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Review

# Complexation of Terpenes for the Production of New Antimicrobial and Antibiofilm Molecules and Their Encapsulation in Order to Improve Their Activities

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**Abstract:** Microbiological risk associated with abiotic surfaces is one of the most important issues worldwide. Surface contaminations by pathogenic bacterial biofilms or adherent cells affect a number of sectors, including medical services, food industries, human services, and the environment. There is a need to synthesize or to set up novel biosource-based antimicrobials. Terpenes such as limonene carvacrol are usually found in essential oils and have potent antimicrobial activities. However, the direct use of these molecules is often inefficient due to their low water solubility, loss of volatile compounds, thermal degradation, oxidation, and toxicity. The organic synthesis of stable metal complexes based on terpene ligands seems to be a promising issue, since it can allow for and promote the use of terpenes and challenge the drawbacks of these molecules. Spray drying could be the most suitable method for encapsulating metal complexes based on terpene ligands to protect and enhance their activity against bacterial biofilms. The goal of this review is to discuss the microbiological risk associated with pathogenic bacterial biofilm and the organic synthesis of novel antimicrobial complexes based on terpene ligands. In addition, this review explores how to improve their bioactivities and characteristics using a formulation based on encapsulation.

**Keywords:** terpenes; metallic ions; complexes; microencapsulation; biofilm



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## 1. Introduction

Pathogenic bacteria have been reported as contaminating microorganisms on equipment surfaces commonly used in both the medical and food sectors. These surfaces are potential reservoirs for the spread of microbial pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. [1,2]. If the environmental conditions are suitable to growth, abiotic surface-adherent bacteria are able to form a complex structure called biofilm [3]. In the food sector, surface contaminations are involved in foodborne infections experienced after the consumption of food and drink contaminated with pathogens. In hospitals, contamination of medical equipment is involved in healthcare-associated infections (HCAIs) [4]. According to the Centers for Disease Control and Prevention, about 1.7 million hospitalized patients in the United States contract HCAIs each year while being treated for other health conditions, and more than 98,000 of these patients die as a result of HCAIs [5].

These infections are generally caused by multidrug-resistant bacteria [6]. The most common microorganisms involved are *Escherichia coli* found in the intestines and *Staphylococcus aureus* bacteria found on the skin and mucous membranes in the nose of healthy

humans. *Pseudomonas aeruginosa* bacteria thrive in soils and wet environments [7,8]. On the other hand, there are food safety concerns that arise frequently in agrifood value chains. Foodborne illness can occur at any stage of food production and distribution. Thus, in food industries, effective cleaning and disinfection of equipment are required to reduce the risks of bacterial contamination such as those related to *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* spp. [9–11].

Therefore, the control of biofilms remains the most important task for many industries to reduce the microbiological risk associated with its persistence in these areas. To reduce the microbiological risk associated with the main bacterial pathogens, the use of plant extracts as biosourced antimicrobials could be a sustainable and eco-friendly strategy. Molecules derived from essential oils (EOs) and plant extracts that are known as antimicrobials could be the best option, as these antimicrobials are efficient and produced by available and renewable resources such as *Pinus pinaster*, *Pistacia lentiscus*, *Calicotome spinosa*, and *Thymelaea hirsute*, as well as food waste like citrus peels. This is one of the principles of green chemistry, which, within the developed approach, leads to an innovative and efficient route for the preparation of high-value-added chemicals with circular economic, environmental, and ethical goals [12–14].

The composition of essential oils from each plant species is unique, with one to three terpenes constituting major components and many minor components [15]. However, their use as antimicrobials is fraught with difficulties due to factors such as their high volatility and solubility in water, as well as their cytotoxicity [16–18]. Several terpene-based ligands and their associated metal complexes have superior antibacterial activity compared to free ligands. Furthermore, some metal complexes have been reported to be water-soluble, as we demonstrated previously [19–21]. This property makes them more useful as antibacterial compounds than terpenes.

Bioinorganic and medicinal chemists have paid close attention to the antibacterial properties of metal ions and their complexes [22]. Metals such as Zn, Fe, Hg, As, Cu, Ag, and Ru have been used as antimicrobials in various forms for thousands of years [22–25]. The use of metals for the treatment of many diseases was mentioned in the Ebers papyrus [26]. Silver has biocidal and bactericidal properties, copper reduces inflammation and is used to treat various *Escherichia coli* and *Pseudomonas* spp. infections, and iron is used to treat anemia. The use of metals as antibacterial agents declined after the discovery of antibiotics in the twentieth century. Antibiotic resistance was discovered shortly thereafter due to the transfer of antibiotic resistance genes, also known as resistance transfer factors. Metal complexes such as  $\{RuCl[(p\text{-cymene})][\text{Aminooxime L3}]^+Cl^-\}$  [21] are promising antimicrobials that have been reported to exhibit stronger antibacterial activity than uncomplicated ligands [27]. The goal of this review is to provide a comprehensive overview of existing data on the microbiological risk associated with pathogenic bacterial biofilms in healthcare sectors and food industries. The organic synthesis of novel antimicrobials metal complexes based on terpene ligands is discussed, demonstrating the potential of this strategy. Their formulation based on spray-drying encapsulation is also discussed as a strategy to improve their antibacterial and antibiofilm activities.

## 2. Healthcare-Associated Infections Related to Adherent Bacteria and Their Biofilms

Healthcare-associated infections (HAIs), also known as nosocomial infections, are a major source of concern for both patients and healthcare workers [28]. An infection of this type can occur in a hospital, nursing home [29], outpatient clinic [30], or other clinical setting. As stated by the Centers for Disease Control and Prevention (CDC), 1 in every 31 hospitalized patients and 1 in every 43 nursing home residents has an HAI [31]. These infections may be caused by self-contamination, such as infections linked to *Staphylococcus* spp. bacteria naturally present in the skin. Such colonizing bacteria may become invasive if natural barriers are broken as a result of surgery or catheter implantation [32]. Cross contamination occurs when one person distributes an infection to another, either directly or indirectly. For instance *Pseudomonas aeruginosa*, can spread from one person to

