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Review

Antimicorbial Potency of Major Functional Foods' Essential Oils in Liquid and Vapor Phases: A Short Review

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Abstract: Due to the increasing risk of chemical contaminations in the application of synthetic fungicides, the use of plant essential oils and extracts has recently been increased. In the present review, the antimicrobial potential of the most active plant-food essential oils in liquid and vapor phases has been reviewed. The volatile isothiocyanates, aldehydes, and phenols, including allyl isothiocyanate, carvacrol, thymol, and eugenol, are considered to be the predominant components of essential oils, possessing significant antimicrobial activities. These components alone or in mixture can be effective. Overall, the antimicrobial activity of aroma compounds depends on the plant species, concentration, and method of application. This review provides useful information about the inhibitory application of the most common plant-foods' essential oils in liquid and vapor phases against the growth of pathogenic microorganisms. Essential oils (EOs) are promising natural antimicrobial alternatives in food processing facilities. Although the food industry primarily uses spices and herbs to impart flavor, aroma, and pungency to foods, potent EOs represent interesting sources of natural products for food preservation.

Keywords: functional foods; essential oils; liquid and vapor phases; antimicrobial potencies

1. Introduction

Medicinal plants are widely consumed in cuisine as foods, additives, spices, and herbal teas. Some of them, including mint, thyme, cinnamon, lavender, and savory are also used in the pharmaceutical industry due to their helpful bioactivities, such as antibacterial, antifungal, and fever- and pain-relief properties. The abovementioned plants are considered aromatic plants and are widely applied in the food industry, for instance, as spices to improve the taste of foods [1]. Essential oils (EOs) are volatile compounds extracted from plants and may have antibacterial and antifungal properties [2]. Many of the used EOs are generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) to consume in the food industry [3]. The emergence of antibiotic-resistant microbial organisms, the spreading of foodborne diseases, and consumers' negative attitudes toward chemical preservatives have led to widespread efforts to find natural food additives. Along with processing technologies, antimicrobial and antioxidant activities can show stability and improve the quality and storage time of perishable food [4]. The continuous application of synthetic fungicides causes resistance in different fungal strains, while resistance to plant EOs will occur more slowly due to the different combination of compounds in EOs [5]. The synergistic effect of two or more natural compounds leads to an increase in antimicrobial potential, since the pathogens cannot easily resist all essential oil constituents [6]. The composition of EOs should be considered in their use as preservatives, because the diversity of chemical compounds may affect their biological activities. In many cases, the main constituents of EOs show bioactivity, while small amounts of EOs play an important and vital role in improving their bioactivity [7]. The type of compounds in the essential oil is one of the things that should be considered in their use as a preservative, because the variability of the chemical composition may biologically affect them. Moreover, it is very difficult to detect the exacerbating effects of some bioactive components on each other because their amounts of EOs vary. In recent decades, numerous studies have investigated the chemical composition of EOs and plant extracts which possess antimicrobial potency [8–13] (Table S1). The susceptibility of different microbial species also differs correlating to the plant's type of EO and its different concentrations [14]. Previous studies have demonstrated that EO constituents are good microbial agents, where the microbial potency decreases in the following order: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [13]. In the present study, we comprehensively reviewed the chemical composition and antimicrobial potential of EOs by searching the keyword "chemical composition and antimicrobial" in the PubMed and Web of Science databases (last search: 25 September 2020).

