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# The Antioxidant and Antimicrobial Potential of Persian Indigenous Herbs as an Alternatives for Nitrate and Nitrite in the Preservation of Meat and Meat Products: An Overview

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*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The consumption of animal products, including meat and meat products, has increased globally with increased household income. Spoilage by microbes, autolytic enzymes, and lipid oxidation can cause the deterioration of meat and meat products, which has a considerable economic and environmental impact. Meat curing, which includes the addition of salt, nitrite, and sometimes

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nitrate to fresh meat cuts, enables a preservative effect by removing moisture and reducing the water activity of the meat. Nitrates and nitrites have been traditionally used as curing agents in the production of cured meat products. Sodium and potassium nitrates and sodium and potassium nitrites are used in meat curing because they stabilize red meat color, inhibit some spoilage and food poisoning anaerobic microorganisms, delay the development of oxidative rancidity, and contribute to flavor development. The beneficial effects of adding nitrates and nitrites to meat products are the improvement of quality characteristics and microbiological safety. However, several studies have indicated that nitrates and nitrites intake should be limited owing to their potential carcinogenic effect on humans. Therefore, the consumer demand for natural or nitrate- and nitrite-free meat products remains high. There is a need to find alternative natural plant material that provides alternative antioxidant and antimicrobial activities since they are noncarcinogenic and reliable; they can substitute or reduce nitrates and nitrites with minimal or no quality compromise of sensory attributes and shelf-life. Hence in this overview, we focused on Persian indigenous herbs, their essential oil and extracts' chemical composition, and their relation to their antioxidant and antimicrobial activity to find out how the essential oils and extracts of the herbs can be applied to meat and meat as a natural substitute.

*Keywords: Curing agents; essential oil; meat products; nitrate; nitrite; plant extract; shelf-life extension.*

## 1. INTRODUCTION

"In affluent societies, consumers increasingly attach importance to all those aspects that improve their quality of life. In diet, the aim is to have balanced, varied diets containing even safer and healthier foods with a pleasant mouthfeel" [1]. "Meat and meat products are important sources of protein, fat, essential amino acids, minerals and vitamins, and other nutrients and are characterized by pH, water activity ($a_w$), and the level of nutrients that cause the easy growth of microorganisms during their storage" [2,3]. "In recent years, the consumers' demands for healthier meat and meat products with a reduced level of fat, and cholesterol, decreased contents of sodium chloride and nitrite, improved composition of the fatty acid profile, and incorporated health-enhancing ingredients are rapidly increasing worldwide" [4,5]. "The extension of consumer demand for natural meals has been pushed by knowledgeable and affluent buyers who are sold the thought of natural and functional as factors of difference in convenience foods. The addition of natural antimicrobials such as plant extracts and the inclusion of new and herbal bacteriocin-producing probiotics as functional meal adjuncts have been recognized as achievable satisfactory indicators for the informed consumer. These adjuncts have also been employed to improve value-added meat products' preservation and shelf-life" [6,7,8]. "Many natural extracts, such as essential oils (EOs) from edible and medicinal plants, herbs, and spices, have been shown to possess an antimicrobial potential and could serve as a source of antimicrobial agents against food spoilage and pathogens. EOs are odorous, volatile products of the secondary metabolism of aromatic flora and are typically shaped in distinctive cells, or groups of cells, in leaves and stems. EOs have been utilized as flavoring agents in food and drinks, and owing to their different content of antimicrobial compounds; they have been practicable as herbal retailers for food preservation" [6,9]. "Their antimicrobial activity is assigned to terpenoids and phenolic compounds, which have also been shown to exhibit antibacterial or antifungal activity in their pure form. The antibacterial properties of these compounds are in phase related to their lipophilic character, leading to their accumulation in membranes and subsequent membrane-associated events, such as energy depletion" [6,10]. "A meat or poultry product may be labeled as "natural" if no artificial ingredients are included and the product has not endured more than minimal processing" [11].

Although various novel food preservation methods, including nano particles-related approaches such as packaging of reinforced nanoparticles, active and intelligent packaging, packaging of biodegradable nanocomposites, etc., have been utilized for the preservation of food products, the role of meat additives is still undeniable [12,13,14]. "Several food additives are added to food to preserve the freshness of food (antioxidants) or slow down or stop the growth of microorganisms (preservative agents). Nitrates and nitrites are present in several food products as naturally occurring compounds, and thus nitrates and nitrites form part of the human diet. Nitrites (sodium nitrite—E249, potassium nitrite—E250) and nitrates (sodium nitrate—E251, potassium nitrate—E252) are authorized as food additives in the European Union under Commission Regulation (EU) No1129/2011" [15]. Research shows that "nitrates and nitrites have both acute and long-term effects on human health. In the past, the high amount of nitrites added to meat products had caused deaths due to intoxication in the early decades of the 20th century in Germany" [16]. However, curing agents, including nitrate and nitrite, are critical elements for cured meats since these compounds are responsible for the unique, special properties that symbolize cured meat products. While either nitrate or nitrite may be used, nitrate is advantageous as a curing agent solely if it is reduced to nitrite. Because nitrate reduction in meat is typically made by microorganisms, ample time and temperature for microbial conversion are necessary.

"Consequently, nitrate is considered useless and superfluous for high-volume cured meat products such as frankfurters, bologna, and most hams cooked within hours of blending with curing agents. Because the authentic curing agent is nitrite, most cured meats are formulated with nitrite as the only curing agent. However, for some merchandise, such as dry sausage and dry-cured hams that are slowly cured over an extended time, nitrate is used to grant a long-term reservoir of nitrite during the prolonged curing time. More recently, a variety of natural and organic meat merchandise have been developed that utilize natural sources of nitrate from vegetables which, when blended with a bacterial starter culture, result in properties characteristic of nitrite-cured products. These natural and organic merchandises represent a new class of cured meats" [17,18,19,20].

"Sodium or potassium nitrite is a light yellow, almost white, crystalline compound that is pretty soluble in water. The appearance of pure crystalline sodium nitrite is very similar to salt, sugar, and a range of other white, crystalline food ingredients and can be effortlessly mistaken for other typically used food ingredients. Since nitrite is a toxic substance, consumption in high concentrations will be associated with severe health problems. There have been cases where salt shakers have been mistakenly filled with sodium nitrite, and nitrite was once used in a punch combination alternatively of citric acid. High doses of nitrite will produce methemoglobinemia in humans, which is not unlike carbon monoxide poisoning. A deadly dose of sodium nitrite for humans has been estimated to be about 1 g" [21,17]. "Sodium nitrite is the most critical preservative and curing ingredient used in the meat industry" [22]. "It is also responsible for meat colour formation and its flavour after curing" [23]. "The primary concern of nitrates or nitrites in meat products is related to the potential of nitrites to form carcinogenic N-nitroso compounds" [24]. "The result of previous studies showed that nitrite use should be limited due to its potential negative influence on human health" [25]. "Considering this, the amount of nitrites added to meat products is legally restricted in the European Union. Thus, nitrites and nitrates are in the spotlight more than ever because consumers have sought products that do not cause health problems" [26]. This concern has led researchers to seek ways to reduce the risk of nitrosamine formation and alleviate potential human health concerns. One such way is the substitution of nitrite with alternative ingredients having comparable characteristics without causing any health hazards. Over the past several decades, studies have been conducted to counter this problematic challenge; however, to date, these attempts remained unsuccessful in identifying an effective single replacement material possessing all the properties of nitrite.

"Concerns over the safety of some chemical preservatives and adverse consumer reactions to preservatives they perceive as chemical and artificial have prompted increased interest in more "natural-green" alternatives for the maintenance or extension of product shelf life" [27,28,29]. "Medicinal herbs and spices contain many phytochemicals, which are potential sources of natural antioxidants, including phenolic di-terpenes, flavonoids, tannins, and phenolic acids" [30]. "These compounds have antioxidant, anti-inflammatory, and anticancer activities. In food systems, they can improve flavor, retard lipid oxidation-induced food deterioration, inhibit the growth of microorganisms, and decrease the risk of some diseases" [31,8]. "Antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality, and extend the shelf-life of products" [32]. This commentary provides an overview of the published data on nitrites and nitrates and potential alternatives among Persian indigenous herbs that commonly grow in the Iranian plateau-extending from East Azerbaijan Province in the northwest of Iran to Afghanistan and Pakistan west of the Indus River and also includes more minor parts of the Republic of Azerbaijan, Iraqi Kurdistan, and Turkmenistan- to entirely or partially replace these salts in meat and meat products.

