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Review Article

Natural Anti-infective Agents: A Promising Front-line Strategy Against Microbial Resistance Mechanisms

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Abstract

The global emergence of drug-resistant strains is one of the major concerns and challenges in human infectious diseases due mainly to antibiotics misuse and dosage leading to inadequate control of microbial infections. Anti-infective agents represent the new generation of natural antibiotics (chalcones, phenolic acids, bacteriocins, antimicrobial peptides). These compounds show diversity in sources, chemical structures, and mechanisms of action. These capabilities make them potential candidates to fight against microbial resistance. The literature on this topic is immense. This review aims to pinpoint general and basic knowledge of natural anti-infective agents (sources, targets, physicochemical properties), all of which constitute key elements that shape their antimicrobial action and make them a hope for more effective treatments.

Keywords: Anti-infective Agents; Bacteriostatic; Bactericidal; Triterpenes; Flavonoids; Gram Positive/Gran Negative Bacteria; Secondary Metabolites; Antimicrobial Peptides

Introduction

Infectious diseases are responsible for millions of deaths every year worldwide. They represent a serious concern and threat to human health. In recent years, it has been reported that around 17% of the total cause of deaths is due to microbial infections [1]. With the rapid dissemination and spread of resistant bacterial, we can mention Multidrug-resistant (MDR) or extremely drug-resistant (XDR) bacterial strains seems to be of the most frightening development. Along with some strains of *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, or *Neisseria gonorrhoeae*, MDR and XDR to methicillin, vancomycin, and the Gram-negative XDR strains. More concern has arisen due to the appearance and spread of other MDR and XDR strains of *Pseudomonas aeruginosa*, *Acinetobacter* sp, and carbapenem-resistant *Enterobacteriaceae* (mostly represented by *Klebsiella pneumonia* [1]. Antibiotic crises have favored that we have turned out to naturally occurring molecules that have become new sources of anti-bacterial and anti-biofilm drugs for clinical use, and so far, it is an urgent priority to search for safe and improved therapeutic treatments.

By definition, an anti-infective agent is a natural compound isolated and extracted from different sources (plants derived, bacteria, fungi, virus, and oceans [1-3] that often display anti-microbial activity, -inhibit growth, replication, or kill the infective agent- toward Gram positive or to Gram negative bacteria and also to any other organism (viruses, fungi) [2].

A list of different classes of natural compounds with antimicrobial activity has been enumerated (Figure 1). These

Figure 1: The natural and immense, rich world of the anti-infective agents. The anti-infective agents are obtained from different sources: plants, animals (insects, bees, snakes), fungi, bacteria, marine environments, that often display antimicrobial activity. Either, secondary metabolites (flavonoids, terpenes, alkaloids) or antimicrobial peptides (produced by bees, insects) provided by nature can have several targets on bacteria. The natural anti-infective agents, extracted from different sources can be divided into two main groups, synthetic antibiotics and secondary metabolites which often display antimicrobial activities. Synthetic antibiotics are still in use however their misuse and over dosage has favored the up rise in microbial resistance development.

natural compounds are isolated and extracted from different natural sources (i.e., plants, bacteria, fungi, viruses, and marine organisms (sponges, marine bacteria and fungi, mollusks, algae, fish, crustaceans) [1-3] that often displays antimicrobial activity.

Different classes of natural compounds with antimicrobial activity are schematized in figure 1, including terpenoids, alkaloids (quinolones, isoquinolines, squalamines, chalcones) [3-6], steroids, peptides (eukaryotic derived), bacteriocins (prokaryotic derived), and phenolic compounds (PC) [6-8], and polyphenols are classic examples of natural plant secondary metabolites necessary for defense against microbial pathogens [11,12]. Moreover, marine peptides rich in nitrogen obtained from the deep sea (algae, fish, mollusks, crustaceans, bacteria, and fungi) possess not only anti-infective properties (antibacterial, antiviral) but unique bioactive properties (immunoinflammatory, antioxidant, antitumor).

