Biological Properties of Essential Oils

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Essential oils (EOs) are a mixture of natural, volatile, and aromatic compounds obtained from plants. In recent years, several studies have shown that some of their benefits can be attributed to their antimicrobial, antioxidant, anti-inflammatory, and also immunomodulatory properties. Therefore, EOs have been proposed as a natural alternative to antibiotics or for use in combination with antibiotics against multidrug-resistant bacteria in animal feed and food preservation.

Keywords: essential oils ; volatile oils ; antimicrobial ; antioxidant ; immunomodulatory ; food preservation ; biofilm

1. Introduction

Foodborne-related diseases are an increasingly major public health problem worldwide [1]. Microbial contamination is one of the factors in developing foodborne diseases and food spoilage [2]. Since ancient times, different methods to preserve food for a longer period have been sought. For this reason, and thanks to the advancement of knowledge and the rapid development of new technologies, different chemical compounds have been developed, commonly known as additives, which extend the life of foods or are used as sweeteners or coloring agents [3]. However, the presence of pathogenic microorganisms continues to result in large economic losses and multiple diseases in humans [4]. On the other hand, the indiscriminate use of antibiotics in both humans and animals against pathogenic microorganisms has contributed to the extension of resistant and even multidrug-resistant bacterial strains [4][5]. In recent years, a tendency to use natural additives, mainly due to the increasing desire for the consumption of minimally processed products, has emerged [4][6]. Therefore, there is a need for alternative natural compounds that can perform the same function of common additives or can be used as an alternative to antibiotics. One such possibility is the use of essential oils (EOs) due to their known antimicrobial, antioxidant, immunomodulatory, and food preservative activities [7]. In line with this, several studies have described the antibacterial activity of EOs, underlying their effective use on multidrug-resistant strains [8][9].

EOs, also known as “volatile oils”, are complex mixtures of volatile compounds that are produced by aromatic plants as secondary metabolites. They are responsible for the aromatic plant’s properties, and for this reason, they are characterized by their strong smells [10]. In general, EOs are liquid, volatile, and soluble in lipids and organic solvents. They can be present in all plant organs, including buds, flowers, leaves, seeds, stems, flowers, fruits, roots, wood, or bark. Different extraction techniques are widely employed for the extraction of EOs such as steam distillation, solvent extraction, and supercritical fluid extraction [11][12]. These EOs are characterized by the presence of variable mixtures of bioactive compounds, mainly terpenoids, especially monoterpenes and sesquiterpenes. Some of them also contain nonterpenic compounds biogenerated by the phenylpropanoid pathway, such as eugenol, cinnamaldehyde, and safrole [13]. These bioactive compounds are responsible for the biological properties of EOs. Among them, terpenoids are the bioactive compounds that have a more important role in pathogen resistance [14]. Specifically, monoterpenoids affect the multiplication and development of microorganisms by interfering with their physiological and biochemical processes during their development and multiplication [15]. Cinnamon bark oil is one of the most effective EOs against common foodborne pathogens [16]. It should be noted that the effect of EOs on bacterial growth will depend on whether they are Gram-positive or Gram-negative bacteria, since the lipopolysaccharide (LPS) layer in Gram-negative bacteria acts as a barrier for macromolecules and hydrophobic compounds such as those present in EOs [15]. Using EOs to extend the shelf life of fish and meat has also been reported in previous studies. Examples include the preservative effect of lemon EOs on salted sardines [17], the effect of chitosan coatings enriched with cinnamon oil on the quality of rainbow trout (*Oncorhynchus mykiss*) during refrigerated storage [18], and the lengthening of the storage period of red sea bass by means of clove, cumin, and peppermint oils or poultry meat in thyme oil [19].

The antioxidant activity of EOs is another biological property of great interest because they may preserve foods from the toxic effects of oxidants [20]. It is noteworthy to mention that EOs have also been shown to possess a wide range of immunomodulatory properties. To date, a few studies dealing with the immunomodulatory effect of EOs have been reported [21][22][23]. In this regard, monoterpenes have been shown to exert a strong immunobiological effect through their effect on tumor necrosis factor (TNF)-α, interleukins (ILs), thromboxane, and leukotriene production [24]. This immunomodulatory activity indicated the possibility of using EOs as ingredients in functional foods.