## 2. RESULTS AND DISCUSSION

### 2.1 Nitrite and Nitrate

#### 2.1.1 Chemical and biological aspects

“Nitrates and nitrites occur naturally as compounds consisting of nitrogen and oxygen, although in different chemical structures. The nitrogen cycle contains both compounds. The chemical difference between nitrate and nitrite lies in one additional oxygen atom. Nitrite is one part nitrogen and two parts oxygen. The nitrogen cycle comprises the oxidation of nitrite, $NO_2$, into $NO_3$, or nitrate” [34]. “Nitrate ($NO_3^-$ ) salts are relatively soluble in water and are regularly determined in groundwater sources due to the use of nitrate fertilizers. Nitrate in cured meat is a pretty inert ingredient and no longer contributes to meat curing till transformed into nitrite” [17]. This transformation can be achieved by bacteria naturally found in meat or by adding microorganisms with nitrate-reducing capabilities, such as starter cultures [35]. *Lactobacillus sakei*, *Lactobacillus plantarum*, *Leuconostoc strains* [36], *Staphylococcus carnosum*, *Staphylococcus xylosus* [37], *Staphylococcus aureus* [38], *Bacillus subtilis* [39] nitrate is utilized as a substrate for anaerobic respiration in bacteria. Nitrite and nitrate are known predominantly as undesired residues in the food chain with potentially carcinogenic effects. “However, from research performed over the past decade, it is now apparent that nitrate and nitrite are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides” [40]. “Nitrate is a fully oxidized nitrogen oxide compound. Nitric acid, $HNO_3$, has a pKa of -1.6, meaning that when nitrate is dissolved in water, nearly all exist as nitrate anions. Nitrite is much more reactive when compared to nitrate. Nitrous acid, HNO2, has a pKa of 3.3, so when nitrite is dissolved in water, it is found mainly as the nitrite anion, NO2” [41,42]. “Once reduced to act as the nitrosating/nitrosylating agent in cured meats, the nitrate ion can occur through several pathways involving endogenous compounds and added ingredients” [16]. “Residual nitrite, the nitrite remaining in cooked meat products, serves a vital role as a reservoir for NO production. Excess residual nitrite can increase the risk of nitrosamine formation” [43]. “Nitric oxide is a potent nitrosylating/nitrosating agent in cured

**Fig. 1. Topographic map of the Iranian plateau (with some changes from [33])**

meats since it is a highly reactive free radical. Depending on the environment, nitric oxide can act as an oxidizing, reducing, or nitrosylating/nitrosating agent" [44]. This use of salt helped preserve the meats, and if these salts contained saltpeter, they also could produce the reddish cured meat color, prolonging the action of preventing the growth of spoilage microorganisms. Saltpeter ($KNO_3$), recognized as a contaminant of salt, enhanced the preservative effect of salt, and the salted meat product then had a red color.

### 2.1.2 Dietary sources

"Various vegetables contain high concentrations of naturally occurring nitrates. Several factors affect the accumulation of nitrate in vegetables and allow for a wide range of nitrate concentrations. Leafy vegetables such as lettuce and spinach tend to have higher nitrate levels than seeds or tubers" [45,46].

Plants typically thrive in a nitrate-rich environment and absorb the greatest quantity of nitrates. According to available data, vegetables and fruit are the major sources of dietary nitrate intake, accounting for 50% to 75% of total dietary intake in both the UK and France [47,46]. Application of fertilizers generally results in a greater uptake of nitrogen in vegetables, resulting in higher nitrate content [34]. "Nitrate uptake, nitrate reductase activity, growth rate, and growth conditions (e.g., soil temperature, the intensity of light, level of rainfall, etc.) all significantly affect the ultimate nitrate content of vegetables. Further, processing methods such as heat treatments and storage conditions can cause the loss of nitrate. For example, increased storage temperatures have been found to decrease the nitrate content of vegetables through increased bacterial-facilitated reduction of nitrate to nitrite" [48]. "Surprisingly, saliva accounts for approximately 93.0 % of the total daily ingestion of nitrite. In contrast, foods account for a tiny portion of the overall daily nitrite intake due to the chemical reduction of salivary nitrate to nitrite by commensal bacteria in the oral cavity. Cured meats have been reported to comprise 4.8 % of daily nitrite intake, and vegetables account for just 2.2 %" [49]. "Reviewing the residual nitrate and nitrite content of commercial cured meat products, it was concluded that consistently lower levels of residual nitrate and nitrite than those from a survey reported by the National Academy of Sciences in 1981 existed" [50]. "Nitrate levels can be significantly high in many green vegetables, such as spinach. As has been reported before, vegetables containing nitrates are commonly utilized in combination with lactic acid starter cultures to produce naturally cured meat products" [51]. Moreover, it has been indicated [52] that pre-converted nitrite from natural sources could maintain the pink color of meat products if used at sufficient levels. Nitrate can be converted to nitrite by microorganisms before formulating meat products in the pre-generation process [53].

### 2.1.3 Daily intake and legislation

The EU's maximum nitrate levels in vegetables have been changed repeatedly. Regulation (EC) No. 1258/2011 establishes the current maximum limits. The Regulation applies to the following foods: spinach, lettuce, rocket, processed cereal-based foods, and infant and young children foods. All maximum values are given in milligrams of nitrate per kilogram of fresh weight [15]. Humans eat between 1.2 and 3.0 mg of nitrite per day, according to the WHO [54]. About 10%–20% of initially added nitrites, referred to as residual nitrites, are typically present in meat products after production, and this amount of residual nitrites slowly declines during the storage period of cured meat products [55]. The average level of residual nitrites in meat products observed is in France (50 $mg.kg^{-1}$), USA (4.7 $mg.kg^{-1}$), Denmark (6 $mg.kg^{-1}$), Belgium (4 $mg.kg^{-1}$), and Iran (13.9 $mg.kg^{-1}$) [56,57,58,59]. A well-known health effect of nitrates (potassium nitrate, E251; sodium nitrate, E252) and nitrites (potassium nitrite, E249; sodium nitrite, E250) in humans is methemoglobinemia, which is the binding of nitrite transformation products to hemoglobin with resulting impairment of oxygen transport capacity. However, the acceptable daily intake (ADI) of nitrite is not based on nitrosamines or methemoglobinemia. The blood rapidly transports absorbed nitrates and selectively secreted by the salivary glands and probably other exocrine glands [24]. The amount of nitrite permitted for use as a food additive in cured meat is currently 150 mg $kg^{-1}$ (expressed as $NaNO_2$), except for somewhat higher levels in some traditionally cured products [55]. "Nitrates and nitrites are listed as official food additives in the corresponding Commission Regulation (EU) No. 1129/2011. To protect consumers and keep the quality characteristics of cured meat products, certain legal limits were set for nitrites and nitrates by various countries. European Union also set limits for nitrates and nitrites for various meat products manufactured and traded throughout the European Union countries by regulating either the ingoing or the residual amounts of these salts in the products, Commission Regulation (EU) No. 601/2014]. However, objections to the limits of residual levels of nitrites were also raised by scientists since the measurement of the residual levels of nitrites in the final product may be of limited value" [60].

## 2.2 Beneficial Effects of Nitrite and Nitrate in Meat Products

### 2.2.1 Antioxidant properties

"One of the most remarkable properties of nitrite is its ability to delay the development of oxidative rancidity effectively. This prevention occurs even in the presence of salt, which is a strong oxidant. Lipid oxidation is a significant reason for the deterioration of quality in meat and poultry products, often resulting in rancidity and subsequent warmed-over flavors" [61]. "Commonly used chemical food preservatives for the inhibition of food microflora growth include butylated hydroxy anisol (BHA), butylated hydroxytoluene (BHT), calcium propionate, nitrates, nitrites, sulfur dioxide ($SO_2$), and sulfites ($SO_3$)" [62]. "The most property of nitrite is its ability to retard the oxidation process during the storage period and the subsequent warmed-over and rancid flavors developed during the thermal processing of meat and meat products" [61,63].

"The antioxidant activity of nitrite is attributed to the potential of nitric oxide to bind to and stabilize heme iron of meat pigments during the meat curing process. Nitric oxide, being a free radical, can also trigger lipid autoxidation by chelate free radicals, including peroxyl radicals. The nitrite binds free irons and stabilizes the heme iron, which can reduce lipid oxidation by limiting the prooxidant activity of iron" [64,65]. Since nitrite can act as a very effective antioxidant at levels permissible under law, its inclusion in cured meat products reduces the need for other antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene.

