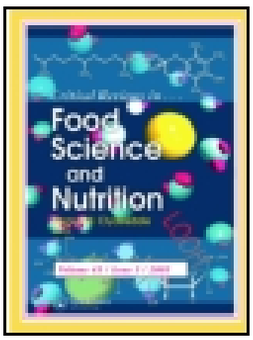

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REVIEW

Synergistic interactions of plant essential oils with antimicrobial agents: a new antimicrobial therapy

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ABSTRACT

The problem of drug resistance of food borne pathogens is becoming more and more serious. Although traditional antimicrobial agents have good therapeutic effects on a variety of food borne pathogens, more effective antimicrobial agents are still needed to combat the development of drug-resistant food borne pathogens. Plant-based natural essential oils (EOs) are widely used because of their remarkable antimicrobial activity. A potential strategy to address food borne pathogens drug resistance is to use a combination of EOs and antimicrobial agents. Because EOs have multi-target inhibitory effects on microorganisms, combining them with drugs can enhance the activity of the drugs and avoid the emergence of food borne pathogens drug resistance. This paper introduces the main factors affecting the antibacterial activity of EOs and describes methods for evaluating their synergistic antibacterial effects. The possible mechanisms of action of EOs and the synergistic inhibitory effects on pathogens of EOs in combination with antimicrobial agents is described. In summary, the combined use of EOs and existing antimicrobial agents is a promising potential new antibacterial therapy.

KEYWORDS

Antimicrobial agents; antimicrobial mechanism; essential oils; foodborne microorganism; synergistic effect

Introduction

In recent years, food safety has become a hot issue of global public health. Inappropriate or overuse of antimicrobial agents leads to the emergence and spread of drug-resistant food borne pathogens, and the emergence of multi-drug resistant pathogens is a global threat (Ayaz et al. 2019; Mikulasova, Chovanova, and Vaverkova 2016).

Food borne pathogens usually refer to the pathogenic bacteria introduced in the process of food processing and circulation. These pathogens can secrete toxic substances, which directly or indirectly lead to disease or poisoning. The common pathogens causing food poisoning are *Escherichia coli*, *Salmonella, Shigella*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Streptococcus haemolyticus* and *Staphylococcus aureus*. In recent years, more and more microorganisms that can cause human disease or poisoning have been found. These microorganisms are not only harmful to human health, but also an important cause of foodborne disease outbreaks (Ayaz et al. 2015).

One of the most effective strategies against multi-drug resistant pathogens is the combined use of plant active compounds and antibiotics. Plant essential oils (EOs) are natural antibacterial agents extracted from plants. They are usually a complex mixture of many different kinds of compounds. Each EOs contains 20–80 different molecular types (Ju et al. 2019; Rao, Chen, and Mcclements 2019; Zhang, Cao, and Liu 2020). A large number of studies have confirmed that EOs have significant antibacterial activity (Cui, Zhao, and Lin 2015; Ju et al. 2019a). The antibacterial activity of specific EOs mainly depends on their concentration and chemical composition, among which terpene, terpenoids, phenols, and aldehydes are the main components with antibacterial activity (Ju et al. 2019; Seow et al. 2014; Reyes-Jurado et al. 2020).

The purpose of combined antimicrobial agents applications is to reduce the drug resistance and toxicity of microorganisms and achieve synergistic antibacterial effects (De Azeredo and Soares 2013; Ju, Xie, Guo, et al. 2020; Liu et al. 2019). Combining natural products and antimicrobial agents reduces minimum inhibitory concentrations (MICs) and increases the sensitivity of multi-drug resistant bacteria to the antibiotics (Chung, Navaratnam, and Chung 2011; Coutinho et al. 2009; Newman and Cragg 2016; Shah et al. 2019). The mechanisms of several compounds have been tested for their ability to improve microbial resistance (Figure 1). Among them, essential oils (EOs) or plant extracts have been found to be effective against almost all targets. For example, thymol and carvol increase the permeability of the bacterial outer membrane; eugenol and citral inhibit β-lactamase; and sage acid inhibits bacterial efflux pumps (Abreu, Mcbain, and Simoes 2012; Hemaiswarya, Kruthiventi, and Doble 2008; Ju, Xie, Yu, et al. 2020).

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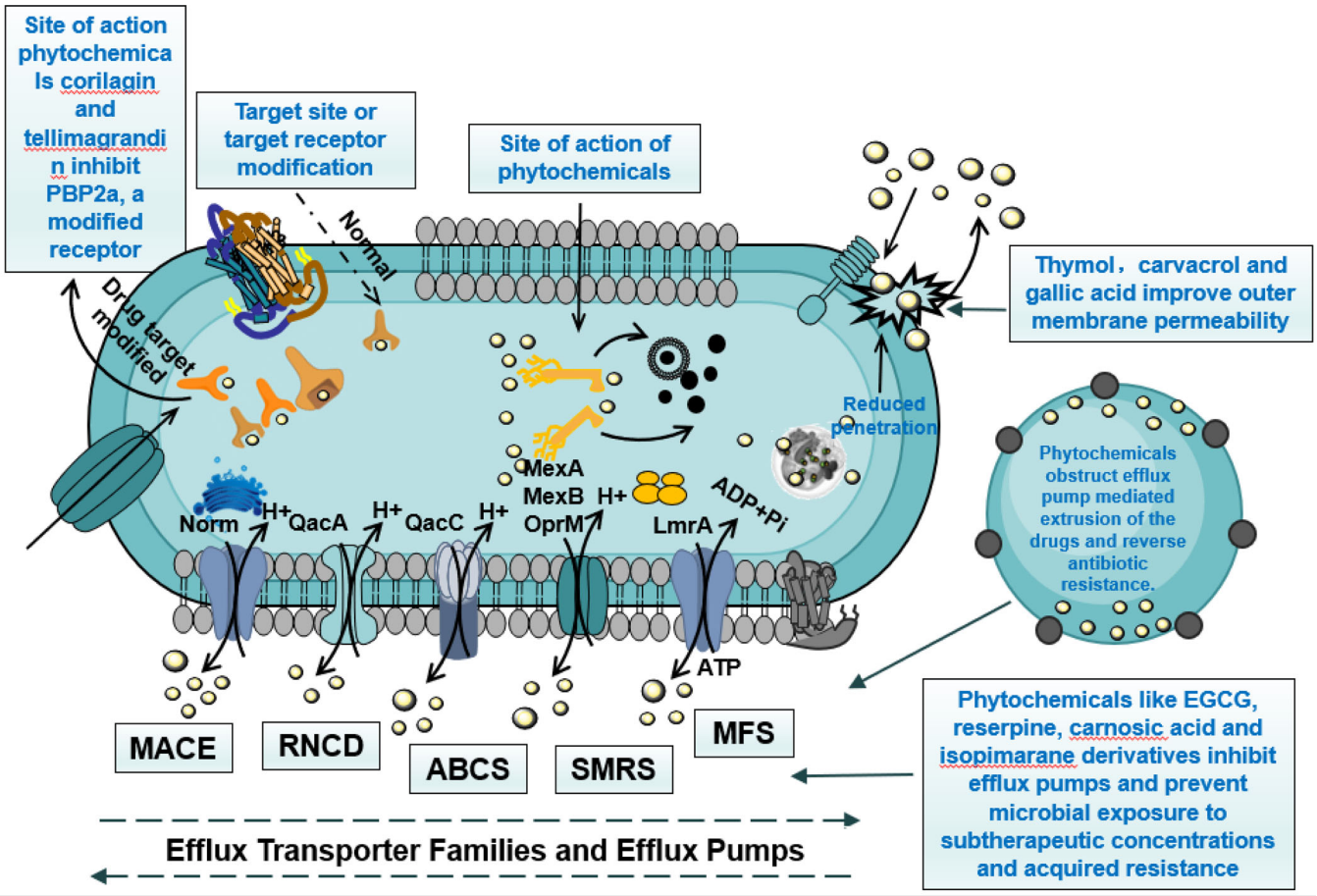

Figure 1. Mechanisms of antibiotic resistance and target sites of natural products. MATE: multidrug and aoxic compound extrusion super-family; RNCD: resistance-nodulation-cell division super-family; ABCS: ATP-binding Cassette super-family; SMRS: small multidrug resistance super-family; MFS: major facilitator super-family.

This paper has three objectives: firstly, to analyze the main factors affecting the antimicrobial activity of EOs; secondly, to comprehensively analyze the antibacterial mechanisms of EOs; and thirdly, to summarize and discuss the efficacy of EOs and their active components in combination with antimicrobial agents against pathogenic microorganisms.

Factors affecting the antimicrobial activity of EOs

Many factors affect the antibacterial activity of EOs, including the type and quantity of microorganisms, culture conditions and the presence of dispersants and emulsifiers (Holley and Patel 2005). Therefore, when evaluating the antimicrobial efficacy of EOs in different systems, it is necessary to provide accurate details of these factors.

Microorganisms

Species of microorganism

In general, the inhibitory effects of EOs or plant extracts are greater on Gram-positive bacteria than on Gram-negative bacteria (Burt 2004; Dung, Kim, and Kang 2008; Klančnik et al. 2011). This may be due to the fact that the outer membrane of Gram-negative bacteria contains more lipopolysaccharide, which is almost impermeable to lipophilic compounds, thereby allowing the bacteria to resist the infiltration of EO active components (Al-Reza, Rahman, and Kang 2009; Kotzekidou, Giannakidis, and Boulamatsis 2008). In addition, hydrolases in the periplasmic space of Gram-negative bacteria help to degrade the active components of EOs. In contrast, Gram-positive bacteria do not have this natural barrier, allowing the lipophilic active molecules in EOs to come into direct contact with the phospholipid bilayer of the cell membrane, increasing the permeability of the cell membrane and causing the contents of the cell to leak (Gao et al. 2011). However, although Gram-negative bacteria have this natural protective shell, EOs can still inhibit or kill them. This is due to the presence of porin in the outer membrane of Gram-negative bacteria, which provides a wide enough channel to allow small molecular weight compounds to pass through (Kotzekidou, Giannakidis, and Boulamatsis 2008; Seow et al. 2014).

