Review

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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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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 to abiotic surfaces is one of the most important issue worldwide. Surface contaminations by pathogenic bacterial biofilms or adherent cell affect a number of sectors including medical services, food industries, human services, and the environment. There is a need to synthesize or to setup novel antimicrobials. Terpenes 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 need for biosourced compounds with antimicrobial activity, low toxicity and low cost promote the search for new stable metal complexes based on terpene ligands. This will enable the manufacture of value-added products with a lower environmental impact, as well as the production of high value-added commodities. The goal of this review is to discuss the development of novel antimicrobial complexes derived from terpenes. In addition, this review explored how to improve their bioactivities and characteristics by using a formulation based on microencapsulation.

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

1. Introduction

Pathogenetic bacteria have been reported as contaminating microorganisms of equipment surfaces, commonly used in both medical and food sectors. These surfaces can be then 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 for growth, the abiotic surfaces adherent bacteria are able to form a complex structure called biofilm [3]. In the food sectors, surface contaminations are involved in foodborne infections after consumption of food and drink contaminated with pathogens. In hospitals, contamination of medical equipment are 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 get HCAIs while being treated for other health conditions each year, and more than 98,000 of these patients die as a result of HCAIs [5].

These infections are generally caused by multidrug-resistant [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 human. Then there's the *Pseudomonas aeruginosa* bacterium, which thrive in soils and wet environments [7,8]. On the other

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hand, there are food safety concerns that arise frequently in agri-food 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 these related to *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp. [9–11] .

Thus, 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 ecofriendly strategy. Molecules derived from essential oils (EO) 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 wastes like citrus peels. This is one of the principles of green chemistry, which leads within the developed approach to an innovative and efficient route for the preparation of high added value 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 as 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. In addition, 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 in many disease treatments was mentioned in the Ebers papyrus [26]. Silver has biocidal and bactericidal properties; copper reduces inflammation and is also used to treat various *Escherichia coli* and *Pseudomonas* spp. infections and iron is used to treat anemia. Metal's use as antibacterial agents declined after the discovery of antibiotics in the twentieth century. Antibiotic resistance was discovered shortly after, due to the transfer of antibiotic resistance genes, also known as resistance transfer factors. Metal complexes, such as {RuCl[(*p*-cymene)][Aminooxime L3]}$^{+}$Cl^{-} [21] are promising antimicrobials, in addition it has been reported that metal complexes have a strong antibacterial activity than uncomplicated ligands [27]. The goal of this review is to provide a comprehensive overview of the existing data on the microbiological risk associated to biofilm in health and food industries. The use of antibacterial biosourced molecules, such as terpenes and their derivatives issued from essential oils and the efficacy of using metal complexes based on terpene ligands as tools to setup new antibacterial molecules with novel properties are highlighted. Their formulation based on microencapsulation is also discussed as strategy to improve their bioactivities and properties.

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 to 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), one in every 31 hospitalized patients and one in every 43 nursing home residents has a HAI [31]. Moreover, detached microorganisms from a biofilm are a major source of bacterial spread and contamination [32]. They cause significant issues in health care sectors and food industries. The National Institutes of Health (NIH) reported that about 65% of all bacterial infections are associated with bacterial biofilms [33]. 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 [33,34]. The global production of biomedical devices and tissue engineering-related materials is estimated to be worth 180 $ billion annually but still medical equipment continue to suffer from microbial contamination and colonization [35,36]. These infections include Central Line-Associated

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BloodStream Infections (CLABSI) (Table 1) when bacteria or fungi enter the bloodstream *via* a central line, Catheter-Associated Urinary Tract Infections (CAUTI), Central Venous Catheter (CVC) (see Table1) or Hemodialysis Catheter, Transcatheter Aortic Valve Replacement (TAVR), Prosthetic Joint Infection (PJI), Pediatric Ventilator-Associated Events (PedVAE) and Ventilator-Associated Pneumonia (VAP) (Table1). Infections can also occur at surgical sites, which are known as Surgical Site Infections (SSI) [38]. Between 2020 and 2021, statistically significant increases in *methicillin-resistant Staphylococcus aureus* MRSA (14%), VAE (12%), CLABSI (7%), and CAUTI (5%) were observed [39] (see Table1).