### 2.2.2 Antimicrobial properties

"Meat is an ideal medium for the growth of microorganisms because of its high moisture content, proteins, minerals, and additional growth factors. In addition, it has some fermentable carbohydrates, usually glycogen, and has a favorable growth pH for the multiplication of most microorganisms. Consequently, meat and meat products are highly perishable unless appropriately preserved and/or stored under conditions designed to retard microbial activity and proliferation" [66,67]. "One of the main concerns about meat products is microbial spoilage because this is responsible for a loss in quality and toxic compounds that can affect human health" [68]. A recent risk-benefit review of nitrite included discussing the antibacterial benefits of nitrite in cured meat products [69]. Generally considered to be more effective against gram-positive bacteria, nitrite has been shown to control the growth of pathogenic bacteria [23]. Nitrites exhibit important bacteriostatic and bactericidal activity against several spoilage bacteria as well as food-borne pathogens found in meat products such as *Salmonella enterica serovar Typhimurium*, *Listeria spp*., and *Clostridium botulinum* and *Staphylococcus aureus* [70,71]. However, most studies concerning the antimicrobial properties of nitrites have been focused on *C. botulinum* in several meat products produced in various countries. The presence of nitrite in processed meats can deter the growth of both *C. botulinum* and *C. perfringens* [72]. "Nitrite was also found to inhibit several enzymes essential to the metabolism of bacteria, such as aldolase.

Moreover, nitrite generally causes a breakdown of the proton gradient in bacteria needed to generate ATP. One method to avoid the direct addition of nitrite to meat is to add instead ingredients that have a naturally high nitrate content. This method is used to produce organic versions of cured meats. Nitrous acid, an uncharged molecule, can then diffuse across the bacterial membrane. In the near-neutral cytoplasm, nitrous acid is converted to nitrite and a proton. The release of free protons leads to intracellular acidification. Such an inhibitory effect of acidified nitrite has first been described for yeasts" [73]. "The inhibitory mechanism that results in nitrite's effects on some bacteria likely differs among bacterial species and is not considered adequate for controlling gram-negative enteric pathogens such as Salmonella and *Escherichia coli"* [74].

## 2.3 Hazardous Aspects

Nitrate and nitrite have been a topic of debate for several decades now. The 1982 National Academy of Sciences report also called for a more thorough evaluation of nitrite in cancer bioassays, and thus FDA nominated it for study in the National Toxicology Program [75,76]. Several epidemiologic studies show strong associations between the consumption of processed red meat and many risks [77], such as pancreatic cancer [78], cardiovascular diseases, and other causes of death [79]. The risk of cancer was estimated to increase by 29% with daily consumption of 100g of red meat and by 21% with daily consumption of 50g of processed meat [80]. Among the compounds responsible for these risks in processed meats are N-nitroso compounds and polycyclic aromatic hydrocarbons [81]. “Nitrite can act as a nitrosating agent in the formation of nitroso compounds. N-nitroso compounds belong to six fundamental categories: volatile N-nitrosamine, non-volatile N-nitrosamine, N-nitrosamide, N-nitrosated heterocyclic carboxylic products, N-nitrosated glycosylarnines, and Amadori compounds. Several epidemiological studies have demonstrated a potential relationship between nitrate, nitrite, N-nitroso compounds, and cancer risk” [82]. The use of high levels of either substance can lead to the production of N-nitrosamines, which are recognized as having carcinogenic effects [83,84,85].

N-nitrosamines are among the most potent and versatile carcinogens [86], which may exhibit potential genotoxicity and increase gastric and colorectal cancer [87]. These harmful substances, which have been detected in meat products, are widely present in meat products and are considered carcinogens and mutagenic [88]. N-nitrosodi-N-butylamine (NDBA), N-nitroso piperidine (NPIP), and N-nitroso pyrrolidine (NPYR) as possibly carcinogenic to humans. It causes the conversion of hemoglobin to metmyoglobin which lacks oxygen-transporting ability [89]. The biological activities of nitric oxide related to secondary products may include pharmacological effects, e.g., high blood pressure and oxidative stress/inflammation induction. However, it is concluded in the literature that available evidence of these effects is inadequate for comprehensive and reliable assessment of optimistic or adverse health effects of nitrate/nitrate, especially long-term effects [90]. These compounds are formed when the temperatures reach above 130 $^{\circ C}$ in meat and meat products. A relationship has been observed between consumption of meat products and appearing childhood leukemia and brain tumors. Although general health risks associated with nitrate are known, Alexander and Cushing (2011) [82] have reported no supportive evidence to prove the relationship between processed meat consumption and cancer risk. Exposure to only high overdoses of nitrite and nitrate from different sources has been associated with increased health risks [91].

## 2.4 Nitrite and Nitrate in Meat Products

“Artificial preservatives are chemical substances that stop microorganisms' growth and activities and help preserve foods for a longer time without affecting their natural characteristics. They include nitrites, benzoates, sulfites, sorbates, nitrates of sodium or potassium, glutamates, and glycerides. Food standards require no more than one chemical preservative in one food item. People consuming or using items containing more than one preservative are at risk of exposure to multiple chemicals” [92]. Natural and synthetic preservatives are further categorized into three types which are antimicrobials, antioxidants, and anti-enzymatic preservatives. Nitrite (E249, E250) and nitrate (E251, E252) are approved food additives in the European Union (EU Regulation No. 1129/2011/EC 2011) and are widely used in meat preservation because of their effect on its organoleptic characteristics (color stabilization, flavor development), oxidative stability of lipids and inhibition of pathogenic microorganisms such as *C. botulinum* or *L. monocytogenes* [93]. In the EU, potassium and sodium nitrite and nitrate are authorized for use in different meat products, and maximum amounts (150 $mg.kg^{-1}$ for nitrite and 300 $mg.kg^{-1}$ for nitrate) are established for all products as well as maximum residual levels for some of them (Directives 95/2/EC and 2006/52/EC), except for somewhat higher levels in some traditionally cured products. Current regulations on the use of nitrite and nitrate in the US vary depending on the curing method used and the product to be cured [94].

### 2.4.1 Natural alternatives to nitrite salts in meat products

Consumers have developed a more robust negative view toward chemical or artificial additives, which has prompted research and industrial interest in finding alternatives that meet consumer demand. There is increased demand for natural additives or preservatives in the meat industry, especially of plant origin, to reduce the nitrite or nitrate content in meat products [93]. The plant ingredients may be used as a natural nitrate/nitrite source. They are chosen for their ability to supply nitrate, but nitrate concentrations vary widely among types of plant parts from 1.0 to 10000 ppm [95]. Natural or synthesized alternatives to nitrite have been developed. For example, Anka rice [96], annatto [97], and tomato paste [98] have been added to meat products to obtain the desired color. However, none of these alternatives has been used widely during meat processing until recently. Li et al. (2013) [99] found that plant polyphenols decreased total N-nitrosamine formation in dry-cured sausage. Polyphenols are widespread compounds in fruits and vegetables such as apples, tomatoes, celery, red beet, etc. [100]. Thus, finding a nitrite/nitrate alternative that reproduces the typically characteristic meat color and maintains the high-quality traits is significant. Some vegetables, such as celery and leafy vegetables, contain significant amounts of nitrate.

EOs contain a mixture of compounds, including terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters, mainly used as food flavorings or functional components in pharmaceuticals [101,102]. Individual components of EOs are also used as food flavorings or antimicrobial compounds [103]. Although the majority of the EOs are classified as GRAS (generally recognized as safe) substances, their use in food as preservatives is often limited due to flavor considerations [104]. Several extracts owing to their phytochemical constituents, have been shown to have antimicrobial activity. The antibacterial activity is most likely due to the combined effects of the adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents and the generation of hydroperoxides from polyphenols [105].

Herbs and spices are rich in phenolic compounds, and besides exerting an antimicrobial effect, they may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity [106,107]. Extracts from oregano, thyme, rosemary, sage, and mint have been used to improve sensory characteristics (taste, odor, appearance) and extend the shelf life of foods, especially meat and meat products such as sausages. Plants extracts and EOs are bioactive sources with antioxidants, antimicrobial effects, and increasing shelf life of products. There is a lot of consumer demand for green food products resulting in high safety and nutritional values. Viuda-Martos et al. [108] suggested that polyphenols and flavonoids reduce the levels of residual nitrite. Thus, reducing residual nitrite levels could be an acceptable alternative for reducing nitrite intake through processed meats to alleviate the potential risk of forming carcinogenic and mutagenic N-nitroso compounds [109]. Vegetables such as celery, spinach, and lettuce contain a considerable amount of nitrates that can be converted to nitrite using nitrate-reducing starter cultures such as *Staphylococcus carnosus* and *Staphylococcus xylosus* [110].

