Chapter 10

Herbal Extracts – Possibility of Preventing Food-Borne Infection

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Additional information is available at the end of the chapter

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Abstract

Despite the high degree of awareness of food preservation methods, there is increasing occurrence of disease outbreaks caused by pathogenic and spoilage microorganisms in foods. Due to consumer awareness and negative perception of artificial preservatives in food, in recent years attention is shifting toward alternatives that the consumers recognize as natural. Thus, herbal extracts are now getting more space in food industry to prevent the propagation of bacteria that affect the spoilage of food or for the spread of so-called food-borne diseases. Herbal extracts, particularly essential oils (EOs), have complex composition that quality and composition depend on the method of extraction. There are now numerous reports of the in vitro antimicrobial activity of EOs in the scientific and medical literature: EOs are found to have broad-spectrum inhibitory activities against various food-borne Gram-positive and Gram-negative pathogenic bacteria. In this chapter, definition, history, and economic importance of aromatic herbs and herbal extracts, particularly EOs, are described. Also, attention has been paid to techniques for extraction, as well as chemical composition and antimicrobial activity of herbal extracts. This chapter demonstrates the possibility of usage of herbal extracts in preventing food-borne infection through literature survey and original results.

Keywords: Aromatic herbs, herbal extracts, food-borne disease, food preservation

1. Introduction

In recent years, there has been a dramatic increase of reported cases of food-borne illness because of consumption of foods contaminated with pathogenic bacteria. The presence of various microorganisms in food followed by inappropriate storage results not only in a reduction of food quality but also in spoilage of food.

Food contaminants, such as harmful parasites, bacteria, viruses, prions, and chemical or radioactive substances, cause more than 200 diseases – ranging from infectious diseases to cancers [1]. Consequently, there is considerable interest in ways to stop this upward trend and reduce the incidence of food poisoning. The development of new and improved methods of food infectious intestinal disease preservation is of utmost importance [2]. The microbiological safety in ready-to-eat products is a cause of big concern not only for the consumers and food industries but also for the regulatory agencies. It can be said that the food industry faces a constant problem in providing the food where there are no food-borne pathogens present [3, 4]. The number of documented outbreaks of food-borne diseases has increased in the last decade, with *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* being responsible for the largest number of outbreaks and deaths [5].

Due to consumer awareness and negative perception of artificial preservatives in food, in recent years attention is shifting toward alternatives that the consumers recognize as natural, for example, herbal extracts. It can be said that herbal extracts have been relatively neglected as soon as modern antibiotics were discovered; they became the primary means of treating bacterial infections. Antibiotics have selective toxicity and that many important bacteria were exquisitely susceptible to them. However, bacterial infections have gone up even after the discovery of many antibiotics, mainly due to the reduced susceptibility to conventional antimicrobial agents shown by many important pathogens. Because of that, studies of the antimicrobial activity of plant extracts are intensified [6] and became prominent in science, this area has suddenly become more important in the scientific literature, and this issue is currently most attractive. Therefore, at the beginning of the 21st century, herbal extracts are getting more space in food industry to prevent the propagation of bacteria that are responsible for the spoilage of food or the spread of so-called food-borne diseases [7].

Herbal extracts, particularly essential oils (EOs) have complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, ethers, and esters). The greatest use of EOs in the European Union (EU) is in food (as flavorings), perfumes (as fragrances and aftershaves), and pharmaceuticals (for their functional properties). The aroma oil is the result of the combination of the main components in the oil, but trace components are also important. Thus, it is significant that the quality and composition of an EO are maintained during isolation from herb matrix, where the method of extraction plays a crucial role [8]. There are now numerous reports of the in vitro antimicrobial activity of EOs in the scientific and medical literature: EOs are found to have broad spectrum inhibitory activities against various food-borne Gram-positive and Gram-negative pathogenic bacteria.

Hydrodistillation has traditionally been applied for EO recovery from plant materials, but, during isolation, heat-sensitive compounds can easily be destroyed and quality is extremely impaired. Thus, the extraction of EO components using solvents at high pressure, or supercritical fluids, has received much attention in the recent years, especially in food, pharmaceutical, and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and steam distillation [9].

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In this study, the antimicrobial properties of herbal extracts obtained by hydrodistillation and supercritical carbon dioxide (CO_2) extraction from plants originated from Montenegro, sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), and savory (*Satureja montana*), were investigated against five pathogenic bacteria important in food industry.

2. Aromatic plants and herbal extracts

Aromatic plants and herbal extracts have been used for thousands of years as incense, perfumes, and cosmetics. After the discovery of plants' medicinal properties, natural flora became a valuable source of health improvement in the ancient civilizations.

Literature dating from around 2000 BC lists over 700 substances, including ginger, myrrh, and coriander, used for therapeutic purposes in India. Chinese ancient herbal tradition was recorded in Yellow Emperor's *Book of Internal Medicine*, dating more than 2000 BC. However, the most famous and richest associations concerning the first aromatic materials are those surrounding the ancient Egyptian civilization. Through thousands of recipes, Egyptian papyruses showed that, for example, coriander and castor oils were used as cosmetics, for medicinal applications, and as preservatives [10].