2. Antimicrobial Activity of Esential Oils

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from EOs due to drug resistance in foodborne bacterial enteric pathogens. Numerous publications have presented data on the antimicrobial properties of EOs [25][26].

A variety of laboratory methods can be used to evaluate the in vitro antimicrobial activity of an EO. The most well-known and basic methods are the disk diffusion and broth or agar dilution methods [27]. The lowest concentration of antimicrobial agent that completely inhibits the growth of the organism is called the minimum inhibitory concentration (MIC). The most appropriate assays for the determination of the MIC value are the dilution methods, as they offer the possibility of a precise estimation of the concentration of the tested antimicrobial agent.

The antibacterial effects based on the MIC determination of several EOs alone or in combination against different food-associated Gram-positive and Gram-negative bacteria have been described. Parsley, lovage, basil, and thyme are a few of the aromatic herbs commonly used in industry with low-cost production. Different parts of these herbs (leaves, flowers, stems, fruits, and seeds) have been used to extract EOs [28]. Parsley and lovage EOs revealed no inhibitory effects against all tested strains. Thyme EO had the highest percentage yield and antibacterial potential from all tested formulations; its use in combination with parsley, lovage, and basil EOs results in a reduction in its antibacterial activity; therefore, thyme EO should be used alone [28]. EOs of cultivated oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) have been shown to exert a potent antimicrobial effect. Among them, the most efficient were the EOs from thyme, followed by those of oregano. With MIC values above 150 mg/mL, sage EOs did not show any antibacterial effect against the majority of the bacterial strains [29]. Three *Origanum* species analyzed, *O. dictamnus* and *O. microphyllum*—both endemic in Greece—and *O. libanoticum*, endemic in Lebanon, were evaluated, but only *O. dictamnus* exerted antibacterial activity [30].

Different bacterial and fungal strains have been used to determine the antibacterial effects of different Eos; these microorganisms comprise strains from *Staphylococcus*, *Bacillus*, *Listeria*, *Helicobacter*, *Micrococcus*, *Pseudomonas*, *Klepsiella*, *Escherichia*, *Salmonella*, *Enterobacter*, and *Candida*. EOs from *Heracleum pyrenaicum* subsp. *orsinii*, *Pistacia vera* L., *Myrcia ovata Cambessedes*, *Thymus bovei*, *Minthostachys verticillata*, *Allium roseum*, *Petroselinum crispum*, *Satureja bachtiarica* Bunge, *Ocimum suave*, *Jatropha gossypifolia* L., and *Juniperus rigida* have been shown to exert antibacterial and anti-yeast effects [31][32][33][34][35][36][37][38][39][40][41]. One of the proposed mechanisms for those effects is the irreversible damage of the bacteria cell wall and membrane, which leads to not only a leakage of proteins but also of DNA and RNA molecules [35][36].

Enteromorpha linza, *Baccharis dracunculifolia*, *Syringa yunnanensis*, *Senecio nutans*, basil, chamomile blue, oregane, thyme, tea tree oil, *Carum copticum*, and *Xanthium strumarium* L. EOs have also been described for their anti-microorganism effects against several bacteria, fungi, and even some pathogens, such as *Vibrio cholerae*. Specifically, *Enteromorpha linza* EO is effective against *B. cereus* and *S. aureus* [42], *Baccharis dracunculifolia* EO is active against *S. aureus* and *E. coli* [43], *Senecio nutans* EO is effective against *V. cholerae* [44], *Syringa yunnanensis* EO is effective against *S. aureus* [45], *Carum copticum* EO is capable of reducing the growth of *E. coli* O157:H7 [46], and *Xanthium strumarium* L. EO is also effective against *S. aureus*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans*, and *A. niger* [47]. In contrast, basil, chamomile blue, oregane, thyme, and tea tree oil EOs were not sufficiently effective against *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* [48].