### 2.4.2 Herbs and vegetable extracts

Herbal plants embrace a wide range of practices and therapies outside the realm of traditional medicine. Although herbal medicines are not risk-free, they can still be safer than synthetic drugs. The potential benefits of herbal medicines include high acceptance by patients, effectiveness, relative safety, and relatively low costs [111].

One of the alternatives to replace chemical additives for cured products are vegetable-based curing ingredients [110]. Among some benefits of the addition of vegetables, extracts can improve products' quality and shelf life without adding other additives [112]. Plant extracts are usually collections of crude mixtures obtained from plant parts and are attested to have inhibitory effects on microorganisms, especially the pathogenic activity; the plant parts could be leaves, stems, flowers, fruits, roots, barks, etc. EOs are aromatic oil liquids from plant parts or (sometimes animals) and are proven to have anti-pathogenic and anti-spoilage properties useful in inhibiting viruses, bacteria, fungi, and some insect parasites.

Many studies have evaluated the addition of different plant and vegetable extracts as nitrite replacements while aiming to preserve and improve the overall quality of meat and meat products [113]. Extracts of basil, broccoli, neem, citrus, and rosemary are better alternatives to preservatives such as benzoic acid, sulfites, nitrates, MSG, BHA, and BHT [114]. “The antioxidant effect prevents rancidity; therefore, stabilizing the fat, besides their similar action to commercial additives, makes vegetable extracts a potentially practical and healthier option for improving meat products. Some examples of vegetable extracts are celery, rosemary, garlic, onion, cumin, ginger, nutmeg, and peppers, commonly used as condiments in the meat industry, besides other sources of vegetable extracts such as yerba mate and Marcela” [115].

“Plant polyphenol extracts have been used as spices, herbs, and natural meat preservatives . They are used as preservatives due to the presence of EOs derived from these plants that contain most of their antimicrobial activity, and they contain a variety of individual components that seem to be able to kill or inhibit the growth of microorganisms” [116].

## 2.5 Persian Indigenous Herbs as an Alternative

The recent fast rise of natural and organic foods has given nitrate in processed meats a new role [117]. Regulatory and labeling regulations prohibit the addition of nitrate or nitrite to natural and organic processed meats. Processors, on the other hand, have devised techniques to include natural sources of nitrate as components, such as vegetable juices and concentrates. Celery, lettuce, and beets are typical sources of nitrate, with values of 1,500–2,800 ppm [50]. It has been revealed that dried vegetable juice powders contain over 2.5% nitrate or more than 25,000 ppm. When added at 0.2–0.4% of a meat product's formulation, Vegetable juice powder offers enough nitrate to create typical cured meat characteristics. Having said that, it is noteworthy that most vegetables and some herbs have components in their extract and EOs that are associated with considerable antimicrobial and antioxidant potential and can be applied in food products for preservation [118]. These plants, which usually include medical plants, are different from region to region, and in this section, some indigenous ones to the Iranian plateau will be mentioned in detail.

### 2.5.1 Thyme (*Thymus vulgaris* L.)

The herb known as thyme, with the botanical name of *Thymus vulgaris* L., a species of the Lamiaceae family, is an important medicinal herb [119]; a perennial herbaceous shrub with a woody root, an erect branching stem, and spreading branches that grow up to 45 cm (2 feet) tall. It has small, evergreen, opposing, gray-green, oval, fragrant, waxy, gland-dotted leaves that are minutely downy. The blooms are pale purple, two-lipped, and have a hairy, glandular calyx. They are produced in loose whorls in axillary clusters with leaf-like bracts [120].

Thyme has about 350 species, of which 14 species are indigenous to Iran. Although this herb is considered a wild herb in Iran, it has been cultivated for medical purposes. *T. vulgaris* has a wide range of non-medicinal applications as well as medicinal ones, including the food and fragrance sectors. *Thymus vulgaris* EO is utilized as a flavor enhancer in a broad range of foods, drinks, and confectionery products, as well as a food preservative due to its antibacterial and antioxidant qualities. Thyme is used to season foods and to neutralize undesirable scents like tri-methylamine odor. The antioxidative activity of

**Fig. 2. A) Plant B) flowers C) leaves D) seeds of *Thymus vulgaris L.* [121]**

thyme EO and T. vulgaris extracts is superior to synthetic antioxidants [122]. Thymol and carvacrol, two main phenolic components of thyme extracts or EO, have potent antioxidant activity that may be higher than the well-known BHT and α-tocopherol antioxidants [123]. The major phenolic components of Thymus vulgaris are carvacrol (5-isopropyl-2-methyl phenol) and thymol (5-methyl-1-2-isopropyl phenol), which make about 20 to 55 percent of the thyme oil extract [119]. The concentration of phenolic chemicals (thymol) and terpene hydrocarbons (γ-terpinene) in thyme EO is correlated to its antibacterial activity [124,125]. P-Cymene, the third major agent in thyme according to its ratio, has a synergistic antibacterial effect when combined with γ-terpinene and thymol [126,127]. Thyme EO has been shown to have efficient radical scavenging capability with an $IC_{50}$ and the ability to scavenge radicals implicated in lipid oxidation [128,129]. Many studies show that thyme volatile oil is one of the most commonly used EOs in cosmetics as antioxidants and preservatives, as well as in food production [130]. The major constituents of thyme EO are phenol isomer carvacrol and its nature terpenoid thymol, which have antimicrobial, antioxidative, antibacterial, antitussive, antispasmodic, and expectorant properties [131]. Thyme also contains phenolic acid, terpenoids, and flavonoid glycosides [132]. Flavonoids (e.g., thymonin, cirsilineol, and 8-methoxycirsilineol), caffeic acid, triterpenoids, aliphatic aldehydes, long-chain saturated hydrocarbons, and "Labiatae tannin" (rosmarinic acid) are also active biochemical components in Thyme species [127]. The antibacterial potential of thyme is determined by its chemical components, particularly thyme EO [127]. Thyme extracts and EOs have been found to have potent antibacterial, antimicrobial, and antifungal properties, as well as anti-inflammatory, spasmolytic, and other functions [133,134,135,136,137]. Thyme EO exhibited antibacterial activity against *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, *Bacillus cereus*, *Campylobacteriosis*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Pseudomonas* [138,139,140]. Moreover, components of thyme have been shown to possess fungicidal (fungistatic) against *Aspergillus oryzae*, *A. brasiliensis*, *A. flavus* as well as *Penicillium*, *Cladosporium*, *Trichoderma*, *Mucor,* and *Rhizopus* [141,142]. Consequently, encapsulated thyme EO can be used as a preservative in hamburger-like meat products; encapsulated thyme EO on the conservation of hamburger-like meat products has shown to possess antioxidant and antimicrobial activity and is effective and can be used as a preservative in hamburger-like meat products [143]. In another study where the effect of potato starch-based antibacterial composite films with thyme oil microemulsion or microcapsule on the shelf life of chilled meat was investigated, it was revealed that slow releasement of the antibacterial active ingredients in thyme oil led to prolonging the antibacterial time of the film and effectively inhibiting the growth and reproduction of *E. coli* and *S. aureus* [144]. In a study where the effect of the joint addition of thyme EO and powdered beet juice to meat sausage, for a partial or total reduction of the addition of nitrates and nitrites, was evaluated, it was revealed that the thyme EO showed a high inhibitory action against *Staphylococcus aureus* and *Escherichia coli* [145].

### 2.5.2 Saffron (*Crocus sativus* L.)

With the botanical name of *Crocus sativus* L., Saffron is a small bulbous perennial herb with a substantial fleshy corm that grows up to 30 cm (1 foot) tall. The corms are 3–5 cm in diameter and produce gray-green leaves. The funnel-shaped flowers are produced in the fall. They have a strong fragrance and vary in color according to location; the throat is generally whitish, with segments ranging from reddish-purple to dark lilac or blue, and very rarely white, and Small, dark brown, globose, and papillose seeds. Crocin is responsible for the vivid red stigma color [146].

