in milk thistle, is known for its hepatoprotective and antioxidant properties. Silymarin helps protect the liver against toxins and regenerate liver cells, being used in the treatment of liver diseases such as hepatitis and cirrhosis [Kren and Walterová, 2005; Loguercio and Festi, 2011; Nicklas et al., 2014; Fekete et al., 2023].

Also, herbs such as valerian (*Valeriana officinalis*) and passiflora (*Passiflora incarnata*) are used to promote relaxation and to relieve symptoms of anxiety and insomnia. Valerian is known for its sedative and anxiolytic effects, having been used for centuries to treat insomnia and anxiety. Studies have shown that valerian extract can improve sleep quality and to reduce the time it takes to fall asleep [Bent et al., 2006; Fernández-San-Martín et al., 2010; Nicklas et al., 2014; Fekete et al., 2023]. Passiflora is also valued for its sedative properties and is traditionally used to treat anxiety and sleep disorders [Akhondzadeh et al., 2001; Miyasaka et al., 2007; Nicklas et al., 2014; Fekete et al., 2023].

#### **15.4. Considerations regarding the dietary supplements customization**

It is important to note that the effectiveness of herbal dietary supplements may vary depending on the biological individuality of each person. Factors such as age, health, diet and lifestyle can influence how the body responds to these supplements. For example, age may affect the absorption and metabolism of supplements, while certain health conditions may require specific adjustments in the dosage and type of supplements used [Todorova, 2021; Liu et al., 2018; Hu et al., 2019; Nicklas et al., 2014; Fekete et al., 2023].

Consultation with a healthcare professional is essential to tailor supplements to specific needs and optimize their benefits. Health professionals can perform detailed assessments to determine individual nutritional needs and to recommend appropriate supplements to meet those needs. They can also monitor the response to supplements and adjust doses or types of supplements based on individual reactions and results [Todorova, 2021; Liu et al., 2018; Nicklas et al., 2014; Fekete et al., 2023].





For example, older people may have different nutritional needs than younger adults and may require supplements to support their bone health, cognitive function, and immune system. On the other hand, athletes may need supplements to support their physical performance and muscle recovery. In both cases, consultation with a specialist can ensure that supplements are properly tailored to maximize benefits [Hu et al., 2019; Kreider et al., 2010; Nicklas et al., 2014; Fekete et al., 2023].

Diet and lifestyle also play a crucial role in how supplements are absorbed and used by the body. For example, a high-fiber diet can affect the absorption of certain minerals, while alcohol consumption and smoking can influence the metabolism and effectiveness of supplements [Liu et al., 2018; Nicklas et al., 2014].

### **15.5. Aspects related to the toxicity of plant-derived substances**

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*

The natural substances in plant extracts can be potent, and when consumed in high concentrations, can lead to toxicity. Overdosing on some herbal supplements can lead to adverse effects, including digestive problems, headaches, or more severe health problems [Brima, 2017; Bent et al., 2006; Stickel and Shouval, 2015; Nicklas et al., 2014; Fekete et al., 2023]. For example, excessive consumption of vitamin A from plant sources such as fish liver oil can cause hypervitaminosis A, which can lead to serious health problems such as liver and vision damage [Hathcock, 1997; Fekete et al., 2023].

Also, green tea extracts, if consumed in very high doses, can cause hepatotoxicity, leading to liver damage and other serious complications [Mazzanti et al., 2015; Nicklas et al., 2014; Fekete et al., 2023]. Kava root, used for its anxiolytic properties, can cause hepatotoxicity if consumed in large amounts or for long term [Stickel and Shouval, 2015; Nicklas et al., 2014].



It is important to follow the recommended dosages for each herbal supplement, as even substances considered safe can become dangerous in large amounts. For example, garlic, known for its cardiovascular health benefits, can cause gastric irritation and blood clotting problems if consumed in excessive amounts [Ried et al., 2016; Nicklas et al., 2014].