3. Food poisoning related to adherent bacteria and their biofilms

Food poisoning is a prevalent, costly and occasionally death disease. The bacteria more often involved in foodborne illness are *Salmonella* spp. ,*Staphylococcus aureus, Escherichia coli and Listeria monocytogens* [40]. In the United States Food poisoning caused 9.4 million illnesses, 55,961 hospitalizations, and 1,351 fatalities each year [41]. 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 [42,43]. Cleaning and disinfection of premises and equipment are among the major measures to control food pathogens in food industries. In addition, as reported one of the five keys to safer food is to keep clean [44]. When premises and equipment are contaminated and the conditions of cell growth are suitable, adherent cell form biofilms [32]. It is established that bacterial cells, under 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 hence may be a source of contamination for foods that come into contact with such food-contact surfaces (countertop, rubber, hand gloves, plastics, etc…) (Table 2) [45,46].

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

Healthcare - associated infections Types	Microorganisms	References
CLABSI - Central line-associated bloodstream infection	*Staphylococcus aureus* *coagulase-negative staphylococci* *Candida* spp. *Methicillin-resistant Staphylococci (MRSA)* *Enterococci,* *Klebsiella* *Enterobacter* *Pseudomonas aeruginosa* *Escherichia coli,* *Acinetobacter* *Candida species*	[47–52]
CVC – Hemodialysis catheter	*Enterobacter cloacae Complex(ECC)* *Candida parapsilosis* *Staphylococcus aureus* *Methicillin-resistant Staphylococcus aureus (MRSA).*	[53–55]
Pediatric Ventilator-associated events (PedVAE)	*Candida albicans* *Staphylococcus epidermidis* *Pseudomonas aeruginosa* *Haemophilus infuenzae*	[56–61]
VAP – Ventilator-associated pneumonia	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Escherichia coli* *Enterobacterales*	[62–64]

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CAUTI – Catheter-associated urinary tract infection	*Staphylococcus aureus* *Escherichia coli* *Proteus mirabilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Enterococcus faecalis* *Candida*	[65–69]
TAVR- Transcatheter aortic valve replacement	*Streptococcus* *Staphylococcus aureus*	[70–76]
Cardiovascular devices	*Staphylococcus aureus* *Coagulase-negative Staphylococcus* *Staphylococcus aureus*	[77–85]
Surgical site infection (SSI)	*Escherichia coli* *Enterobacter* spp. *Staphylococcus aureus* *Streptococcus* spp. *Klebsiella pneumoniae* *Streptococcus pneumoniae* *Pseudomonas aeruginosa* *Enterococcus faecalis* *Proteus* spp. *Methicillin-resistance of S. aureus (MRSA)* *CoNS*	[86–93]
Prosthetic joint infection (PJI)	*Methicillin-resistant Staphylococcus* *Staphylococcus aureus* *Staphylococcus lugdunensis* *Staphylococcus* spp. *Pseudomonas aeruginosa* *Streptococcus gordonii*	[94–104]

Table 2. Pathogenic bacterial diversity in the agro-food ecosystem.

Foodborne pathogen	Food environment processes	Food equipment isolation	Food Product	References
Listeria spp.	Fine cutting loin	Apron Conveyor belt Loin ripping board	Meat	[105]
		Packaging film Hooks		
	Meat cutting	Saw Conveyor belt		
		Drains Floors Freezers Aprons Door handles Taps	Smoked salmon Raw salmon	[106]

		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	[107]
			Mushroom	[108]
			Iceberg lettuce	[109]
			Poultry meat Raw beef	[110]
			Chicken cold cuts	[111]
	Cold storage		Pork meat	[112]
	3D Food Printing Systems		Food Ink Capsules	[113]
		Bulk Tank Milk Milk Filter	Raw milk	[114]
		Fish-processing plants		[115]
	Food-service establishments		Enoki mushrooms	[116]
	Cutting room		Meat	[117]
		Floor Mixing trough Separating machines Cutter Transport belt Mixing machine Dicing machine Knifes	Saucisse Saucisson Rosette Chorizo	[118]
Escherichia coli			Sliced cooked cured ham Sliced cooked cured sausage Sliced cooked Meats veal pie and calf liver pâté	[119]
	Refrigerated Storage		Kale	[120]
			Fresh Beef	[121]
		Countertop Draining board	Chicken	[122]
Staphylococcus spp.			Milk	[123,124]
			Pastries Cereals	[125]
			Quail breast	[126]
			Kazak cheese	[127]