Of relevance is that these compounds constitute a potentially safe and non-toxic alternative for microbial pathogens that have acquired antibiotic resistance. PC and the polyphenols constitute a classical example of plant natural secondary metabolites that are important as a defense against microbial pathogens [8-13] (Figure 1). Thus, the vast repertoire of natural anti-infective agents, the secondary metabolites which often display antimicrobial properties, as well as the natural antimicrobial peptides obtained from eukaryotes and prokaryotes organisms, represent novel natural sources that should be undertaken as the novel, natural, and a promising frontline strategy with enhancer antimicrobial activities and with less resistance development and/or [13] better outcome against microbial resistance mechanism [14].

Today's current investigation of anti-infective agents is addressed precisely on this property that we are sure will

lead and give the welcome to the new era of the new natural antibiotics for emergent and re-emergent infectious diseases that need eradication (poliomyelitis, leprosy, measles) and/or for "untreatable infections" ((*Staphylococcus. aureus, Mycobacterium tuberculosis* (MTb). *Helycobacter pylori (H. pylori) Haemophilus influenza (H. influenza); Enterococcus faecalis (E. faecalis), Listeria monocytogenes (L. monocytogenes), Clostridium tetanus (C. tetanus), Escherichia coli (E. coli), Salmonella typhi (S. typhi), Pseudomonas aeruginosa* (*P. aeruginosa*)) for which there are still no vaccines or effective treatments [15-17]. Furthermore, the recent SARS-CoV2 pandemic has led to search and optimize different drugs, vaccines, to avoid such amounts of deaths and to be able to overcome evasion and microbial resistance mechanisms.

One current question is how the scientist of the different disciplines can approximate this sanitary emergencies, by bacterial or viruses. It has been proposed that the arsenal of natural like antibiotics -the universe of natural anti-infective agents - (Figure 1) can be used strategically as a Trojan horse to target any of the structural, physiological, and molecular components of microorganisms. In fact, searching Pubmed data from inception to 2021, we have found that chemical structures of the natural products families serve as precursors and scaffolds of new molecules with biological function. Furthermore, and of relevance is that these secondary metabolites derived from these family of natural products can be used either alone, in synergism (among different compounds) or adjuvanted by other types of compounds to overcome microbial resistance mechanism.

Properties of the natural anti-infective agents. The chemical crosstalk of the anti-infective agents with the microorganism and the virus

One of the most outstanding properties of the natural anti-infective agents is their chemical structure. Because for an efficient interaction with the microorganisms, with microbial surfaces, external membranes, a physicochemical environment is necessary provided either by the chemical groups of the anti-infective agents [18-21]. Thus, is the presence of the chemical groups, such as hydroxyls and/or prenyl units enable access to the site of action (periplasmic space) and interact with their targets in the bacterial cell membrane. In fact, hydroxyl groups in the molecular structure of alkaloids could provide bactericidal than bacteriostatic properties and increasing the ability to permeabilize lipidic bilayers. Indeed, in the nature exist several families of compounds, that possess several hydroxyl conjugated to rings, show this ability and allow their pharmaceutical application [13,18-21]. Moreover, aliphatic chains and aromatic rings give the compounds their hydrophobic character [7,20,21], and some studies have shown that they also shape their anti-infective activity [23]. Indeed, it has been reported that anti-infective agents obtained from the marine environment have a particular structure, formed by heterocyclis, and hydroxlated, methoxylated, as well as aliphatic chains binding the heteroatoms the rings, is a cyclic structure, amphipathic in some way, and possibly this can allow to interact and cross talk with their targets. An example of this is the bisquinolizidine alkaloid, petrosin and a series of bis-1-oxaquinolizidime, xestospongins which possess di-heteroatom rings (C. D, E. F. G. H. I and J). These compounds were obtained from the ethyl acetate extract of the sponge *Oceanopia* sp. Petrosin along with xestopongin and exhibited moderate to high activities against some microorganism and clinical isolates.

Of relevance the chemical crosstalk of the family of natural anti-infective agents with the microbial surfaces. As example are the POLYPHENOLS. The chemical structure of the polyphenosl is characterized by a carbon skeleton of diphenyl propanes and two benzene rings (rings A and B) joined by a central three cargon chain [3,23]. The pyran ring (ring C) is formed by an A benzene ring and by a central three carbon chain.

