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Essential oils: Mode of antimicrobial activity and potential application in food systems

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ABSTRACT: *Trend of production and use of healthy safe food without synthetic chemical compounds is becoming more and more emphasized, thus consumers increasingly require the use of natural products, the so-called "green chemicals". The food industry is driven with the trend towards "Green Consumerism" gradually incorporating natural antimicrobials from plant or microbial sources into food products to replace the more traditionally used synthetic chemical preservatives.*
Taking into account that medicinal plants are generally considered as the most important source of natural antimicrobial agents, this review will summarize the published data on the antibacterial activity of those essential oils and their components that could be considered suitable for application in/on foods. Antimicrobial activity mechanisms of some natural antimicrobial compounds will be also highlighted.

ESSENTIAL OILS

Essential oils (EOs) are aromatic and volatile liquids obtained from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant, mainly by steam distillation. They usually contain between twenty and sixty, but sometimes even more than sixty individual components. The concentration of components is quite different, and major components can constitute up to 85% of the EOs, while other components can be found only in traces. These major components usually determine the biological properties of the EOs. The most common components with antibacterial properties of a number of EOs are presented in Table 1. The chemical composition, as well as the content of EO in aromatic plants, are subjected to seasonal variations and depend on phenophase of plant. In addition to the development phase of the plant, chemical composition and content of EO can be under an impact of geographical origin, climate, plant material collection time and the technique of distillation (1).

Table 1. Major components of selected[a] EOs that exhibit antibacterial properties

Common name of EO	Latin name of plant	Major components	Approximate composition (%)[b]	Reference
Oregano	*Origanum vulgare*	Carvacrol Thymol γ-Terpinene *p*-Cymene	64.50 3.50 10.80 10.90	(2)
	Origanum heracleoticum	Carvacrol Thymol γ-Terpinene *p*-Cymene	69.0 7.94 2.86 10.50	(3)
Thyme	*Thymus vulgaris*	Thymol Carvacrol γ-Terpinene *p*-Cymene	49.1 3.5 4.2 20.0	(4)
	Thymus serpyllum	Thymol Carvacrol γ-Terpinene *p*-Cymene	38.5 4.7 7.2 8.9	(4)
Rosemary	*Rosmarinus officinalis*	Camphor 1,8-Cineole Verbenone α-Pinene Borneol	17.66 16.11 13.84 12.45 9.22	(5)
Sweet basil	*Ocimum basilicum* L.	Linalool epi-α-Cadinol α-Bergamotene γ-Cadinene	56.7- 60.6 8.6 - 11.4 7.4 - 9.2 3.2 - 5.4	(6)

Clove	*Syzygium aromaticum*	Eugenol β-E-caryophyllene Acetyl eugenol α-Humulene	54.9 - 63.6 23.2 - 31.4 2.4 - 5.9 2.9 - 4.2	(7)
Cinnamon	*Cinnamonum* spp.	Cinnamaldehyde Benzaldehyde	92.4 1.5	(8)

[a] EOs which have been shown to exert antibacterial properties *in vitro* and for which the composition could be found in the literature. [b] Percentages of total volatiles rounded up to the nearest whole number.

ANTIMICROBIAL ACTIVITY

Essential oil constituents are a diverse family of low molecular weight organic compounds with large differences in antimicrobial activity. According to their chemical structure, the active compounds can be divided into several groups: alcohols, phenols, aldehydes, ketones, hydrocarbons and ethers.

Phenols exhibit the largest antimicrobial effect and they are mainly present in the highest percentage in the EOs, followed by alcohols, aldehydes, ketones, ethers, whereas the antibacterial effect of hydrocarbons is very low (9). Although the antimicrobial effect of EO is mainly attributed to phenols, the influence of components present in traces should not be neglected because of the potential interactions that may affect the antimicrobial activity. In a number of studies it has been shown that EO or a mixture of EO components may have a greater antimicrobial effect compared to the individual active components of EOs (10-12). These studies suggest that the antimicrobial activity of the EOs is a result of interactions between different classes of compounds present in the EO, although in some research activity of the EO is closely associated with the activity of the main components of EO (13). In terms of interactions between different classes of compounds present in the EO three effects can be highlighted: additive, antagonistic and synergistic. The additive effect occurs when the combined effect of the components is equal to the sum of the individual effects. Synergism is registered when the activity of the combined substances is higher than the sum of the individual activities. In contrast, the antagonistic effect is registered when the activity of components in combination is inferior in comparison with their separate application. (14). Important characteristics responsible for the antimicrobial action of EOs include hydrophobic components that allow the participation of lipids from the bacterial cell membrane, which disturbs cell structures and make them more permeable. Selected MICs (Minimal Inhibitory Concentration) of essential oils and their components tested *in vitro* against food borne pathogens are presented in Table 2 and Table 3.