Some herbal supplements may contain active compounds that can interact with medications or other supplements, amplifying or diminishing their effects. Therefore, it is essential to consult a healthcare professional before starting a new supplement, especially if other drug treatments are also being used [Izzo and Ernst, 2009; Nicklas et al., 2014; Fekete et al., 2023].

In addition to dosage, the method of administration can significantly influence the absorption and effects of herbal substances. For example, supplements administered in the form of capsules or tablets may have different absorption rates compared to liquids or powders, thus affecting the plasma concentrations of the active compounds and, implicitly, their effects on the body [Gaby, 2006; Nicklas et al., 2014].

It is also important to take into account the natural variability of concentrations of active compounds in plants, which may vary depending on environmental factors, harvesting method and processing. Standardizing herbal supplements can help ensure consistent and safe dosing [Gafner, 2018; Nicklas et al., 2014].

*Calculation examples for plant-derived substances:*

a) Vitamin A:

- o Recommended Daily Dose (RDD) for adults: 700-900 micrograms ( $\mu\text{g}$ ) retinol equivalents (RE).
- o Upper safety limit: 3000  $\mu\text{g}$  RE per day.
- o Examples: 1 gram of cod liver oil can contain about 1000  $\mu\text{g}$  RE. Consuming 3 grams would reach the upper safe limit (3 grams  $\times$  1000  $\mu\text{g}/\text{gram}$  = 3000  $\mu\text{g}$  RE), risking toxicity.

b) Green tea extract:

- o Catechins are the main active compounds.



- o RDD: there is no specific value, but green tea consumption is considered safe at 3-5 cups per day.
  - o Upper safe limit for supplements: about 800 mg of catechins per day.
  - o Examples: a supplement containing 400 mg of catechins per capsule. Consuming two capsules per day (2 capsules x 400 mg/capsule = 800 mg) reaches the upper safety limit, and consuming three capsules would exceed it, risking hepatotoxicity.
- c) Kava root:
- o Main active compounds: kavalactone.
  - o Recommended dose: 60-120 mg of kavalactone per day.
  - o Examples: a supplement containing 60 mg of kavalactone per capsule. Consuming two capsules per day (2 capsules x 60 mg/capsule = 120 mg) reaches the recommended dose. Consuming four capsules would double the recommended dose, risking hepatotoxicity.
- d) Garlic (*Allium sativum*):
- o Main active compounds: allicin.
  - o Recommended dose for cardiovascular benefits: about 600-1200 mg of powdered garlic per day.
  - o Examples: a supplement containing 300 mg of garlic per tablet. Consuming four tablets per day (4 tablets x 300 mg/tablet = 1200 mg) reaches the recommended dose. Consuming eight tablets would double the recommended dose, risking gastric irritation and clotting problems.

In conclusion, dosage and concentration are critical factors in using herbal supplements. Adherence to dosage directions and consultation with health professionals are essential to maximize the benefits of these supplements and minimize the risks of toxicity.

### *Allergenic Reactions*

Allergic reactions to plant compounds are a common problem, even when these compounds are generally considered safe. These reactions can vary significantly from



mild skin irritation to severe, life-threatening anaphylactic responses [Shahali, 2018; Asero, 2000; Sicherer, 2011; Pumphrey, 2004; Haahtela et al., 2013].

Exposure to plant allergens can cause various allergic symptoms, including contact dermatitis, allergic rhinitis, and gastrointestinal symptoms [Haahtela et al., 2013]. In some cases, inhalation of pollen or other plant particles can trigger severe asthmatic symptoms (Pumphrey, 2004).