Therapeutic uses of herbal plants were described by several scholars, during Greek and Roman period, namely Hippocrates, Galen, Dioscorides, and many others [11]. Romanians are known for their use of medicinal herbs since very long; thus, Herodotus was the first to mention the method of distillation of turpentine, in about 425 BC. These great Graeco-Roman works were translated into Persian, Arabic, and other languages, and at the end of Byzantine Empire, their knowledge was passed to the Arab world. However, in 1975, archeological expedition in Pakistan found perfectly preserved perfume containers and terracotta apparatus similar to distillation apparatus, from about 3000 BC. This discovery suggests that the Arabs simply revived or improved upon a process that had been known for over 4000 years. In 19th century, herbal products were introduced by Romanian pharmacopoeia, whereas in 1904 the first institute of medicinal herbs was established in Cluj city [10].

2.1. History and definition of bioactive compounds

The use of aromatic plants and herbal extracts in the past demonstrates the history of bioactive compounds usage. Typically, bioactive compounds of plants are produced as secondary metabolites [12]. Traditionally, secondary plant metabolites have been defined as all compounds synthesized by the plant that do not appear to be essential for plant growth and development and/or those compounds without an obvious function [13]. In different words, secondary metabolites are those metabolites that are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), have unusual chemical structures, and are often formed as mixtures of closely related members of a chemical family [14]. They are not universally synthesized in all plants. In contrast, primary

metabolites are produced by all plants, usually are part of essential metabolic processes of growth and development, such as proteins, carbohydrates, lipids, and amino acids.

In recent years, as interest as well as investigation of secondary metabolites grow, this artificial and naive definition is changing. Natural functions of many secondary metabolites are unknown because they have never been investigated; this lack of evidence or knowledge is then interpreted as lack of function [15]. Recently, it was discovered that they can help plant to increase their overall ability to survive and overcome local challenges by allowing them to interact with their surroundings [16, 17]. Thus, floral species synthesize aroma to attract insect for their pollination and fertilization while synthesis of toxic chemical has evolved to pathogens as well as to herbivores for suppressing the growth of neighboring plants [18].

Among secondary metabolites, some of these substances have effect on biological systems, which are considered as bioactive. Thus, bioactive compounds in plants can be defined as follows: secondary plant metabolites eliciting pharmacological or toxicological effects in humans and animals [19]. Bioactive compounds of plants could be divided into three main categories: (1) terpenes and terpenoids (approximately 25,000 types), (2) alkaloids (approximately 12,000 types), and (3) phenolic compounds (approximately 8000 types) [13].

2.1.1. EOs as bioactive compounds

EOs are natural, volatile, complex plant compounds, oily or lipid-like in nature, and frequently characterized by strong fragrance [20, 21]. They have been known as herbal extracts to mankind for hundreds of years, even millenniums, but there are different opinions about historical origin of EO production [22]. According to some authors, China has been the cradle of hydrodistillation [23], whereas others claim, as already mentioned, that Arabs invented this process about 3000 BC. Also, there are other opinions that distillation as a method of producing EOs was first used in the East (Egypt, India, and Persia) more than 2000 years ago and improved in the 9th century by the Arabs [23, 24]. However, Villanova (ca. 1235–1311), a Catalan physician, was the first scientist who performed distillation of EOs and left authentic written account [23].

By the 13th century, EOs were being made by pharmacies and their pharmacological effects were described in pharmacopoeias [24], but their use does not appear to have been widespread in Europe until the 16th century when they were traded in the City of London [20]. In "Grete Herbal," published in 1526, some of the illustrations of the retorts and stills used for the extraction of volatile oils are presented [25].

While knowledge of the science of EOs did not increase during 17th century when pharmacies generally used 15–20 different oils, 18th century brought about only small progress in the design of equipment and in refinements of the techniques used [23]. At the end of the 18th century, the use of tea tree oil for medical purposes has been documented in Australia [26]. In 19th century, the first movable apparatus appeared, cooling methods were improved, and double-wall distillation plants appeared. The medical properties and application of increasing number of new EOs were analyzed and recorded by the pharmacists, and the first experimental

measurement of the bactericidal properties of EOs were performed [23]. With the scientific revolution in this century, chemists were able to identify for the first time the various constituents of EOs, and the most important investigation was performed by O. Wallach, an assistant of Kekule [27]. In 1876, Haarman and Reimer started the first production of synthetic aroma chemicals: vanillin, coumarin, anisaldehyde, heliotropin, and terpineol. These researches, partly, laid the ground for the development of the EOs' synthetic counterparts and the growth of the modern drug industry. By the middle of 20th century, the use of EOs in medicine gradually became secondary to their use for flavor and aroma in perfumes, cosmetics, and foodstuffs [28].

2.1.2. Taxonomy of EO-producing plants

EOs are complex mixture of volatile compounds produced by living organisms, isolated by steam distillation, hydrodistillation, or solvent extraction from a whole plant or plant part of known taxonomic origin [29]. Around 3000 EOs have been produced from around 2000 plant species that belong to various genera distributed to around 60 families. It is well known that all plants possess the ability to produce volatile compounds, however, quite often only in traces. Only 300 EOs are important from the commercial point of view, and "EO-bearing plants" in particular are those plant species delivering an EO of commercial interest [20]. Some of those plants have specialized anatomic structures (secretory cells, glandular trichomes, and cavities/ducts), which leads to accumulation of volatiles and higher concentration in the plant [30]. Others, such as rose (*Rosa* spp.), jasmine (*Jasminum sambac*), or tuberose (*Polyanthes tuberosa*), produce and emit the volatiles by the epidermal layers of their petals [31, 32]. Their EO yield is exceptionally small so special techniques have to be applied to recover volatile fragrance compound.