Moisture 8.5–9.5 %, starch 13 %, fixed oil 8–13 %, total ash 1.2 %, EO 0.4–1.5 %, 2% of picrocrocin, crocin, carotenoids, flavonoids, and vitamins B1 and B2 are among the active components of saffron [148,149]. The perianth, stamen, and corm contain lauric acid, hexadecanoic acid, 4-hydroxydihydro-2(3H)-furanone, and stigmasterol [150]. The main components are crocin, crocetin, picrocrocin, safranal, and stigmasterol. Catechol, vanillin, salicylic acid, cinnamic acid, p-hydroxybenzoic acid, gentisic acid, syringic acid, p-coumaric acid, gallic acid, t-ferulic acid, and caffeic acid are among the other components identified [151,152]. Over 100 biologically active chemicals, mostly terpenes, flavonoids, anthraquinones, and anthocyanins, were extracted from C. sativus stigmas. Saffron stigmas have been shown to have a wide range of biological properties, including antioxidant, cytotoxic, antibacterial, depressive, hypolipidemic, antiparasitic, and more. Mental

**Fig. 3. Crocus sativus and potential parent species. (A) C. *cartwrightianus*, (B) C. *hadriaticus*, (C) C. *oreocreticus*, (D) C. *pallasii*, (E) C. *thomasii*, (F) C. *sativus* [147]**

illnesses, neurological diseases, learning and memory dysfunctions, cardiovascular diseases, atherosclerosis, hyperlipidemia, diabetes mellitus, hypertension, ulcer, fatty liver disease, epilepsy, and convulsions have all been found to benefit from the stigmas of *C. sativus* [153,154]. Several bacterial food-borne pathogens, including *E. coli*, *Bacillus subtilis*, *Pseudomonas florescens*, *Serratia marscens*, *Citrobacter frendii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Proteus vulgaris*, were tested against saffron extract and other spices, and it was determined that saffron's antibacterial property was relatively low [155]. Antibacterial activity was shown in a methanolic extract of saffron petal against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli*, and *Shingella dysenteriae* [156]. Previous research has found that stamens had the highest scavenging action for peroxyl LOO(•) and hydroxyl OH(•) radicals, surpassing that of the food antioxidant propyl gallate. Saffron flowers, tepals, stamens, styles, and floral bio-residues all exhibited scavenging activity for LOO(•), OH(•), and ABTS(•-) radicals, whereas stigmas showed scavenging activity for LOO(•) and ABTS(•-) radicals [157]. Nanoencapsulation of saffron extract in zein nanofibers aimed at the preservation of sea bass fillets extends the fillets' shelf life and delays their spoilage by applying its antimicrobial and antioxidant activity [158]. Another examining effect of saffron nano-emulsion on the shelf-life of shrimp showed that the EO and nano-emulsions of saffron might be employed as a natural protective resource in the food sector due to their antibacterial and protective properties [159]. The utilization of saffron in Chicken (Breast) meat stored in a refrigerator was reported to reduce fat oxidation in chicken breast meat during storage and extend its shelf life [160].

### 2.5.3 Savory (*Satureja hortensis* L.)

Savory, known as summer savory with the botanical name of *Satureja hortensis* L., and Satureja, known as winter savory with the botanical name of *Satureja montana* L., are the genus of aromatic plants of the Lamiaceae family. Around 200 species of aromatic and medicinal herbs belong to the genus *Satureja*, which grows naturally in the Middle East and Mediterranean European areas, as well as in West Asia, North Africa, and South America [161,162].

**Fig. 4. L) The branched stems of *Satureja hortensis* L., R) *Satureja montana* L. [163,164]**

It has been found that the chemical composition of the EOs of the *Satureja* subspecies differs significantly. The primary components of *Satureja* species, according to phytochemical research, include volatile oils, tannins, phenolic compounds, sterols, acids, gum, mucilage, and pyrocatechol. Phenols, carvacrol, thymol, *p*-cymene, *β*-caryophyllene, linalool, monoterpenes, sesquiterpenes, alcohols, phenolic acids, labiatic acids, and flavonoids are the primary components of EOs in most species [165]. Several investigations on *S.hortensis* volatile oils and, in some instances, extracts revealed the chemical composition of summer savory. Fresh leaves include moisture (72%), protein (4.2%), fat (1.65%), sugar (4.45%), fiber (8.60%), and ash (2.11%). On a dry weight basis, the volatile oil (up to 5%), triterpenic acids, tannins (up to 8%), mucilage, resins, sugars, mineral salts, and other substances are the dominant source of bioactive chemicals [166,167]. Carvacrol, thymol, phenols, and flavonoids are important components of the volatile oil extracted from summer savory. Several investigations have discovered thymol (0.3–28.2%), γ-terpinene (15.30–39%), carvacrol (11–67%), and p-cymene (3.5-19.6%) as major components in volatile oils [168,167]. In comparison to EOs, extracts derived from *S. hortensis* aerial parts have been the focus of far fewer investigations. Rosmarinic acid (24.9 mg/g), caffeic acid (1.3 mg/g), naringenin (1.1 mg/g), isoferulic acid (220 µg/g), and apigenin (165 µg/g) were found to be the most abundant compounds in the methanolic extract produced obtained by maceration. Other flavones (luteolin) and their glycosides (apigetrin and vitexin) were found, as well as flavonol (quercetin), flavonol glycosides (isoquercitrin, astragalin, quercitrin), and coumarin derivatives (aesculin and aesculetin) were also detected [167,169]. The whole savory (*Satureja montana* L.) EO, as well as its various fractions or pure constituents having a hydroxyl group, demonstrated relatively significant antioxidant activity in both TBARS and -carotene–linoleic acid methods [170]. On the other hand*,* *S. hortensis* EO has antioxidant action, as evidenced by in vitro experiments, due to the presence of polyphenolic components. Different assays, such 2,20-diphenyl-1-picrylhydrazyl (DPPH), 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS), ferric thiocyanate and -carotene bleaching, have shown that commercial EO from Iran has a significant antioxidant effect [171]. Furthermore, it was observed that adding Iranian commercial *S. hortensis* EO to kappa-carrageenan film improved the antioxidant properties of the film [172]. The antibacterial activity of *S. hortensis* EO was assessed using 23 bacteria, 15 fungi, and yeast species. The EO derived from this plant has potent antibacterial activity against all bacteria and fungi tested [173]. The antibacterial activity of *S. hortensis* EO extracted from Iranian plants was assessed against various microorganisms, including *Candida glabrata* and *Pseudomonas aeruginosa* showed good antimicrobial activity [174]. Iranian commercial *S. hortensis* EO incorporated with kappa-carrageenan improved the antimicrobial characteristics of the produced film against *S. aureus*, *E. coli*, *B. cereus*, *S. typhimurium,* and *P. aeruginosa* [172]. Evaluating the savory extract effect on the shelf-life of fresh chicken meat at refrigeration temperature showed that meat oxidation and its microbial load significantly decreased [175]. In another study where *Satureja hortensis* L. EO was utilized to reduce the survival of *S. typhimurium* in minced poultry meat during refrigerated storage, it was reported that summer savory EO significantly decreased Salmonella count compared to the control group [176]. Applying *Satureja montana* L. EO as antioxidants in precooked pork chops during chilled storage showed to be effective in oxidation stability and warmed-over flavor formation [177]. It has also been reported that the addition of winter savory (Satureja montana L.) extract to cooked pork sausages resulted in a significant reduction of thiobarbituric acid reactive substances and inhibition of microbial growth [178].

### 2.5.4 Sideritis (*Stachys lavandulifolia*)

The Lamiaceae family includes the genus *Stachys* L., which is a prominent member of the family. Stachys species have simple petiolate or sessile leaves and grow as annual or perennial herbs or tiny bushes. The number of verticillate flowers varies from four to numerous, generally forming a spike-like inflorescence at the apex. Calyx tubes are tubular-campanulate, 5 or 10 veined, regular or weakly bilabiate with five subequal teeth. The upper lip of the Corolla is flat or hooded and typically hairy, while the lower lip is 3-lopped and glabrous to hairy. The nutlets are oblong to ovoid shape, with a rounded apex [179]. Stachys is represented by thirty-one species in the Iranica generic flora. Many regions of Iran, Iraq, and Anatolia are habitats for *Stachys lavandulifolia* [180].