EOs from plants from different regions of the world have been studied. In this sense, EOs derived from *Aloysia citriodora Palau*, which is harvested in different regions of Morocco, showed significant antimicrobial activity against both Gram-negative and Gram-positive bacteria [49]. The EOs of *Peperomia pellucida*, an herbaceous plant from the Amazon region, exhibited strong antibacterial activities against six different bacteria strains [50]. Salem et al. evaluate the biological activity of the EOs derived from *Corymbia citriodora* leaves and *Cupressus macrocarpa* from Egypt. While the antibacterial activity of EO from *C. citriodora* leaves has MIC values ranging from 0.06 to 0.20 mg/mL, EO from *C. macrocarpa* branchlets showed less activity against bacterial strains [51].

In recent years, there has been a dramatic increase in resistance to antimicrobial drugs against *Salmonella* Enterica and *Campylobacter* spp. *Campylobacter* spp. is one of the most common causative agents of gastroenteritis in the world, whereas salmonellosis is a major foodborne disease worldwide. Bacteria can be transmitted to humans by the consumption of contaminated poultry, eggs, beef, milk, juices, fruits, and vegetables. Several studies have shown that EOs could be used as alternative therapeutics to treat antibiotic-resistant *Salmonella*. In this regard, *Ruilopezia bracteosa* EO has been described as being effective against *S. aureus* and *E. faecalis* compared with several antibiotics [52]. Similarly, Ashraf et al. studied the effect of *Nigella sativa* (Black seed) oil against antibiotic-resistant isolates by a well diffusion and microbroth dilution method, and they concluded that *N. sativa* had in vitro activity against *Salmonella* Enterica [53]. Chiboub et al. evaluated the biological activity of the EOs of two varieties of *Foeniculum vulgare* in the growth of *Salmonella* Enterica, and the results showed a significant antimicrobial activity [54]. Aghraz et al. showed that EOs from *Cladanthus arabicus* and *Bubonium imbricatum* contain a potent activity against the tested *Salmonella strain*, with MIC values between 200 and 800 μg/mL for *C. arabicus* and from 400 to 1600 μg/mL for *B. imbricatum* [55]. The evaluation of the synergistic effect of mixed EOs was also investigated. To increase the sensitivity against the *Salmonella* Typhimurium strain, a mixture of *Thymus vulgaris* L., *Rosmarinus officinalis* L., and *Myrtus communis* L was used. EOs were used in combined treatment using an experimental design methodology [56]. A mixture of 55% of *T. vulgaris* L. and 45% of *M. communis* L. EOs, respectively, can be considered for the increase of *Salmonella* Typhimurium sensitivity. Mutlu-Ingok et al. studied the antibacterial activities of cardamom, cumin, and dill weed EOs against *Campylobacter jejuni* and *Campylobacter coli*. The results indicated that EOs might be effective inhibitors by directly acting at the bacterial membrane integrity level [57]. It is important to highlight that EOs derived from oregano, thyme, clove, and arborvitae also showed a very strong antibacterial activity against other bacteria causing foodborne disease; therefore, they can be used as antimicrobial agents [58].

One important concern in the food industry is the presence of biofilms. Bacteria can be suspended in liquid food, usually living planktonically, although they can easily adhere to the surface of food materials and food processing equipment, forming a bacterial biofilm. Biofilms are microbial communities that are characterized by their adhesion to solid surfaces and the production of a matrix of exopolymeric substances; the matrix consists of polysaccharides, proteins, DNA, and lipids, which surround the microorganisms, proffering structural integrity and a unique biochemical profile to the biofilm [59]. Biofilms can exist on all types of surfaces in food plants ranging from plastic, glass, metal, and wood, to food products [60], resulting in food spoilage and economic losses for the producers [60]. Several studies revealed how EOs can inhibit biofilm formation [61][62][63][64][65]. *Cinnamomum zeylanicum* oil may be a useful approach to impair the biofilm produced by Gram-negative bacteria [61]. According to Porfirio et al., *Lippia Alba* EOs have a strong inhibition of *S. aureus* biofilm formation [62]. Likewise, EOs derived from parsley and basilic can inhibit and eradicate the mature biofilm formed by *Vibrio* strains on a polystyrene surface even at low concentrations. These two EOs could be used to prevent and eradicate the contamination of sea products by these strains [64]. It has been described that quorum sensing (QS), the process through which bacterial cells communicate with each other by releasing, sensing, and responding to small diffusible signal molecules [66], is involved in biofilm formation. QS has been inhibited by the EOs of several plants, such as *Thymus daenensis* and *Satureja hortensis*. Consequently, EOs act as anti-biofilm and QS inhibitor agents against bacteria [63].