The term "EO" groups together a wide range of chemical compounds on the basis of their historic use and method of isolation and belies the variety and complexity of compounds found within them [33]. Some plant families are particularly well known for their oil-bearing species and ability to produce EOs of medicinal and industrial value. These include *Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rosaceae, Rutaceae, Santalaceae, Zingiberaceae*, and *Zygophyllaceae* [30, 34]. Those plant families are not restricted to one specialized taxonomic group but are distributed among all plant classes. Gymnosperms, for example, the families Cupressaceae (cedarwood, cedar leaf, juniper oil, etc.) and Pinaceae (pine and fir oils, etc.), produce EOs with significant biological activities [34] as well as angiosperms (*Magnoliopsida*, *Rosospida*, and *Liliospida* [35–37]).

2.1.3. Composition of EOs

EOs as herbal extracts are not simple compounds or even simple mixtures of several individual compounds. They may comprise up to approximately 100 components, but usually between 20 and 60 components [38–40]. Major components can constitute up to 85% of the EO, whereas other components are present only as a trace [24, 41]. Numerous publications have presented data on the composition of the various EOs [20, 21, 42–48].

The major volatile constituents are hydrocarbons (e.g. pinene, limonene, and bisabolene), alcohols (e.g. linalol and santalol), acids (e.g. benzoic acid and geranic acid), aldehydes (e.g. citral), cyclic aldehydes (e.g. cuminal), ketones (e.g. camphor), lactones (e.g. bergaptene), phenols (e.g. eugenol), phenolic ethers (e.g. anethole), oxides (e.g. 1,8 cineole), and esters (e.g. geranyl acetate) [45]. They are defined as substances composed of isoprene (2-methylbutadiene) hydrocarbons joined together in a repetitive head-to-tail manner (known as the isoprene rule) [46, 49]. Leopold Ruzicka, recipient of the 1939 Nobel Prize in Chemistry, proposed biogenetic isoprene rule [50], which emphasizes the single biochemical origin of terpenes.

The composition of EOs are influenced by many factors such as plant ecotype or variety, genetic variation, plant nutrition, application of fertilizers, geographic location of plants, surrounding climate, seasonal variation, stress during growth or maturity, and postharvest handling, and also by isolation technique applied [51–53].

The major components of EOs isolated from aromatic herbs examined in this chapter are presented in Table 1.

Common name of EO	Latin name of plant source	Major components	Approximate % composition	References
Sage	*Salvia officinalis* L.	Camphor	6-23%	[55-57]
		B-pinene	2-10%	
		1,8-cineole	6-14%	
		α-thujone	20-42%	
Rosemary	*Rosmarinus officinalis*	α-pinene	2-51%	[57-59]
		camphor	2-32%	
		Bornyl-acetate	0-17%	
		1,8 cineole	3-89%	
		Limonene	4-15%	
Oregano	*Origanum vulgare*	Carvacrol	2-80%	[55, 60-62]
		Thymol	1-64%	
		γ-terpinene	2-52%	
		p-cymene	Trace-52%	
		sabinene	1-48%	
Savory	*Satureja montana*	Thymol	4-38%	[54, 63,64]
		Carvacrol	5-96%	
		γ-terpinene	1-31%	
		p-cymene	3-27%	

Table 1. Major components of selected EOs

2.1.4. Economic importance of EOs

Use of herbal extracts, particularly EOs, for perfumery, additives in food/confectionary as well as for pharmaceuticals and cosmetics is a growing market trend. The huge production of EOs (>70,000 tons per annum) with estimated market value of more than 700 million $US indicates

that production and consumption of EOs is increasing all over the world [45]. This production is achieved mainly by major cultivators and producers like the United States, Brazil, India, and China; although some other countries are important contributors of EOs; for example, vetiver/khus, clove, lemongrass, basil, and celery oils are mainly produced in India, whereas Spain and France are major producers of rosemary oil [52, 60].

Today, aromatherapy and use of herbal extracts as "natural" products are fast developing segment of the industry, and this is a return to what was common practice in ancient and medieval times [64].

The largest world consumer of EOs is the flavor industry, especially for soft drinks. However, this is limited to a few EOs, mainly citrus (orange, lemon, grapefruit, and lime), ginger, cinnamon, clove, and peppermint. Similar oils are used in confectionery, bakery, desserts, and dairy products, besides some fruity products and spices. Alcoholic beverage industry is the one of the largest consumers of various EOs as well as dairy, desserts, sweet bakery, confectionery, and cream manufacturers. The fast food and processed food industries use EOs with spicy and herbal flavors: oregano, basil, fennel, pepper, dill, etc [65].

3. Techniques for extraction of bioactive compounds from aromatic plants

When considering quality and composition of an EO, the method of extraction plays a crucial role. EO are extracted from different part of the plants – flowers, buds, seeds, leaves, bark, herbs, wood, fruits, and roots – and different extraction techniques should be used in different conditions for understanding the extraction selectivity.

Furthermore, separation, identification, and characterization of bioactive compounds are only possible by an appropriate extraction process of plant matrix.

Different techniques could be used to extract bioactive compounds; many of them remain almost same through hundreds of years. All these techniques have some common objectives: (a) to extract targeted bioactive compounds from complex plant sample, (b) to increase selectivity of analytical methods, (c) to increase sensitivity of bioassay by increasing the concentration of targeted compounds, (d) to convert the bioactive compounds into a more suitable form for detection and separation, and (e) to provide a strong and reproducible method that is independent of variations in the sample matrix [66].

3.1. Conventional extraction techniques

Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing. To obtain bioactive compounds from plants, the existing classical techniques are maceration [19, 67], expression [25], solvent extraction [19], and hydrodistillation.