	Dairy farms	Hand Bulk farm milk Pooled udder milk Milking container Bulk container Teat Overall	Milk Water for cleaning teat and hands	[128]
		Dish cloth hands Refrigerator handle Oven handle Countertop Draining board	Chicken	[122]
	Slaughter hall Cutting room		Meat	[117]
	Dairy staff	The hands Anterior nares	Raw Milk Minas Frescal cheese Food handlers	[129]
***Salmonella* spp.**			Chicken Breeds	[130]
			Pet food	[131]
		Plastic (Tote) Plastic (Bucket elevator) Stainless steel Concrete Rubber (Belt) Rubber (Tire)		[132]
	Domestic Kitchen Surfaces	-	Chicken carcasses	[133]
			Tomatoes	[134]
	Individual production chains		Poultry food Chicken gizzards	[135]
	3D Food Printing Systems		Food Ink Capsules	[113]
		Bulk Tank Milk Milk Filter	Raw milk	[114]
			Fresh Beef	[121]
		Dish cloth Countertop	Chicken	[122]

4. Biofilm

4.1. Biofilm formation

A biofilm is a structured microbial cells community, enclosed in a self-extracellular produced polymeric matrix, and adherent to a surface, to interface, and to each other [136,137]. The biofilms protect the bacteria and allows them to survive in hostile environmental conditions. Bacterial biofilms can resist to host immune response and are much more resistant to antibiotics and disinfectants treatments than planktonic bacterial cells [138]. Biofilm formation occurs in several steps according to a well-established pattern (Figure 1). First, bacteria adhere to a surface (1) and start to develop into irreversible attachment (2) The bacteria will then clump, multiply, and form microcolonies (3). During the biofilm maturation stage, bacteria synthesize and secrete the polymeric matrix constituents (Figure 2), which play an important role in biocidal resistance (4). The detachment or dispersion of bacterial cells, which can then colonize new surfaces, is the final step in biofilm formation (5). Thus, the detachment and dispersion of bacterial cells from a biofilm play an important role in the transmission of bacteria and in the spread of cross contaminations and infection [139,140].

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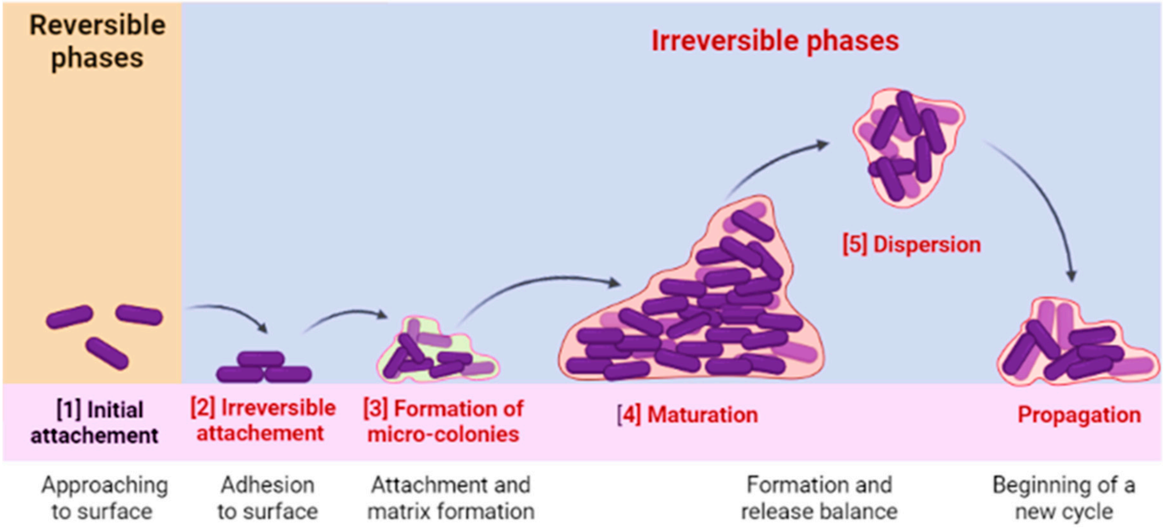

Figure 1. Different steps of biofilm formation.