3. Antioxidant Activity of Essential Oils

The excessive amounts of reactive oxygen species (ROS) can lead to the peroxidation of lipids, glycation/oxidation/nitration of proteins, inactivation of enzymes, DNA damage, and other alterations in the cellular organelles [67][68].

In recent years, food oxidation and food spoilage caused by microorganisms form one of the most important issues facing the food industry and consumers. Accompanied by growing consumer interest in natural food additives, the search for effective antioxidants and antibacterial agents from natural resources as alternatives to suppress food deterioration is now focused on edible plants, since they present with fewer side effects than the synthetic chemicals used in today's foods [69]. There has been an increasing realization in recent years that several plant-derived EOs may possess antioxidant, antimicrobial, anticancer, and apoptosis-inducing properties [70].

Cyperus rotundus L. is a smooth and perennial weed that is widely distributed in tropical and warmer temperate regions worldwide [71]. The antioxidant properties of the *C. rotundus* rhizome were determined. In addition, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging activity, ferric-reducing antioxidant power (FRAP), and oxidative DNA damage protective effect induced by Fe^{2+} and 2,2′ -azobis (2-methylpropionamidine) dihydrochloride (AAPH) were also determined. *C. rotundus* rhizomes possessed an excellent antioxidant activity, as evidenced by in vitro DPPH, ABTS, and FRAP assays. In addition, EOs exhibited a protective effect against DNA oxidative damage induced by Fe^{2+} and AAPH, respectively [71].

An antioxidant combination effect of bay leaf, black pepper, coriander (seed and leaf), cumin, garlic, ginger, mustard, onion, and turmeric EOs was assessed by the DPPH free radical scavenging method. Only the coriander/cumin seed oil combination exhibited antioxidant activity in a synergistic interaction. Bioactive compounds responsible for this antioxidant capacity were linalool from coriander seed oil and p-coumaric acid from cumin seed oil [72]. DPPH radical scavenging activity assay, β-carotene bleaching test (BCBT), and ABTS assay were determined in *Melissa officinalis* and *Dracocephalum moldavica* EOs. Both EOs showed a strong activity in terms of the maintenance of β-carotene molecules. The ABTS radical scavenging of the EOs was dose-dependent and increased with the increase in the EOs concentration [73]. The antioxidant activity of the EO of *Ruta chalepensis* was tested by DPPH using Trolox as a reference compound. Percentages of inhibition for *R. chalepensis* collected from Jerusalem, Hebron, and Jenin were 69.56%, 61.53%, and 24.12%, respectively [74]. *Achillea millefolium* L., *Anethum graveolens* L., and *Carum copticum* L. EOs were selected to evaluate their antioxidant properties using DPPH, FRAP, BCBT, and total phenolic content assays. *A. millefolium* EO had the highest antioxidant activity in all conducted assays [75]. With a similar methodology, *Foeniculum vulgare*, *Petroselium crispum*, and *Lavandula officinalis* EOs, six different populations of *Origanum heracleoticum* L. from Calabria (Italy) Eos, and *Pelargonium asperum* and *Ormenis mixta* were analyzed. *Petroselium crispum* had the highest phenolic content and the best antioxidant profile [76], EO samples from Bagaladi and Longobucco were the most active in DPPH and BCBT assays [77], and only *Ormenis mixta* EO displayed an effective antioxidant ability, as tested by DPPH assay [78].