another through contact and activities such as meetings or sharing rooms, medical equipment, and cutlery [33,34]. Infections propagate through vehicles such as tap water, hospital foods, and intravenous drugs [35]. Moreover, detached microorganisms from a biofilm are a major source of bacterial spread and contamination [36]. They cause significant issues in healthcare sectors and food industries. The National Institutes of Health (NIH) reported that about 65% of all bacterial infections are associated with bacterial biofilms [37]. In addition, infections caused by biofilm growth are notoriously challenging to treat. As reported in Table 1, biofilms frequently form on the inert surfaces of devices like catheters, prosthetic heart valves, and joint replacements [38,39]. The global production of biomedical devices and tissue-engineering-related materials is estimated to be worth 180 USD billion annually, but medical equipment continue to suffer from microbial contamination and colonization [40,41]. These infections include central line-associated bloodstream infections (CLABSIs) (Table 1), which occur when bacteria or fungi enter the bloodstream via a central line; catheter-associated urinary tract infections (CAUTIs); central venous catheter (CVC) (see Table 1) or hemodialysis catheter infections; transcatheter aortic valve replacement (TAVR) infection; prosthetic joint infection (PJI); pediatric ventilator-associated events (PedVAEs); and ventilator-associated pneumonia (VAP) (Table 1). Infections can also occur at surgical sites, which are known as surgical site infections (SSI) [42]. Between 2020 and 2021, statistically significant increases in *methicillin-resistant Staphylococcus aureus* MRSA (14%), VAE (12%), CLABSI (7%), and CAUTI (5%) were observed [43] (see Table 1).

**Table 1.** Most frequently isolated microorganisms discovered in biofilm-related HACIs.

Healthcare-Associated Infection Types	Microorganisms	References
Central line-associated bloodstream infection (CLABSI)	<i>Staphylococcus aureus</i> , <i>coagulase-negative staphylococci</i> , <i>Candida</i> spp., <i>methicillin-resistant Staphylococci (MRSA)</i> , <i>Enterococci</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Acinetobacter</i> , and <i>Candida species</i>	[44–49]
CVC/hemodialysis catheter infection	<i>Enterobacter cloacae complex (ECC)</i> , <i>Candida parapsilosis</i> , <i>Staphylococcus aureus</i> , and <i>methicillin-resistant Staphylococcus aureus (MRSA)</i>	[50–52]
Pediatric ventilator-associated events (PedVAEs)	<i>Candida albicans</i> , <i>Staphylococcus epidermidis</i> , <i>Pseudomonas aeruginosa</i> , and <i>Haemophilus influenzae</i>	[53–58]
Ventilator-associated pneumonia (VAP)	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , and <i>Enterobacteriales</i>	[59–61]
Catheter-associated urinary tract infection (CAUTI)	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Proteus mirabilis</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , and <i>Candida</i>	[62–66]

Table 1. Cont.

Healthcare-Associated Infection Types	Microorganisms	References
Transcatheter aortic valve replacement (TAVR) infection	<i>Streptococcus</i> and <i>Staphylococcus aureus</i>	[67–73]
Cardiovascular devices	<i>Staphylococcus aureus</i> , <i>coagulase-negative Staphylococcus</i> , and <i>Staphylococcus aureus</i>	[74–82]
Surgical site infection (SSI)	<i>Escherichia coli</i> , <i>Enterobacter</i> spp., <i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp., <i>Klebsiella pneumoniae</i> , <i>Streptococcus pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , <i>Proteus</i> spp., and <i>methicillin-resistant S. aureus (MRSA) CoNS</i>	[83–90]
Prosthetic joint infection (PJI)	<i>Methicillin-resistant Staphylococcus</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus lugdunensis</i> , <i>Staphylococcus</i> spp., <i>Pseudomonas aeruginosa</i> , and <i>Streptococcus gordonii</i>	[91–101]

### 3. Food Poisoning Related to Adherent Bacteria and Their Biofilms

Food poisoning is a prevalent, costly, and occasionally deadly disease. The bacteria most often involved in foodborne illnesses are *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogens* [102]. In the United States, food poisoning causes 9.4 million illnesses, 55,961 hospitalizations, and 1351 fatalities each year [103]. The majority of pathogens involved in foodborne disease are frequently detected in the intestines of mammals, reptiles, and birds. These pathogens are transmitted to humans through the consumption of foods of animal origin, such as eggs, meat, and milk. These bacteria are able to adhere to abiotic surfaces, and they have the ability to form biofilms on almost all utensil surfaces and under almost all environmental conditions encountered in food production plants [104,105]. The cleaning and disinfection of premises and equipment are among the major measures to control food pathogens in food industries. Furthermore, as reported, one of the five keys to safer food is keeping clean [106]. When premises and equipment are contaminated and the conditions of cell growth are suitable, adherent cells form biofilms [36]. It has been established that bacterial cells in a biofilm state are more resistant than planktonic cells to cleaning and disinfection procedures. Biofilm cells in a food processing unit are typically not eliminated by standard cleaning procedures and therefore may be a source of contamination for foods that come into contact with food contact surfaces (countertops, rubber, gloves, plastics, etc.) (Table 2) [107,108]. Food industries use physical, chemical, and biological treatments as biofilm inhibition techniques. However, traditional cleaning methods usually fail to remove or destroy germs found in the inner layers of the biofilm. Novel strategies based on hurdle technology using safe biochemical agents such as enzymes, essential oils, etc., show efficient antibiofilm activities and are now under development and drawing increasing attention as potentially safe and environmentally friendly biochemical procedures [10,109–113].

**Table 2.** Pathogenic bacterial diversity in the agrifood ecosystem.

Foodborne Pathogen	Food Environment Processes	Food Equipment Isolation	Food Product	References
<i>Listeria spp.</i>	Fine cutting of loin	Apron Conveyor belt Loin ripping board	Meat	[114]
		Packaging film Hooks		
	Meat cutting	Saw Conveyor belt		
		Drains Floors Freezers Aprons Door handles Taps	Smoked salmon Raw salmon	[115]
		Knives Mincing machine Deriding machine Bowl cutter Vacuum packaging machine Slicing machine Scales Stainless-steel tables Sticks for hanging the products Cutting boards Stainless steel trolley	Pork, beef, chicken, and sheep meat	[116]
			Mushroom	[117]
			Iceberg lettuce	[118]
			Poultry meat Raw beef	[119]
			Chicken cold cuts	[120]
	Cold storage		Pork meat	[121]
	3D Food Printing Systems		Food ink capsules	[122]
		Bulk tank milk Milk filter	Raw milk	[123]
		Fish processing plants		[124]
	Food service establishments		Enoki mushrooms	[125]
	Cutting room		Meat	[126]
	Floor Mixing trough Separating machines Cutter Transport belt Mixing machine Dicing machine Knives	Saucisse Saucisson Rosette Chorizo	[127]	

Table 2. Cont.