Research has reported that the EOs of *S. lavandulifolia* Vahl consist of main constituents of the EOs were α-thujone (0.3%–32.3%), α-pinene (trace to 37.3%), myrcene (0.5%–15.9%), β-phellandrene (1.1%–37.9%), germacrene D (0.4%–11.3%), δ-cadinene (trace to 11.6%) and 1, 4-methano-1 H-indene (trace to 10.1%) [182]. Another study regarding the composition of the EO of *S. lavandulifolia* Vahl. from Iran has revealed that the major components found in the oil are α-pinene (20.1%), β-pinene (12.1%) and spathulenol (7.2%) [183]. In a similar study, the EO composition of *S. lavandulifolia* from Iran was studied the germacrene D (17.1%), 4-hydroxy-4-methyl-2-pentanone (12.3%), 7-epi-α-selinene (8.3%), bicyclogermacrene (6.7%), β-caryophyllene (6.2%), and α-pinene (5.9%) were reported as constitutes [184]. In a study where five extracts, namely n-hexane, dichloromethane, methanol, methanol with Soxhlet apparatus and ethanol 70% extract from the aerial parts of *S. lavandulifolia* prepared for evaluation their antioxidant activity, using carotene bleaching test, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and Ferric Reducing Antioxidant Power (FRAP) assays, it was revealed that ethanol 70% and methanol extracts, showed the highest radical scavenging activity against ABTS radicals, whereas the methanol extract Soxhlet apparatus was the most active in the DPPH method. In the carotene bleaching test, the methanol and ethanol extract demonstrated a more robust activity. Moreover, they studied the antioxidant activity of bioactive secondary metabolites; arbutin, acteoside, monomelittoside, melittoside, 5-allosyloxy-aucubin, and stachysolone, reporting that in both DPPH and ABTS assays, the most active compounds were arbutin [185]. The antibacterial activity of methanol extracts of the dried flowering aerial parts of *S.byzantina*, *S. inflata*, *S.lavandulifolia*, and *S.laxa* against bacteria test showed dose dependant. Gram-positive bacteria, *Streptococcus sanguis,* and *Staphylococcus aureus*, were more susceptible to methanol extracts [186]. Another research reported that EOs from aerial parts of the S. lavandulifolia Vahl showed antimicrobial activity against *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* [187].

**Fig. 5. *Stachys lavandulifolia* picture taken from the countryside of Marand (East Azerbaijan-Iran) [181]**

**Fig. 6. Asafoetida growing wild in Mount Telesm, Kermanshah, Iran [189]**

### 2.5.5 Asafoetida (*Ferula assa-foetida* L.)

Asafoetida is derived from the Persian *aza*, which means mastic or resin, and the Latin *foetidus*, which means stinking. With a fleshy taproot, highly dissected leaves, and inconspicuous yellow flowers grown in complex umbels, this erect perennial herb can grow up to 3 m (9 ft) high. The plant has a fusiform perennial root with a coarse, hairy top, similar to parsnip. The bark is wrinkled and dark, with thick alliaceous fluid inside. The glossy leaves have oblong and obtuse lobes. They are scarce and occur in the autumn. Herbaceous, firm, smooth, and covered with membrane sheaths, the stem is herbaceous. Thin, flat, foliaceous, and reddish brown with vittae [188]. There are about 150 species of ferula, with the majority of them found in the Mediterranean and Central Asia. This plant is a member of the Apiaceae family, which has 275 genera and 2850 species. Sesquiterpenes, sesquiterpene coumarins, and sulfur-containing compounds were among the bioactive chemicals identified from ferula. Germacranes, humulanes, carotanes, himachalanes, and guaiaanes are the main components of this species, according to research findings from over 70 species [8].

EO from the fruit of the asafoetida has fifty-four components, comprising 96.9% of the total oil, which was identified.epi-α-Cadinol (23.15 %), germacrene B (10.98 %), α-gurjunene (6.18 %), (Z)-1-propenyl sec-butyl disulfide (5.89 %), 5-epi-7-epi-α-eudesmol (4.89 %), δ-cadinene (4.78 %), δ-cadinene (3.36 %) and germacrene D (3.09 %) were found to be the major constituents of the oil [189]. EO from asafoetida -derived oleo gum resin has revealed the major components are included (E)-1-propenyl sec-butyl disulfide (13.66–49.35%), β-pinene (1.06–21.18%), (Z)-1-propenyl sec-butyl disulfide (2.02–15.29%), α-pinene (2.04–17.61%), thiophene (0.03–36.81%), and thiourea (0.08–9.63%) [190]. Regarding the chemical composition of the aerial part of asafoetida, twenty-three components representing 97.06% of the total EO were identified, and (E)-1-propenyl sec-butyl disulfide (53.77%), (Z)-1-propenyl sec-butyl disulfide (35.6%), and a-pinene (3.4%) were identified as major components [191]. Previous studies on the antioxidant evaluation of asafoetida extract and EO have shown their potential [192,193]. The hydroalcoholic extract of asafoetida leaves and EO have shown that the EO had higher free radical scavenger and may act as a primary antioxidant, which can react with free radicals by donating hydrogen. However, this scavenging potential was lower than BHT [192]. Another study reported that the aerial parts of the asafoetida extract have good but varying levels of antioxidant activity in all of the models tested. The extracts demonstrated high $Fe^2$ chelating, DPPH radical scavenging, and nitric oxide scavenging properties [194]. The antibacterial activity of EOs extracted from asafoetida -gum-resins at various periods revealed that all EOs greatly decreased the growth of both Gram-positive and Gram-negative bacteria, including

*S. typhi*, *E. coli*, *S. aureus*, *B. subtilis*, *A. niger*, *C. albicans*, with Gram-positive bacteria being more effective at the doses utilized [195]. EO of Ferula assa-foetida showed to have considerable antifungal properties against various *Candida* genus including *albicans*, *dubliniensis*, *glabrata*, *krusei*, *tropicalis* and *neoformans* as well as growth inhabotion and eliminiation effect on different molds such as *Aspergillus* (*flavus*, *fumigatus*, *oryzae*, *clavatus*), *Pseudallescheria boydii*, *Exophiala dermatitidis*, *Penicillium marneffei*, *Microsporum gypseum*, *Microsporum canis*, *Trichophyton rubrum* [196]. The study that investigated the incorporation effect of asafoetida and Adhatoda Vasica extract on N,O-carboxymethyl chitosan films reported that the extract of both plants possesses the antimicrobial potential and preservative impact of generated film improved by the addition of the extracts [197].

### 2.5.6 Fennel (*Foeniculum vulgare Mill*.)

It is a perennial plant that grows to a height of 2 m (6 ft). It has delicate feathery green leaves with golden blooms that bloom in umbels. The thick stems are wrapped in sheaths formed by the leaf stalks. Bitter fennel (*var. vulgare*) and sweet fennel (*var. dulce*) are two medicinally important sub-sp. *vulgare* varieties. The seeds are oval, yellowish to greenish brown, and slightly curved [198].

The EO obtained from fennel seeds has been reported to include 28 components identified, representing 95.8% of the total amount. Trans-anethole (68.53%), a phenylpropanoid, was found as the main component. Estragole (10.42%) was identified to be the second most abundant phenylpropanoid in fennel oil, followed by limonene (6.24%), fenchone (5.45%), and other minor components in the EO of fennel seeds [200]. According to another study, estragole (84.8%), limonene (7.8%), fenchone (3.1%), and α-pinene (1.3%) constitute the basic components of the EO derived from seeds of *Foeniculum vulgare Mill* [201]. Furthermore, 60 compounds representing 90.1–98.7% of the EOs derived from leaves of Florence fennel were identified. The major constituent of the EOs was trans-anethole (59.8–90.4%). In addition, the fennel EOs also contained minor amounts of various constituents as limonene (0.1–21.5%), neophytadiene (0–10.6%), (E)-phytol (0.1–6.0%), exo fenchyl acetate (0.3–3.8%), estragole (0.1–2.5%) and fenchone (0.1–3.1%) [202]. As revealed in a study, Fennel EO and extracts had strong DPPH radical scavenging action, with IC50s of 32.32 and 23.61–26.75 mg/ml, respectively, and peroxidation inhibition of 45.05 and 48.80–70.35 percent [203]. Regarding antimicrobial activity, fennel EOs showed significant antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger*, *Fusarium solani*, and *Rhizopus solani*, particularly Gram-positive bacteria demonstrating the most activity [203]. Furthermore, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, and *Shigella flexneri* are all susceptible to aqueous fennel extract [204]. In addition, research on the antifungal effect of funnel found that it has considerable antifungal activity against fungi found in food waste, such as *Aspergillus niger* and *Fusarium oxysporum* [199]. In a study on the practical application of this plant for meat and meat product preservation, it was shown that the fresh-keeping impact of pork meat patties was enhanced by an edible nanoemulsion of fennel EO/cinnamaldehyde, which increased the shelf life from 6 to 10 days [205]. In another study where fennel EO loaded porous starch-based microencapsulation as an efficient delivery system aimed at improving the quality of ground pork, it was reported that the EO micro-capsules exhibited good antibacterial and antioxidant activities, delayed the oxidation of fat and protein, reduced the total viable counts, total volatile-base nitrogen and methemoglobin [206]. Generating biodegradable film based on polylactic acids and polyhydroxybutyrate amalgamated with fennel oil showed to possess antimicrobial activity by inhibiting the growth of *E. coli* and *Staphylococcus* and prolonged the shelf-life of the packaged oysters with these films [207].