2. Major Essential Oil Components

Terpenes, comprising over 7000 derivatives, are considered to be major EOs compounds and are divided into several groups. Despite their wide chemical diversities, based on their hydrocarbon structure they are classified into two major groups: terpenoids and phenylpropanoids. Both groups contain phenolic compounds, which in most cases constitute the main composition of most EOs [15]. Terpenoids are divided into alcohol (e.g., geraniol, menthol, linalool, citronellol), ester, aldehyde (e.g., geranial, neral), ketone (e.g., carvone, camphor), and phenol (e.g., thymol, carvacrol) derivatives. Terpenes possess a hydrocarbon skeleton, which is classified by the number of isoprene units (C_5H_8), including monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20). Moreover, the major phenylpropanoids include cinnamaldehyde, cinnamyl alcohol, chavicol, eugenol, estragole, methyl eugenol, and methyl cinnamate [16,17]. In Table 1, the major volatile terpenoid compounds which are potent against fungi/bacteria pathogens are mentioned. Figure 1 exhibits the main chemical structures of the essential oil constituents.

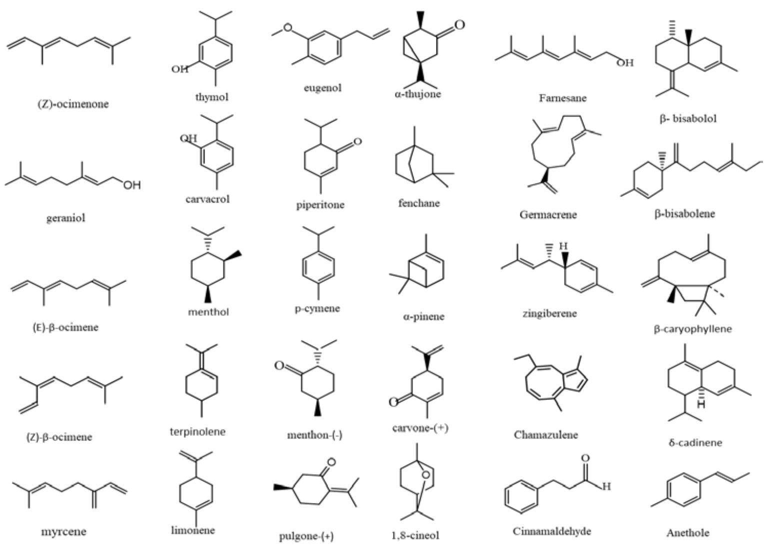

Figure 1. Chemical structures of the main essential oil constituents.

Table 1. Major terpene constituents of essential oils documented in the literature.

Monoterpene	acyclic	hydrocarbons	o-cymene, β-myrcene, β-ocimene
		alcohols	geraniol, linalool, nerol
		aldehyde	geranial, citronellal
	monocyclic	aldehyde	puelgone, perillaldehyde, phellandral, carvenone
		alcohols	menthol, limonene, terpinolene
		phenol	carvacrol, thymol, eugenol
	bicyclic	caranes	δ-carvone
		thujanes	sabinene, α-thujone
		pinenes	α-pinene, β-pinene
		camphanes	camphene
		fenchanes	fenchyl alcohol
Sesquiterpenes	acyclic	farnesanes	farnesane
	monocyclic	bisabolanes	zingiberene, β-bisabolene, α-bisabolol
		germacranes	germacrene
		elemanes	elemane
	polycyclic	caryophyllenes	caryophyllene, cadinene
		eudesman	chamazulene,
		furanoeudesmas	humulene
Diterpenes	acyclic	phorbasins	subersin
	monocyclic	zoapatanols	zoapatanol
	polycyclic	labdanes	labdane
		clerodanes	clerodane, cascarillins, teughrebin

2.1. Monoterpenes

Monoterpenes possessing 10 carbons in their structures ($C_{10}H_{16}$) are divided into three main groups, including acyclic (linear), monocyclic, and bicyclic. The most linear and mono-cyclic monoterpene structures are based on 10-carbon and para-methane structures, respectively. Bicyclic monoterpenes are very structurally diverse, mainly comprising caranes, thujanes, pinenes, camphanes, and fenchanes. Further variability can be gained by adding double bonds (oxidation) or removing them (reduction), and also by combining with oxygen in the forms of alcohol (-OH), ketone (-CO), aldehyde (-CHO), and ester (-OCO) compounds [18].