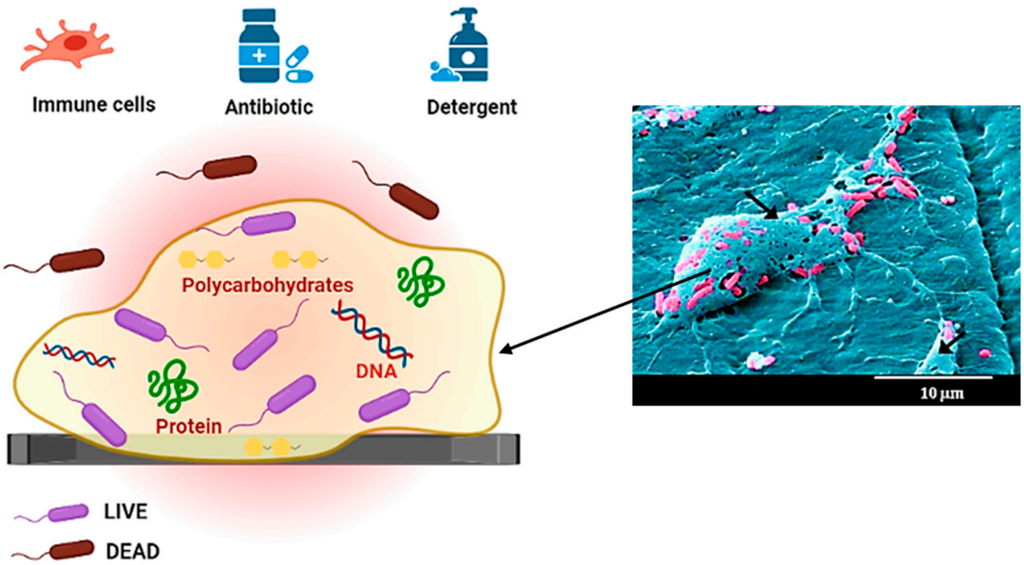

Figure 2. Effect of the biofilm's extracellular matrix on pathogenicity.

4.1. 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 scanning electron micrograph of an Escherichia coli biofilm enclosed in an extracellular matrix [32]. The 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 [141,142]. 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[143]. The ECM is made up of extracellular polymeric substances (EPS) that have been identified, highlighting the matrix's versatility with various roles (Table 3) [144,145].

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Table 3. Main roles of the ECM [144].

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
The 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, in addition EPS matrix's stability and functionality are critical in the development of a robust and resilient microbiome community. It also helps 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 [146,147]. Biofilms can withstand antibiotics at concentrations 10 to 1 000 times higher than planktonic bacteria, and it has been reported that the matrix acts as a diffusion barrier for toxic molecules [148].The presence of low or no oxygenated zones in the biofilm's deep layers may also contribute to resistance to some biocides, which may be inactivated under these conditions or are ineffective against metabolically inactive bacteria. Eventually, an increasing body of experimental evidence suggests that resistance is linked to the expression of specific genetic mechanisms. All of these characteristics suggest that the biofilm is a favorable way of life for bacteria, to the point of constituting a default mode of life for certain bacterial species [149,150].

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 health care sectors or in food industries. They are used as antimicrobial in hospitals, dental offices, kitchens, and bathrooms, in food premises and equipment. The today challenge is to set up new products to avoid toxic ones by attempting to use bio-based antibacterial agents [151]. 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 [152,153]. Essential oil, 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 [154]. The chemical composition of essential oils reveals that they are composed primarily of complex mixtures of two groups of organic compounds: terpenes and phenylpropane derivatives (terpenoids and phenylpropanoids) [155]. Terpenes are naturally occurring hydrocarbons with cyclic or acyclic structures that are made up of a multiple of 5 carbon atoms and have 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 essential oils antibacterial, antioxidant, and insecticidal properties [156]. It is primarily about 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 -pinene), and monoterpene ethers [157,158]. 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

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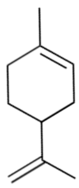
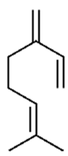
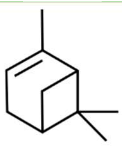
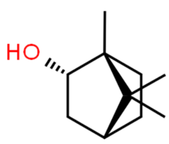
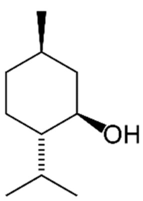
have been reported that terpenes and their derivatives are active against various micro-organisms (Table 4) [159]. The majority of terpenes have a greater impact on Gram-positive bacteria than they do on Gram-negative bacteria [160,161].