Phenolic compounds comprise two main groups: Flavonoids (flavans, flavanones, flavanol, anthocyanidins) and Non-flavonoids (coumarins, lignans) [3,26]. The chemical groups (OH, N, S, rings, C) contribute and shape their antimicrobial activity. The chemical features of these flavonoids are characterized by aromatic rings of six carbons, heteroatoms cyclic rings, hydroxyl groups, and some methyl groups of five and/or six carbon atoms they can exert a bactericidal action at minimal bactericidal action (MBC). Other examples are baicalin, flavanone, diplacol, diplacone flavonoids. In some cases it has found that these compounds can induce aggregates and reduce the number of CFUs [21,25,27]. On referring to one of the most studied phenolic compounds, chalcones, insert a characterized by an open heterocyclic ring that can serve as a template for modifications with improved antimicrobial activity [13,19-22]. Furthermore, chalcone, (2E)-1,3-diphenylprop-2-en-1-one) is characterized by an open heterocyclic ring, that can be closed and transformed in a flavonone without losing its antimicrobial action. Indeed, by doing this its action can be enhanced. Indeed, it has been reported that by adding hydroxyls groups and/or certain degrees of lipophilicity at certain position in ring A or B of chalcone enhance antibacterial properties [12,25,27]. It is highly possible

that certain position (4' and 5' position) are more favoured to the interaction with membranes, enhancing antibacterial potential. In addition, other hydroxyls substitutions in other positions in the ring B of chalcone, 2' and 4' or 2' and 6', and at the 5' and 7' positions of the A-ring, significantly increases antibacterial activity of flavanones even against the MRSA strains. The enhancement of antimicrobial potential can be achieved also by substituting the 6' or 8' position with a long aliphatic chain. Indeed, the presence of phenolic groups with high protein binding, affinity may inhibit microbial enzymes and simultaneously increase affinity to cytoplasmic membranes, thus,, leading to increased antibacterial activity. The substitution of the hydroxyl group at the 3'carbon of the C-ring of flavonoid increases the antibacterial activity. In addition the O-acyl or O-alkyl chains in the above position also augment antibacterial activity, especially of flavanols and flavanols. There are also reports that the substitution of sulfur or nitrogen in 4'position of the C-ring also ameliorate antimicrobial activity [27,28]. Furthermore, synthetic modifications of natural flavonoids increase their antibacterial activity. For example, the addition of the N-heterocyclic ring in 7' position of the ring A of chrysin yields 16-32 fold increase din antimicrobial activity compared to the chyrsin itself, due to its greater lipophilicity. Liu., *et al*. showed that quercetin and chrysin possess a stronger antimicrobial activity against *Staphylococcus aureus* ATCC 6538 strain (minimal inhibitory one centration, (MIC) = 6.25 micrograms/m, than luteolin (40 microgram/ml and other 140 glycosides derivatives (100-400 microgram/ml because of their relatively low polarity. The antibacterial activity of flavonoids is suggested to be to their ability to bind to the lipid bilayer of the bacterial plasma membrane [27]. This last compound, along with non-flavonoids, coumarins, lignoid, synergin, inhibits viral proteases enzymes, as PLpw enzyme of SARS-CoV [25,27], or toward PLpro enzyme [27,28].