The antioxidant properties of EOs from the fruits *Dennettia tripetala* G. Baker as ripe and unripe fruit oil were tested. The ripe fruit EO has shown higher antioxidant strength than unripe fruit EO and vitamin C, but a lower activity compared to BCBT. The EOs also demonstrated strong ability in terms of scavenging three other different radicals (ABTS, lipid peroxide, and nitric oxide radicals) in a concentration-dependent manner [79]. With a similar methodology, *Jatropha gossypifolia* L and *Peperomia pellucida* (L.) Kunth were tested. The EOs effectively reduced oxidants to neutral molecules in a concentration-dependent manner [50][37].

Ferulago angulata—collected from natural habitats in the alpine regions of southwestern Iran—balsam fir (*Abies balsamea* (L.) Mill.), black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce (*Picea glauca (Moench) Voss*), tamarack (*Larix laricina (Du Roi) K. Koch)*, jack pine (*Pinus banksiana Lamb.*), eastern white cedar (*Thuja occidentalis L.*), Labrador tea *(Ledum groenlandicum L.)*, *Mentha spicata* EOs, and the EO of the *Pistacia vera* L. variety Bronte were analyzed using DPPH assay. The highest antioxidant activity was obtained from the EO of the Kallar population [80]; in contrast, balsam fir, black spruce, white spruce, tamarack, and eastern white cedar oils again exhibited very poor antioxidant activities [81]. The antioxidant ability of the spearmint oil was 3 μg/mL, in comparison to 11.5 μg/mL for the standard compound. This interesting biological activity can be explained by the presence of the monoterpenes limonene, terpinolene, γ-terpinene, 1,8-cineole, and carvone in the EO [82]; the *Pistacia vera* L. variety Bronte showed little affect against the DPPH test [38].

The antioxidant properties of aerial parts of *Glycyrrhiza triphylla* Fisch. and CA Mey and parsley, lovage, basil, and thyme EOs were investigated with DPPH and BCBT assays. *G. triphylla* EO exhibited a high antioxidant activity only in terms of the DPPH radical scavenging activity [83]. Parsley and lovage had a weak antioxidant activity, whereas basil showed a moderate antioxidant activity. Finally, thyme EO showed the highest antioxidant capacity [84].

Bergamot and lemon EOs extracted from the fruit peel of several citrus varieties were analyzed to determine their antioxidant activity through a thiobarbituric acid reactive substances (TBARS) test in a fish model (sardine). Samples of sardine treated with the bergamot EO displayed greater antioxidant activity than lemon EO [85].

4. Immunomodulatory Activity Effects of Essential Oils in Cells and Animals

Inflammation is a complex immune response against different types of harmful factors. Pathogenic microorganisms, irritants compounds, or damaged tissue induce an acute inflammatory response that can persist for a short period of time, which is beneficial for the host. In spite of this, if resolution of the inflammation is not adequate or the stimulus persists, then it is called chronic inflammation, which predisposes the hosts to different diseases such as cancer, cardiovascular disease, neurological disease, and metabolic disorders. During a chronic inflammation response, different signaling pathways are activated, leading to the overexpression of pro-inflammatory genes and proteins such as the NF-κB transcription factor and cytokines including IL and TNF-α. This inflammation is also related to an increased release and accumulation of ROS and reactive nitrogen species (RNS). When ROS production is greater than the cellular antioxidant capacity, oxidative stress can harm lipids, proteins, and DNA [86]. In this sense, EOs are of the greatest interest because of their anti-inflammatory and antioxidant properties, which are a potential source for the development of functional foods.