2.2. Sesquiterpenes

Sesquiterpenes have a 15-carbon skeleton ($C_{15}H_{24}$). Sesquiterpene alcohols, known as farnesols, are divided into three main groups, including linear (e.g., farnesane (*E*,*E*)), mono-cyclic (e.g., β-bisabolene, cyclofarnesane), and polycyclic (e.g., caryophyllene, humulane) derivatives [19].

2.3. Diterpenes

One of the most important terpenoid compounds is composed of four isoprene units. These terpenoids contain 20 carbons in their skeleton ($C_{20}H_{32}$). Its component is geranylgeranyl diphosphate, which is formed by the binding of an IPP (isopentenyl pyrophosphate) group to farnesyl diphosphate through a head-to-tail reaction [20].

3. The Assessment Methods of Essential Oils' Antimicrobial Potency

The antimicrobial activity of essential oils has been previously evaluated by disc diffusion and microbroth dilution assays.

3.1. Disk-Diffusion Agar Method

This method can be designed by applying paper disks, which are placed on agar media or through wells created in agar media. The effect of essential oil on the desired microorganism is mainly evaluated through the halo zones created around the disk. This method has some drawbacks, including the volatility of the compounds during heating and the lack of the proper release of compounds with antimicrobial properties in the agar media [21].

3.2. Microbroth Dilution Method

This method has been considered as one of the most common assays for testing the antimicrobial potency of various materials, quantitatively as well. The MIC value is determined through the study of apparent growth, light density or opacity, colorimetric methods, and microorganism counting [22].

3.3. Vapor Method (Essential Oil Application in Vapor Phase)

In diffusion assays, EO components are partitioned through the agar according to their affinity with water, whilst in dilution methods a low water solubility has to be overcome by the addition of emulsifiers or solvents (such as Tween 80, DMSO, ethanol), which may alter the activity [23]. The EOs components and their relative volatilities determine the characteristics of their vapors, which in turn has an impact on the antimicrobial potential [21]. The EO in vapor phase could highly be effective against food-borne pathogens and spoilage bacteria at relatively lower concentrations than in the liquid phase, thereby causing a minimum effect on the organoleptic properties [10], though it should be noted that EO consumption in high concentrations should not affect the taste.

EO application in vapor form at low concentrations is more effective than in the liquid phase, therefore it has no unpleasant impact on the quality characteristics and taste [24]. The antimicrobial properties of EOs are usually reported in high concentrations of these compounds, which change the taste and smell of food and affect its quality properties; to reduce this impact, vapor is an alternative to the direct use of these compounds [25]. The distance application of EOs in gas form may control pathogens without having any negative influence on food quality [26]. The EO application of thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), and lavender (*Lavandula pubescens*) in vapor phase demonstrated a higher inhibitory effect than liquid, explaining that the diminished activity of the EO in liquid media was due to the presence of detergents and lipophilic materials [27].

4. Antimicrobial Activities of Plant-Food EOs in Liquid and Vapor Phases

4.1. Antifungal Activities of EOs in Liquid and Vapor Phases

The antimicrobial power of EOs describes with parameters such as the minimum inhibitory concentration (MIC) and the minimum fungal concentration (MFC) [21] (Table S1). Plant EOs antifungal properties are correlated with their constituents. Lemongrass (*Cymbopogon martinii*) EO with 88% carbonyl compounds, such as geraniol (48.14%) and neral (38.32%), compared to *Citrus aurantifolia* and *Citrus limon* EOs, with 91% and 79% monoterpene hydrocarbons and possessing a much lower content of carbonyl groups, which showed more fungicidal properties against *Botrytis cinerea* [28,29].