The chemical structure of TERPENES based on "prenyl units" which are also the primary structures of lipids (cholesterol, sterols) and other types of molecules (retinal, carotenoids, quinones) that contribute to their cellular function. The presence of the prenyl units confer the hydrophobic character to terpenoids, enable them to have a better interaction with cell membranes of Gram positive than Gram negative bacteria (Figure 2) [7,28]. The length of the hydrocarbon chains in terpenes structure connected to the hydroxyl group, have been suggested that play a role in antimicrobial activities of these compounds. The mechanism of action could be most bactericidal than bacteriostatic due to their lipophilic character by the presence of the long aliphatic chains (higher than 10) that leads to increase in the hydrophobicity properties and caused a leaky membrane, The presence of aliphatic chains and hydroxyl as in the case of terpens alcohols (linalool, geraniol, farnesol, phytol, geranyl geraniol), the terpene carvone and the pentacyclic triterpenes (ursolic acid and alpha-amyrin) favored also inhibitory actions in the cell growth of *S. aureus* (Figure 2) [28]. In addition, diterpenoids, sesquiterpenoids, betulinic acid triterpene also have virustatic action against SARS-CoV2 (inhibit viral replication) [28]. Moreover, to the triterpenoid, *Quillaga saponaria,* described in 1782, a saponin with antimicrobial properties (bactericidal foam formation) and as vaccine adjuvant characterized by its amphiphilic structure. In general saponins are bidesmosides, and contain a triterpenic aglycone, that belongs to the beta-amyrin series, most frequently, quillaic acid [12]. These authors reported among others, 27 bidesmosidic triterpenic saponins, with either quillaic acid, gypsogenin, phytoalaccininc acid, or 23-O-acetyl-phytolaccinic acid as aglycones. The chemical characteristics of this triterpenoids, in particular of aquillaic acid, favour the affinity toward cholesterol presente in the membranes, and as detergent can make permeable to the membrane and therefore facilitating the ion flux, the permeability of the membrane, and such a way that can also act as immunostimulant in their interacton with the immune cells [18,27]. The aglycone part of the saponins structure (*Quillaga saponaria*) reside the affinity with the membrane cholesterol [12,27]. The antimicrobial action, the crosstalk of saponins with the viral surfaces of, Herpes Simple virus type 1, vaccinia virus, Human Immunodeficienciy viruses 1 and 2, Varicella zoster virus, rhesus rotavirus (RRV), and reovirus is mediated by inhibition of the specific binding of viral receptors. Very low concentration of the extract were able to prevent the virus from infecting their host cells and were still able to maintain their blocking activity and stop the virus binding to the cell for up to 16 h after their removal from the culture medium. Both extracts studied prevented the binding of RRV and reovirus to host cells pretreated with them, also inhibiting the propagation of infectious particles to the uninfected neighboring cells. This cell protecting effect persisted for up to 16 h after the extracts were removed from the cell monolayers. A pharmacological use of *Quillaga saponaria*, that has been tested and showed antiviral activity *in vitro* and *in vivo*, is a formulation of the Ultra Dry 100 Q (a spray-dried purified aqueous extract), that consisted of 65% saponins, and Vax Soap (purified medical grade material), with saponin content greater than 90% [13,27]. Furthermore, studies have demonstrated antiviral action against

other virus, such as vaccinia virus, reovirus, as well against others microorganisms, *Staphylococcus aureus*, *Salmonella typhimurium*), antifungal (*Penicillium roguefortii*, *Aspergillus ochraceus*, *Alternaria solani, Fusarium oxysporum, Verticillium dahlia, and Pythium ultimum* [27].

Figure 2: The chemical crosstalk of the natural anti-infective agents. One of the most outstanding properties of the natural anti-infective agents are their chemical structure. Why is this?. Because through their chemical groups (OH, CHO, COOH, rings, prenyl units) can establish a fruitful interaction with the microbial surfaces of the bacteria and also with the virus surfaces. Moreover, these chemical groups often constitute scaffolds of antibiotics, of pharmacology drugs. The chemical structure of the anti-infective agents as well as the physicochemical environment in the host, plays a pivotal role that shape and define the main mechanism of action of these compounds. The diversity in chemical structure allow to act through three main types of mechanism of action (bactericidal, bacteriostatic, or a combination of both, and as immunomodulators (antimicrobial peptides). Natural anti-infective agents considered also as natural antibiotics have the capacity to interfere with bacterial cell growth, bacterial metabolism, cell wall or protein synthesis, secretion systems, efflux pumps (ion channels, porines), bacteria killing.