### 2.5.7 Sage (*Salvia officinalis* L.)

Sage is a perennial shrub that grows up to 80 cm (2 feet). It features a long spindle-shaped root, a woody stem with straight branches, silver oval wooly leaves on opposing sides, and enormous appealing violet blooms. The leaves are oblong to spear-shaped, grayish-green to slightly silvery green, glossy, and covered with small hairs [208].

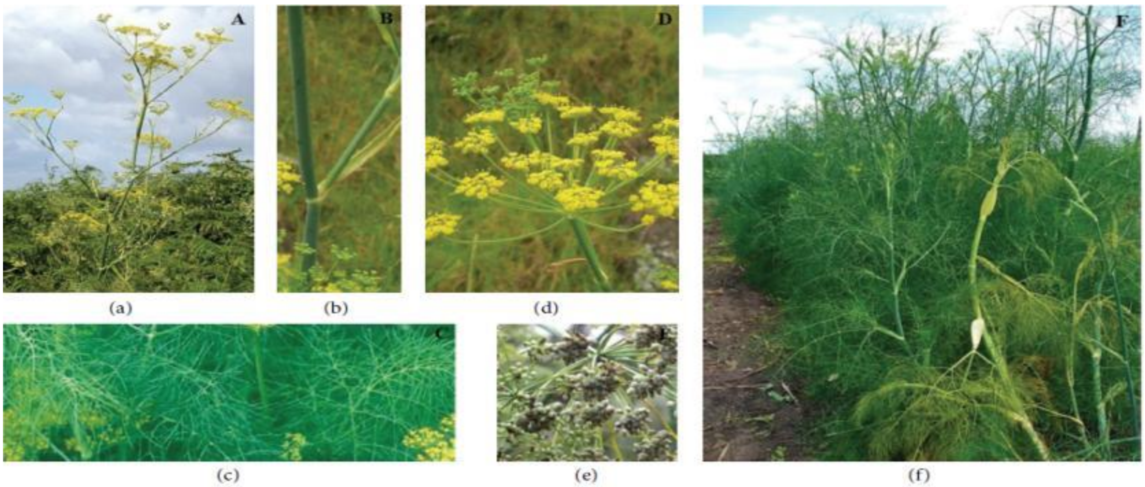

**Fig. 7. *Foeniculum vulgare* Mill (a) in its natural habitat; (b) stem; (c) leaves; (d) inflorescences and flowers; (e) fruits; and (f) population of *F. vulgare* Mill. [199]**

**Fig. 8. *Salvia officinalis* L.: flowers (a) and leaves (b) [209]**

There were 14 compounds identified in sage Eos, which the most abundant compounds in all the samples were a-thujone (7.8–20.1%), camphor (8.4–20.8%), borneol (2.5–16.9%), cmuurolene (2.9–13.8%) and sclareol, a bicyclic diterpene alcohol with a sweet, balsamic scent (5.9–23.1%). Although these compounds were commonly found in the EO, season, geographic origin, environmental factors, extraction methods, plant organ, phonological stage, sampling techniques, and genetic differences all contributed to the percentage of these compounds in the EO of *S. officinalis* leaves [210,211]. In another study, the main components of sage EO, 1,8-cineole, α and β-thujone, camphor, viridiflorol, and manool showed significant variation with drying methods [212]. Sage EO is utilized in food and pharmaceutical goods [210]. Multiple studies have indicated that EO's content varies, which might have significant implications; for the example given, sage extracts were found to be a superior antioxidant to BHT in the oxidation of rapeseed oil in prior studies [213]. Several substances in sage EO and extract were discovered to show antibacterial action against vancomycin-resistant enterococci, *S. pneumoniae*, and MRSA, including carnosol, carnosic acid, oleanolic acid, ursolic acid, uvaol, betulinic acid, and betulin [214,215]. EO extracted from aerial parts of a cultivated sage showed high antibacterial activity against *C. albicans* [216]. In addition, Sage EO has antibacterial efficacy against *E. coli*, *Salmonella typhi*, *Salmonella enteritidis*, and *Shigella sonnei*, as well as antifungal activity against six fungi [217,218]. In a study looking into the antimicrobial potential of plastic films incorporating sage extract on chicken meat, it was observed that coatings containing sage extracts of various viscosities were effective as antimicrobial adhesives in food packaging films and could be used commercially to extend the storage of chicken breast meat without compromising its quality [219]. Investigating the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food-borne pathogens showed that the oil may serve as an auxiliary factor to prolong the shelf life of vacuum-packed low-pressure mechanically separated meat from chickens stored frozen since it could inhibit lipid oxidation and restrict the bacterial growth [220]. In another study, it was reported that the antibacterial efficacy of EO nanoemulsions against food-borne bacteria was found to be superior to pure EO against *E. coli*, *S. dysentery*, and *S. typhi* [221].

### 2.5.8 Fenugreek (*Trigonella foenum-graecum* L.)

Fenugreek is an annual plant with a well-developed taproot and a fibrous root structure that spreads widely. The stem is smooth, green to purple, and grows up to 140 cm (1.5 ft). The three oval leaflets of the light-green leaves are alternating and pinnate. The inflorescence is a terminal, compound umbel. Flowers range in color from white to whitish-yellow. With 20–30 tiny, smooth brownish seeds, the fruit is light green to golden brown, ovoid-cylindrical, and slightly curved [222].

A total of 23 chemical components were found and identified in fenugreek seed oil, which made up 99 percent of the total oil in the study. Linoleic acid (54.13 %), palmitic acid (16.21 %), pinene (4.56 %), 4-Pentyl-1-(4-propylcyclohexyl)-1-cyclohexene (3.87 %), and linoleic acid methyl ester (3.19 %) were the most abundant components in the extracted oil [224]. In another study where a methanolic extract of fenugreek aerial parts was investigated, it was shown eleven different flavonol glycosides (quercetin, kaempferol, and vitexin), and five novel components were identified for the first time in fenugreek aerial parts [225]. In a study, it was reported that the fenugreek seed extract with methanol, ethanol, dichloromethane, acetone, hexane, and ethyl acetate have a radical scavenging activity possesses radical scavenging activity [226]. Furthermore, the protective effect of fenugreek on lipid peroxidation and enzymatic anti-oxidants has been reported [227]. Regarding the antimicrobial activity of fenugreek, it has been reported *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Aspergillus niger* are all susceptible to fenugreek oil. Fenugreek seeds and oil are efficient food preservatives and can also be employed in the pharmaceutical sector [228]. In a study, it was reported that *Pseudomonas spp*., *E. coli*, *Shigella dysenteriae*, and *Salmonella typhi* are all susceptible to fenugreek [229]. In another study antimicrobial activity of *T. foenum-graecum* leaves were reported against some microorganisms, including two bacteria *viz*., *Serratia marcescens* and *Bacillus cereus,* as well as all the fungal strains [230]. Evaluating the antioxidant and antimicrobial potential of fenugreek for quality preservation in burgers made of mutton and beef cattle meat showed that adding crud fenugreek leaves to mutton and beef cattle meat resulted in antibacterial and antioxidant activities [231]. Investigating the

**Fig. 9. *A.* Fenugreek *(Trigonella foenum-graecum L.)* crop; B. Plant showing characteristic curved legumes (or pods); C. Fenugreek seed; D. Hydroponic generated fenugreek seedlings in the lab [223]**

**Fig. 10.** ***Glycyrrhiza glabra*** **(a) flowers, (b) fruits and (c) bark [234]**

effect of ethanolic extracts of fenugreek seeds amalgamated with chitosan coating as natural preservatives for pacific white shrimp during refrigerated storage revealed that it can significantly decrease the Total Volatile Bases Nitrogen, thiobarbituric acid reacting substances, total bacterial count, pH and consequently assist in quality maintenance during refrigerated storage [232].

### 2.5.9 Licorice (*Glycyrrhiza glabra* L.)

It's a perennial herb or bushy herb with oblong leaves and tiny blue-violet blooms that grow up to 2 m (6 ft). The leaves are separated into leaflets in pairs. The fruits are reddish pods that are tiny in size. The rhizomes have a fibrous texture and are grayish brown on the exterior and yellow on the inside. Spanish licorice, Persian licorice, Russian licorice, and Chinese licorice are some of the variations and species of licorice [233].