**Table 15.2. Table with examples of allergic reactions to plant compounds**

PLANT COMPOUNDS	COMMON SYMPTOMS	SEVERITY
AMBROSE POLLEN	Sneezing, runny nose, irritated eyes	Moderate
POISONOUS IVY	Rash, itching, swelling	Moderate to severe
LATEX (OF RUBBER TREES)	Rash, itching, anaphylaxis	Severe
PEANUT PROTEIN	Urticaria, stomach cramps, anaphylaxis	Severe
SOY PROTEIN	Rash, itching, stomach cramps	Moderate to severe
WHEAT GLUTEN	Urticaria, stomach cramps, anaphylaxis	Severe
PROTEINS FROM NUTS (EX. ALMONDS, WALNUTS)	Rash, itching, anaphylaxis	Severe
CRUSH PROTEIN	Urticaria, stomach cramps, anaphylaxis	Severe
GRASS POLLEN	Sneezing, runny nose, irritated eyes	Moderate
MOLD SPORE	Sneezing, coughing, wheezing	Moderate to severe
CAT HAIR (CONTAINS VEGETABLE PROTEINS)	Sneezing, runny nose, irritated eyes	Moderate
DOG HAIR (CONTAINS VEGETABLE PROTEINS)	Sneezing, runny nose, irritated eyes	Moderate
PROTEIN FROM SUNFLOWER SEEDS	Urticaria, itching, anaphylaxis	Severe
SESAME SEED PROTEIN	Urticaria, itching, anaphylaxis	Severe
MUSTARD SEED PROTEIN	Urticaria, itching, anaphylaxis	Severe



PROTEIN FROM CELERY ROOT	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM GARLIC	Rash, itching, stomach cramps	Moderate to severe
ONION PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PAPAYA ENZYME (PAPAIN)	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM KIWI FRUIT	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM TOMATOES	Rash, itching, stomach cramps	Moderate to severe
CARROT PROTEIN	Rash, itching, stomach cramps	Moderate to severe
BANANA PROTEIN	Rash, itching, stomach cramps	Moderate to severe
STRAWBERRY PROTEIN	Rash, itching, stomach cramps	Moderate to severe
APPLE PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM PEACHES	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM CHERRIES	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM GRAPES	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM ORANGES	Rash, itching, stomach cramps	Moderate to severe
LEMON PROTEIN	Rash, itching, stomach cramps	Moderate to severe
LIME PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM AVOCADO	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM PINEAPPLE	Rash, itching, stomach cramps	Moderate to severe



MANGO PROTEIN	Rash, itching, stomach cramps	Moderate to severe
COCONUT PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM PEPPER	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM SPINACH	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM BROCCOLI	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM CAULIFLOWER	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM BRUSSELS SPROUTS	Rash, itching, stomach cramps	Moderate to severe
CABBAGE PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM SALAD	Rash, itching, stomach cramps	Moderate to severe
RADISH PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM CUCUMBERS	Rash, itching, stomach cramps	Moderate to severe
PUMPKIN PROTEIN	Rash, itching, stomach cramps	Moderate to severe
ZUCCHINI PROTEIN	Rash, itching, stomach cramps	Moderate to severe
SWEET POTATO PROTEIN	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM TUPPIES	Rash, itching, stomach cramps	Moderate to severe
PROTEIN FROM PARSLEY	Rash, itching, stomach cramps	Moderate to severe
BEET PROTEIN	Rash, itching, stomach cramps	Moderate to severe

*Interactions*



Herbal supplements can interact with medications or other supplements, leading to unexpected side effects or decreased effectiveness. It is essential to consult a health care professional before adding new supplements to a person's regimen, especially if prescription medications are also being used [Sprouse, 2016; Williamson, 2003; Izzo and Ernst 2009; Gurley et al., 2012; Bent, 2008; Shi and Klotz, 2012].

For example, St. John's wort (*Hypericum perforatum*) can reduce the effectiveness of oral contraceptives and anticoagulants, increasing the risk of unplanned pregnancy and bleeding [Izzo and Ernst, 2009]. Ginkgo biloba may interact with anticoagulants and antiplatelet agents, increasing the risk of bleeding [Williamson, 2003]. Echinacea can also influence the metabolism of certain drugs, affecting their blood levels and their potential effects [Gurley et al., 2012].