Number of microorganisms

The antimicrobial activity of EOs depends, to some extent, on the number of microorganisms present, although no correlation has been found between the number of microorganisms and the bioactivity of EOs. Generally speaking, the greater the amount of the microorganism inoculated, the higher the MIC of the EO, and some active components tend to have better biological activity at low concentrations of microorganisms (Kalemba and Kunicka 2003; Lambert

et al. 2001). In addition, the tolerance of microorganisms to EOs also differs at different growth stages. In general, microbes in the exponential growth phase are more tolerant to EOs. Therefore, it is usually necessary to select strains in the exponential growth phase when exploring the antimicrobial activity of EOs.

Stress response of microorganisms

The stress response of microorganisms mainly refers to changes that occur in microorganisms when they are affected by physical, chemical or biological factors. There have been few studies on the effects of EO-related stress on the survival of microorganisms, and those studies that do exist have reached different conclusions. Tea tree EO has been reported to have a significant inhibitory effect on *Escherichia coli*, *S. aureus* and *Salmonella* (Mcmahon et al. 2007). However, exposure for 3 days to sublethal concentrations increased the resistance of these microorganisms to gentamicin. In contrast, it has been reported that tea tree EO did not alter the resistance of *S. aureus*, *E. coli* or *Staphylococcus epidermidis* to antibiotics (Katherine, Christine, and Carson 2012). In addition, it has also been reported that the treatment of *Listeria monocytogenes* with oregano *S. epidermidis* stress did not lead to resistance (Luz, Gomes Neto, Tavares, Magnani, et al. 2012).

Although the authors of these studies have come to different conclusions, this does not mean that microorganisms do not initiate their own protective mechanisms under stress. The stress response often leads to cross-resistance of microorganisms (Luz, Gomes Neto, Tavares, Nunes, et al. 2012). Therefore, EOs are still an option to replace some antibiotics to not only target microorganisms but also reduce resistance in some drug-resistant bacteria.

Dispersants and emulsifiers

Most EOs are insoluble in water, and only more water-soluble components can be uniformly diffused into different systems (Remmal et al. 1993; Sokovic et al. 2009). Therefore, in order to improve the solubility of EOs, many dispersants and surfactants, such as anhydrous ethyl alcohol, Tween, dimethyl sulfoxide, agar and lecithin, have been used to ensure maximum contact with microorganisms. Emulsifiers can slow the separation of EOs and water, thereby increasing the contact area between EOs and bacteria (Burt and Reinders 2003; Mann and Markham 1998). For example, it has been reported that the dispersion of citral, geraniol and linalool in Tween-20 can improve their antimicrobial activity (Kim et al. 1995). Ethanol and dimethyl sulfoxide are both polar solvents, which can enhance contact with other polar materials and improve the antibacterial activity of EOs (Chalova, Crandall, and Ricke 2010).

Cultivation conditions

Time. Longer exposure times usually result in stronger antimicrobial activity. However, there may be a maximum, that is, a point at which antibacterial activity no longer increases over time. EOs can be divided into two types depending on the length of time that they produce antibacterial activity: slow-acting and fast-acting. Some antimicrobial agents, such as cinnamaldehyde, carvanol and geraniol, are considered fast-acting because they can inactivate *E. coli* O157:H7 and *Salmonella* in as little as 5 min (Friedman et al. 2004), whereas slow-acting compounds usually take 30–60 min to show antibacterial activity.

Temperature. The effect of temperature on the antibacterial activity of EOs cannot be ignored. This is because different microbial species have different optimal growth temperatures, above or below which the activity of the microorganism is reduced. For example, the same concentration of lemon EO completely inhibits the growth of bacteria at 30 °C for 30 days, but is only active for 2–3 days at 20 °C (Moleyar and Narasimham 1992). In contrast, it has been reported that lowering the culture temperature seems to improve inhibitory effects on *E. coli* (Rivas et al. 2010).

Oxygen content. Oxygen also has an effect on the antimicrobial activity of EOs. On one hand, oxygen causes a series of chemical reactions with the active components in EOs. For example, under aerobic conditions, thymol exhibits stronger antibacterial activity. However, tea tree EO is converted into stronger antibacterial compounds under anaerobic conditions (Kalemba and Kunicka 2003). On the other hand, different oxygen concentrations may affect the growth and metabolism of microorganisms. EOs combined with modified atmosphere packaging at high concentrations of carbon dioxide (40%) have been shown to significantly inhibit the growth of bacteria (Matan et al. 2006).

Acidity and alkalinity. In general, the sensitivity of bacteria to EOs increases with decreasing pH. However, fungi do not seem to be as sensitive as bacteria. The combination of low pH and EOs may have a synergistic inhibitory effect on bacteria. This may be due to the fact that, at lower pH values, EOs do not decompose and have stronger hydrophobic effects, making it easier for them to combine with bacterial cell membranes (Rivas et al. 2010). However, acidic conditions are not always effective for all microbes, with the exception of *E. coli* O157:H7 (Friedman et al. 2004).

Antibacterial mechanism of essential oil

The antibacterial activity of EOs depends on their main components or synergism among components (Alessandra et al. 2019; Rao, Chen, and Mcclements 2019). The active components in common EOs are shown in Figure 2. The antibacterial mechanisms of different antimicrobial agents may be different. Therefore, the antibacterial mechanism of EOs is usually not a single mode of action, but multiple modes of action coexisting. Several sites in microorganisms are the main targets of EOs (Ju et al. 2019a; Khorshidian et al. 2017). Figure 3 is a schematic diagram of the possible mechanism of action of EOs (Ju et al. 2019).

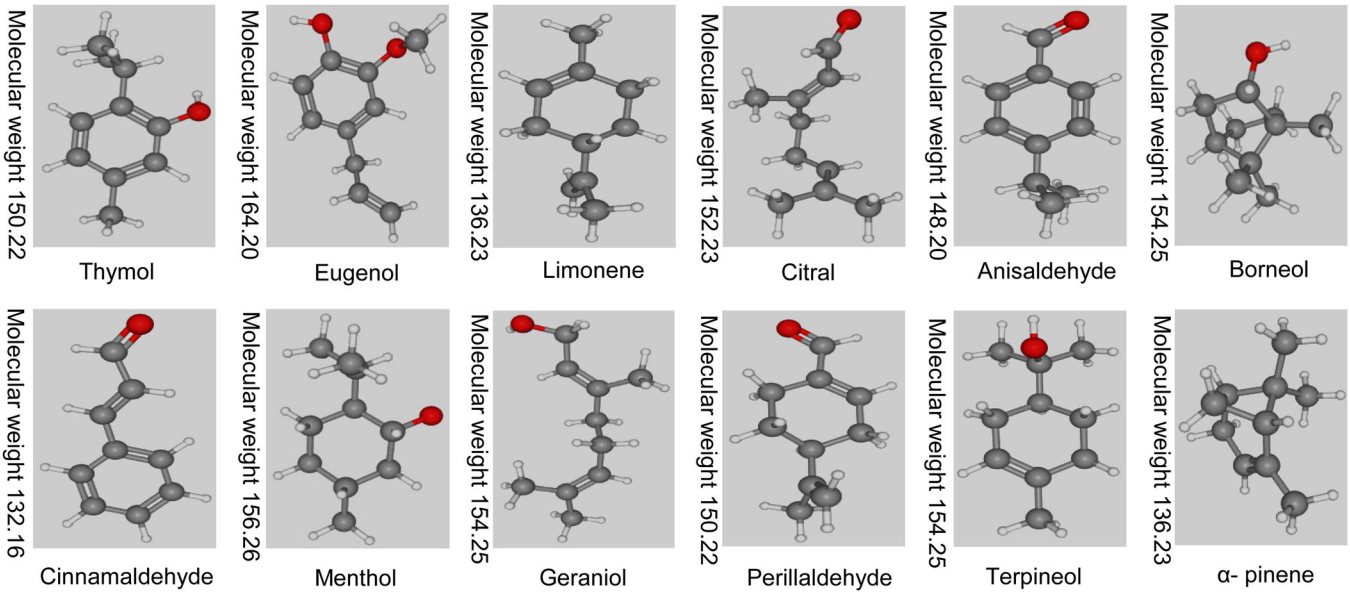

Figure 2. Molecular formula of active components in common essential oils.