Foodborne Pathogen	Food Environment Processes	Food Equipment Isolation	Food Product	References
<i>Escherichia coli</i>			Sliced cooked and cured ham Sliced cooked and cured sausage Sliced cooked meats Veal pie and calf liver pâté	[128]
	Refrigerated storage		Kale	[129]
			Fresh beef	[130]
		Countertop Draining board	Chicken	[131]
			Milk	[132,133]
			Pastries Cereals	[134]
<i>Staphylococcus spp.</i>		Hand Bulk farm milk Pooled udder milk Milking container Bulk container Teat Overall	Milk Water for cleaning teat and hands	[137]
		Dish cloth Hands Refrigerator handle Oven handle Countertop Draining board	Chicken	[131]
	Slaughter hall cutting room		Meat	[126]
	Dairy staff	Hands Anterior nares	Raw milk Minas Frescal cheese Food handlers	[138]
			Chicken breeds	[139]
<i>Salmonella spp.</i>		Plastic (tote) Plastic (bucket elevator) Stainless steel Concrete Rubber (belt) Rubber (tire)	Pet food	[140]
	Domestic kitchen surfaces	-	Chicken carcasses	[142]

Table 2. Cont.

Foodborne Pathogen	Food Environment Processes	Food Equipment Isolation	Food Product	References
			Tomatoes	[143]
	Individual production chains		Poultry food Chicken gizzards	[144]
	3D food printing systems		Food ink capsules	[122]
		Bulk tank milk Milk filter	Raw milk	[123]
			Fresh beef	[130]
		Dish cloth Countertop	Chicken	[131]

### 4. Biofilm

#### 4.1. Biofilm Formation

A biofilm is a structured community of microbial cells enclosed in a self-produced extracellular polymeric matrix that are adherent to the a surface, the interface, and each other [145,146]. Biofilms protect the bacteria and allow them to survive in hostile environmental conditions. Bacterial biofilms can resist the host immune response and are much more resistant to antibiotics and disinfectant treatments than planktonic bacterial cells [147]. Biofilm formation occurs in several steps according to a well-established pattern, as shown in Figure 1. First, bacteria adhere to a surface (1) and start to develop an irreversible attachment (2). The bacteria then group together, multiply, and form microcolonies (3). During the biofilm maturation phase, bacteria begin to synthesize extracellular polymeric substances (EPS), which are made up of polysaccharides, proteins, extracellular DNA (eDNA), and lipids. Within hours of accumulation of EPS, bacteria are trapped in a complex protective extracellular matrix (Figure 2), forming a mature biofilm that offers a protective environment against antibacterial agents (4) [148,149]. The final stage in biofilm formation is the detachment or dispersal of bacterial cells, which can then colonize new surfaces (5) (Figure 1). Thus, these bacterial cells have the ability to adhere to new surfaces and reform a biofilm and can contribute to biological dispersion, which plays an important role in the transmission of bacteria and the spread of cross contamination and infection [111,150].

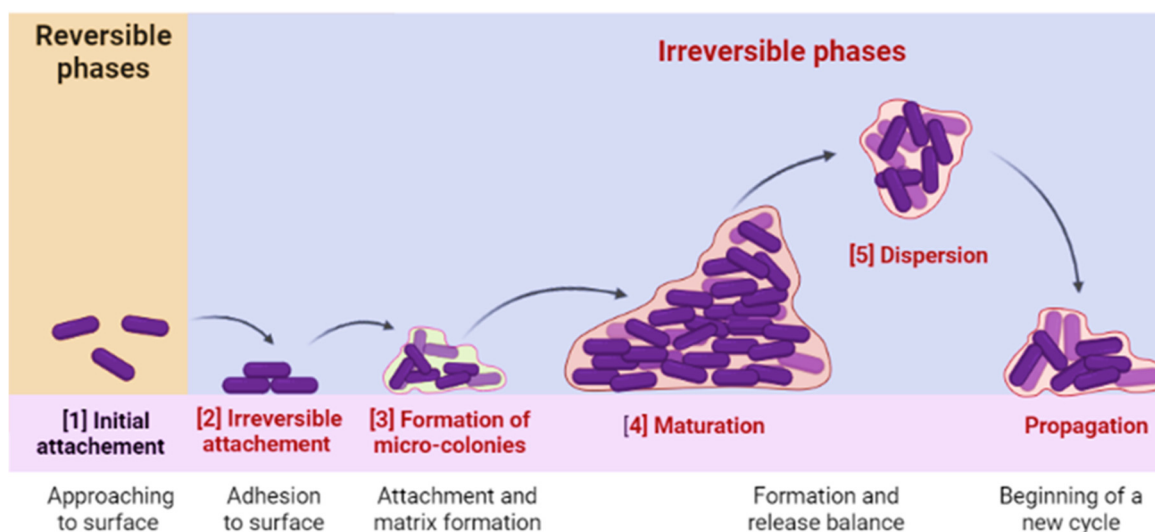
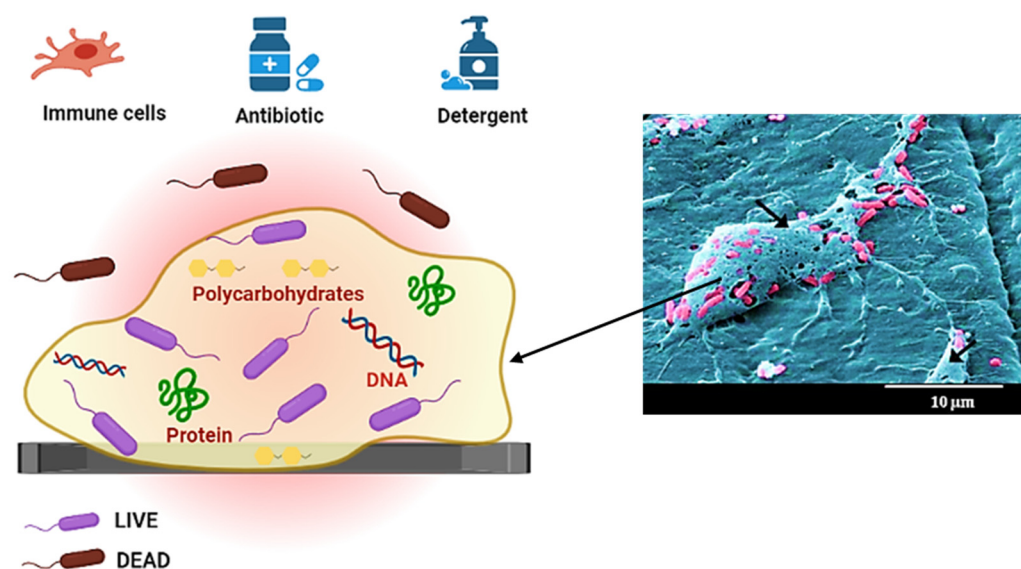