The ALKALOIDS, a family of compounds that exist in nature in thousands, among them, pyrrolidines, isoquinolines, purines, imidazoles, indoles. These compounds, which characteristic is the presence of a heteroatom, are usually nitrogen (N). The hydroxyl groups in the molecular structure of alkaloids can increase the ability to permeabilize lipidic bilayers of pathogenic microorganisms. Moreover, the aliphatic chains and aromatics ring shapes the hydrophobic properties of alkaloids, leading to the disruption of the external membranes and, therefore, bacterial lysis [7,27,28]. Around 18000 compounds have been discovered. extracted from plants, animal, bacteria or fungi [28,29]. Their diversity and the bioactive properties of the alkaloids is thought to reside either in the nitrogen (be part of primary, secondary or terciary amine) and the indol rings, the polyamins (Squalamine, Sanguinarine, Quinines, Indolizidine, Bis-indole, Isoquinolines, Berberine, Cocsoline, Azomycin, hydratine alkaloid) with antibacterial activity against *Staphylococcus* sp, *Streptococcus* sp, *Campylobacter* sp, *Enterobacteriaceae* sp. Of relevance is that Chloroquine alkaloid [29,30], and/or N-acetylglucosamine, alkaloid [31] have a virustatic action (inhibition of viral replication). Ongoing clinical trials using natural Alkaloids has been well described by Khalifa., *et al*. 2021 [31].

By another hand, antimicrobial peptides (AMPs) and tannins [30,31] show a similar behavior. Their amphiphilicity and hydrophilic properties give them the ability to penetrate and interact with lipid bilayers [18,23,24]. In summary, the chemical structure of the anti-infective agents properties can circumvent these natural barriers, due to their OH, CHO, COOH, isoprene units present in most of the

natural anti-infective agents [7,24,25]. Indeed, the contribution of which along with the membrane cellular physicochemical environment favoured the antimicrobial action for example against *Gram us* sp., *Streptococcus* sp., *Mycobacterium* sp., *Clostridium* sp.) and Gram-negative bacteria (e.g., *Enterobacteria* sp., *Pseudomonas* sp., *Bordetella* sp) [8,25,26]. Currently, there is an urgent need to approach the vast natural resources, an urgent need to optimized extraction methodologies, gain from the knowledge in biotechnology, omics technologies, to profit at the most possible from the wealth source of natural agents with antimicrobial properties. In particular referring to the COVID 19 pandemic, a recent report update on the pharmacological therapy against SARS-CoV2 [27,29-31]. Checking the structures of these pharmacological anti-infective agents, in comparison with flavonoids, triterpenes, anti-SARS-CoV2 are characterized by heterocyclic rings (N, O) of four to five carbon atoms, conjugated with hydroxyl, amino groups, aliphatic chains with double bonds. Several natural anti-infective compounds that are active or in clinical trials process (recruiting persons), Cannabidiol, Baricitinib, Brensocatib, Colchicine, Dexamethazone, Ivermetin, Azithromycin, Amantadine, Remdesivir, hydroxychloroquine [32,33]. These author revisited and make detailed of the clinical trials with these compounds. In another report, it is also possible to see that the chemical features of the ant-infective agents, antiviral agents, are also very similar to those with antibacterial action. It is also suggested that their increase in lipophilicity and hydroxyl action this can potentially favoured the interaction with the phospholipid bilayer. Khalifa., *et al*. [31] in other report made a very good and extensive review and list of the most promising antiviral, anti-SARS-CoV2 drugs.

From the studies reports iin the literature on cliinical trials using natural compounds against COVID-19. The chemical structure of these compounds such as Chloroquine, Prednisolone is characterized by aromatic rings combined with aliphatic chains double bonds, that can allow a crosstalk with the virus components, and even some of them have the ability to inhibit viral replication [32,33].

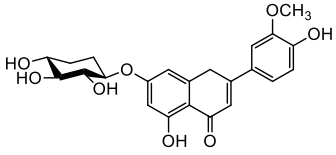
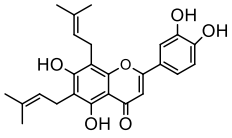
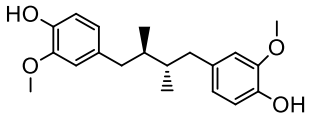
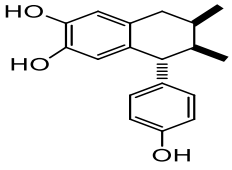
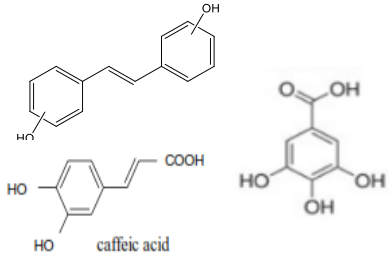
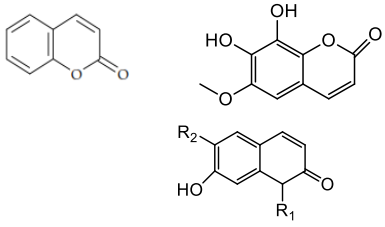
Targets and antimicrobial activity of the natural anti-infective agents