Hydrodistillation is a traditional method for extraction of bioactive compounds and EOs from plants. Organic solvents are not involved, and it can be performed before dehydration of plant

materials. There are three types of hydrodistillation: water distillation, water and steam distillation, and direct steam distillation [68]. In hydrodistillation, first, the plant materials are packed in a still compartment; second, water is added in sufficient amount and then brought to boil. Alternatively, direct steam is injected into the plant sample. Constituents that are insoluble in the water but volatile enough to be driven off by the steam come over and are cooled, condensed, and collected in the receiving vessel. The condensed mixture flows from the condenser to a separator, where oil and bioactive compounds separate automatically from the water [69]. At a high extraction temperature, some volatile components may be lost. This drawback limits its use for thermolabile compound extraction. EOs extracted by hydrodistillation need further purification, especially drying, to remove water.

3.2. Nonconventional extraction techniques

It was found that the major drawbacks of conventional extraction are necessity of costly and high purity solvent, solvent or water removal, low extraction selectivity, thermal decomposition of thermolabile compounds and long extraction time [70]. In recent years, to overcome these limitations, so-called nonconventional extraction techniques are presented: ultrasound-assisted extraction [71], pulsed electric field–assisted extraction [19, 72], enzyme-assisted extraction [19, 73], microwave-assisted extraction [19, 74], and supercritical fluid extraction (SFE).

SFE was systematically investigated over the past decades [48]. SFE is performed by using fluids in supercritical state at temperature higher than their critical temperature and under the pressure higher than their critical pressure. Supercritical fluid possesses gas-like properties of diffusion, viscosity, and surface tension and liquid-like density and solvation power [7]. These characteristics enable easy penetration of the fluid in supercritical state into herbal material and the extraction of secondary herbal metabolites.

For several practical reasons, more than 90% of all analytical SFE is performed with carbon dioxide (CO_2). Apart from having relatively low critical pressure (74 bar) and temperature (32°C), CO_2 is relatively nontoxic, nonflammable, available in high purity at relatively low cost, and easily removed from the extract. The main drawback of CO_2 is its lack of polarity for the extraction of polar compounds [75]. The limitation of low polarity of carbon dioxide has been successfully overcome by the use of chemical modifier [76].

A basic SFE system consists of the following parts: a tank of mobile phase, usually CO_2; a pump to pressurize the gas; co-solvent vessel and pump; an oven that contains the extraction vessel; a controller to maintain the high pressure inside the system; and a trapping vessel. Usually, different type of meters such as flow meter and dry/wet gas meters could be attached to the system. The major variables influencing the extraction efficiency are temperature, pressure, particle size, packing density and moisture content of feed material, extraction time, flow rate of CO_2, and solvent-to-feed-ratio [9, 77]. By varying the extracting conditions, it is possible to obtain extract with the maximum content of desired active substances. The only disadvantage of industrial application of SFE, as opposed to conventional methods, is the larger investment in equipment due to working in conditions of elevated pressure. The costs of production,

however, are significantly reduced; the process is simpler and more cost-efficient; and quality of the final product good [7].

Supercritical carbon dioxide (SC_{CO2}) is known as a good solvent for a wide range of natural bioactive principles. In the last 10 years, studies on the extraction of classical compounds like essential and seed oils from various sources: seeds, fruits, leaves, flowers, rhizomes, etc., with or without the addition of a co-solvent, have been published [78]. Extraction of bioactive compounds from plant material using SC_{CO2} has been indicated as a favorable technique for producing solvent-free extracts suitable for wide use in pharmaceutical, biomedical, cosmetic, and food industries [79].

4. Herbal extracts as antimicrobial agent

Even before the role of microorganisms in disease pathogenesis was understood, plant-based medicines were used for treating such illnesses. It is recognized that plant molecules have antimicrobial properties; especially, EOs exhibit broad spectrum inhibitory activities against various Gram-positive and Gram-negative bacteria pathogens [20, 38, 57].

However, herbal extract medicine diminished as soon as modern antibiotics were discovered. Renewed recent interest in their use has been attributed to several factors, including the desire for antimicrobial compounds with even better safety and toxicity profiles [80]. Also, severity of bacterial infections has gone up even after the discovery of many antibiotics, mainly due to the reduced susceptibility to conventional antimicrobial agents shown by many important pathogens. Therefore, infectious diseases caused by bacteria are still one of the leading causes of deaths [57, 81]. In addition, toxicity due to side effects limits the prolonged use of high concentrations of available antibacterial drugs [57].

Most EOs possess at least some degree of antibacterial activity. Generally, EOs with phenolics and aldehydes exhibit better antibacterial efficacies [38]. In few cases, a main component of the oil has been observed to possess activity better than the EO. For example, carvacrol and eugenol from clove oil, terpinen-4-ol in *Melaleuca alternifolia* (tea tree oil), or thymol from oregano oil displays greater efficacy than specific oil. Many of the plant molecules are effective against drug-sensitive as well as drug-resistant strains [58, 82].

The methods used for establishing antibacterial activities are, usually, disc diffusion methods or agar or broth dilution methods. Although disc diffusion methods are popular, the data they offer are less useful than others. Agar and broth dilution methods, in which serial dilutions of the test oil in agar or broth media are inoculated with a known concentration of test organism, allow minimum inhibitory concentrations (MICs) to be determined [83]. The MIC is generally defined as the lowest concentration of EO that inhibits growth of the test organism. Although solubilization or dispersion in these systems may be problematic, MICs can help establish safe and effective final concentrations in formulated products.