Figure 1. Different steps of biofilm formation.





**Figure 2.** Effect of the biofilm's extracellular matrix on pathogenicity.

Biofilm resistance is linked to a microbial cell-to-cell communication system called quorum sensing (QS). When a bacterial community reaches a high level, signaling molecules are synthesized. The expression of QS molecules differs biochemically between Gram-negative and Gram-positive cells. In Gram-positive bacteria, the main function of the QS system is to synthesize intracellular molecules called autoinducer peptides (AIPs). However, in Gram-negative bacteria, autoinducer molecules are secreted from parent molecules called N-acyl homoserine lactones (AHLs). These molecules can enter the intracellular environment to regulate gene expression in a manner dependent on the extracellular environment. This ability helps bacteria to survive environmental stressors [151–153].

Inhibition of QS or quorum quenching [154] is therefore a preferred strategy to fight against microbial infections. This strategy attenuates the pathogenicity of microbes and increases the sensitivity of microbial biofilms to antibiotics. This happens by degrading the communication molecules involved in quorum-sensing or -blocking receptors for the same molecules [154]. These mechanisms are implemented by certain organisms, such as plants. In this respect, various bioactive molecules, notably terpenoids, flavonoids, and phenolic acids, exhibit numerous anti-QS mechanisms via inhibition of autoinducer release, sequestration of QS-mediated molecules, and deregulation of QS gene expression [151,155,156].

#### 4.2. Biofilm Matrix

The production of the extracellular matrix (ECM) by bacteria in biofilms provides protection against hostile environments such as antimicrobial agents and the host immune system. Figure 2 shows a scanning electron micrograph of an *Escherichia coli* biofilm enclosed in an extracellular matrix [36]. ECM contributes to pathogenicity by increasing antibiotic tolerance and promoting immune evasion. The production of the extracellular matrix is central to the development of bacterial biofilm architecture [157,158].

The matrix is highly hydrated, with up to 97% water content, and is rich in polysaccharides, proteins, and extracellular microbial DNA. It can be composed of one or more microbial species (bacterial or fungal). The matrix is a hydrated mucilaginous layer that prevents bacteria from drying out [159]. The ECM is made up of extracellular polymeric substances (EPS) that have been identified, highlighting its versatility, with various functions (Table 3) [160,161].

**Table 3.** Main roles of the ECM [160].

EPS Elements	Role
Polycarbohydrates, proteins, and DNA	Adhesion
Neutral and charged polycarbohydrates, proteins (such as amyloids and lectins), and DNA	Cohesion
Polycarbohydrates and proteins	Barrier of defense
Potentially all the components of EPS *	Source of nutrients
Hydrophilic polycarbohydrates and eventually proteins	Water retention
Extracellular DNA	Genetic information exchange

\* EPS: extracellular polymeric substances.

EPS promote microbe adhesion to biotic and abiotic surfaces, The stability and functionality of the EPS matrix are critical in the development of a robust and resilient microbiome community, in addition to aiding in the tolerance of these multicellular communities to various antimicrobial agents (Figure 2). Biofilm bacteria are more resistant to external aggressions such as pH, temperature, and antimicrobial agents than planktonic bacteria [162,163]. Biofilms can withstand antibiotics at concentrations 10 to 1000 times higher than planktonic bacteria, and it has been reported that the matrix acts as a diffusion barrier for toxic molecules [164]. The presence of zones of low or no oxygenation in the biofilm's deep layers may also contribute to resistance to some biocides, which may be inactivated under such conditions or are ineffective against metabolically inactive bacteria. An increasing body of experimental evidence suggests that resistance is linked to the expression of specific genetic mechanisms. All of these characteristics suggest that biofilm is a favorable way of life for bacteria, to the point of constituting a default mode of life for certain bacterial species [165,166].

### 5. Terpenes and Their Derivatives as Good Candidates to Fight against Adherent Bacterial Cells and Biofilm (Antimicrobial and Antibiofilm Effect)

Chemical substances or compounds are used as disinfectants to inactivate or to destroy pathogenic microorganisms on inert surfaces used in healthcare sectors or in food industries. They are used as antimicrobials in hospitals, dental offices, kitchens, bathrooms, and food premises, as well as on equipment. The current challenge is to set up new products and avoid toxic by attempting to use biobased antibacterial agents [167]. Green chemistry is a branch of chemistry and chemical engineering that focuses on the development of products and processes that reduce or eliminate the use of hazardous substances [168,169]. Essential oil, which is derived primarily from herbs and citrus fruits, is a commercially important product with health-promoting properties due to the presence of terpenes and limonoids, as well as other bioactive components such as flavonoids, carotenoids, and coumarins [170]. Essential oils reveals are composed primarily of complex mixtures of two groups of organic compounds: terpenes and phenylpropane derivatives (terpenoids and phenylpropanoids) [171]. Terpenes are naturally occurring hydrocarbons with cyclic or acyclic structures that are made up of a multiple of five carbon atoms and with the general formula  $(C_5H_8)_n$ . The basic molecule is isoprene (2-methyl-1,3-butadiene:  $C_5H_8$ ). This family includes monoterpenes (10 carbon atoms), sesquiterpenes (15 carbon atoms), diterpenes (20 carbon atoms), sesterpenes (25 carbon atoms), triterpenes (30 carbon atoms), and polyterpenes ( $5n$  carbon atoms). Essential oils, on the other hand, only contain the most volatile terpenes, such as monoterpenes, sesquiterpenes, and (very rarely) diterpenes. Monoterpenes are primarily responsible for the antibacterial, antioxidant, and insecticidal properties of essential oils [172], primarily comprising alcohols (carveol, menthol, linalool, alpha-terpineol, citronellol, nerol, and geraniol), phenol derivatives (carvacrol and thymol), aldehyde (citral), ketone (carvone), hydrocarbons ((*R*)- and (*S*)-limonene, and  $\alpha$ -pinene), and monoterpene ethers [173,174]. The synthesis and properties of coordination compounds with chiral ligands based on terpenes are the subject of considerable attention.