One of the principles of antimicrobial effect of EOs is based on their hydrophobic feature, owing to easier incorporation within the lipid bilayer cellular membranes of bacteria causing disturbances in its structure, permeability and flow of protons with a decline in membrane potential, intracellular pH and synthesis of ATP. When osmotic cell equilibrium is disturbed in this manner, the secondary effect is cell death (15). This mode of action of EOs has been confirmed by electron microscopy in *Escherichia coli* (16).

Even though the mechanism of antimicrobial effect of EOs was the subject of numerous studies during the decades, the link between their antimicrobial activity and chemical structure is still not completely clear. Considering the large number of different chemical compounds constituents of EOs, it can be assumed that the antimicrobial activity of EOs is not based on just one specific mechanism. Until now there are several target positions described and their mechanisms of antimicrobial action: degradation of the cell wall, damage to membrane proteins, damage to cytoplasmic membrane, leakage of cell contents, coagulation of cytoplasm and depletion of the proton motive force (Figure 1)(13).

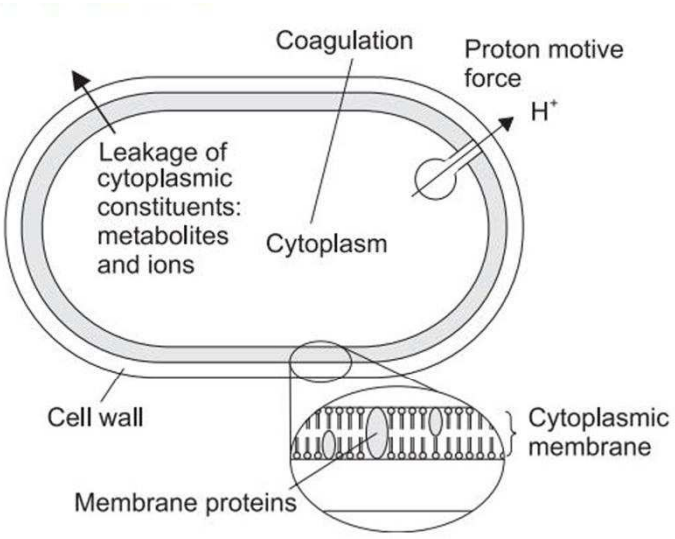

Figure 1. Location and the mechanisms of antimicrobial action of essential oils on bacterial cell (13)

MODE OF ANTIBACTERIAL ACTION

Considering that the most of antimicrobial effect is exhibit by phenols, considerable research is directed towards testing their antimicrobial activity and mechanisms of action. Also, it has been postulated that most of the active antimicrobial components of the EOs are phenolic compounds, and their mechanism of action should be similar to phenols. The antimicrobial activity of phenols has been shown to be concentration dependent; at lower concentrations they may inhibit enzyme activity while at high concentration they cause protein denaturation. Phenolic compounds also have the ability to alter the bacterial cell membrane permeability leading to the loss of macromolecules thereby negatively affecting the microbial growth and energy production, leading to cell death (17).

Carvacrol is a monoterpenoid phenol, with a hydroxyl group and delocalized electron system that contribute to its antimicrobial effect. The mode of action of carvacrol, one of the major components of oregano and thyme oils, appears to have received the most attention from researchers. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring (Figure 2). Both substances appear to make the cell membrane permeable (18). The mechanism of action of carvacrol has been hypothesized by Ultee et al. (19) and according to this theory carvacrol acts as the transmembrane holder of monovalent cations. The carvacrol in undissociated form passes through the cytoplasmic membrane into the cytoplasm, dissociates by releasing a proton, which results in the attraction of potassium ions or other ions. After binding of this ion, carvacrol migrates into undissociated form through the cytoplasmic membrane outside the cell. Efflux of potassium ions and influx of hydrogen ions impairs intracellular pH, which leads to reduced synthesis of ATP. As a result in this way disturbed homeostasis leads to cell death (Figure 2).