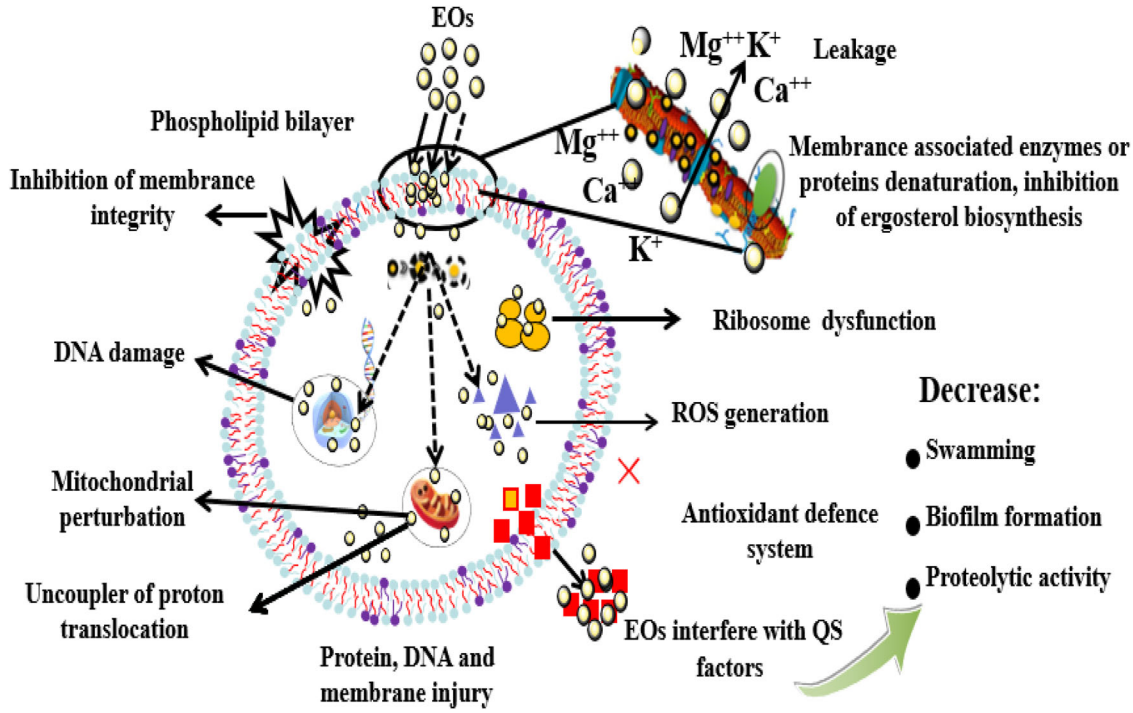

Figure 3. Schematic diagram of possible action mechanism of essential oil.

Effect on cell walls and cell membranes

The barrier function of the microbial cell wall reduces the sensitivity of microorganisms to antimicrobial agents (Meychik et al. 2011). The main components of the cell wall and the enzymes related to the cell wall are important sites of action for EOs molecules (Sun et al. 2020). The active components in EOs destroy the peptidoglycan structure or inhibit its synthesis, which damages the cell wall and deforms or kills the bacteria (Cezar, Maraschin, and Di Piero 2015). In addition, the transpeptidase needed to compete with the bacteria to synthesize the cell wall inhibits the connection between d-alanine and the pentapeptide bridge on the side chain of tetrapeptides, so the bacteria cannot synthesize a complete cell wall (Meng et al. 2020).

Cell membranes play an important role in maintaining the normal activities of microorganisms. The cellular structure of Gram-positive bacteria allows hydrophobic molecules to penetrate into the cytoplasm relatively easily. The cell walls of Gram-negative bacteria are more complex; thus, providing greater resistance to hydrophobic natural extracts or EOs (Ju et al. 2019b; Marchese et al. 2016).

EOs affect the cell membrane structure by changing the permeability of the cell membrane. For example, cinnamaldehyde easily dissolves in the fatty acyl chain of the cell membrane and destroys its outer membrane, resulting in an

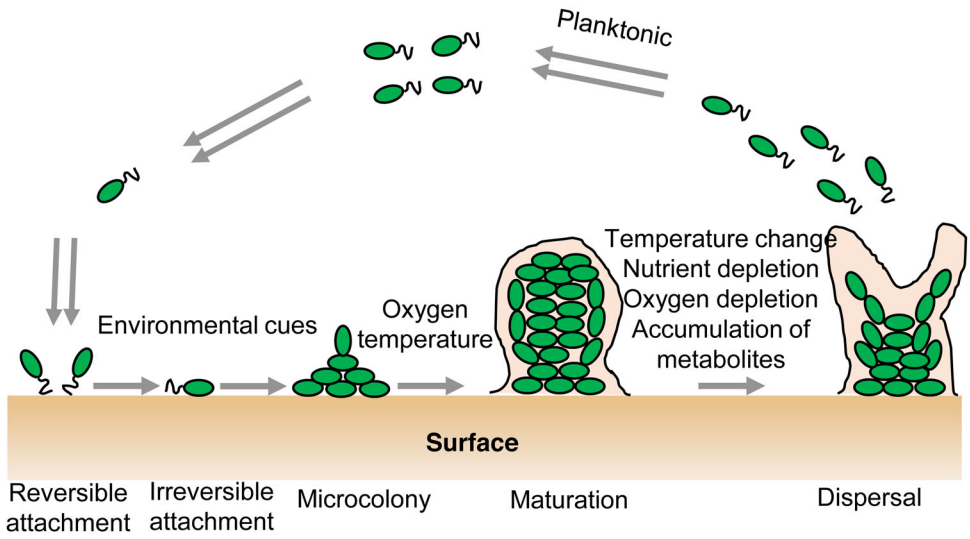

Figure 4. Schematic diagram of biofilm formation process.

increase in the permeability of the cell membrane, outflow of adenosine triphosphate, and cell death. Secondly, EOs interact with phospholipid molecules, thus changing the proportion and structure of fatty acids in the membrane. Third, EOs inhibit the synthesis of ergosterol. Consistent with this conclusion, eugenol inhibits the synthesis of ergosterol by microorganisms; thus, destroying the integrity of the cell membrane. Finally, EOs molecules can pass through porins on the cell membrane, reduce the expression of membrane porin-related genes, and destroy amino acid transporters.

Effects on respiration and energy metabolism

Respiratory metabolism is the power for microorganisms to produce energy, which is essentially oxidative decomposition of carbohydrates. The main pathways include the glycolytic pathway, the pentose phosphate pathway, and the tricarboxylic acid cycle pathway. The normal metabolic processes of the organism are hindered when oxidation and decomposition of sugars are inhibited, which can lead to death (Ju, Xie, Yu, et al. 2020). Antimicrobial agents inhibit or even prevent energy production in pathogenic bacteria. These agents can inhibit the growth and reproduction of pathogenic bacteria by blocking the absorption and transport of nutrients (Ulanowska et al. 2006). In addition, when the activities of key regulatory enzymes in the tricarboxylic acid cycle are inhibited, they can disrupt normal metabolism cycle; thus, inhibiting normal growth of the microorganism (Ju, Xie, Yu, et al. 2020). For example, thyme EOs inhibits ATP synthase activity in *Salmonella typhimurium*, disrupting the tricarboxylic acid cycle pathway (Pasqua et al. 2010).

Effect on genetic material

The genetic material (DNA or RNA) of microorganisms plays a leading role in their growth, development, reproduction, and mutation. The accuracy and stability of self-replication ensure the continuity of inheritance between parents and children). In addition, genetic material controls the synthesis and metabolism of proteins. Therefore, the destruction of genetic material will affect the normal reproduction and self-replication of microorganisms (Ya-Ru et al. 2014).

Inhibition of exercise ability and biofilm formation

Exercise ability plays a key role in the reproduction, transmission, and interaction with the host of microorganisms. The ability of pathogenic bacteria to transmit, colonize, and produce toxin includes a number of programs, such as signal transduction, chemotaxis, and flagellar movement (Josenhans and Suerbaum 2002). In addition, the regulation of cell movement may also affect the formation of complex structures in the biofilm (Picioreanu et al. 2007). The formation of a biofilm is not a single action process but a coordinated collective behavior (Jiménezfernández et al. 2016).

The basic process of biofilm formation includes microbial colonization and attachment to the external (abiotic or biological) surface (Rouse et al. 2007). Then, the biofilm changes from a planktonic state to a fixed state. An extracellular polysaccharide matrix is produced through intercellular aggregation and proliferation, and the cells are gradually bonded together to form small colonies (Chen and Wang 2020; Thien-Fah and Mah 2001; Park et al. 2019). With the continuous proliferation of microorganisms, a three-dimensional (3D) biofilm structure slowly forms (Figure 4). As the biofilm matures, it gradually degrades or separates the planktonic cells for the next cycle of biofilm formation (Vasconcelos, Croda, and Simionatto 2018). The growth of biofilms by microorganisms has many advantages, such as preventing self-drying, cooperating with each other through metabolism, and improving genetic diversity through gene transfer. Previous studies have shown that hexanal inhibits biofilm formation in *Erwinia* and *Pseudomonas fluorescens* (Brackman et al. 2011). In addition, cinnamaldehyde also has an anti-biofilm effect on *S. aureus*, suppurative *Streptococcus*, *Escherichia coli*, *Pseudomonas aeruginosa*,