Two principal reasons for performing the in vitro tests are as follows:

- Identification of antimicrobially active compounds

- Control of microbial susceptibilities toward approved antibiotics and antimicotics

The procedure from identification of antimicrobially active compounds for their use in humans to treat infectious disease is a multistep pathway, which includes pharmacological (concentration of the active compound at the site of action, half-life time, serum levels, dose–response relationship, etc.) and toxicological (e.g. toxicity, allergic responses, and interactions) aspects [84].

4.1. Mode of action of bioactive compounds

The health benefits of medicinal plants are ascribed to their bioactive compounds, known as phytochemicals. It has been estimated that 74% of pharmacologically active plant-derived compounds were discovered after following up on ethnomedicinal use of the plants [85]. Various phytochemicals are recognized to possess antimicrobial, anti-inflammatory, analgesic, anesthetic, antioxidant, neuroprotective, and antitumor activity, thus providing medicinal plants with great therapeutic as well disease-preventive potential [86].

However, detailed knowledge about the mode of action of EOs and other bioactive compounds is still lacking. In general, the mechanism of action of EOs is to alter the structure of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents [87, 88]. The mode of action of phytochemicals depends on the type of microorganism and is generally related to their outer membrane arrangement as well as cell wall structure. For example, antimicrobial action of EOs depends on their hydrophilic or lipophilic character. Certain components of EOs can act as uncouplers, which interfere with proton translocation over a membrane vesicle and subsequently interrupt ADP phosphorylation [89]. Phytochemicals may also modulate transcription factors, redox-sensitive transcription factors, redox signaling, and inflammation [90].

4.1.1. Terpenoids

Specific terpenoids with functional groups, such as phenolic alcohols or aldehydes, interfere with membrane-integrated or associated enzyme proteins stopping their production or activity. It was found that some phenolic alcohols (e.g. carvacrol and thymol) cause a disruption of the lipopolysaccharide outer layer followed by partial disintegration of the outer membrane [89]. The interaction of thymol with the membrane affects membrane permeability and results in the release of K+ ions and ATP [91]. In some cases, thymol can induce the release of lipopolysaccharides, but it does not affect chelating cations [92]. Thymol integrates within the polar head groups of the lipid bilayer, inducing alterations of the cell membrane. Zengin and Baysal [93] found that α-terpineol 1,8-cineole and linalool alter the function of cell membranes and the permeability of outer membranes of *Staphylococcus aureus* and *E. coli*. Bard *et al.* [94] reported that geraniol enhances the permeability of whole cells of *Candida albicans* and also increases the fluidity of both *C. albicans* membranes and dipalmitoyl phosphatidylcholine DPPC liposomal membranes. Mendanha *et al.* [95] revealed that all the tested terpenoids (nerolidol, menthol, pulegone, carvone, (+)-limonene, α-terpineol, and 1,8-cineole) increase the fluidity of cell membrane and exert cytotoxic effects on fibroblast cells. Yin *et al.*

[96] found that borneol increases the fluidity of DPPC bilayer membranes. Terpenoid, isolated from purple prairie clover, petalostemumol, has significant activity against *Bacillus subtilis* and *S. aureus* [97]. Carvone is capable to disrupt pH gradient and membrane potential of cells. With increasing amount of carvone, Oosterhaven *et al.* [98] reported a decrease in the growth rate of *E. coli*, *Streptococcus thermophilus*, and *Lactococcus lactis* was caused by disturbing the metabolic energy status of cells.

4.1.2. Phenolics

Phenolic toxicity to microorganism is often explained by enzyme inhibition with the oxidized compounds, probably through reaction with sulfhydryl groups or in more nonspecific interactions with the proteins. The induced defense response includes formation of a lesion that limits the growth of the pathogen, where polyphenols and other antibiotic compounds accumulate [85].

Hydroxyl groups number as well sites in phenolics are thought to be related to their relative toxicity to microorganisms (increased hydroxylation results in increased toxicity) [85]. Thus, eugenol is considered bacteriostatic against both fungi and bacteria [88]. It was found that eugenol alters the membrane, affects the transport of ions and ATP, and changes the fatty acid profile of different bacteria [99]. It also acts against different bacterial enzymes, including ATPase, histidine carboxylase, amylase, and protease [100, 101]. Cinnamaldehyde is usually less powerful than eugenol [102], but extremely effective against *E. coli* and Salmonella *typhimurium* [92]. Catechin acts on different bacterial strains belonging to different species (*E. coli*, *Salmonella choleraesis*, *Klebsiella pneumonie*, *Serratia marcescens*, *B. subtilis*, *Pseudomonas aeruginosa*, and *S. aureus*) by generating hydrogen peroxide and by altering the permeability of the microbial membrane [85, 103]. It was found that polyphenols obstruct bacterial quorum sensing, that is, the production of small signal molecules by *E. coli*, *Pseudomonas putida*, and *Burkholderia cepacia* cells that trigger the exponential growth of a bacterial population [104, 105].

4.1.3. Other compounds

Flavonoids have been shown in vitro to be effective antimicrobial substances against a wide array of microorganisms [106]. Their activity is probably due to their ability to disrupt microbial membranes or to complex with extracellular and soluble proteins in bacterial cell walls [107, 108]. Moreover, Arora *et al.* [109] demonstrated that some flavonoids (naringenin and rutin) and isoflavonoids (genistein) decrease the membrane fluidity. Selvaray *et al*. [110] correlated bioactivities of flavonoids to their membrane localization and their induced changes in membrane fluidity. Thus, catechins inhibited in vitro *Vibrio cholerae*, *Streptococcus mutans*, *Shigella*, and other bacteria and microorganisms [97]. Several studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (from licorice), and chrysin against multiple viruses [97, 106, 111]. It was found that membrane-interacting properties of flavonoids to modify permeability of cellular membranes could inhibit *E. coli* growth [112].