**Table 15.3. Examples of interactions between herbal supplements and drugs**

PLANT BASED SUPPLEMENT	COMMON INTERACTIONS	POTENTIAL EFFECTS
<b>HYPERICUM PERFORATUM</b>	It reduces the effectiveness of oral contraceptives and anticoagulants	Unplanned pregnancy, increased bleeding
<b>GINKGO BILOBA</b>	Increases the risk of bleeding of anticoagulants and antiplatelets	Increased bleeding
<b>ECHINACEA</b>	It affects the metabolism of certain drugs	Variable blood drug levels
<b>GINSENG</b>	May increase the stimulant effects of caffeine	Agitation, insomnia
<b>KAVA</b>	May interact with anxiety and antidepressant medications	Drowsiness, increased adverse effects
<b>VALERIAN</b>	It can intensify the effects of sedatives	Excessive sedation
<b>SAW PALMETTO</b>	May interact with prostate medications	Low effectiveness of prostate treatment
<b>YARROW</b>	May interact with anticoagulants	Increased bleeding
<b>LICORICE (GLYCYRRHIZA GLABRA)</b>	May cause sodium retention and potassium loss when combined with diuretics	Hypertension, electrolyte imbalances
<b>GINGER (ZINGIBER OFFICINALE)</b>	May increase the risk of bleeding with anticoagulants	Increased bleeding



<b>TURMERIC (CURCUMA LONGA)</b>	May interact with diabetes medications	Hypoglycemia
<b>MILK THISTLE (SILYBUM MARIANUM)</b>	May influence liver drug metabolism	Variable levels of liver medications
<b>GARLIC (ALLIUM SATIVUM)</b>	Increases the risk of bleeding of anticoagulants	Increased bleeding
<b>ALOE VERA</b>	It may reduce the effectiveness of diabetes medications	Hypoglycemia
<b>FENUGREEK</b>	May affect iron absorption	Low absorption of iron
<b>EVENING PRIMROSE OIL</b>	May interact with anticoagulants	Increased bleeding
<b>BLACK COHOSH</b>	May interact with hormonal medications	Variable hormonal effects
<b>GOLDENSEAL</b>	It can influence the metabolism of drugs	Variable medication levels
<b>PEPPERMINT</b>	May interact with antacids	Reducing the effectiveness of antacids
<b>CHAMOMILE</b>	It can intensify the effects of sedatives	Excessive sedation
<b>ST. JOHN'S WORT</b>	It may reduce the effectiveness of oral contraceptives and anticoagulants	Unplanned pregnancy, increased bleeding
<b>HAWTHORN</b>	May interact with heart medications	Variable cardiac effects
<b>FEVERFEW</b>	May interact with anticoagulants and antiplatelets	Increased bleeding
<b>GREEN TEA EXTRACT</b>	May interact with blood pressure medications	Variable blood pressure
<b>BILBERRY</b>	May interact with anticoagulants and antiplatelets	Increased bleeding
<b>CRANBERRY</b>	May interact with urinary tract infections medication	Treatment ineffectiveness for urinary tract infections
<b>DANDELION</b>	May influence the effects of diuretics	Increased diuresis
<b>ELDERBERRY</b>	May interact with antiviral medications	Low effectiveness of antivirals
<b>NETTLE</b>	May interact with allergy medications	Low efficacy of allergy treatment
<b>RHODIOLA</b>	May interact with depression and anxiety medications	Variable effectiveness of treatment



### *Purity and Contamination*

The source and quality of herbal supplements are critical. Poor quality or contaminated products can introduce toxins or harmful substances into the body, leading to adverse effects [Ratajczak, 2020; Ghosh et al., 2013; Posadzki et al., 2013; Navarro et al., 2017; Cohen, 2019].