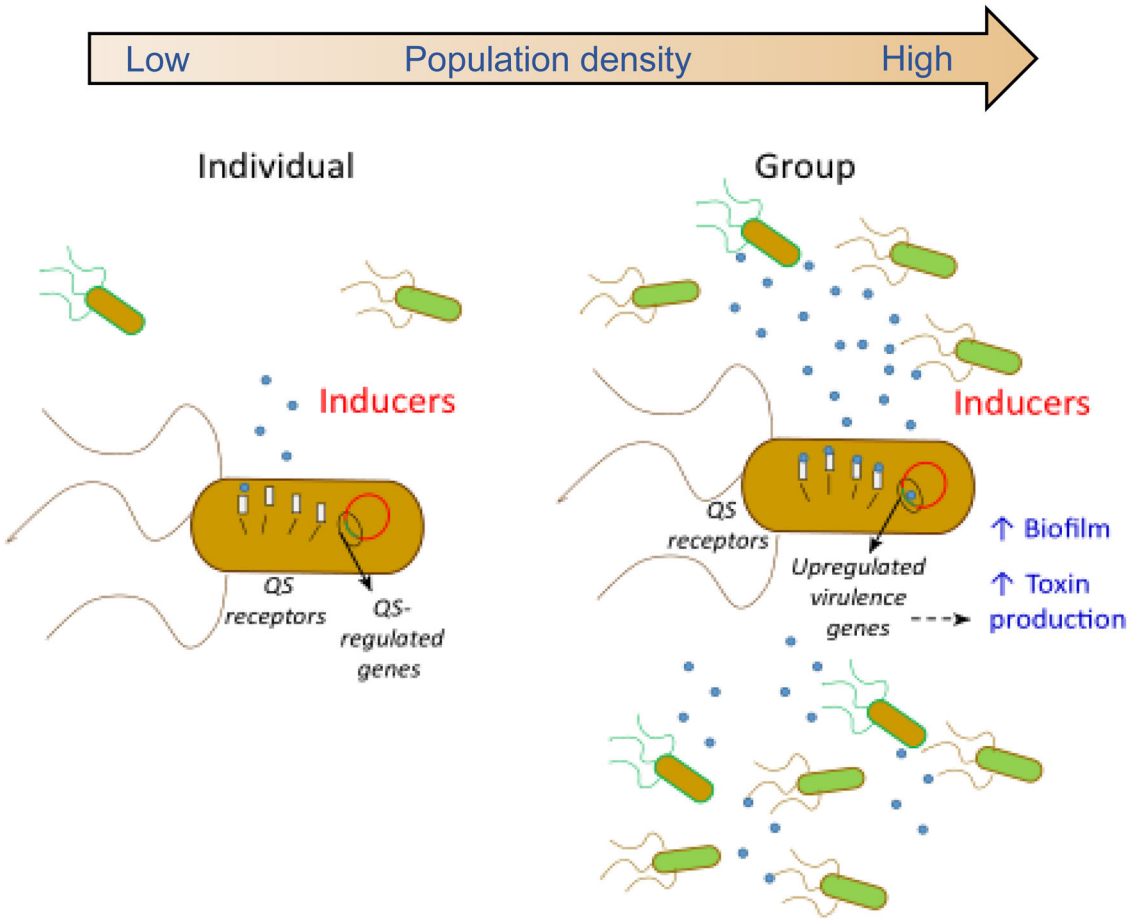

Figure 5. Schematic diagram of quorum sensing.

Listeria monocytogenes, and *Salmonella* (Brackman et al. 2009; Niu and Gilbert 2004; Upadhyay et al. 2013).

Anti-quorum sensing

Quorum sensing (QS) is an information exchange program discovered during the study of the luminous mechanism of *Vibrio fischeri* in the 1880s (Choudhary and Sandhu 2009). QS is the behavior that bacteria perceive bacterial density and the environment outside the cell by synthesizing and secreting an autologous inducer called a signal molecule (Figure 5) (Niu, Afre, and Gilbert 2006). The concentration of signal molecules also increases as bacterial density increases. When the concentration reaches a certain threshold, bacteria turn on the expression of cell-dependent genes, and regulate bacterial population behavior. Inhibiting QS regulates a variety of physiological and biochemical functions of bacteria, such as bioluminescence, biofilm formation, extracellular enzyme secretion, antibiotic production, and other metabolites and virulence factors (Takayama and Kato 2016). Some studies have confirmed that cinnamaldehyde downregulates the expression of bcsA and luxR in *E. coli*, and both are involved in the QS reaction. In addition, cinnamaldehyde also significantly inhibits the QS reaction in *P. aeruginosa* and *Streptococcus pyogenes* (Brackman et al. 2011; Brackman et al. 2009). Different EOs may have different inhibitory effects on QS of different cell types. Finding new ways to inhibit QS in bacteria is an interesting strategy for developing new antibiotics (Rasko et al. 2008). An anti-QS compound could overcome the pathogenicity of bacteria without causing drug resistance.

The action mechanism of EOs against microorganisms may not coexist, so it is difficult to appear alone. Therefore, it is necessary to further explore the action targets of different EOs or their active components on different microorganisms, and further explore the action mechanism of EOs at the molecular level to improve the specific antibacterial effect of antimicrobial agents on target microorganisms.

Methods for evaluating the synergistic effect of drugs

Because it is very difficult to develop new antibiotics, the synergistic effects of different combinations of antimicrobials provide a promising strategy to solve the problem of microbial drug resistance. Combined EOs and antibiotics may have one of three different effects: additive, synergistic or antagonistic (Delaquis et al. 2002). Additive effects provide the basis for quantitative analysis of the effects of combined drugs. Synergistic effects of drug combinations exceed those of the individual drugs. Antagonism refers to weakened effects as a result of the combination of drugs. However, it is worth noting that different evaluation criteria or evaluation methods may produce different results. Table 1 summarizes some

Table 1. The evaluation method of drug combination and its advantages and disadvantages.

Evaluation method	Advantages	Disadvantages	References
Algebraic sum	The method is very simple, and it is easy to judge the nature of the combination of drugs.	It can only be used to judge the combination of drugs with simple linear relationship.	Levine (1978)
Q value method	This method can be directly used to compare the original dose effect level and the operation is simple.	The amount of information is small, only qualitative.	Abt and Paul (1972)
Burgi formula method	This method is commonly used to measure the final effect of combined drugs.	Because it does not need to consider the dose-response relationship and the mode of action of the combination of drugs, its application is limited.	Guo, Huo, and Jiang (2005)
Chou-Talalay	It can be used in combination of multiple drugs or in combination of unsteady dose ratio, and can be described qualitatively and quantitatively.	This method cannot give the drug map with non-constant ratio, only the equivalent line map after standardization.	Chou and Rideout (1987)
Finney Harmonic average method	When the combined action of the two drugs is similar, the equivalent line can be derived by this method.	It may be too simple to determine the experimental results by probability operation, unless a large number of experiments are carried out, the results are not very reliable.	Finney (1952)
Webb Fractional product method	It is the simplest method and is widely used at present.	It is only suitable for the additive calculation of non repellent drugs.	Ribo and Rogers (2010)
Reaction surface method	It can display two-dimensional and three-dimensional atlas with reliable results. According to the spectrum, the best joint mode can be obtained.	The mathematical model is complex and the workload is heavy. It needs relevant statistical knowledge and S-curve relationship of drug effect.	Minto et al. (2000)
Relative effect method	The combined effect is determined by the value of the two drugs acting alone and the actual value of the single drug.	The confidence interval can not be calculated. In most cases, it lacks reliability and can only be analyzed qualitatively.	Pradhan and Kim (2014)
Mapping analysis	This method does not need to consider the type of action between the two drugs. It is a relatively general analysis method.	A large amount of data is needed to draw dose-response curve, and a fixed ratio of drug combination is needed, so it will be limited in practical application.	Zheng and Sun (2000)
Parametric method	Data can be analyzed systematically. Abundant data and reliable results.	It needs high statistical knowledge and a lot of work. It is suitable for data that can be fitted by Hill equation.	Wang et al. (2014)
Logistic Regression model analysis	This method needs to design appropriate experimental factors, and the method is not mature, and the relevant reports of using this method are rare.	With the help of computer, it is easy to judge the nature of combined drug use.	Gennings et al. (2002)
Weight matching method	This method can be used to judge the interaction between six drugs and six concentrations, and can show the intensity of the interaction between drugs.	It is necessary to carry out pre experiment. When the effective dose range of the drug is small, the experiment design should be changed.	Qing and Rui (1999)
Orthogonal t-value method	The principle is clear, easy to understand, simple to calculate, and easy to analyze the synergistic or antagonistic effect between the two drugs.	The combination of drugs can be made according to or without drugs, and then the nature of the combination can be judged. It is limited in practical application.	Jin and Gong (2011)

common methods and their advantages and disadvantages. Researchers can choose one or more methods according to the needs of the experiment.

The interaction between EOs and its active components

The synergism or antagonism of EOs combinations depends on the types of EOs or the microorganisms. Different EOs or microorganisms may have different effects. Thymol and carvol have synergistic inhibitory effects on *E. coli O157:H7, S. aureus, L. monocytogenes, Saccharomyces cerevisiae*, and *Aspergillus niger* (Guarda et al. 2011). In another study, the combination of cinnamon and clove EOs antagonized the growth of *E. coli* and showed a synergistic effect on *L. monocytogenes*, *Bacillus cereus*, and *Yersinia enterocolitica* (Moleyar and Narasimham 1992). Therefore, it is difficult to directly predict the antimicrobial efficacy of an EOs mixture. However, some studies have concluded that when mixed in proportion, the whole EOs shows stronger antibacterial activity than the main components. For example, Ocimum basilicum EOs has a stronger inhibitory effect on *Lactobacillus campylobacter* and *S. cerevisiae* than its main components linalool or methyl piperol (Lachowicz et al.1998). The antibacterial activity of conifer EOs against *L. monocytogenes* was significantly higher than that of its main components (Mourey and Canillac 2002). This finding shows that the trace components in the EOs may be very important for microbial activity to produce a synergistic effect.

Combined application of EOs and antimicrobial agents

The main causes of bacterial drug resistance

There are two main causes of drug resistance in bacteria. First, when bacteria come into contact with drugs, they produce enzymes or receptors to protect themselves (Ayaz et al. 2019), rendering them resistant to the antimicrobial agents.