Due to their capacity to alter cell envelope, cytoplasmic stability, and they lead to cell damage, they have a harmful effect on microbes [162,163]. 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 affect this activity [164,165]. Terpenes and terpene derivatives have a multi-target impact, which is one of the reasons that they are a good 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 are only different in the position of their hydroxyl groups [166,167]. As a result of their multi-target effects, most of terpenes and their derivatives are known to be potent antibacterial agents against multidrug-resistant organisms, particularly bacteria and fungus, like methicillin-resistant *staphylococcus aureus* (MRSA), this particular strain is resistant to a number of different antibiotics. The terpene derivatives have several target sites and methods of action, therefore no microbial resistance has yet been created in opposition to them [168–170].

Table 4. Main antibacterial constituents of terpene derivatives.

Terpene derivatives	Structure	Bacteria	References
Limonene		*Staphylococcus aureus*	[171]
		Escherichia coli	[172]
		Pseudomonas aeruginosa *Staphylococcus aureus*	[173]
		Enterococcus faecalis	[174]
		Escherichia coli *Staphylococcus aureus* *Enteroccocus faecalis* *Listeria monocytogenes*	[21]
Myrcene		*Escherichia coli* *Salmonella enterica* *Staphylococcus aureus*	[175]
α-Pinene		*Staphylococcus aureus* *Escherichia coli*	[176–178]
Borneol		*Staphylococcus aureus,* *Escherichia coli* *Pseudomonas aeruginosa*	[179]
Menthol		*Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella pneumonia* *Staphylococcus aureus*	[180]
		Escherichia coli *Staphylococcus aureus* *Listeria innocua,* *Saccharomyces cervicea*	[181]
Thymol		*Enterobacter sakazakii*	[182]

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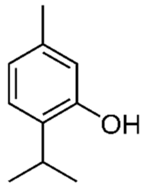
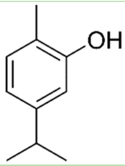
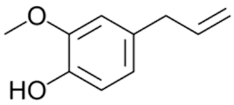
		*Salmonella Enteritidis*	[10]
		Aeromonas hydrophila	[183]
		Methicillin-resistant Staphylococcus aureus	[184]
Carvacrol		*Escherichia coli* *Pseudomonas aeruginosa* *Salmonella* spp.	[185]
		Pseudomonas aeruginosa *Enterococcus faecalis*	[186]
Eugenol		*Listeria monocytogenes CECT 933* *Escherichia coli* ATCC *35218* *Pseudomonas aeruginosa PAO1* *Staphylococcus aureus ATCC 6538*	[187]
		Escherichia coli ATCC *25922* *Escherichia coli* *Pseudomonas aeruginosa* ATCC *9027* *Pseudomonas aeruginosa* *Staphylococcus aureus* ATCC *25923* *Staphylococcus aureus* *Streptococcus mutans* ATCC *0446*	[188]

6. Metal complexes based on terpene ligands and their biological activities.

6.1. The reactivity of terpenes.

To address the microbiological risk associated to 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, formulation to setup the best formula to enhance the antibacterial and the antibiofilm activities and to go through the 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 [189,190]. The today's challenge is to find out highly reactive ligands for the synthesis, that's why it's of importance to study the coordination behavior of biologically active chiral ligands containing N and O donor atoms towards metal ions [191].

The two chiral forms of a molecule, also known as enantiomers, have opposing spatial geometries and thus interact with their environment differently. This feature is extremely important in medicinal chemistry [192,193]. 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 will not necessarily have the same biological activity because they will not react with the same receptors [194,195]. It is known that 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, which can induce chirality and reactivity (Figure 5) due to the presence of double bonds in their structure, as well as perform addition [196], rearrangement [197], cyclization, isomerization (Figure 3) and ozonolysis (O_3) (Figure 4). Dehydration reactions can also occur in the compounds derived from terpenes with alcohol functions.