Citrus sinensis EO indicated an MIC value of 1600 mg/L in direct application and 800 mg/L in vapor application against *Aspergillus flavus*, and the main fragrant compounds were identified as limonene, β-myrcene, α-, β-pinene, as well as (*Z*)-, (*E*)-citral. Of these, limonene represented 96.62% [10,30]. Aguilar-González et al. (2015) reported the MIC of *Syzygium aromaticum* EO with a 75.39% eugenol content as 92.56 $\mu L/L_{air}$, while for *Brassica nigra* EO with 98% allyl isothiocyanate it was 15.42 $\mu L/L_{air}$ against *Botrytis cinerea* [31]. Mejia-Garibay et al. (2015) reported that 41.1 $\mu L/L_{air}$ of *Brassica nigra* EO with 98.88 allyl isothiocyanate delayed the growth of *Penicillum citrinum*, *Aspergillus ochraceus*, and *Aspergillus niger* [32]. Another study reported the positive effect of *Origanum dictamnus* EO in preventing *Botrytis cinerea* growth, possessing an MIC of 100 μL/L [33]. The high sensitivity of fungal species to oregano (*Lippia berlandieri*), thyme (*Thymus vulgaris*), or mustard (*Brassica nigra*) EOs with an

MIC of 0.012–1.0 µg/mL of air has been reported. The major volatile compounds are allyl isothiocyanate in mustard EO; *p*-cymene, linalool, and thymol in thyme EO; and *p*-cymene and carvacrol in oregano EO [34].

Raeisi et al. (2019) also showed that the antimicrobial activity of *Mentha piperita* and *Zataria multiflora* EOs is correlated with the presence of monoterpenes such as menthol (39.18%), menthone (21.64%), carvacrol (36.81%), and thymol (33.04%) [35]. The amount of monoterpene hydrocarbons in *Mentha piperita* EO in the vapor phase was analyzed as 62.8%, three-fold that of the liquid phase. The MIC and MFC values varied from 1.13 to 2.25 mg/mL and 2.25 to 4.5 mg/mL for *Penicillium digitatum*, *Aspergillus flavus*, *Aspergillus niger*, *Mucor* spp., and *Fusarium oxysporum.* The rapid solubility of these compounds in the cell membrane in the gaseous phase is due to the strong antimicrobial properties of this EO [25].

The antifungal effect of *Satureja bachtiarica, S. khuzistanica,* and *S. rechingeri* EOs was studied by Saharkhiz et al. (2016), with an MIC 0.062 µL/mL, where the main chemical constituents of EOs are identified as monoterpenoid derivatives in the majority of thymol (87.7%) and carvacrol (28%) [36]. In a similar study, the potent antifungal properties of EOs in three *Satureja* species (*S. hortensis, S. spicigera,* and *S. khuzistanica*) were reported in the control of fungi microorganisms *Botrytis cinerea*, while high concentrations of phenolic monoterpenes, including carvacrol and thymol, have been documented in those EOs. Considering that these components are produced by the metabolic pathway of the auto-oxidation of γ-terpinene and *p*-cymene, they are assumed as precursors of oxygenated derivatives. In this study, the MIC values of *Satureja* species EOs have been measured as between 1200 and 600 µL/L against *Botrytis cinerea* [11].

Gamma-terpinene and cumin aldehyde are reported as the predominant constituents of *Cuminum cyminum* EO. The fungicidal properties of *C. cyminum* are mostly associated with volatile phenolics such as cuminaldehyde, *p*-cymene, β-pinene, and α-terpinene [37]. *Eucalyptus globulus* EO, mainly containing the potent ingredients of 1,8-cineole, limonene, *p*-cymene, terpinene, α-pinene, and α-terpineol, shows a higher antifungal activity in vapor phase compared to direct application on spoilage microorganisms. The MIC amounts varied from 2.25 to 9 mg/mL for fungal strains and from 1.13 to 2.25 mg/mL for yeast strains [25].