A common example is the alternative penicillin binding protein (PBP2a) produced by methicillin-resistant *S. aureus* (MRSA). This altered protein is encoded by the *mecA* gene and reduces affinity for β-lactam, penicillins and cephalosporins (Leonard and Markey 2008). A second cause of bacterial drug resistance is β-lactamase, which catalyzes the hydrolysis of penicillins, cephalosporins and other β-lactam antibiotics (Ubukata et al. 1989). More than 200 kinds of β-lactamases have been described in the literature and are widely found in a variety of Gram-positive and Gram-negative bacteria. This enzyme can hydrolyze both penicillins and cephalosporins (Shimizu et al. 2001).

The potential of combined EOs and food additives

In general, the use of a small number of multiple preservatives is more effective than the use of a large number of single preservatives. Because this can increase the action targets of preservatives on microorganisms, and at the same time avoid the emergence of drug-resistant microorganisms. The combination of different EOs and food additives may be an effective method for food preservation (Ju, Xu, et al. 2018). It has been reported that the combination of peppermint EOs and methylparaben has a synergistic inhibitory effect on *Pseudomonas aeruginosa* (Parsaeimehr et al. 2010). In addition, oregano EOs combined with propyl hydroxybenzoate showed synergistic inhibitory effect on *Staphylococcus aureus* (Patrone et al. 2010).

Related to the application of food preservation industry, the combination of thyme EOs and chitosan can significantly inhibit the growth of *Pseudomonas aeruginosa*, yeast and mold, and prolong the shelf life of ready-to-eat chicken (Giatrakou, Ntzimani, and Savvaidis 2010). Similarly, the combination of thyme EOs and nisin has a synergistic inhibitory effect on *Listeria monocytogenes* and can effectively prolong the shelf life of beef (Solomakos et al. 2008). In addition, the combination of tea polyphenols and nisin can effectively prolong the shelf life of chilled sea bass (Antonio et al. 2009). The use of electrolytic NaCl solution and thymol (0.5%) on carp fillets showed stronger antibacterial and antioxidant effects (Mahmoud et al. 2006). All this implies the infinite possibility of combining chilled sea bass with various food additives.

The potential of combined EOs and antibiotics

The combined use of EOs and antibiotics is a potential strategy to solve the problem of microbial drug resistance. Phytochemicals can inhibit or kill bacteria at low concentrations, thereby minimizing any potentially toxic effects. It has been reported that the combination of thyme EO with fluconazole has a significant synergistic inhibitory effect on *Candida albicans*, suggesting that a lower dose of the drug can be used in combination with EOs. Active plant components extracted from *Artemisia argyi* were used against *S. aureus* in combination with ciprofloxacin and norfloxacin. The results showed that the MICs of ciprofloxacin and norfloxacin decreased by 5- and 10-fold, respectively. Synergistic effects of geranium EO and prickly ash EO with fluoroquinolone antibiotics have also been demonstrated. In addition, salicylic acid, a phenolic compound found in many plants, has been shown to significantly inhibit the growth of *S. aureus* when combined with ciprofloxacin. Volatile oil from myrtle leaves and amphotericin B also have synergistic inhibitory effects on *C. albicans* and *Aspergillus*. EOs have also been shown to be effective against antibiotic-resistant bacteria. When oxacillin was used in combination with *A. argyi* EO against oxacillin-resistant *S. aureus* CCARM3511, the MIC of *A. argyi* EO decreased significantly by 4- to 8-fold. The combination of *Polygonum multiflorum* EO and vancomycin also had synergistic inhibitory effects on MRSA.

Tetracycline antibiotics, including chlortetracycline, oxytetracycline, tetracycline, doxycycline and minocycline, are relatively cheap and effective broad-spectrum antibiotics produced by actinomycetes. However, with the emergence of drug resistance in bacteria, the role of these antibiotics is gradually decreasing. *S. aureus* employs two mechanisms to resist these antibiotics: (1) active efflux of tetracycline from the cell via the transporting proteins Tet (K) or Tet (L) belonging to the major facilitator superfamily (MFS) and (2) the ribosomal protection proteins Tet (M) and Tet (O) (Trzcinski et al. 2000). In contrast, three mechanisms are involved in erythromycin resistance: (1) use of energy-dependent efflux, (2) production of inactivating enzymes and (3) alteration of 23S rRNA methylases (Wang et al. 2008). The first mechanism involves the macrolide efflux pumps Msr(A) and/or Msr(B), which belong to the ATP-binding cassette (ABC) transporter family and export 14-membered macrolide antibiotics from bacterial cells (Leclercq 2002).

In a study in which an ethanol mixture extracted from mango peel was combined with erythromycin and tetracycline to explore the effect of these combinations on drug-resistant *S. aureus*, it was observed that the MICs of erythromycin and tetracycline decreased 4-fold (de Oliveira et al. 2011). Similarly, baicalein isolated from thyme leaves significantly decreased the MIC of tetracycline against MRSA from 4 to 0.06 μg/ml after 16 h (Fujita et al. 2005). The antibacterial activity of tetracycline was also significantly increased 8-fold by rosemary EO. These results are comparable to those reported for reserpine (Oluwatuyi, Kaatz, and Gibbons 2004).

Aminoglycosides, such as kanamycin, streptomycin, gentamicin and amikacin, are broad-spectrum antibiotics used to treat *S. aureus* infections (Ramirez and Tolmasky 2010). Mechanisms of resistance to these antibiotics involve the production of aminoglycoside-modifying enzymes. It has been shown that there is a significant enhancement effect between *Zanthoxylum bungeanum* EOs and aminoglycosides (Ramirez and Tolmasky 2010). More recently, studies have focused on inhibiting the synergistic effects of drug-resistant pathogenic microorganisms (Dhara and Tripathi 2020; Gao et al. 2020). Therefore, synergistic combinations of drugs may be beneficial to the public, especially young and elderly patients with low immune functioning. In addition to the above EOs, we also list some commonly used phytochemicals that have synergistic antibacterial activity with antibiotics in Figure 6.

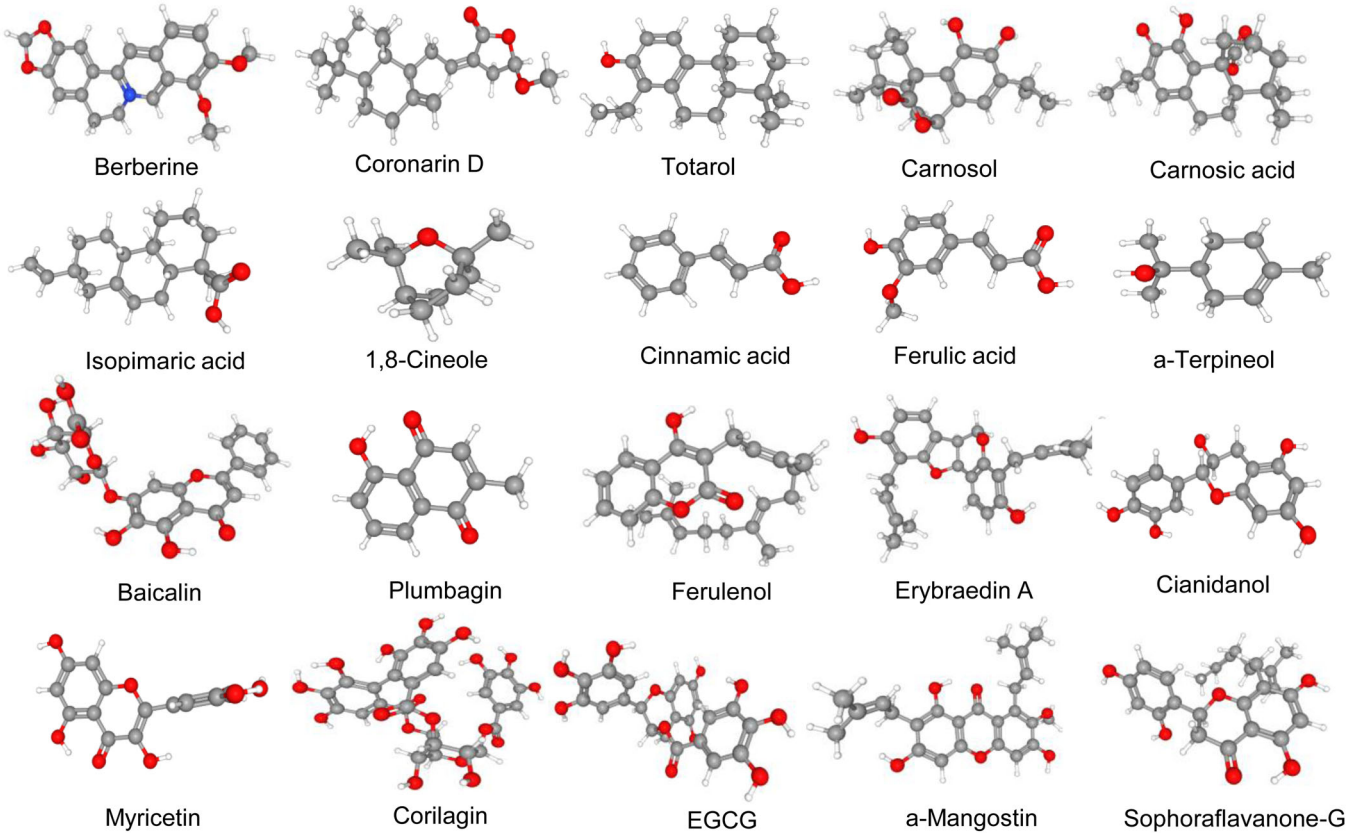

Figure 6. List of plant chemical components with synergistic antibacterial effect combined with antibiotics.