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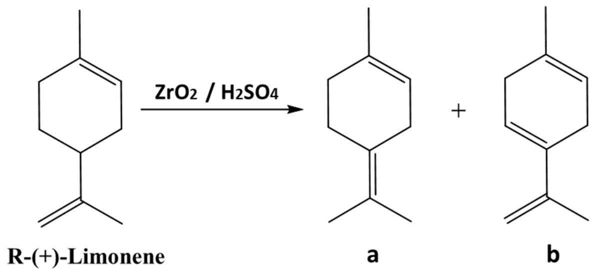

Figure 3. The (*R*)-(+)-limonene isomerization process was carried out at 60 °C in the presence of the impregnated with H_2SO_4 zirconium oxide (ZrO_2).The mixture of terpinolene (a) and terpinene (b) was obtained as a result of this isomerization [198].

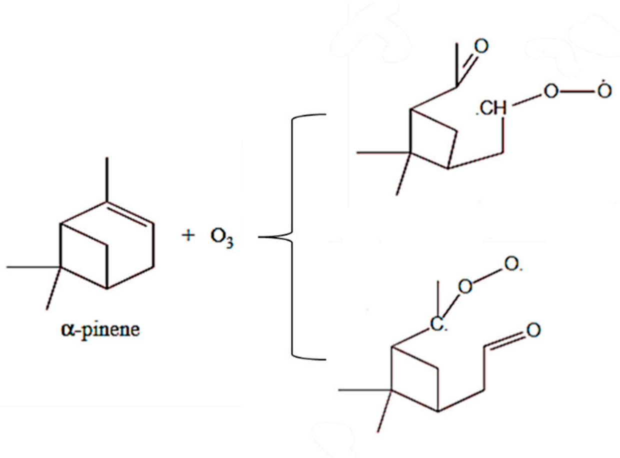

Figure 4. Reaction of O_3 with *α*-pinene [199].

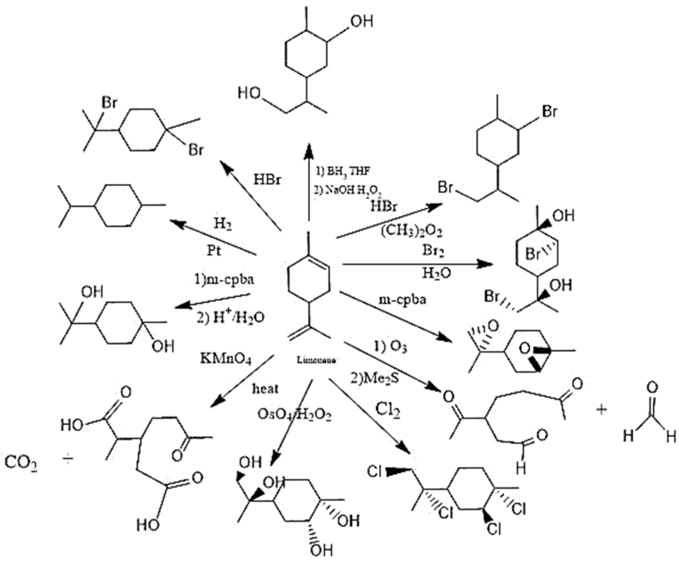

Figure 5. limonene reactions [200].

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 [201,202]. 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. Because of

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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, enzymatic activity, altering membrane stability and function, damaging DNA, and generally inhibiting plankton growth [203,204]. 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 to preserve food and disinfect water by Indians, Egyptians, Persian kings, Phoenicians, Greeks, and Romans [205]. A variety of metal-coated surfaces have antibacterial capability against *Staphylococcus aureus, Escherichia coli,* and *Listeria monocytogenes*, including silver, titanium, copper, iron, molybdenum, zinc...[206] 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 [207–209]. 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 [210]. Silver ions disrupt the function of membrane bound enzymes and respiratory enzymes leading to the complete destruction of the bacterial cell [211].

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 [212]. Metals have a wide range of properties and almost infinite combinations of ligands to form complexes, with a number of coordination ranging from 1 to 20 [213,214], 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 a 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 [207,215–217].

Metal ions, 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 [218], 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 [219]. Biosourced terpenes might be used to synthesize complexes with antibacterial and antibiofilm activities, such as the complex [(*p*-cymene)][aminooxime L3] $^{+}Cl^{-}$ (RuL3) is based on (*R*)-Limonene, which is miscible in water, therefore complexation overcomes the problem of solubility while also making it more stable. We had 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 [220–222]. 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 [223–226].

Table 5. Selected studies describing the complexes' various biological activities.