4.2. Antibacterial Activities of EOs in Liquid and Vapor Phases

Many studies have shown that EOs possess antibacterial properties against a wide range of bacterial strains [35–37] (Table S1). The *D*-values on exposure to *Mentha piperita* EO were analyzed as 2.14 and 2.8 min and 1.4 and 12.8 min, against *Escherichia coli* and *Staphylococcus aureus*, respectively; in this study, the antimicrobial potency of *M. piperita* EO was correlated with its major constituents, such as α-terpinene (19.7%), isomenthone (10.3%), piperitone oxide (19.3%), and β-caryophyllene (7.6%). In a study carried out by Yadegarinia et al. (2006) on *Origanum vulgare* EO, among 22 identified compounds pulegone (31.54%), 1,8-cineole (15.89%), menthofuran (11.8%), and *cis*-isopulegone (9.74%) were the major constituents, and the MIC values against *Staphylococcus aureus* was ranged from 75 to 1200 µg/mL.

In an investigation of *Lavandula tenuisecta* EO, 38 components (88.6%) were characterized, where the major constituents were identified as camphor (26.9%), fenchone (22.7%), and 1,8-cineole (18.1%); the EO was effective against almost all studied bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*, with MIC and MBC values ranging from 6.25 to 50 µg/mL [38]. In a study carried out on *Tanacetum walteri* EO, thymol (22.5%), 1,8-cineole (8.2%), umbellulone (6.9%), α-bisabolol (6.3%), and camphor (5.3%) were characterized as the principal constituents; the highest antimicrobial activity of the EO was observed against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* with MIC values of 0.63 mg/mL [39].

Hajlaoui et al. (2010) showed that the EO of *Cuminum cyminum* mostly contains cuminaldehyde (39.48%), γ-terpinene (15.21%), o-cymene (11.82%), β-pinene (11.13%), 2-caren-10-al (7.93%), *trans*-carveol (4.49%), and myrtenal (3.5%), indicating MIC values of 0.078 mg/mL for Gram-positive bacteria and from 0.078 to 0.15 mg/mL for Gram-negative bacteria [8]. In one study, the major constituents of fennel (*Foeniculum vulgare*) EO were identified as *trans*-anethole (63.16%), l-fenchone (15.53%), estragole (6.43%), limonene (4.69%), and α-pinene (4.33%). In this experiment, the MIC amounts were determined by the microbroth dilution method and were in the range of 50–100 µL/mL, depending on the studied bacteria and the tested yeast *C. albicans* [40]. The antibacterial activities of *Satureja* species (*S. hortensis, S. spicigera,* and *S. khuzistanica*) EOs against Gram-positive and Gram-negative bacteria was studied by Saharkhiz et al. (2016), and the MIC value was determined to be 0.031 and 0.062 µL/mL, respectively [36]. The antibacterial effects of *Mentha piperita* EOs against *Staphylococcus aureus* and *Escherichia coli* microorganisms were in agreement with those of linalool, carvone, and *p*-menthan-1-ol contents, while the MIC value was analyzed as 0.1% [41].

The stronger activity of cinnamon (*Cinnamomum verum*) EO on *Escherichia coli* and *Staphylococcus aureus* may be due to cinnamaldehyde, the major identified constituent in its EO (92.40%). The MIC value was similar for both bacteria (1.0 mg/mL), while the MBC values were found to be 4.0 and 2.0 mg/mL against *E. coli* and *Staphylococcus aureus*, respectively [42]. Other research revealed that the EOs of garlic (*Allium sativum*), onion (*Allium cepa*), and cinnamon (*Cinnamomum cassia*) show an effective activity against *Listeria monocytogenes*. Sulfides (80%) were the most abundant compounds present in onion and garlic essential oils, while cinnamaldehyde (76.54%) is rich in cinnamon EO. In this study, the MIC values were 0.025 mg/mL for onion EO and 0.100 mg/mL for cinnamon and garlic [43].