Terpenes are widely used and exhibit high enantiomeric purity and biological activity, which has led to their use in medicine. It has been reported that terpenes and their derivatives are active against various microorganisms (Table 4) [175]. The majority of terpenes have a greater impact on Gram-positive bacteria than on Gram-negative bacteria [176,177].

Due to their capacity to alter the cell envelope and cytoplasmic stability and to lead to cell damage, they have a harmful effect on microbes [178,179]. Although aromatic substances like carvacrol, thymol, and eugenol exhibit a stronger inhibitory action, the antimicrobial activity of monoterpenes has demonstrated that neither the amount of double bonds in a structure nor the existence of an acyclic structure significantly affects this activity [180,181]. Terpenes and terpene derivatives have a multitarget impact, which is one of the reasons that they are a potent antimicrobial agent. Carvacrol is known to have an adverse effect on the outer membrane by causing the release of lipopolysaccharides (LPS) or by increasing the permeability of the cytoplasmic membrane. Thymol also causes structural and functional changes to the inner or outer cytoplasmic membrane, interactions with membrane proteins, and effects on intracellular targets. Thymol and carvacrol only differ in terms of the position of their hydroxyl groups [182,183]. As a result of their multitarget effects, most terpenes and their derivatives are known to be potent antibacterial agents against multidrug-resistant organisms, particularly bacteria and fungi, like methicillin-resistant *staphylococcus aureus* (MRSA), a strain that is resistant to a number of different antibiotics. Terpene derivatives have several target sites and methods of action; therefore, no microbial resistance has yet been created in opposition to them [184–186].

**Table 4.** Main antibacterial constituents of terpene derivatives.

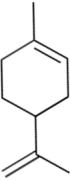
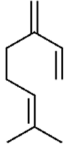
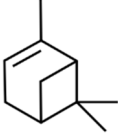
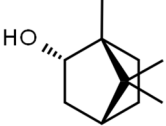
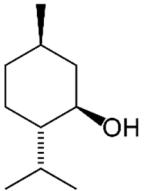
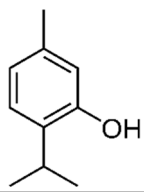
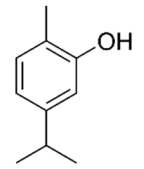
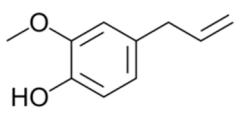
Terpene Derivative	Structure	Bacteria	Reference
Limonene		<i>Staphylococcus aureus</i>	[187]
		<i>Escherichia coli</i>	[188]
		<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	[189]
		<i>Enterococcus faecalis</i>	[190]
		<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Enterococcus faecalis</i> <i>Listeria monocytogenes</i>	[21]
Myrcene		<i>Escherichia coli</i> <i>Salmonella enterica</i> <i>Staphylococcus aureus</i>	[191]
$\alpha$ -Pinene		<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	[192]
Borneol		<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i>	[193]

Table 4. Cont.

Terpene Derivative	Structure	Bacteria	Reference
Menthol		<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Klebsiella pneumoniae</i> <i>Staphylococcus aureus</i>	[194]
		<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Listeria innocua</i> , <i>Saccharomyces cerevisiae</i>	[195]
Thymol		<i>Enterobacter sakazakii</i>	[196]
		<i>Salmonella Enteritidis</i>	[10]
		<i>Aeromonas hydrophila</i>	[197]
Carvacrol		<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Salmonella spp.</i>	[199]
		<i>Pseudomonas aeruginosa</i> <i>Enterococcus faecalis</i>	[200]
Eugenol		<i>Listeria monocytogenes</i> CECT 933 <i>Escherichia coli</i> ATCC 35218 <i>Pseudomonas aeruginosa</i> PAO1 <i>Staphylococcus aureus</i> ATCC 6538	[201]
		<i>Escherichia coli</i> ATCC 25922 <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> ATCC 9027 <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> ATCC 25923 <i>Staphylococcus aureus</i> <i>Streptococcus mutans</i> ATCC 0446	[202]

## 6. Metal Complexes Based on Terpene Ligands and Their Biological Activities

### 6.1. The Reactivity of Terpenes

To address the microbiological risk associated with adherent pathogenic bacteria and their biofilms in hospital and in food environments, many scientific disciplines have to be combined: organic chemistry to synthesize new molecules with antibacterial and antibiofilm effects; microbiology to assess their biological activity; and formulation to set up the best formula to enhance the antibacterial and antibiofilm activities and to deal with challenges related to these molecules such as solubility, volatility, and eco- and cytotoxicity. Coordination chemistry of transition metals and biologically active ligands is an active field of modern chemistry that incorporates contributions from asymmetric synthesis, metal complex catalysis, biochemistry, medicinal chemistry, and pharmacology [203,204]. The current challenge is to set up highly reactive ligands for organic synthesis. Therefore, it is important to first study the coordination behavior of biologically active chiral ligands containing N and O donor atoms towards metal ions [205].