In general, EOs did not produce any cytotoxic effect when they were used at low concentrations; indeed, in human blood-isolated lymphocytes from healthy donors, *Pistacia vera* L. EOs significantly increased cell viability [38]. However, a high dose can have a negative effect on cell viability. In the case of malignant cells, it has been described that EOs derived from *Heracleum pyrenaicum* subsp. *orsinii* inhibited cell growth, which is in agreement with the established criteria from the National Cancer Institute (NCI), whereas they showed no toxic side effects on normal MRC-5 cells [41]. In line with these results, *Pituranthos tortuosus* EO is able to inhibit cell proliferation in a concentration-dependent and time-dependent manner on B16F10 melanoma cancer cells, which is likely by an increased apoptotic pathway [87]. Likewise, in human colonic adenocarcinoma cancer cell lines (HT29-D4 and Caco-2 cell), *Allium roseum* L. EO has a growth-inhibitory effect in a dose-dependent manner, without being cytotoxic. This effect has been attributed to the presence of sulfurous compounds as the major constituents of this EO [40]. Conversely, *Cirsium japonicum* DC EOs could promote cell proliferation in the human pulmonary adenocarcinoma A549 cell line [88].

On the other hand, in LPS-stimulated murine macrophage RAW264.7 cells, treatment with EOs derived from *Trachydium roylei*, *Artemisia argyi*, and *Chmaecyparis obtusa* has been shown to inhibit the secretion of pro-inflammatory cytokines, whereas treatment with EOs derived from *Trachydium roylei* also increased the secretion of IL-10, which is an anti-inflammatory cytokine. Therefore, the regulation of cytokines in this cell model may be one of the mechanisms by which EOs have an anti-inflammatory effect [22][89][90][91]. In the case of *Artemisia argyri* EOs, the regulation of NF-κB and AP-1 translocation has been proposed as a possible mechanism for its anti-inflammatory effect. In addition, a significant phosphorylation of JAK2 and STAT1/3 was also observed, but not the activation of NF-κB and mitogen-activated protein kinase (MAPK) cascades [22]. Other important mediators in inflammation are the production of nitric oxide (NO), secretion of prostaglandin E2 (PGE2), and the production of ROS. EOs have been shown to affect the expression of inducible nitric oxide synthase (iNOS) and cycloxygenase-2 (COX-2) expression; therefore, they might affect the secretion of NO and PGE2. In line with this, *Artemisia argyri* and *Trachydium roylei* EOs have been described to alter iNOS and COX-2 gene and protein expression, and to inhibit NO and PGE2 secretion and ROS production [22][91]. In immune human cells, there are studies showing that EOs exert their anti-inflammatory effects through the regulation of cytokine secretion and ROS production [92][93].

Similarly, in C57BL/6 mice, treatment with EOs has been shown to be efficient in reducing the levels of pro-inflammatory mediators [94][90]. In the contact hypersensitivity response, treatment with *Litsea cubea* L. EOs was able to inhibit the immune response [94]. In one interesting article, Sutili et al. described the use of *Hesperozygis ringes* and *Ocimun americamun* in silver catfish exposed to *Aeromonas hydrophila*, where this EO significantly decreases the hematocrit values and increases the plasma cortisol level and complement system activity. These results indicated a potential use of EOs in the treatment of infected fish [95].

One possible use of EOs is in poultry production as a supplement in the diet to improve production and to decrease the use of antibiotics. Their use in broiler chicken has been shown to improve animal growth. One interesting point is the effect of EOs in gastrointestinal microbiota composition, where supplementation with them has been shown to exert a positive effect—decreasing the pathogenic microorganism while increasing the number of probiotic bacteria such as *Lactobacillus* spp. [96][97][98]. Correspondingly, in weaned piglets, EOs or their main active compounds positively modulated gastrointestinal microbiota [23][99][100]. In addition, the use of carvacrol and thymol enriched protein biosynthesis, amino acids, and lipid metabolism [100].

Owing to this, EOs could be useful to inhibit pathogenic bacteria without affecting gastrointestinal commensal bacteria. Using static batch culture systems inoculated with human feces, Thapa el al. have shown that several EO compounds selected for their effectiveness against gastrointestinal pathogen need not have a toxic outcome on commensals bacteria at concentrations that would probably suppress pathogen bacterial growth. In this regard, the relative proportion of bifidobacteria was increased, while *Bacteroidetes* and *Clostridium* clusters IV and XIVa were not significantly affected. In terms of fermentation, except for high concentrations of thymol and geraniol, the essential oil compounds had no effects [101].

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