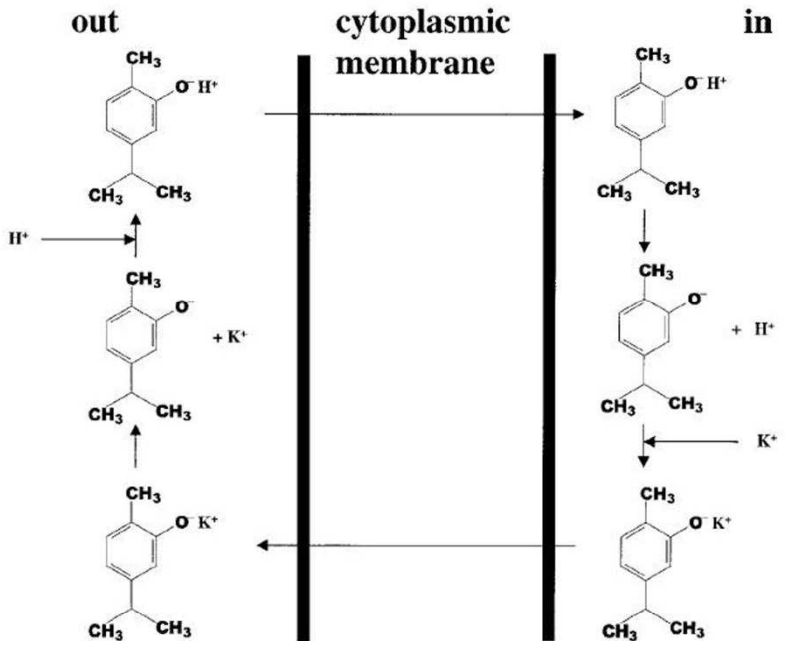

Figure 2. Schematic overview of the hypothesized activity of carvacrol (19).

In the case of phenolic compounds it has been proven that the presence of hydroxyl groups and systems of delocalized electrons in the phenol ring has influence on the antimicrobial activity, while the relative position of these groups has no significant impact. Comparing the antimicrobial effect of carvacrol to his isomer thymol, which also possesses a hydroxyl group, with the system of the delocalized electrons (the double bond) at meta position, there are no determined differences in their antimicrobial activity, while in the case of the methyl ester of carvacrol comprising the ethyl ester instead of the hydroxyl group, and p-cymene who lacks hydroxyl group, antimicrobial effect was not determined. Significance of the hydroxyl groups and system of delocalized electrons can be viewed at a much lower activity of menthol with respect to carvacrol. Namely, menthol possesses hydroxyl group in its ring, but its antimicrobial activity was not established. It is assumed that the absence of antimicrobial activity is consequences of the lack of a system of delocalized electrons (double bonds), due to its hydroxyl group that is not able to dismiss proton (19).
This coincides with the findings of Veldhuizen et al. (20) who was comparing the activity of carvacrol and 2-amino-p-cymene, analogue of carvacrol, in order to determine the importance of the hydroxyl groups on the activity of carvacrol. This study revealed a three-fold greater activity of carvacrol compared to 2-amino-p-cymene, and thus it is shown that the hydroxyl group influences the antimicrobial activity of carvacrol. Studies have shown

that phytophenols affect the proteins of the cytoplasmic membrane and transport of the protein through the channels. Interaction of these compounds with a membrane proteins leads to reduction or complete inhibition of their activity. Two possible mechanisms have been suggested whereby cyclic hydrocarbons could act on proteins: 1. Accumulation of lipophilic molecules in the lipid bilayer, which causes the distortion of this layer thus disrupting the lipid-protein interactions; 2. The direct interaction of a lipophilic component with a hydrophobic part of the protein (13).

Hydrocarbon monoterpenes p–cymen and γ-terpinen are biochemical precursors of carvacrol and thymol that do not exibit antimicrobial activity, but it has been noticed that their presence enhances the antimicrobial effect of carvacrol and thymol (21). p-Cymene is hydrophobic, and treatment of bacteria with these hydrocarbon monoterpenes leads to a stronger swelling of the cytoplasmic membrane with respect to treatment with only carvacrol. As the antimicrobial activity of the mixture is greater than the antimicrobial effect of the individual components, it is clear that between these two compounds exists synergism.

p-Cymene appears to incorporate into the cytoplasmic membrane causing its swelling and very likely facilitates transport of carvacrol across the cytoplasmic membrane. Result of joint action of two compounds leads to destabilization of the cytoplasmic membrane, the fall of membrane potential, intracellular pH reduction and disruption of ATP synthesis causing cell death. p-Cymene in the absence of carvacrol, except swelling of the cytoplasmic membrane, causes only a slight decrease of membrane potential (19, 22). Eugenol and cinnamaldehyde are phenylpropenes synthesized from phenylalanine and contain a six-carbon phenol group. Eugenol has similar bactericidal activity as thymol and carvacrol, incorporating into the cellular membrane and altering surface and structural proteins. Both compounds are believed to inhibit cellular metabolism and potentially serve as ATPase inhibitors, while cinnamaldehyde may also act through membrane disruption (23, 24). Further theories describing the potential antibacterial mechanisms of EOs include the inhibition of enzymatic production or activity required for energy generation, disruption in the generation process of ATP, or depletion of ATP already present within the cell (25).