More than 400 phytochemicals have been identified from the Glycyrrhiza genus. Saponins, flavonoids, chromenes, coumarins, dihydrostilbenes, coumestans, benzofurans, and dihydrophenanthrenes are instances of these compounds [235]. Naphthalene, Decahydro-4a-Methyl (15.62%); 2, 6-Octadiene-1-ol, 3, 7-Dimethyl (6.96%); Butanoic Acid, 3, 7-Dimethyl-2 (5.79%), Lavandulyl Acetate (4.93%), 3-Hexene-1-ol, Benzoate (3.45%) are being reported to be among constituents of *G. glabra* [236]. In a study, the presence of the three active components was discovered in a crude extract from a specific plant species of licorice using the ABTS$^+$ -HPLC on-line radical scavenging detection system, which allows for the quick identification of antioxidants in a natural product [237]. Both aqueous and ethanol licorice extracts are efficacious against *S. mutans* and *L. acidophilus*, with methanol extract being particularly bactericidal against *S. mutans* and also selectively potent against *P. falciparum* and *P. berghei* [238,239,240]. *C. albicans* standard strain and two clinical isolates were both suppressed by fresh aqueous extract [241]. The antibacterial activity of ethanol extract against *P. acnes* is remarkable [242]. In a study in this regard, the combination of chitosan and citric acid or licorice extract had substantial antioxidant effects on ovate pompano fillets. It was shown that they have the potential to be used as natural antioxidant preservatives in seafood and other meat products [243]. Another research reported that Incorporating licorice extract into chicken patties is a cost-effective way to increase oxidative stability, qualitative features, and shelf life with a preservation impact [244]. Investigating the effect of licorice extracts on fatty acid oxidation in rabbit meat during storage showed that licorice extracts possess effective DPPH scavenging activity and slow down fatty acid oxidation [245].

**Fig. 11. Different parts (roots, leaves, seeds, and flower) of the coriander plant [246]**

### 2.5.10 Coriander (*Coriandrum sativum* L.)

An intensely scented, upright, herbaceous annual plant with a hollow stem that grows to about 1.5 m (5 ft.) in height. It features brilliant green leaves with a lustrous sheen and umbels of little white or pale-pink flowers. The globular coriander fruits (seeds) are light brown in color, spherical, and have two pericarps with a warm pleasing odor [222].

Linalool (57.5–75.1%), geranyl acetate (8.9–24.5%), α-pinene (2.3–23.2%), terpineol (0.08–5.3%), geraniol (0.5–2.3%) and citronellol (0.6–1.6%) are indicated as major constituents of Coriander seeds EO. EO content of leaves varies between 0.1 and 0.29%, and the major constituents of which are (E)-dec-2-enal, (E)-dodec-2-enal, decanol, dodecanol, n-tetradecanol and decanal [247]. According to another study, in the leaves EO of *Coriandrum sativum* L., 46 compounds were identified, accounting for 90.17 % of the aroma, including 1-decanol (17.85%), decanal (11.04%), trans-2-dodecen-1-ol (7.87%), menthone (6.71%), 2-decen-1-ol, trans- (5.44%), dodecanal (4.76%), trans-tetradec-2-enal (3.14%), sedanolide (3.02), and thymol (3.01%) [248]. Coriander has been shown to have potent antioxidant activity, including radical scavenging activity, lipoxygenase inhibition, phospholipid peroxidation inhibition, iron chelating activity, hydroxyl radical scavenging activity, superoxide dismutation, glutathione reduction, and anti-lipid peroxidation [249,250]. Coriander leaves revealed more antioxidant activity than coriander seeds. Nevertheless, extracts from coriander leaves are more potent than extracts from coriander seeds, and compounds with a medium polarity appear to be the most active, even if their overall antioxidant contribution to the plant is minor [251]. Coriander oil was shown to have strong antibacterial action against *S. pyogenes* and *S. aureus* (MRSA) bacteria, and it might be used as an antiseptic for the prevention and treatment of Gram-positive bacteria skin infections [252]. Coriander EO has been proven to have high antifungal action against Candida spp. and may be effective in treating candidiasis [253]. Adding EOs of coriander to ground beef restricted the growth of Enterobacteriaceae as well as other groups of microorganisms [254]. Evaluation of active edible coating of sodium alginate incorporated with coriander seed EO for shelf life extension of chicken fillets showed significant decrees in peroxide formation and microbial load of the fillets [255].

### 2.5.11 Nigella (*Nigella sativa* L.)

Nigella is an annual plant that grows up to 60 cm (1.5 feet) high and has a well-developed yellow-brown taproot. The stem is densely branched, hollowing out as it ages, and light to dark green. The feathery leaves are generally green, but as they age, they become brown or red. When the blooms are small, they are pale green, and when they grow become light blue. When fully grown, the fruit is a capsule that is yellow or brownish. The seeds have an oily white interior and are small, pitted, and wrinkled [233].

**Fig. 12. *Nigella sativa* L. green seeds (a), matured seeds (b) [256]**

It has been reported that *Nigella sativa* L. seed EO consists of *p* -cymene (34.67%), α-thujene (11.55%), trans-4-methoxythujane (5.81%), β-pinene (4.66%), methylcyclohexane (3.11%), α-pinene (2.82) and longifolene (2.55%) [257]. In another study, it was reported that the major components of *Nigella sativa* seed oil were detected as palmitic acid (10.48%), linoleic acid (8.05%), *o*-cyemene (7.11%), 3,5-dimethyl cyclohexanol (6.68%), thymoquinone (6.44%), *p*-tert-buthyl catechol (6.28%) and 8-methyl-1-undecene (3.28%) [258]. The biological features of *N. sativa* oil include antifungal, antibacterial, and antioxidant potential. In a rapeseed oil model system, the oil had a superior antioxidant potential than synthetic antioxidants. The oil also had a higher antiradical activity when it came to the DPPH radical. *N. sativa* oil's industrial and commercial value stems from the wide range of food and non-food uses to which it may be used. Gram-negative and Gram-positive bacteria, such as *Penicillium citrinum*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were completely inhibited by *N. sativa* oil [259]. It has been shown that MDR Gram-negative isolates were more resistant to aqueous extract of *Nigella sativa* than Gram-positive isolates [260]. Its volatile oil was significantly active against *S. aureus*, and moderately active against *E. coli*, *S. typhi*, *P. aeruginosa*, *B. subtilis* and *C. albicans* [261]. Alcoholic extracts of the *Nigella sativa* seed oil were shown to have antibacterial activity against *E. coli* and *M. pyogenes var.aureus* [262]. In a study where cold-pressed Nigella sativa seed oil was incorporated into ground beef meat, ground beef meat was extended with low microbial loads [263]. Another research aiming at determining the effect of the addition of different levels of *Nigella sativa* L. oil to minced pork on the quality and shelf life of pork patties showed an improvement in the meat quality of pork patties and effectively delayed lipid oxidation potential [264]. In addition, examining the effects of nigella and synthetic antioxidants on the sensory and physicochemical quality of beef patties during refrigerant storage showed that Nigella extract possesses an excellent antioxidant activity over BHA in terms of sensory [265].

## 3. CONCLUSION

The rising global consumption of animal products, particularly meat and meat products, has brought to light the challenges posed by spoilage, enzymatic activity, and lipid oxidation, which collectively impact both economies and the environment. The practice of meat curing, involving the introduction of salts, nitrites, and nitrates to fresh meat, has traditionally served as an effective means of preservation by reducing moisture content and lowering water activity. Nitrates and nitrites, integral to cured meat production, offer benefits such as color stabilization, spoilage inhibition, oxidative rancidity delay, and flavor enhancement. However, concerns regarding potential carcinogenicity in humans have led to a surge in consumer demand for nitrate- and nitrite-free alternatives, prompting a quest for natural substitutes that retain the same antimicrobial and antioxidant properties.

This overview has delved into the realm of Persian indigenous herbs, exploring the chemical composition of their essential oils and extracts and their intricate relationship with antioxidant and antimicrobial activities. The potential of these natural plant materials to serve as reliable, noncarcinogenic alternatives to nitrates and nitrites has been investigated, with a focus on maintaining sensory attributes and extending shelf life. As the drive for healthier, more sustainable meat products intensifies, the integration of these indigenous herbs and their derivatives emerges as a promising avenue for enhancing product quality and safety.

By harnessing the inherent potential of these herbs, it is possible to pave the way for a new era in meat production that addresses consumer health concerns and the environmental impact of traditional preservation techniques. As research continues to unravel the complexities of natural additives, it is evident that innovation driven by traditional knowledge can lead to advancements that redefine the meat industry, promoting a harmonious balance between human nutrition, culinary satisfaction, and ecological consciousness.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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