Metal	Ligand	Activity	References
Ru(II)	Based on limonene	Antibacterial Anticancer	**[21]**
Zn(II) **Fe(III)**	Monodentate Schiff base	Antifungal Antioxidant Antibacterial	**[227]**
Fe (II) **Co(II)** **Zn(II)** **Ru (II)**	Azo dye	Enzyme inhibitions Antioxidant	**[228]**
Zn(II)	PhenanthrolineIndomethacin	Anti-Breast Cancer	**[229]**
Zn(II) **Sn(II)** **Ce(III)**	Gemifloxacin and Glycine	Antifungal Antioxidant Antibacterial	**[230]**
Cu(II) **Ni(II)** **Co(II)** **Fe(II)**	bis-pyrazole	Antibacterial Antifungal	**[231]**
Co(II) **Fe(II)** **Ni(II)** **Mn(II)**	N-heterocyclic	Antitumor	**[232]**
Ru(II)	triazolopyrimidine in liposomes	Anticancer	**[233]**
Pt(II)	*cis*-diaminodichloro	Anticancer	**[234]**
Co(II) **Cu(II)** **Zn(II)**	Diimine– glycinate	Anticancer	**[235]**
Cu(II) **Zn(II)**	Bidentate-morpholine based	Antibacterial	**[236]**
Co(II), **Ni(II),** **Cu(II)** **Zr(IV)** **Pd(II)** **Cd(II)**	combination of Metformin and 1,4-Diacetylbenzene	Antifungal Antibacterial	**[237]**
Cr(III) **Fe(III)** **Cu(II)**	Multi-substituted aryl imidazole	Antibacterial Anticancer	**[238]**
Mn(II), **Co(II)** **Ni(II)** **Cu(II)** **Zn(II)** **Cr(III)**	Moxifloxacin–imidazole	Antifungal Antibacterial	**[239]**

7. Encapsulation of terpene derivatives for improving 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, stability, increases bioavailability, masks flavors, and reduces contamination risk [240]. Microencapsulation is a technique by which 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 having diameters comprised 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. In fact, different types of capsules can be produced from a wide range of wall materials (polysaccharides, proteins, monomers…) and by a large number of different processes such as: spray-drying (Figure 6), freeze-drying, extrusion, coacervation, liposome entrapment, interfacial polymerization.

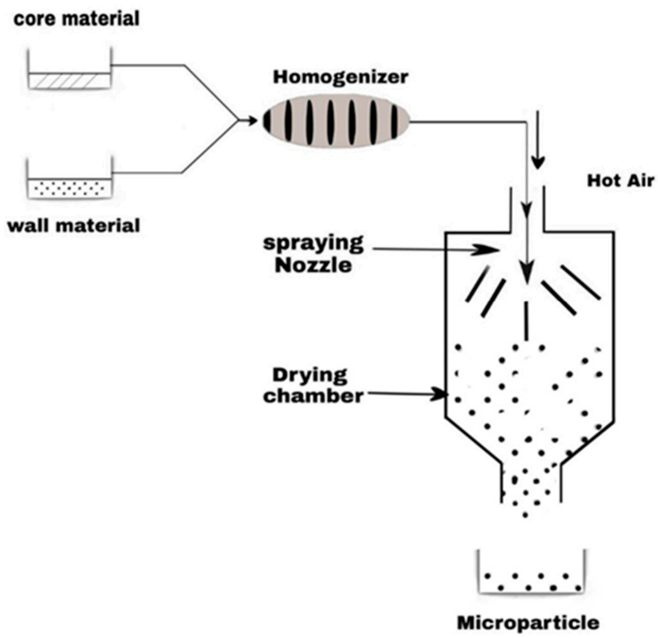

Figure 6. Schematic representation of the spray drying microencapsulation process**.**

Among these techniques, spray-drying is the most common technology used in food industry due to low cost and available equipment. Depending on the core material and the characteristics desired in the final product, wall materials could 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 [241]. It is possible to prepare mixtures of natural antimicrobials and biopolymers. The wet suspensions/emulsions will then be converted to powders by evaporating the majority of the water using this appropriate dehydration method. The resulting powder will be evaluated for activity, density, flow, size, stability, and re-dispersion.