Table 2. Selected MICs of essential oils tested *in vitro* against food borne pathogens

Plant from which EO is derived	Species of bacteria	MIC, approximate range ($\mu L/mL^{-1}$)[a]	Reference
Origanum heracleoticum	*Salmonella* Enteritidis	0.025 - 0.625	(26, 27)
Origanum vulgare	*Salmonella* Typhimurium	1.2 - 0.312	(27 - 29)
Ocimum basilicum	*Escherichia coli* *Staphylococcus aureus* *Bacillus subtilis* *Pseudomonas aeruginosa*	1.6 - 2.6 0.9 - 1.5 0.8 - 1.4 1.7 - 2.3	(6)
Cinnamomum spp.	*Escherichia coli* *Staphylococcus aureus*	1.0 1.0	(8)
Rosmarinus officinalis	*Escherichia coli* *Salmonella* Enteritidis *Salmonella* Typhimurium *Salmonella* Choleraesuis *Staphylococcus aureus*	1.25 0.63 0.63 0.63 0.63	(5)
Syzygium aromaticum	*Staphylococcus aureus*	0.39	(30)

[a] In the references MICs have been reported in the units mg/mL^{-1} and $\mu g/mL^{-1}$. For ease of comparison these have been converted to $\mu L/mL^{-1}$, whereby it was assumed that EOs have the same density as water.

Table 3. Selected MICs of essential oil components tested *in vitro* against food borne pathogens

Essential oil component	Species of bacteria	MIC, approximate range ($\mu L/mL^{-1}$)[a]	Reference
Thymol	*Salmonella* Enteritidis *Staphylococcus aureus* *Escherichia coli*	0.2 0.31 0.4 - 5.0	(31, 32)
Carvacrol	*Salmonella* Typhimurium *Escherichia coli*	0.2 0.4	(11, 31)
Eugenol	*Escherichia coli* *Staphylococcus aureus*	1.6 0.2	(11, 30)
Cinnamaldehyde	*Escherichia coli* *Staphylococcus aureus*	0.4 0.19	(11, 30)
Linalool	*Escherichia coli* *Staphylococcus aureus* *Bacillus subtilis* *Pseudomonas aeruginosa* *Salmonella* Choleraesuis	0.4 0.9 0.3 0.9 0.4	(6, 33)

[a] In the references MICs have been reported in the units mg/mL^{-1} and $\mu g/mL^{-1}$. For ease of comparison these have been converted to $\mu L/mL^{-1}$, whereby it was assumed that essential oil components have the same density as water.

LIMITATIONS AND PERSPECTIVES IN APPLICATION OF EOs IN FOOD SYSTEMS

Despite the demonstrated antimicrobial properties of EOs and their constituents *in vitro*, their use as preservatives in food has been limited due to the fact that high concentrations are needed in order to reach sufficient antimicrobial activity. A number of previous studies have revealed that food composition and structure have a significant effect on the final outcomes of antimicrobial activity of EOs. Until now, numerous *in vitro* trials have shown that antimicrobial activity of EOs might be impaired by certain food components (fats, carbohydrates, proteins, water, salt, antioxidants, preservatives, other additives) and pH. Some extrinsic factors (temperature and the level of microbial contamination) may also affect antimicrobial potential of EOs. Namely, high concentrations of fats and proteins in foodstuffs may protect bacteria. Previous research indicated that they may provide a protective layer and absorb EOs, thus decreasing their concentration and effectiveness in the aqueous phase. Furthermore, high water and/or salt levels appear to facilitate the action of EOs. Other limiting factors for the antimicrobial activity of EOs, such as the physical structure of foods, also may reduce and affect the antibacterial activity of EOs (13).

Undoubtedly, one of the main limitations of their application as antimicrobial agents is intense flavour and odour. These limitations could be overcome with alternative approaches such as use of EOs in active packaging that is encapsulated in polymers of edible and biodegradable coatings (34). From a food safety point of view, very important is to emphasize that the majority of EOs are classified as Generally Recognized As Safe (GRAS).

CONCLUSION

Considering all explanations based on antimicrobial properties possessed by EO components, we can say that application of EOs in order to control food spoilage and pathogenic microorganisms can satisfy the requirements of trend called "Green Consumerism". Application of EOs in food could provide a more natural and attractive alternative to industry, meaning an additional barrier to inhibit the growth and survival of microorganism in food. Chemical preservatives can be replaced with EOs; this provides the opportunity for "Green labelling" to which consumers are attracted by their 'natural image'. This is highly relevant since food quality and safety are of prime importance in the current world.

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