The two chiral forms of a molecule, also known as enantiomers, have opposing spatial geometries and therefore interact with their environment differently. This feature is extremely important in medicinal chemistry [206,207]. In the case of limonene, which is abundant in citrus fruit essential oils and has two enantiomers—one with a lemon smell ((S)-(-)-limonene) and one with an orange smell ((R)-(+)-limonene)—the two enantiomers do not necessarily exhibit the same biological activity because they do not react with

the same receptors [208,209]. An enantiomer of a medical molecule can have beneficial properties while the other is highly harmful. Nature is a high-level synthetic chemist, producing a wide range of chiral substrates with high stereochemical purity, particularly ligands based on natural molecule derivatives. Terpenes are among these molecules and can induce chirality and reactivity (Figure 3) due to the presence of double bonds in their structure, in addition to the ability to perform addition [210], rearrangement [211], cyclization, isomerization (Figure 4), and ozonolysis ( $O_3$ ) (Figure 5). Dehydration reactions can also occur in compounds derived from terpenes with alcohol functions.

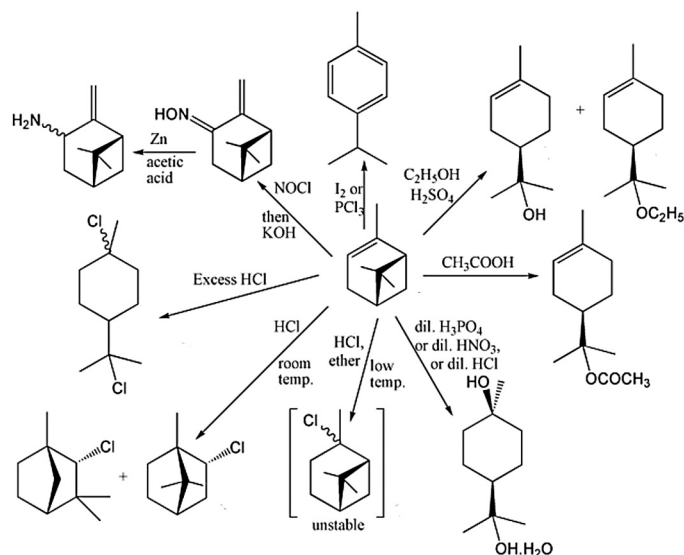


Figure 3.  $\alpha$ -Pinene reactions [212].

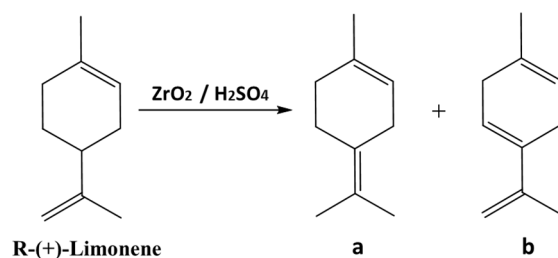


Figure 4. The (*R*)-(+)-limonene isomerization process was carried out at 60 °C in the presence of the  $H_2SO_4$  impregnated zirconium oxide ( $ZrO_2$ ). A mixture of terpinolene (a) and terpinene (b) was obtained as a result of this isomerization [213].

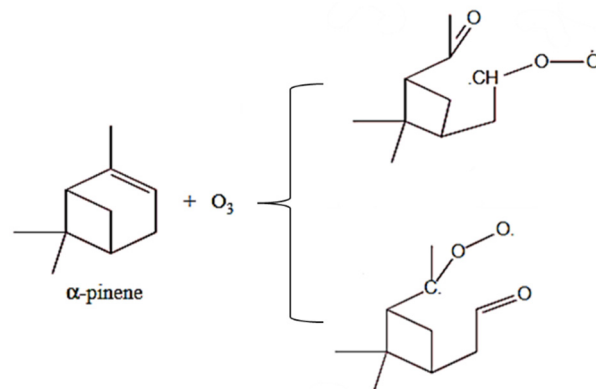


Figure 5. Reaction of  $O_3$  with  $\alpha$ -pinene [214].

## 6.2. Oligodynamic Effect

The oligodynamic effect enables certain metals to self-clean by destroying microorganisms with their metal ions, which would otherwise be toxic to many bacteria [215,216]. This effect can be seen in brass door handles, water tanks on certain aircraft, and silverware. The simple composition of metal surfaces provides protection against bacteria, even in the absence of a disinfectant. Owing to the changing nature of bacteria, the study of antimicrobials based on metals and metal ions has been slow and difficult, but it has been demonstrated after several experiments that mineral compounds disrupt biofilm production and synergistically exert antimicrobial effects by inhibiting biofilm production and enzymatic activity, altering membrane stability and function, damaging DNA, and generally inhibiting plankton growth [217,218]. Metals have been used as antimicrobial agents for thousands of years, dating back to the Egyptians' use of copper salts as an astringent. Copper and silver were also used by Indians, Egyptians, Persian kings, Phoenicians, Greeks, and Romans to preserve food and disinfect water [219]. A variety of metal-coated surfaces have antibacterial capability against *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*, including silver, titanium, copper, iron, molybdenum, zinc, etc. [220]. In fact, some metal compounds, especially those that do not show substantial metal complexation might just serve as vehicles for metal ions. Given that metals are used to kill bacteria, it is not surprising that research has looked at the direct antibacterial applications of these metals, particularly in the form of nanoparticles. It has been shown that the metal ions released by these nanoparticles are involved in their antibacterial activity [221–223]. Copper has a high affinity for carboxyl (COOH) and amine groups present on the cell surface. Released Cu ions can bind to DNA and disrupt the helical structure by cross linking nucleic acid strands. It also disrupts the biochemical processes of bacterial cells [224]. Silver ions disrupt the function of membrane-bound enzymes and respiratory enzymes, leading to the complete destruction of the bacterial cell [225].

## 6.3. Antimicrobial Activity of Metal Complexes Based on Terpene Ligands

A metal complex, as opposed to freely solvated metal ions or metal nanoparticles, is a well-defined arrangement of ligands centered on one or more metal centers. These compounds are distinguished by the fact that their characteristics can be modified in a manner similar to that used in conventional medication development [226]. Metals have a wide range of properties and almost infinite combinations of ligands to form complexes, with the number of coordinations ranging from 1 to 20 [227,228], resulting in a rich and three-dimensional variety of chiral metal complex structures. In comparison, the geometric diversity of organic compounds is lower because carbon normally forms no more than four bonds.

In biology, chirality is particularly significant, since it can change the characteristics and therapeutic actions of molecules. When a chiral chemical is used in a compound of medical treatments or antimicrobial agents, one of the enantiomers may be effective on the organism while the other is ineffective. This potential enables us to create substances with three-dimensional structures, as the use of chiral centers correlates with higher target selectivity and lower off-target effects [221,229–231].