5. EOs Mechanism of Action in Inhibiting Growth of Microorganisms

Thus far, many studies have been conducted to investigate the mechanisms of EOs in the growth inhibition of microorganisms. Since terpenes have been recognized as primary antimicrobial agents, a practical mechanism of these compounds has been attributed to the EOs [21]. The high activity of terpenes, such as thymol, carvacrol, and eugenol, is most probably correlated to their phenolic structures. The action mode of phenolic compounds is usually in the form of interference with the action of the cytoplasmic membrane, including protein stimulation and active transportation [17]. The EO inhibition capacity can be attributed to the presence of an aromatic ring attached to the polar group [44]. The widespread use of phenols and related compounds as disinfectants can be considered as evidence. For example, due to the hydroxy phenolic group in the structure of thymol, hydrogen bonding can easily be performed with active sites of enzymes; consequently, a high activity is dedicated to this substance. Borneol and geraniol have a lower inhibitory effect compared to thymol due to the lack of an aromatic ring [21].

Cinnamon (*Cinnamomum verum*) essential oil, possessing a high content of cinnamaldehyde, disrupts the function of proteins and inhibits the amino decarboxylase enzyme, thus the lipophilic part of the EO combines directly with the hydrophilic parts of the protein [42]. In the case of volatile aldehyde constituents, the growth inhibition of microorganisms is mainly due to the reactions of aldehydes with sulfhydryl groups that are effective in fungal growth, and in other cases sulfhydryl compounds are not effective in inhibiting aldehydes [21]. By calculating the molecular orbital energy of aldehydes, it was found that the antifungal activity of these compounds is related to the energy of the lowest molecular orbital, which is empty, thus the lower energy level leads to a higher antifungal activity [21]. These results indicated that the antifungal actions and activities of aldehydes, in addition to their reaction with the sulfhydryl group, are probably due to their ability to form charge transfer complexes with electron donors [45]. The examination of the fungicidal mechanism of *Melaleuca alternifolia* EOs showed that it disrupts the mitochondrial and tricarboxylic acid cycles, therefore, accumulating reactive oxygen species and disrupting the *Botrytis cinerea* fungal activity [46].

Lipids are one of the most important components in cell membranes and responsible for membrane stability and the regulation of membrane permeability to water-soluble substances [3]. Prashar et al. (2003) reported that the fat structures of cells are broken down by terpenes, since fat saturation is increased [47]. The destruction of the *Shigella dysenteriae* cell membrane by *Foeniculum vulgare* EO was confirmed [48].

Due to the hydrophobic properties of EOs, they are dispersed in the lipid part of the mitochondrial and cell membrane of microorganisms; subsequently to the increase in permeability, they lead to a leak of intracellular materials and the disruption of many biological activities [49]. Moreover, Prashar et al. (2003) believes that the lipid structures of cells are broken down by terpenes via their increasing saturation [47]. Damage to the cell wall and membrane can lead to macromolecular leakage and cell deterioration. Gram-positive bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* are more sensitive to EOs than Gram-negative bacteria such as *Escherichia coli* and *Salmonella enteritidis* [50].

In general, it is supposed that EOs possess more antimicrobial activity against Gram-positive bacteria due to the direct interaction of water-repellent chemical components with their cell membrane [51]. Gram-negative bacteria are more resistant to EOs due to their hydrophilic cell walls. This membrane prevents the penetration of hydrophobic compounds (Figure 2) [52]. Meanwhile, one study found that, after cinnamon (*Cinnamomum cassia*) treatment, the metabolic activity of *Listeria monocytogenes* was reduced by 60.26% and the secretion of extracellular polysaccharides and proteins was significantly inhibited [53]. The gaseous *Citrus limon* targets the *Listeria monocytogenes* cell wall and plasma membrane [54].