The mode of antimicrobial action of tannins could be related to their capability to inactivate enzymes, cell envelope, transport proteins, microbial adhesins, etc. Previous studies have

shown that tannins can be toxic to filamentous fungi, yeasts, and bacteria. It was found that condensed tannins prevent growth and protease activity by binding the cell walls of ruminal bacteria [97].

The potential range of quinones' antimicrobial effects is great, due to its ability to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. Possible targets in the microbial cell are cell wall polypeptides, surface-exposed adhesins, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism [85]. Hypericin, an anthraquinone from *Hypericum perforatum*, has received much attention lately as an antimicrobial agent [97].

Several alkaloids have the properties to interact with artificial and biological membranes to change the fluidity in association with their pharmacological effects [113]. Berberine is one of isoquinoline alkaloids, which have been considered to possess anti-inflammatory and antimicrobial effects [114]. Also, it has been found that alkaloid sanguinarine possesses antibacterial activity against bacteria and pathogenic fungi [115].

The bacterial resistance is conferred by multidrug resistance pumps (MDRs), membrane translocases that extrude structurally unrelated toxins from the cell. These protect microbial cells from both synthetic and natural antimicrobials [116]. The mechanism of action of EOs depends on their chemical composition, amount of the single components, and their antimicrobial activity is not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell [20]. Also, the synergistic effects of antibiotics and herbal extracts can provide successful therapy against drug-resistant bacteria. The use of herbal extracts and phytochemicals can be of great significance in therapeutic applications and could help control the problem of multidrug-resistant organisms [85].

4.2. Effect of some herbal extracts on selected food-borne microorganisms

Here, some original results of antibacterial activity of EO and supercritical extracts from aromatic herbs (from Montenegro) – sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), and savory (*Satureja montana*) – against some pathogenic bacteria important in food industry are presented.

Leaves from selected herbs were collected in the central southern part of Montenegro, air-dried and stored in double-layer paper bags at the room temperature, until further analysis.

The selected test organisms, used to evaluate the antimicrobial activity of the herbal extracts, were as follows: Gram-positive (*Bacillus cereus* ATCC 11778, *S. aureus* ATCC 25923, and *Listeria innocua* ATCC 33090) and Gram-negative (*Salmonella enteritidis* ATCC 13076 and *E. coli* ATCC 25922).

To obtain EOs, herb material was subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours according to Yugoslav Pharmacopoeia IV. Supercritical CO_2 extraction (SCE) procedure is previously described in detail [117], while the extraction conditions were: temperature 40 °C, pressure 100 bar, extraction time 360 min; and CO_2 flow rate 0.3 kg CO_2/h.

MIC values were determined for extracts displaying antimicrobial properties in screening studies, using a modified microdilution broth method [118]. Briefly, the extracts were first dissolved in DMSO, then diluted in sterile water and tested over a range of concentrations from 0.09 to 25 mg/ml against overnight broth cultures of selected bacteria grown to a population of 10^6 CFU/ml in tryptic soy broth (TSB). Microplates were incubated at optimum growth temperature for each bacterial strain, and growth was monitored by measuring absorbance at 600 nm every 45 min over 18 hours, using a microplate reader.

The antibacterial activity, for investigated herbal extracts against some pathogenic bacteria important in food industry, are summarized in Table 2.

Herb material	*Salvia officinalis*	*Rosmarinus officinalis*	*Origanum vulgare*	*Satureja montana*
Method of extraction	HD/SCE	HD/SCE	HD/SCE	HD/SCE
Bacterial strain	**MIC (mg/ml)**			
Bacillus cereus ATCC 11778	0.09/0.09	0.36/0.72	0.09/0.18	0.18/0.36
Staphylococcus aureus ATCC 25923	0.09/0.09	0.36/0.72	0.36/0.72	0.18/0.36
Listeria innocua ATCC 33090	0.18/0.18	3.13/6.25	0.18/0.36	1.57/3.13
Salmonella enteridis ATCC 13076	3.13/3.13	3.13/6.25	3.13/3.13	3.13/6.25
Eschericia coli ATCC 25922	0.18/0.18	0.36/0.72	0.36/0.36	1.57/3.13

Table 2. Antibacterial activity of isolates obtained by hydrodistillation and SCE from selected herbs against some pathogenic bacteria important in food industry

According to the results presented in Table 2, sage extracts had the highest antibacterial efficiency against tested bacteria strains (MIC=0.09–3.13 mg/ml) followed by oregano (MIC=0.09–3.13 mg/ml) extracts. In this study, the carriers of antimicrobial activity of the sage oil were probably α-thujone and camphor. High efficiency of oregano extracts could be attributed to the high content of compounds with known antimicrobial activity in examinated samples, such as phenolic components, thymol and its isomer carvacrol, as well as its precursors, γ-terpinene and p-cymene. Rosemary extracts showed somewhat smaller activity than expected, probably because rosemary is a cultivated herb, whereas all other examined herbs were wild growing.

Among tested bacteria, *B. cereus* and *S. aureus* were the most sensitive to presence of all tested extracts, especially to presence of sage extracts. Thus, Gram-positive bacteria seemed to be more susceptible to all tested herb extracts.