It is essential that supplements are sourced from trusted suppliers and tested for purity and contamination. Common contaminants include heavy metals, pesticides, and pathogenic microorganisms, which can compromise the health of users [Navarro et al., 2017]. For example, heavy metals such as lead, mercury, and cadmium may be present in herbal supplements and lead to serious poisoning if consumed long-term [Ghosh et al., 2013].

Some herbal supplements may be contaminated with synthetic drugs that are not declared on the product label. This can lead to dangerous interactions with other medications a person is taking or unexpected side effects [Posadzki et al., 2013]. Pesticide contamination can also cause severe allergic reactions and other long-term health problems [Cohen, 2019].

Moreover, pathogenic microorganisms such as bacteria, fungi, and viruses can contaminate herbal supplements, leading to infections and other health problems [Navarro et al., 2017]. For example, *E. coli* and *Salmonella* are common bacterial contaminants that can cause severe food poisoning.

To avoid these risks, it is important that herbal supplements are produced and packaged under strict hygienic conditions and undergo rigorous quality control testing. Regulatory organizations in different countries have set standards for the purity and safety of dietary supplements, but compliance with these standards can vary. Therefore, consumers should look for products that have been certified by independent organizations to ensure they meet quality and safety standards.



Table 15.4. Examples of contaminants in herbal supplements

PLANT BASED SUPPLEMENT	POTENTIAL CONTAMINANTS	ADVERSE EFFECTS
AYURVEDIC SUPPLEMENTS	Heavy metals (lead, mercury, arsenic)	Lead, mercury or arsenic poisoning; abdominal pain, weakness, anemia
GINKGO BILOBA	PESTICIDES	Allergic reactions, toxicity
GINSENG	PESTICIDES	Allergic reactions, toxicity
ECHINACEA	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
ALOE VERA	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
TURMERIC (CURCUMA LONGA)	PESTICIDES	Allergic reactions, toxicity
GARLIC (ALLIUM SATIVUM)	PESTICIDES	Allergic reactions, toxicity
ST. JOHN'S WORT	Synthetic drugs	Dangerous drug interactions
SAW PALMETTO	Heavy metals	Heavy metal poisoning
PEPPERMINT	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
CHAMOMILE	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
VALERIAN	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
MILK THISTLE (SILYBUM MARIANUM)	PESTICIDES	Allergic reactions, toxicity
BLACK COHOSH	Heavy metals	Heavy metal poisoning
GOLDENSEAL	PESTICIDES	Allergic reactions, toxicity
GINGER (OFFICIAL ZINGIBER)	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
FENUGREEK	PESTICIDES	Allergic reactions, toxicity
EVENING PRIMROSE OIL	PESTICIDES	Allergic reactions, toxicity
LICORICE (GLYCYRRHIZA GLABRA)	Heavy metals	Heavy metal poisoning
NETTLE	Bacteria (E. coli, Salmonella)	Gastrointestinal infections
CRANBERRY	PESTICIDES	Allergic reactions, toxicity
DANDELION	PESTICIDES	Allergic reactions, toxicity
ELDERBERRY	PESTICIDES	Allergic reactions, toxicity
RHODIOLA	PESTICIDES	Allergic reactions, toxicity
ASHWAGANDHA	PESTICIDES	Allergic reactions, toxicity
MACA	Heavy metals	Heavy metal poisoning
GREEN TEA EXTRACT	PESTICIDES	Allergic reactions, toxicity
BILBERRY	PESTICIDES	Allergic reactions, toxicity
FEVERFEW	PESTICIDES	Allergic reactions, toxicity
HAWTHORN	PESTICIDES	Allergic reactions, toxicity

### *Lack of Regulation*

The dietary supplement industry is not as tightly regulated as pharmaceuticals, leading to variations in quality and safety between different products. It is important to choose reputable brands that follow good manufacturing practices [Dwyer, 2018; Cohen, 2020; Geller et al., 2015; Marcus and Grollman, 2016; Navarro et al., 2017].