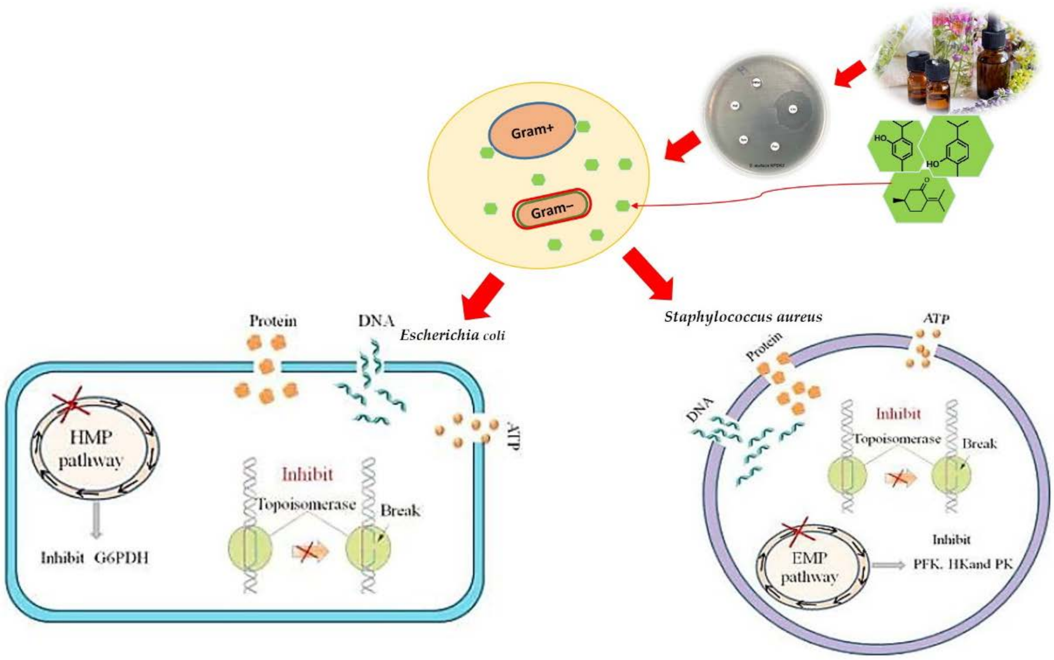

Figure 2. Mechanism of action of essential oils on Gram-positive and Gram-negative bacteria.

The EOs are able to penetrate and disrupt the cell wall and cytoplasmic membrane of the fungus and ultimately damage the mitochondrial membrane [21]. It has been reported that carvacrol reacts with cell membranes by altering the permeability of H^+/K^+ channels; thus, changing an ion gradient leads to the cessation and disruption of basic cell functions [55]. Several studies have studied the effect of EOs on fungal strains. The enhancing of nucleic acid leakage by increasing the concentration of tea tree and lemongrass EOs has been reported against *Botrytis cinerea* [3,28]. Kalemba and Kunicka (2005) attributed the morphological changes when eugenol and carvacrol were used against *Cladosporium* to the action of these compounds on several cell wall enzymes, such as chitinases [56]. Furthermore, Soylu et al. (2006) attributed the morphological changes in the mycelium to a defect in the cell wall followed by plasma membrane permeability [57].

6. Conclusions and Prospective

The inhibitory activity of EOs against microorganism growth in vapor phase is mainly higher than in liquid form and highly attributed to their major constituent and strain types. Different values of MIC, MFC, and MBC are also correlated to the different assays used for the evaluation of their antimicrobial activity. In general, EOs indicate more antibacterial activity against Gram-positive bacteria due to possessing similar membrane structures (hydrophobic) and permeability effects. Regarding the performed antimicrobial investigations on EOs derived from medicinal plants, specifically agents that are promising in vapor phase, more studies should be performed to explore safe and potent volatile natural candidates as alternatives for synthetic chemicals in the food processing industry.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/22/8103/s1: Table S1: Antimicrobial activities of essential oils of the main functional foods in both liquid and gas phases. References [58–87] are cited in the supplementary materials.

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