Metal ions, as well as terpenes and their derivatives, have well-known antimicrobial properties. However, terpenes and their derivatives are insoluble in water, necessitating the use of organic solvents such as ethanol, chloroform, diethyl ether, and DMSO to examine their activity against living organisms [232]. There is also the issue of volatility, despite the fact that monoterpenes are relatively stable. Sesquiterpenes and oily diterpenes, on the other hand, are less stable because they have more oxygen functional groups, making them biodegradable [233]. Biosourced terpenes might be used to synthesize complexes with antibacterial and antibiofilm activities, such as the [(*p*-cymene)] [aminoxime L3]<sup>+</sup>Cl<sup>−</sup> (RuL3) complex based on (*R*)-limonene, which is miscible in water; therefore, complexation overcomes the problem of solubility while also making it more stable. We previously demonstrated that the minimum inhibitory concentration (MIC) values for limonene were

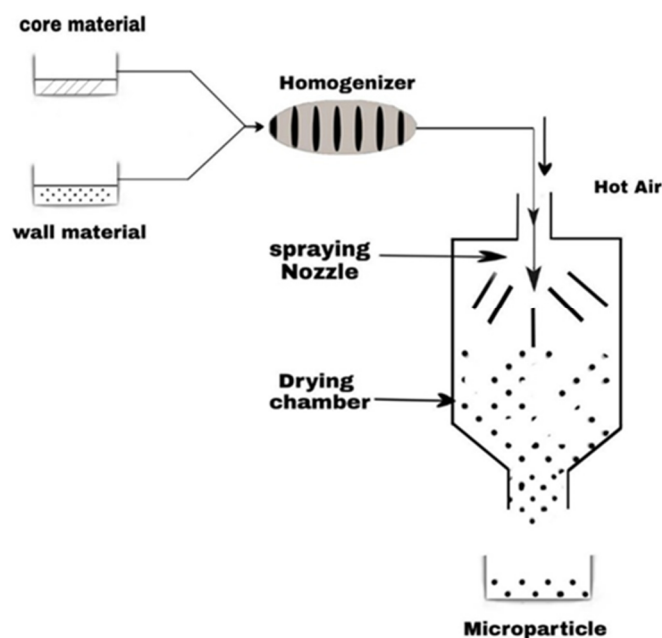
12.5 mg/mL when tested against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis*. In comparison, the MIC of the RuL3 complex (0.4 mg/mL) was approximately 30 times lower. Thus, limonene complexation with ruthenium increased its antibacterial effectiveness and appears to be a promising way to decreasing the amount of antimicrobial used against bacteria and biofilms [21]. Although there are many metal complexes based on terpene ligands, little is known about their biological activity [234–236]. Nonetheless, based on existing evidence, transition metal complexes with terpene ligands or Schiff base ligands are effective antimicrobial, anticancer, antifungal, and antioxidant agents (Table 5). Complexation protects against environmental variables, improves stability, avoids terpene component volatilization, and enhances antibacterial activity [237–240].

**Table 5.** Selected studies describing the various biological activities of metal complexes.

Metal	Ligand	Activity	Reference
Ru (II)	Based on limonene	Antibacterial Anticancer	[21]
Zn (II) Fe (III)	Monodentate Schiff base	Antifungal Antioxidant Antibacterial	[241]
Fe (II) Co (II) Zn (II) Ru (II)	Azo dye	Enzyme inhibition Antioxidant	[242]
Zn (II)	PhenanthrolineIndomethacin	Anti-breast cancer	[243]
Zn (II) Sn (II) Ce (III)	Gemifloxacin and glycine	Antifungal Antioxidant Antibacterial	[244]
Cu (II) Ni(II) Co (II) Fe (II)	Bis-pyrazole	Antibacterial and antifungal	[245]
Co (II) Fe (II) Ni (II) Mn (II)	N-heterocyclic	Antitumor	[246]
Ru (II)	Yriazolopyrimidine in liposomes	Anticancer	[247]
Pt (II)	Cis-diaminodichloro	Anticancer	[248]
Co (II) Cu (II) Zn (II)	Diimine–glycinate	Anticancer	[249]
Cu (II) Zn (II)	Bidentate–morpholine-based	Antibacterial	[250]
Co (II) Ni (II) Cu (II) Zr (IV) Pd (II) Cd (II)	Combination of metformin and 1,4-diacetylbenzene	Antifungal Antibacterial	[251]
Cr (III) Fe (III) Cu (II)	Multisubstituted aryl imidazole	Antibacterial Anticancer	[252]
Mn (II) Co (II) Ni (II) Cu (II) Zn (II) Cr (III)	Moxifloxacin–imidazole	Antifungal Antibacterial	[253]

## 7. Encapsulation of Terpene Derivatives to Improve Their Stability and Antimicrobial Activity

Encapsulation of terpene derivatives is a promising approach to overcome the aforementioned challenges by protecting them from heat, light, and oxygen. It promotes their solubility and stability, increases bioavailability, masks flavors, and reduces contamination risk [254]. Microencapsulation is a technique whereby liquid droplets, solid particles, or gas compounds are entrapped into thin films of food-grade encapsulating agents called wall material. The retention of the encapsulated compounds depends on their chemical structure, solubility, polarity, and volatility. Most microcapsules are small particles with diameters between a few micrometers and a few millimeters. The size and shape of the microcapsules depend on the materials and processes used to prepare them. Different types of capsules can be produced from a wide range of wall materials (polysaccharides, proteins, monomers, etc.) and by a large number of different processes, such as spray drying (Figure 6), freeze drying, extrusion, coacervation, liposome entrapment, and interfacial polymerization.



**Figure 6.** Schematic representation of the spray-drying microencapsulation process.

Among these techniques, spray drying is the most common technology used in the food industry due to the low cost and availability of equipment. Depending on the core material and the characteristics desired in the final product, wall materials can be selected from a wide variety of natural and synthetic polymers or monomers. This process can produce powdered microcapsules from a liquid in a single simple and scalable operation [255]. It is possible to prepare mixtures of natural antimicrobials and biopolymers. The wet suspensions/emulsions are then converted to powders by evaporating the majority of the water using an appropriate dehydration method. The resulting powder is evaluated for activity, density, flow, size, stability, and redispersion.