The results of the bioassays show that tested extracts obtained by SC-CO_2 extraction from different pretreated herb matrices exhibited the same or weaker antimicrobial activity when compared to the EO obtained by hydrodistillation.

The presented results in this study confirm the facts that plant molecules have significant antibacterial activity and therefore can be used as a strong antimicrobial agent. The use of EOs

in foods as preservatives is limited due to toxicological aspects, but also possible reasons for this limitation may be the strong smell and taste of these substances when used at effective doses. The SC-CO_2 extracts bear the closest natural smell and taste of original material, thus further investigation should point to various combinations of investigated extracts, which should improve the level of inhibition due to synergistic effects.

5. Potential application of herbal extracts in foods

The microbiological safety in ready to eat products is a cause of big concern not only for the consumers and food industries but also for the regulatory agencies. It can be said that the food industry faces a constant problem in providing the food where there are no food-borne pathogens present [3, 4]. The number of documented outbreaks of food-borne diseases has increased in the last decade with *Salmonella* spp., *L. monocytogenes*, and *E. coli* being responsible for the largest number of outbreaks and deaths [5]. The different diseases, caused by food-related pathogenic bacteria, such as listeriosis, hemorrhagic colitis, campylobacteriosis, and salmonellosis, are still reported. For example, in meat and meat products, spoilage bacteria could shorten the shelf life by causing off-odors, discoloration, gas production, etc. Recently, we have trend in reducing the level of synthetic antimicrobial agents, as well as salt levels, in ready meals because of the proven development of hypertension and increased risk of cardiovascular disease. In recent years, consumers prefer fewer chemicals and more natural foods. Therefore, there is growing interest in using natural antimicrobial compounds, including extracts of herbs and spices, as salt replacers or alternatives to synthetic compounds for food preservation [119].

As already emphasized, in addition to providing flavor and fragrance, spices and herbs also have antimicrobial potential and thus can be used for preventing food deterioration and shelf life extension. It was found that, however, well herbal extracts perform in antibacterial assays in vitro, generally, a higher concentration is required to obtain the same efficacy in foods. [120]. The basic properties of the food (fat/protein/water content, antioxidants, preservatives, pH, salt, and other additives) are the most relevant in this respect, although, the extrinsic elements (temperature, packaging in vacuum/gas/air, and characteristics of microorganisms) can also influence bacterial sensitivity [20, 121].

Utilization of packaging materials containing these herbal extracts as antimicrobial compounds is also becoming an attractive option in the food industry. If herbal extracts were to be more widely applied as antibacterials in foods, the organoleptic impact would be important.

5.1. Meat, meat products, and fish dishes

Activity of oregano, thyme, basil, marjoram, lemongrass, ginger, and clove EOs against bacterial strains inoculated experimentally in irradiated minced meat and against natural microbiota found in minced meat samples was tested [122]. $MIC_{90\%}$ values ranged from 0.05%v/v (lemongrass oil) to 0.46%v/v (marjoram oil) to Gram-positive bacteria and from 0.10%v/v (clove oil) to 0.56%v/v (ginger oil) to Gram-negative strains.

Eugenol and coriander, clove, oregano, and thyme oils were found to be in inhibiting *L. monocytogenes, Aeromonas hydrophila*, and autochthonous spoilage flora in meat products, sometimes causing a marked initial reduction in the number of recoverable cells [123] whereas mustard, cilantro, mint, and sage oils were less effective or ineffective [124].

Carvacrol vapor antimicrobial activity was established against *S. enteritidis* on pieces of raw chicken [125]. The effectiveness of oils and vapors of lemon, sweet orange, and bergamot against *L. monocytogenes*, *S. aureus*, *B. cereus*, *E. coli* O157, and *Campylobacter jejuni* was investigated on chicken [126]. The results indicate that bergamot was the most inhibitory EO due to high content of citral and linalool.

The antimicrobial effect of extracts from *S. officinalis* L. and berries of *Schinus molle* L. against *Salmonella anatum* or *S. enteritidis* inoculated on minced beef meat was studied [127]. It was found that use of 0.1% or 1.5% *S. officinalis* with 6% or 4% NaCl or 0.1% or 1.5% *S. molle* with 4% or 8% NaCl could effectively eliminate *S. anatum* from refrigerated raw beef.

Effectiveness of eight EOs as antimicrobial agents for fish preservation on 18 genera of bacteria, which included some important food pathogen and spoilage bacteria, was investigated. Clove EO showed the highest inhibitory effect, followed by rosemary and lavender [128]. It was found that citrus EO incorporated into different edible biopolymer film has the potential to preserve fish fillets [129]. Also, using EO in a coating for shrimps appears effective in inhibiting the respective natural spoilage flora [130].

The antimicrobial effect of nine EOs on *Photobacterium phosphoreum* on the shelf life of modified atmosphere-packed cod fillets was determined. The antimicrobial effect of EO was studied in a liquid medium and in product storage trials. Oils of oregano and cinnamon had strongest antimicrobial activity, followed by lemongrass, thyme, clove, bay, marjoram, sage, and basil oils, whereas oregano oil (0.05%, v/w) reduced the growth of *P. phosphoreum* in naturally contaminated cod fillets and extended shelf life from 11–12 days to 21–26 days at 2°C [131].

5.2. Fruit and vegetable

The shelf life of unpasteurized fruit juices is limited by microbial enzymatic spoilage; moreover, these products could be contaminated by some pathogens. Some EOs could be used to prevent this kind of problem. Lemongrass and geraniol have been found effective against *E. coli*, *Salmonella* sp., and *Listeria* spp. in apple, pear, and melon juices [47, 132].