In many countries, dietary supplements are regulated as foods and not as drugs, which means they do not have to go through the same rigorous testing for efficacy and safety before being marketed. For example, in the United States, the Food and Drug Administration (FDA) does not approve dietary supplements before they are sold on the market. Instead, the responsibility for ensuring product safety and quality rests with the manufacturers. This creates an environment where products on the market can vary greatly in terms of purity, potency and safety.

Manufacturers of dietary supplements should follow good manufacturing practices (GMP) to minimize the risk of contamination and ensure product consistency. GMP includes standards for factory hygiene, control of raw materials, testing of finished products, and handling of consumer complaints [Geller et al., 2015]. However, compliance with these practices is not always strictly monitored or enforced, which can result in products that do not meet the necessary standards of safety and efficacy.

**Table 15.5. Regulatory issues of dietary supplements**

REGULATORY ISSUES	DESCRIPTION
<b>CONTAMINATION</b>	Products may be contaminated with heavy metals, pesticides, pathogenic microorganisms or other undesirable substances. This can lead to serious adverse effects on the health of users [Navarro et al., 2017].
<b>ADULTERATION</b>	Supplements may contain undeclared ingredients or synthetic drugs that are not listed on the label. This can lead to dangerous drug interactions or unexpected side effects [Cohen, 2020].
<b>CONTENT VARIABILITY</b>	Products may vary in the concentration of active ingredients. This may mean that a product does not provide the intended benefits or, in extreme cases, may be toxic [Marcus and Grollman, 2016].
<b>MISLEADING ADVERTISING</b>	Supplements can be promoted with health claims unsupported by sound scientific evidence, misleading consumers [Dwyer, 2018].



To safely navigate the dietary supplement market, consumers should look for products that have been certified by independent testing organizations such as the US Pharmacopeia (USP), NSF International, or ConsumerLab. These organizations test supplements to verify purity, potency, and the absence of contaminants.

It is also essential that consumers consult a healthcare professional before starting any supplement regimen, especially if they are already taking prescription medications. Professionals can provide advice on the safety and effectiveness of supplements, as well as potential interactions with other medications.

In conclusion, substances in dietary supplements derived from plants and plant extracts may provide a number of health benefits, but their effectiveness and safety depend on various factors, including dosage, purity, individual tolerance, and potential interactions with other substances.

Plant-derived substances such as polyphenols, alkaloids, terpenoids, glycosides, tannins, phytonutrients, essential fatty acids, polysaccharides, lignans and essential oils have recognized beneficial properties such as antioxidant activity, anti-inflammatory activity, eye health support and cholesterol reduction. The effectiveness of these substances can be influenced by the synergy between the compounds and their bioavailability, as well as by the adaptogenic properties of some plant extracts (eg ashwagandha and rhodiola) that help the body adapt to stress.

Excessive consumption of natural substances can lead to toxicity. Supplements such as vitamin A, green tea extract, or kava root should be taken in recommended doses to avoid severe adverse effects. Allergies to plant compounds are common and can range from mild irritation to severe anaphylactic responses. Allergies can be caused by various plants and proteins in common foods. Herbal supplements may interact with medications or other supplements, leading to unexpected side effects or decreased effectiveness of treatment. It is essential to consult with a healthcare professional before adding new supplements to your treatment regimen.

The quality of herbal supplements is critical, and contaminants such as heavy metals, pesticides, and pathogenic microorganisms can lead to severe health problems. Supplements must be tested for purity and safety. The supplement industry is not as tightly regulated as pharmaceuticals, which can lead to wide variations in the





quality and safety of products available on the market. Consumers should choose products certified by independent organizations and consult health professionals before starting any supplement regimen.

It is essential that individuals approach these supplements with caution, do their research, consult with health professionals, and pay close attention to product quality and sourcing to ensure they receive potential benefits while avoiding toxicity and side effects.

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