Spray drying is the most common method of encapsulating bioactive ingredients. It can produce powdered microcapsules from a liquid in a single simple and scalable operation [256]. As represented in Figure 6, spray drying of hydrophobic compounds comprises four steps: (1) emulsion preparation by high-pressure homogenization, (2) wall material addition, (3) dispersion in small droplets by atomization, and (4) dehydration of the atomized particles. This method entails creating an emulsion containing the wall material and the core, then spraying it into a drying chamber with circulating hot air; when



the water comes into contact with the hot air, it evaporates immediately, and the core is entrapped into the wall material matrix [257–259].

Since almost all spray-drying processes in the food industry are carried out with an aqueous feed formulation, the wall material must be soluble in water and should possess good emulsification, film-forming, and drying properties, and the concentrated wall solutions should have low viscosity. Many available wall materials possess these properties, but the number of materials approved for food uses is limited.

Despite the beneficial effects of terpenes and their derivatives (antimicrobial, anti-fungal, anticarcinogenic, and pharmacological properties), they are subject to a number of drawbacks, such as their low miscibility in water (which lowers their bioavailability) and their degradation by light and temperature, as they are sensitive to environmental conditions and undergo volatilization. To overcome the chemical instability of certain terpenes, encapsulation of these compounds has been used to address this issue [233,260]. Antimicrobial activity is typically unaffected by covering the “active” ingredient (which may include volatile substances) in a protective matrix. The used polymers are basically inert to the encapsulated material and can offer excellent protection against degradation or evaporation. On the other hand, nano-sized delivery methods can improve passive cellular absorption mechanisms because of their subcellular size, which lowers mass transfer resistance and boosts antibacterial action [261–263]. Free carvacrol dissolved in DMSO had a high MIC of  $5 \text{ mg}\cdot\text{mL}^{-1}$  against *P. aeruginosa*. Nevertheless, the results after encapsulation demonstrated that encapsulated carvacrol suppressed bacterial growth at a concentration four times lower than that of F-CARV ( $1.25 \text{ mg}\cdot\text{mL}^{-1}$ ), indicating that encapsulation increases antibacterial action [200]. The enhancement of antibacterial performance may be primarily attributable to the smaller particle size and higher surface-to-volume ratio, which make it easier for encapsulated chemicals to diffuse into microbial cells [113]. Encapsulation is a tool used to control and reduce volatile terpene emissions, overcoming the issue of immiscibility in water and thereby improving stability, antimicrobial, antibiofilm, antiaflatoxigenic, and antioxidant activities, protecting enzymes and terpenes and enhancing their properties, as well as lowering cytotoxicity and ecotoxicity, as shown in Table 6.

**Table 6.** The use of encapsulation as a tool to enhance the activities and control the release of terpenes and their derivatives.

Compound	Composition	Encapsulation Effect	Reference
Ginger Essential Oil	Gingerol Curcumene Zingiberene	Controls and reduces the emissions of terpenes	[264]
Sacha Inchi Oil (Plukenetia huayllabambana)	-	Protects sachal inchi oil against oxidation	[265]
Flaxseed Oil	-	Improves oxidative stability	[266]
Sichuan Pepper Essential Oil (SPEO)	-	Responds to SPEO problems such as poor stability and low water solubility	[267]
Lycopene (Tetraterpene)	-	Enhances stability	[268]
Gaultheria Procumbens L. Essential Oil (GPEO)	-	Improves antimicrobial and antiaflatoxigenic activity and the stability	[269]
{RuCl [(Paracymene) [Aminooxime L3]] <sup>+</sup> Cl <sup>-</sup> Complex	Ruthenium metal (R)-limonene-based ligand	Increases antibacterial activity against biofilms of food-pathogenic bacteria while decreasing cytotoxicity	[21]
Oregano Oil	Thymol Carvacrol	Preserves the majority of antibacterial action and enhances the stability of oregano essential oils	[270,271]

Table 6. Cont.

Compound	Composition	Encapsulation Effect	Reference
Carvacrol	-	Overcomes insolubility and increases antibacterial activity against pathogenic bacterial biofilms while minimizing the amount used	[200]
D-Limonene	-	Preserves and possibly improves antimicrobial activity in order to evaluate the preservation of juice against inoculated spoilage microorganisms	[262]
Carvacrol Thymol	-	Improves antibacterial activity against Salmonella Enteritidis biofilms and reduces ecotoxicity against Daphnia magna	[10,272]
Peppermint Oil (PO) Green Tea Oil (GTO)	-	Enhances thermal stability, as well as antioxidant and antibacterial activities	[273]
Pepsin Trypsin Carvacrol	-	Protects enzymes and terpenes and boosts their antibacterial activities	[112]
Origanum vulgare	-	Overcomes stability-related restrictions, extending shelf life and maintaining its antioxidant, antimicrobial, and sensory-preserving properties	[274]

## 8. Conclusions

The eradication of pathogenic microorganisms biofilms is a major issue in medical sectors and food industries. Today, the most important need is to set up biosource-based antimicrobials to kill bacteria structured under a biofilm state. In this context, the use of terpene derivatives obtained from essential oils as biosource-based antimicrobial agents represents a promising strategy, since it is sustainable and innovative. In addition, the use of biosourced antimicrobials is in accordance with the concept of a circular economy. The present work shows that the setup of stable metal complexes based on terpene ligands seems to be a good strategy to mitigate the drawbacks of terpenes, such as water solubility and volatility. In addition, this study shows that encapsulation can be used to protect and enhance the stability and efficacy of terpenes or complexes against pathogenic microorganisms and their biofilms.

## 9. Future Perspectives

The setup of stable metal complexes based on terpene ligands seems to be a promising issue, that can allow for and promote the use of terpenes. This issue seems to be a source of novel antimicrobials and could help to challenge the increase in antibiotic resistance among bacteria. More studies have to be carried out to assess their cytotoxicity and ecotoxicity. Microencapsulation and nanoencapsulation based on spray drying could be a way to reduce the amount used and to increase their efficiency against bacterial biofilms. A hurdle technology-based strategy using metal complexes based on terpene ligands and other antibiofilm molecules such as enzymes could be an efficient way to fight bacterial biofilms.

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