Concluding remarks and future perspectives

In this paper, the main factors affecting the antibacterial activity of EOs and the possible antibacterial mechanism of EOs were introduced in detail. And then analyzed the potential applications of combined EOs and antimicrobial agents to address the problem of microbial drug resistance. However, future research needs to establish the validity of each combination for different microbial species, improve the efficacy and specificity of each combination and develop high-throughput screening methods. In order to develop more effective combinations, it will be necessary to optimize the proportion and dose of each combination. In addition, more sophisticated instruments combined with computer technology are needed to identify and evaluate the effects of synergistic drug combinations and their corresponding contribution rates. Finally, safer, cheaper and more effective natural drugs to against food borne pathogens may be developed using genetic engineering, genomics, proteomics and metabolomics.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

Abreu, A. C., A. J. Mcbain, and M. Simoes. 2012. Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Product Reports* 29 (9):1007–21. doi: 10.1039/c2np20035j.

Abt, K., and F. Paul. 1972. Acute toxicity of drug combinations: A method for evaluating the interaction of the active components. *Excerpta Medica* 10 (2):192–5.

Alessandra, P., S. M. Ameen, M. Maria, and C. Giorgia. 2019. Antimicrobial substances for food packaging products: The current situation. *Journal of Aoac International* 101 (4):942–7.

Al-Reza, S. M., A. Rahman, and S. C. Kang. 2009. Chemical composition and inhibitory effect of essential oil and organic extracts of Cestrum nocturnum L. on food-borne pathogens. *International Journal of Food Science & Technology* 44 (6):1176–82. doi: 10.1111/j.1365-2621.2009.01939.x.

Antonio, C. M., H. Abriouel, R. L. Lopez, N. B. Omar, E. Valdivia, and A. Galvez. 2009. Enhanced bactericidal activity of enterocin AS-48 in combination with essential oils, natural bioactive compounds and chemical preservatives against Listeria monocytogenes in ready-to-eat salad. *Food and Chemical Toxicology*47 (9):2216–23. doi: 10.1016/j.fct.2009.06.012.

Ayaz, M., F. Ullah, A. Sadiq, F. Ullah, M. Ovais, J. Ahmed, and H. P. Devkota. 2019. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. *Chemico-Biological Interactions* 308:294–303. doi: 10.1016/j.cbi.2019.05.050.

Ayaz, M., F. Subhan, J. Ahmed, A. Khan, F. Ullah, I. Ullah, and S. Hussain. 2015. Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. *Journal of Biological Research* 22 (1):4.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – A review. *International Journal of Food Microbiology* 94 (3):223–53. doi: 10.1016/j.ijfoodmicro.2004.03.022.

Burt, S. A., and R. D. Reinders. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology* 36 (3):162–7. doi: 10.1046/j.1472-765x.2003.01285.x.

Brackman, G., S. Celen, U. Hillaert, S. Van Calenbergh, P. Cos, L. Maes, H. J. Nelis, and T. Coenye. 2011. Structure-activity relationship of cinnamaldehyde analogs as inhibitors of AI-2 based quorum sensing and their effect on virulence of Vibrio spp. *PLoS One* 6 (1): e16084. doi: 10.1371/journal.pone.0016084.

Brackman, G., U. Hillaert, S. V. Calenbergh, H. J. Nelis, and T. Coenye. 2009. Use of quorum sensing inhibitors to interfere with biofilm formation and development in Burkholderia multivorans and Burkholderia cenocepacia. *Research in Microbiology* 160 (2): 144–51. doi: 10.1016/j.resmic.2008.12.003.

Cezar, D. R. N. A., M. Maraschin, and R. M. Di Piero. 2015. Antifungal activity of salicylic acid against penicillium expansum and its possible mechanisms of action. *International Journal of Food Microbiology* 215:64–70. doi: 10.1016/j.ijfoodmicro.2015.08.018.

Chalova, V. I., P. G. Crandall, and S. C. Ricke. 2010. Microbial inhibitory and radical scavenging activities of cold-pressed terpeneless Valencia orange (*Citrus sinensis*) oil in different dispersing agents. *Journal of the Science of Food and Agriculture* 90 (5):870–6. doi: 10.1002/jsfa.3897.

Chen, J., and Y. Wang. 2020. Genetic determinants of salmonella enterica critical for attachment and biofilm formation. *International Journal of Food Microbiology* 320:108524. doi: 10.1016/j.ijfoodmicro.2020.108524.

Choudhary, D. K., and S. S. Sandhu. 2009. Quorum sensing (QS) as etiological phenomenon: The bacterial paradigm. *Peoples Journal of Scientific Research* 2 (2):41–4.

Chou, T. C., and D. Rideout. 1987. *Synergism and antagonism in chemotherapy*, 37–64. New York: Academic Press.

Chung, P. Y., P. Navaratnam, and L. Y. Chung. 2011. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. *Annals of Clinical Microbiology and Antimicrobials* 10 (1):25. doi: 10.1186/1476-0711-10-25.

Coutinho, H. D. M., J. G. M. Costa, E. O. Lima, V. S. Falcão-Silva, and J. P. Siqueira. 2009. Herbal therapy associated with antibiotic therapy: Potentiation of the antibiotic activity against methicillin-resistant *Staphylococcus aureus* by Turnera ulmifolia L. *BMC Complementary and Alternative Medicine* 9 (1):13. doi: 10.1186/1472-6882-9-13.

Cui, H., C. Zhao, and L. Lin. 2015. The specific antibacterial activity of liposome-encapsulated Clove oil and its application in tofu. *Food Control* 56:128–34. doi: 10.1016/j.foodcont.2015.03.026.

De Azeredo, C. M., and M. J. Soares. 2013. Combination of the essential oil constituents citral, eugenol and thymol enhance their inhibitory effect on *Crithidia fasciculata* and *Trypanosoma cruzi* growth. *Journal of Pharmacognosy* 23 (5):762–8.

de Oliveira, S. M. S., V. S. Falcão-Silva, J. P. Siqueira-Junior, M. J. Costa, and M. D. Diniz. 2011. Modulation of drug resistance in *Staphylococcus aureus* by extract of mango (*Mangifera indica* L., Anacardiaceae) peel. *Revista Brasileira de Farmacognosia* 21 (1): 190–3. doi: 10.1590/S0102-695X2011005000014.

Delaquis, P. J., K. Stanich, B. Girard, and G. Mazza. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology* 74 (1–2):101–9. doi: 10.1016/S0168-1605(01)00734-6.

Dhara, L., and A. Tripathi. 2020. Cinnamaldehyde: A compound with antimicrobial and synergistic activity against esbl-producing quinolone-resistant pathogenic enterobacteriaceae. *European Journal of Clinical Microbiology & Infectious Diseases* 39 (1):65–73. doi: 10.1007/s10096-019-03692-y.

Dung, N. T., J. M. Kim, and S. C. Kang. 2008. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food and Chemical Toxicology* 46 (12):3632–9. doi: 10.1016/j.fct.2008.09.013.

Finney, D. J. 1952. *Probit analysis*. 2nd ed., 146–53. Cambridge: Cambridge University Press.

Fujita, M., S. Shiota, T. Kuroda, T. Hatano, T. Yoshida, T. Mizushima, and T. Tsuchiya. 2005. Remarkable synergies between baicalein and tetracycline, and baicalein and betalactams against methicillin-resistant *Staphylococcus aureus*. *Microbiology and Immunology* 49 (4): 391–6. doi: 10.1111/j.1348-0421.2005.tb03732.x.

Friedman, M., P. R. Henika, C. E. Levin, and R. E. Mandrell. 2004. Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. *Journal of Agricultural and Food Chemistry* 52 (19):6042–8. doi: 10.1021/jf0495340.

Gao, C., C. Tian, Y. Lu, J. Xu, J. Luo, and X. Guo. 2011. Essential oil composition and antimicrobial activity of Sphallerocarpus gracilis seeds against selected food-related bacteria. *Food Control* 22 (3–4): 517–22. doi: 10.1016/j.foodcont.2010.09.038.

Gao, L., Y. Sun, M. Yuan, M. Li, and T. Zeng. 2020. In vitro and in vivo study on the synergistic effect of minocycline and azoles against pathogenic fungi. *Antimicrobial Agents and Chemotherapy* 64 (6):e00290-20.

Gennings, C., W. H. Carter, J. A. Campain, D. S. Bae, and R. S. H. Yang. 2002. Statistical analysis of interactive cytotoxicity in human epidermal keratinocytes following exposure to a mixture of four metals. *Journal of Agricultural, Biological, and Environmental Statistics* 7 (1):58–73. doi: 10.1198/108571102317475062.

Giatrakou, V., A. Ntzimani, and I. N. Savvaidis. 2010. Combined chitosan-thyme treatments with modified atmosphere packaging on a ready-to-cook poultry product. *Journal of Food Protection* 73 (4): 663–9. doi: 10.4315/0362-028x-73.4.663.

Guarda, A., J. F. Rubilar, J. Miltz, and M. J. Galotto. 2011. The antimicrobial activity of microencapsulated thymol and carvacrol. *International Journal of Food Microbiology* 146 (2):144–50. doi: 10.1016/j.ijfoodmicro.2011.02.011.

Guo, J. Y., H. R. Huo, and T. L. Jiang. 2005. Evaluation of research methods to measure the effect of combination drugs. *Pharmacology and Clinic of Traditional Chinese Medicine* 21 (3):60–4.