Carvacrol and cinnamaldehyde were very effective at reducing the viable count of the natural flora on kiwifruit but less effective on honeydew melon. It is possible that this difference is due to difference in pH between the fruits: the lower the pH, the more effective EOs and their components generally are [20, 133].

The antimicrobial activity of basil, caraway, fennel, lemon balm, marjoram, nutmeg, oregano, parsley, rosemary, sage, and thyme EO against food-borne pathogens and key spoilage bacteria pertinent to ready-to-eat vegetables was evaluated [134]. On a carrot model product, basil, lemon balm, marjoram, oregano, and thyme EOs were deemed organoleptically acceptable, but only oregano and marjoram EOs were deemed acceptable for lettuce. It was found

that selected EOs may be useful as natural and safe additives for promoting the safety and quality of ready-to-eat vegetables [135]

Listeria strains were more sensitive than spoilage bacteria, and oregano and thyme were the most active EOs using food model media based on lettuce, meat and milk [136]. This work shows that EOs might be more effective against food-borne pathogens and spoilage bacteria when applied to foods containing a high protein level at acidic pH, as well as moderate levels of simple sugars.

The antimicrobial potential of oregano EO on *S. typhimurium* ATCC 13311 on tomatoes was tested. Tomatoes treated with 100 ppm oregano oil resulted in 2.78 log reduction, after 20 min [137]. Oregano oil was effective at inhibiting *E. coli* O157:H7 and reducing final populations in eggplant salad compared to the untreated control. Also, it was found that, in vegetable dishes, the antimicrobial activity of oil decrease in storage temperature and/or a decrease in the pH increases [138].

5.3. Cereals and dairy products

Nielsen *et al*. [139] found that cinnamon, mustard, garlic and clove EO have strong effect in active packaging to prevent bread from fungal contamination. Although oregano oleoresin weakly prevent the growth of most important spoilage fungi of bread, vanilla EO had no preventative effect against these fungi [139].

It was found that sage oil was ineffective against *B. cereus* in rice was while carvacrol was very effective at extending the *B. cereus* lag phase, reducing the final population compared to a control [20, 140, 141].

Orange, lemon, grapefruit, madrine, terpeneless lime, orange, D-limonene, terpineol, and geraniol were tested against *Salmonella, E. coli, S. aureus*, and *Pseudomonas* spp. in different types of milk. Terpineol was the most effective oil in vitro, thus it was used in combination with orange oil for a validation in milk [142]. For mint oil, it was found that it is effective against *S. enteritidis* in cucumber salad and low-fat yoghurt [143].

6. Legal aspects of the herbal extracts use in foods and safety data

The new Regulation (EC) N°1334 of the European Parliament and of the Council on flavorings and certain food ingredients with flavoring properties for use in and on foodstuffs entered into force in 2009 [138].

The new Regulation stipulates new labeling requirements for both flavoring manufacturers and (final) food manufacturers. These include labeling as "natural flavoring substance(s)" may only be used for flavorings where the flavoring part contains exclusively natural flavoring substances.

The risk management of certain substances naturally present in certain food ingredients with flavoring properties (e.g. herbs, spices) and/or flavorings is based on the "major contributor

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approach": maximum levels are established for the presence of these undesirable substances in food, which contribute most to the human intake of these substances.

European Commission has been registered numerous herbal extracts' components for use as flavorings in foodstuffs. The flavorings registered are considered to present no risk to the health of the consumer and include, among other, carvacrol, carvone, cinnamaldehyde, p-cymene, eugenol, limonene, menthol, and thymol.

New flavorings may only be evaluated for registration after carrying out serious toxicological and metabolic studies, which could require a considerable financial expense. Also, the legislative is different in different countries, for example, estragole and methyl eugenol were deleted from the European Commission list in 2001 due to their being genotoxic. However, since today, estragole is on the EAFUS (Everything Added to Food in the United States) list. The EAFUS list is a list of substances that the United States Food and Drug Administration (FDA) has classified the as generally recognized as safe (GRAS) or as approved food additives.

Till today, many researchers found that a significant number of herbal extracts' components are GRAS and/or approved food flavorings. However, some research data indicate irritation and toxicity: cinnamaldehyde, carvacrol, carvone, and thymol appear to have no significant effects in vivo, whereas in vitro they exhibit mild-to-moderate toxic effects at the cellular level [20]. Also, it was found that eugenol, menthol, and thymol, when applied in root canal treatments, could cause irritation of mouth tissues [144]. Some oils used in the fields of medicine, paramedicine, and aromatherapy have been shown to exhibit spasmolytic or spasmogenic properties [145].

7. Conclusion

Due to consumer awareness and negative perception of artificial preservatives in food, in recent years attention is shifting toward alternatives that the consumers recognize as natural. Thus, herbal extracts, particularly EOs, are getting more space in food industry to prevent the propagation of bacteria that are responsible for the spoilage of food or for the spread of so-called food-borne diseases.

Herbal extracts have antimicrobial potential and thus can be used for preventing food deterioration and shelf life extension. However, if herbal extracts were to be more widely applied as antibacterials in foods, the cytotoxic property and organoleptic impact are extremely important issues to consider. They may vary according to extract composition where the method of extraction plays a crucial role.

Therefore, herbal extracts should be used very carefully and with considerable precautions about the concentrations and product application, target consumer, major constituents of the oil, and toxicology profile.

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