Since almost all spray-drying processes in the food industry are carried out from aqueous feed formulation, the wall material must be soluble in water and should possess good properties of emulsification, film forming, and drying and the wall concentrated 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, antifungal, anticarcinogenic, and pharmacological properties), they have 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 [219,242]. Antimicrobial activity is typically unaffected by covering the "active" ingredient (which may include volatile substances) in a protective matrix. The polymer used 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 sub-cellular size, which lowers mass transfer resistance and

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boosts antibacterial action [243–245]. Free carvacrol dissolved in DMSO had a high MIC of 5 mg. mL^{-1} against *P. aeruginosa.* Nevertheless, the results after encapsulation demonstrated that encapsulated carvacrol suppressed bacterial growth at a concentration 4 times lower than that of F-CARV (1.25 $mg.mL^{-1}$), indicating that encapsulation increases antibacterial action [186]. 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 [246]. Encapsulation is a tool for controlling and reducing volatile terpene emission, overcoming the issue of immiscibility in water and thus improving stability, antimicrobial, anti-biofilm, antiaflatoxigenic, and antioxidant activities, protecting enzymes and terpenes and enhancing their properties, as well as lowering cytotoxicity and ecotoxicity, as shown in the (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's effect	Reference
Ginger essential oil	Gingerol Curcumene Zingiberene	Controlling and reducing the emission of terpenes	**[247]**
Sacha Inchi Oil (Plukenetia huayllabambana)	-	Protecting sacha inchi oil against oxidation	**[248]**
Flaxseed oil	-	Improve the oxidative stability	**[249]**
Sichuan pepper essential oil (SPEO)	-	Facing SPEO problems such as poor stability and low water solubility	**[250]**
Lycopene (Tetraterpene)	-	Enhancing the stability	**[251]**
Gaultheria procumbens L. essential oil (GPEO)	-	Improving the antimicrobial and antiaflatoxigenic activity and the stability	**[252]**
Complex {RuCl[(Para-Cymene)][Aminooxime L3]}$^{+}$Cl^{-}	Ruthenium metal (*R*)-limonene-based ligand	Increasing antibacterial activity against biofilms of food-pathogenic bacteria while decreasing the cytotoxicity.	**[21]**
Oregano Oil	Thymol Carvacrol	Preserving the majority of antibacterial action and enhancing the stability of oregano essential oils.	**[253,254]**
Carvacrol	-	Overcoming insolubility and increasing antibacterial activity against pathogenic bacterial biofilms while minimizing the amount used	**[186]**
D-Limonene	-	Preserve and possibly improve the antimicrobial activity in order to evaluate the preservation of the juice against inoculated spoilage micro-organisms	**[244]**
Carvacrol Thymol	-	Improving antibacterial activity against Salmonella Enteritidis biofilms and reducing ecotoxicity against Daphnia magna	**[10,255]**
Peppermint oil (PO) Green Tea oil (GTO)		Enhancing thermal stability, antioxidant and antibacterial activities	**[256]**
Pepsin, Trypsin Carvacrol	-	protecting enzymes and terpenes and boosting their antibacterial activities	**[257]**
Origanum vulgare	-	Overcoming stability-related restrictions, extending shelf life, and maintaining its	**[258]**

	antioxidant, antimicrobial, and sensory preserving properties.

8. Conclusions

The prevention / eradication of pathogenic microorganisms is a major goal for the food and medical industries because they are the primary cause of infectious diseases worldwide. These two sectors can promote favorable conditions to the formation of biofilms, which are responsible for major illnesses such as nosocomial and food-borne diseases. Nowadays, the most important need is the discovery of natural substances that can either eradicate or inhibit the development of these microorganisms. In this context, the use of terpenes and terpene derivatives obtained from essential oils as antimicrobial agents will have a number of advantages for the economy and the environment. These antimicrobials will be extracted from leftover food. This relates to sustainability and innovation and it's an essential part of the circular economy concept, which aims to reduce waste and pollution and restore natural systems. However, the use of these molecules is posing challenges through their low water solubility, volatility, thermal degradation, oxidation and cytotoxicity. Thus, the need to find bio-based compounds with antimicrobial and antibiofilm activity, water solubility, low toxicity and low cost is leading to the research of novel stable metal complexes based on terpene ligands. Terpenes and their derivatives can produce chirality and reactivity because they contain double bonds in their structure as well as the existence of N and O atoms which function as metal ion donors. This review also shows that encapsulation can be used to protect and enhance the stability and efficacy of terpenes or complexes against pathogenic microorganisms and their biofilms.

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