Hemaiswarya, S., A. K. Kruthiventi, and M. Doble. 2008. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine : international Journal of Phytotherapy and Phytopharmacology* 15 (8):639–52. doi: 10.1016/j.phymed.2008.06.008.

Holley, R. A., and D. Patel. 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology* 22 (4):273–92. doi: 10.1016/j.fm.2004.08.006.

Jin, W., and W. Gong. 2011. Disintegration of orthogonal t value method on prescription of anti-fatigue health wine. *Ra Harmaal Jornal* 120–31.

Jiménezfernández, A., A. Lópezsánchez, L. Jiménezdíaz, B. Navarrete, P. Calero, A. I. Platero, and F. Govantes. 2016. Complex Interplay between FleQ, Cyclic Diguanylate and Multiple σ Factors Coordinately Regulates Flagellar Motility and Biofilm Development in Pseudomonas putida. *Plos One* 11 (9):e0163142.

Ju, J., X. Chen, Y. Xie, H. Yu, Y. Guo, Y. Cheng, H. Qian, and W. Yao. 2019. Application of essential oil as a sustained release preparation in food packaging. *Trends in Food Science and Technology* 92: 22–32. doi: 10.1016/j.tifs.2019.08.005.

Ju, J., Y. Xie, Y. Guo, Y. Cheng, H. Qian, and W. Yao. 2019a. Application of edible coating with essential oil in food preservation. *Critical Reviews in Food Science and Nutrition* 59 (15):2467–80.

Ju, J., Y. Xie, Y. Guo, Y. Cheng, H. Qian, and W. Yao. 2019b. The inhibitory effect of plant essential oils on foodborne pathogenic

bacteria in food. *Critical Reviews in Food Science and Nutrition* 59 (20):3281–92. doi: 10.1080/10408398.2018.1488159.

Ju, J., Y. Xie, Y. Guo, Y. Cheng, H. Qian, and W. Yao. 2020. Application of starch microcapsules containing essential oil in food preservation. *Critical Reviews in Food Science and Nutrition* 60 (17): 2825–36.

Ju, J., Y. Xie, H. Yu, Y. Guo, Y. Cheng, R. Zhang, and W. Yao. 2020. Major components in Lilac and Litsea cubeba essential oils kill *Penicillium roqueforti* through mitochondrial apoptosis pathway. *Industrial Crops and Products* 149:112349.

Ju, J., X. Xu, Y. Xie, Y. Guo, Y. Cheng, H. Qian, and W. Yao. 2018. Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. *Food Chemistry* 240:850–5. doi: 10.1016/j.foodchem.2017.07.120.

Josenhans, C., and S. Suerbaum. 2002. The role of motility as a virulence factor in bacteria. *International Journal of Medical Microbiology* 291 (8):605–14.

Kalemba, D., and A. Kunicka. 2003. Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry* 10 (10):813–29. doi: 10.2174/0929867033457719.

Katherine, A., F. Christine, and V. R. Carson. 2012. Effects of melaleuca alternifolia (tea tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility. *Antimicrobial Agents and Chemotherapy* 56 (2):909–15. doi: 10.1128/AAC.05741-11.

Kim, J. M., M. R. Marshall, J. A. Cornell, J. F. Preston III, and C. I. Wei. 1995. Antibacterial activity of carvacrol, citral, and geraniol against Salmonella typhimurium in culture medium and on fish cubes. *Journal of Food Science* 60 (6):1364–8. doi: 10.1111/j.1365-2621.1995.tb04592.x.

Khorshidian, N., M. Yousefi, E. Khanniri, and A. M. Mortazavian. 2017. Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science & Emerging Technologies* 45: 62–72.

Klančnik, A., S. Piskernik, S. S. Možina, L. Gašperlin, and B. Jeršek. 2011. Investigation of some factors affecting the antibacterial activity of rosemary extracts in food models by a food microdilution method. *International Journal of Food Science & Technology* 46 (2): 413–20.

Kotzekidou, P., P. Giannakidis, and A. Boulamatsis. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. *LWT - Food Science and Technology* 41 (1): 119–27. doi: 10.1016/j.lwt.2007.01.016.

Lachowicz, K. J., G. P. Jones, D. R. Briggs, F. E. Bienvenu, and M. J. Coventry. 1998. The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum* L.) against acidtolerant food microflora. *Letters in Applied Microbiology* 26 (3):209–14.

Lambert, R. J., P. N. Skandamis, P. J. Coote, and G. E. Nychas. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology* 91 (3):453–62. doi: 10.1046/j.1365-2672.2001.01428.x.

Leonard, F. C., and B. K. Markey. 2008. Meticillin-resistant *Staphylococcus aureus* in animals: A review. *Veterinary Journal* 175 (1):27–36. doi: 10.1016/j.tvjl.2006.11.008.

Leclercq, R. 2002. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases of America* 34 (4):482–92. doi: 10.1086/324626.

Levine, R. R. 1978. *Pharmacology: drug actions & reactions*, 2nd ed., 284–5. Boston: Little Brown Co.

Liu, R., H. Gao, H. Chen, X. Fang, and W. Wu. 2019. Synergistic effect of 1-methylcyclopropene and carvacrol on preservation of red pitaya (*Hylocereus polyrhizus*). *Food Chemistry* 283:588–95. doi: 10.1016/j.foodchem.2019.01.066.

Luz, I. D. S., N. J. Gomes Neto, A. G. Tavares, M. Magnani, and E. L. De Souza. 2012. Exposure of listeria monocytogenes to sublethal amounts of *Origanum vulgare* L. essential oil or carvacrol in a food-based medium does not induce direct or cross protection. *Food Research International* 48 (2):667–72. doi: 10.1016/j.foodres.2012.05.026.

Luz, I. D. S., N. J. Gomes Neto, A. G. Tavares, P. C. Nunes, M. Magnani, and E. L. De Souza. 2012. Evidence for lack of acquisition of tolerance in salmonella enterica serovar typhimurium atcc 14028 after exposure to subinhibitory amounts of origanum vulgare L. essential oil and carvacrol. *Applied and Environmental Microbiology* 78 (14):5021–4. doi: 10.1128/AEM.00605-12.

Mann, C. M., and J. L. Markham. 1998. A new method for determining the minimum inhibitory concentration of essential oils. *Journal of Applied Microbiology* 84 (4):538–44. doi: 10.1046/j.1365-2672.1998.00379.x.

Matan, N., H. Rimkeeree, A. J. Mawson, P. Chompreeda, V. Haruthaithanasan, and M. Parker. 2006. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *International Journal of Food Microbiology* 107 (2):180–5. doi: 10.1016/j.ijfoodmicro.2005.07.007.

Mahmoud, B. S. M., K. Yamazaki, K. Miyashita, Y. Kawai, I.-S. Shin, and T. Suzuki. 2006. Preservative effect of combined treatment with electrolyzed NaCl solutions and essential oil compounds on carp fillets during convectional air-drying. *International Journal of Food Microbiology* 106 (3):331–7. doi: 10.1016/j.ijfoodmicro.2005.09.010.

Marchese, A., I. E. Orhan, M. Daglia, R. Barbieri, A. Di Lorenzo, S. F. Nabavi, O. Gortzi, M. Izadi, and S. M. Nabavi. 2016. Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chemistry* 210:402–14. doi: 10.1016/j.foodchem.2016.04.111.

Meychik, N. R., Y. I. Nikolaeva, O. V. Komarynets, and I. P. Ermakov. 2011. Barrier function of the cell wall during uptake of nickel ions. *Russian Journal of Plant Physiology* 58 (3):409–14. doi: 10.1134/S1021443711030137.

Meng, D., B. Garba, Y. Ren, M. Yao, and X. Xia. 2020. Antifungal activity of chitosan against aspergillus ochraceus, and its possible mechanisms of action. *International Journal of Biological Macromolecules* 158:1063–70.

Mcmahon, M. A., I. S. Blair, J. E. Moore, and D. A. Mcdowell. 2007. Habituation to sub-lethal concentrations of tea tree oil (*Melaleuca alternifolia*) is associated with reduced susceptibility to antibiotics in human pathogens. *The Journal of Antimicrobial Chemotherapy* 59 (1):125–7. doi: 10.1093/jac/dkl443.

Mikulasova, M., R. Chovanova, and S. Vaverkova. 2016. Synergism between antibiotics and plant extracts or essential oils with efflux pump inhibitory activity in coping with multidrug-resistant staphylococci. *Phytochemistry Reviews* 15 (4):651–62.

Minto, C. F., T. W. Schnider, T. G. Short, K. M. Gregg, A. Gentilini, and S. L. Shafer. 2000. Response surface model for anesthetic drug interactions. *Anesthesiology* 92 (6):1603–16. doi: 10.1097/00000542-200006000-00017.

Moleyar, V., and P. Narasimham. 1992. Antibacterial activity of essential oil components. *International Journal of Food Microbiology* 16 (4):337–42. doi: 10.1016/0168-1605(92)90035-2.

Mourey, A., and N. Canillac. 2002. Anti-Listeria monocytogenes activity of essential oils components of conifers. *Food Control* 13 (4–5):289–92. doi: 10.1016/S0956-7135(02)00026-9.

Newman, D. J., and G. M. Cragg. 2016. Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products* 79 (3): 629–61. doi: 10.1021/acs.jnatprod.5b01055.

Niu, C., and E. S. Gilbert. 2004. Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. *Applied and Environmental Microbiology* 70 (12):6951–6. doi: 10.1128/AEM.70.12.6951-6956.2004.

Niu, C., S. Afre, and E. S. Gilbert. 2006. Subinhibitory concentrations of cinnamaldehyde interfere with quorum sensing. *Letters in Applied Microbiology* 43 (5):489–94. doi: 10.1111/j.1472-765X.2006.02001.x.

Oluwatuyi, M., G. Kaatz, and S. Gibbons. 2004. Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. *Phytochemistry* 65 (24):3249–54. doi: 10.1016/j.phytochem.2004.10.009.

Park, E. J., M. S. Hussain, S. Wei, M. Kwon, and D. H. Oh. 2019. Genotypic and phenotypic characteristics of biofilm formation of emetic toxin producing bacillus cereus strains. *Food Control* 96: 527–34. doi: 10.1016/j.foodcont.2018.10.008.

Parsaeimehr, M., A. A. Basti, B. Radmehr, A. Misaghi, A. Abbasifar, G. Karim, N. Rokni, M. S. Motlagh, H. Gandomi, N. Noori, et al. 2010. Effect of *Zataria multiflora* Boiss. essential oil, nisin, and their combination on the production of enterotoxin C and alpha-hemolysin by *Staphylococcus aureus*. *Foodborne Pathogens and Disease* 7 (3): 299–305. doi: 10.1089/fpd.2009.0416.

Patrone, V., R. Campana, E. Vittoria, and W. Baffone. 2010. In vitro synergistic activities of essential oils and surfactants in combination with cosmetic preservatives against Pseudomonas aeruginosa and *Staphylococcus aureus*. *Current Microbiology* 60 (4):237–41. doi: 10.1007/s00284-009-9531-7.

Pasqua, R. D., G. Mamone, P. Ferranti, D. Ercolini, and G. Mauriello. 2010. Changes in the proteome of Salmonella enterica serovar Thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics* 10 (5):1040–9. doi: 10.1002/pmic.200900568.

Picioreanu, C., J. U. Kreft, M. Klausen, J. A. J. Haagensen, T. Tolker-Nielsen, and S. Molin. 2007. Microbial motility involvement in biofilm structure formation – A 3D modelling study. *Water Science and Technology* 55 (8–9):337–43. doi: 10.2166/wst.2007.275.

Pradhan, A. M. S., and Y. T. Kim. 2014. Relative effect method of landslide susceptibility zonation in weathered granite soil: A case study in deokjeok-ricreek, South Korea. *Natural Hazards* 72 (2):1189–217. doi: 10.1007/s11069-014-1065-z.

Qing, S. Z., and Y. S. Rui. 1999. Quantitative design of drug compatibility by weighted modification method. *Acta Pharmacologica Sinica* 20 (11):1043–51.

Rao, J., B. Chen, and D. J. Mcclements. 2019. Improving the efficacy of essential oils as antimicrobials in foods: Mechanisms of action. *Annual Review of Food Science and Technology* 10 (1):365–87. doi: 10.1146/annurev-food-032818-121727.

Rasko, D. A., C. G. Moreira, D. R. Li, N. C. Reading, J. M. Ritchie, M. K. Waldor, N. Williams, R. Taussig, S. Wei, M. Roth, et al. 2008. Targeting QseC signaling and virulence for antibiotic development. *Science (New York, N.Y.)* 321 (5892):1078–80. doi: 10.1126/science.1160354.

Ramirez, M. N., and M. E. Tolmasky. 2010. Aminoglycoside modifying enzymes. *Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy* 13 (6):151–71. doi: 10.1016/j.drup.2010.08.003.

Reyes-Jurado, F., A. R. Navarrocruz, C. E. Ochoavelasco, E. Palou, A. Lopezmalo, and R. Ávila-Sosa. 2020. Essential oils in vapor phase as alternative antimicrobials: A review. *Critical Reviews in Food Science and Nutrition* 60 (10):1641–50.

Remmal, A., T. Bouchikhi, A. Tantaoui-Elaraki, and M. Ettayebi. 1993. Inhibition of antibacterial activity of essential oils by tween 80 and ethanol in liquid medium. *Journal de Pharmacie de Belgique* 48 (5): 352–6.

Rivas, L., M. J. McDonnell, C. M. Burgess, M. O'Brien, A. Navarro-Villa, S. Fanning, and G. Duffy. 2010. Inhibition of verocytotoxigenic *Escherichia coli* in model broth and rumen systems by carvacrol and thymol. *International Journal of Food Microbiology* 139 (1–2):70–8. doi: 10.1016/j.ijfoodmicro.2010.01.029.

Ribo, J. M., and F. Rogers. 2010. Toxicity of mixtures of aquatic contaminants using the luminescent bacteria bioassay. *Environmental Toxicology* 5 (2):135–52.

Rouse, M. S., B. Afessa, S. S. Talsma, and R. Patel. 2007. In vitro assessment of microbial colonization and biofilm formation on silver coated endotracheal tubes post extubation. *Chest* 132 (4):576C–6. doi: 10.1378/chest.132.4_MeetingAbstracts.576c.

Seow, Y. X., C. R. Yeo, H. L. Chung, and H.-G. Yuk. 2014. Plant essential oils as active antimicrobial agents. *Critical Reviews in Food Science and Nutrition* 54 (5):625–44. doi: 10.1080/10408398.2011.599504.

Shah, S., F. Ullah, M. Ayaz, A. Sadiq, S. Hussain, A-u-H. Ali Shah, S. A. Ali Shah, N. Ullah, F. Ullah, I. Ullah, et al. 2019. Benzoic acid derivatives of Ifloga spicata (Forssk.) sch.bip. as potential anti-leishmanial against leishmania tropica. *Processes* 7 (4):208. doi: 10.3390/pr7040208.

Shimizu, M., S. Shiota, T. Mizushima, H. Ito, T. Hatano, T. Yoshida, and T. Tsuchiya. 2001. Marked potentiation of activity of beta-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. *Antimicrobial Agents and Chemotherapy* 45 (11):3198–201. doi: 10.1128/AAC.45.11.3198-3201.2001.

Solomakos, N., A. Govaris, P. Koidis, and N. Botsoglou. 2008. The antimicrobial effect of thyme essential oil, nisin, and their combination against Listeria monocytogenes in minced beef during refrigerated storage. *Food Microbiology* 25 (1):120–7. doi: 10.1016/j.fm.2007.07.002.

Sokovic, M. D., J. Vukojevic, P. D. Marin, D. D. Brkic, V. Vajs, and L. J. van Griensven. 2009. Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. *Molecules (Basel, Switzerland)* 14 (1):238–49. doi: 10.3390/molecules14010238.

Sun, Q., J. Li, Y. Sun, Q. Chen, L. Zhang, and T. Le. 2020. The antifungal effects of cinnamaldehyde against *Aspergillus niger* and its application in bread preservation. *Food Chemistry* 317: 126405. doi: 10.1016/j.foodchem.2020.126405.

Takayama, Y., and N. Kato. 2016. Switch of spnr function from activating to inhibiting quorum sensing by its exogenous addition. *Biochemical & Biophysical Research Communications* 477 (4):993–7.

Thien-Fah, C., and G. P. Mah. 2001. Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology* 9 (1):34–9. doi: 10.1016/S0966-842X(00)01913-2.

Trzcinski, K., B. S. Cooper, W. Hryniewicz, and C. G. Dowson. 2000. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 45 (6):763–70. doi: 10.1093/jac/45.6.763.

Ubukata, K., R. Nonoguchi, M. Matsuhashi, and M. Konno. 1989. Expression and inducibility in *Staphylococcus aureus* of the mecA gene, which encodes a methicillin-resistant S. aureus-specific penicillin-binding protein. *Journal of Bacteriology* 171 (5):2882–5. doi: 10.1128/jb.171.5.2882-2885.1989.

Ulanowska, K., A. Tkaczyk, G. Konopa, and G. Wegrzyn. 2006. Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. *Archives of Microbiology* 184 (5):271–8. doi: 10.1007/s00203-005-0063-7.

Upadhyay, A., I. Upadhyaya, A. Kollanoor-Johny, and K. Venkitanarayanan. 2013. Antibiofilm effect of plant derived antimicrobials on Listeria monocytogenes. *Food Microbiology* 36 (1): 79–89. doi: 10.1016/j.fm.2013.04.010.

Vasconcelos, N. G., J. Croda, and S. Simionatto. 2018. Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial Pathogenesis* 120:198–203. doi: 10.1016/j.micpath.2018.04.036.

Wang, Y., C. Wu, L. Lu, G. N. Ren, X. Cao, and J. Shen. 2008. Macrolide–lincosamide-resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Veterinary Microbiology* 130 (1–2):118–25. doi: 10.1016/j.vetmic.2007.12.012.

Wang, Y., E. W. M. Ma, T. W. S. Chow, and K. L. Tsui. 2014. A two-step parametric method for failure prediction in hard disk drives. *IEEE Transactions on Industrial Informatics* 10 (1):419–30. doi: 10.1109/TII.2013.2264060.

Ya-Ru, L. I., L. Y. Zhou, L. I. Shu-Rong, Z. Z. Cao, L. Zhang, M. Wei, and C. H. Peng. 2014. Antibacterial activity and mechanism of action of plant essential oils and their main components from fruits and vegetables: A review. *Journal of Food Science* 35 (11):325–9.

Zheng, Q. S., and R. Y. Sun. 2000. Analysis of drug interaction in combined drug therapy by flection method. *Acta Pharmacologica Sinica* 21 (2):183–7.

Zhang, W., X. Cao, and S. Q. Liu. 2020. Aroma modulation of vegetable oils – A review. *Critical Reviews in Food Science and Nutrition* 60 (9):1538–51.