The targets of the natural anti-infective agents are the structural components of bacteria [cell wall (biosynthesis, including enzymatic system) external membrane (protein synthesis), fimbriae], physiological processes [cell growth, DNA replication, energy metabolism, motility, secretion system (efflux pumps, porins)]; molecular components (DNA, RNA, peptidoglycan, lipopolysaccharide (LPS), lipoarabinomannan (LAM), teichoic acid (TAC), mycolic acid (MAC), and glycoproteins) [9,22,24] (Figure 2).

In general, antimicrobial agents primarily have two different mechanisms against microorganisms to stall their pathogenesis (Figure 2): The first mechanism is called "bacteriostatic" which interferes with synthesizing main bacterial components. Thus, hampers inhibit or suppress bacterial cell growth, DNA replication and repair, protein synthesis, cell wall biosynthesis, or metabolic pathways. However, these are reversible effects that do not cause bacterial cell death, keeping bacteria in the stationary phase of growth and prevent microbial resistance mechanisms. b). The second mechanism, so-called "bactericidal" can inhibit the cell wall formation of bacteria, which is an irreversible effect that damages the bacterial cell wall and membrane, causing microbial death [4-7,34].

Natural bacteriostatic agents

The chemical structure of for example phenol and phenolic acids—quercetin, flavonoids, triterpenes, tannins, and volatile oils [9,10] (Figure 2) allow them to inhibit bacterial DNA replication [35,36] biofilm formation [36], and promote autophagosome formation [36] (Table 1). In the case of flavonoids, it has been suggested that their ability to bind to the lipid bilayer of the bacterial plasma membrane [37,38] (Figure 2) (Table 1). Among the polyphenols, flavanols, and phenolic acids possess the highest antibacterial activity thanks to the ability to: Inhibit bacterial virulence factors such as enzymes and toxins; interact with cytoplasmic membrane and suppress biofilm formation and exert a synergistic effect with antibiotics. Moreover, the transport system ABC of *Staphylococcus aureus* and/or *M. tuberculosis* can be repressed by the action of natural compounds such as lignane [39]. In other words, the anti-infective agent can exert their action at the level of membrane, affecting the proteins that form part of the transport system. Other compounds such as epicatechin gallate and epigallocatechin gallate act by gene suppressing. In synergy with beta-lactam antibiotics, can decrease MICs enhancing antibacterial properties. Furthermore, biotechnology combined with natural anti-infective agents have yielded some promising results, through the use of nanoparticles. This is of relevance since in this way it can be targeted the natural secondary metabolites to reach and exert their antimicrobial action a low doses [39].

Natural product	Chemical Structure	Mechaninsm of action	Reference
Phenolic compounds **Flavonoids** -Chalcone -Naringenin -Apigenin -Quercetin -Kaempherol -Myrcetin Resveratroñ	Chrysoeriol-7-O-β-D- xyloside   6,8-diprenyleriodictyol	Damage to the bacterial membrane -Inhibition of virulence factors (toxins, enzymes, biofilm formation) -synergistic effect when combined with chemotherapeutics Inhibit DNA replication Inhibit protein synthesis	Jamal et al., 2018 Wang et al., 2018 Adamczak et al., 2019¨ Mahapatra et al., 2015 Farhadi FB., 2019 Echeverria et al., 2017
Non flavonoids (lignans, stilbenes, phenolic acids)	 meso-dihydroguaiaretic acid (MDGA)	Synthesis of coenzyme A transferase and disrupt celular membrane	Jamal et al., 2018 Khameneh et al., 2019
Lignans	 3'-demethoxy-6-O demethylisoguaiacin	ATP-binding cassette (ABC) transport system (cell membrane)	
Stilbenes **-Phenolic acids** -Benzoic acid, -cinnamic acid	 caffeic acid	Bacterial membrane permeability, causing electrolyte leaking, depolarization of the membrane. -Inhibition of respiratory activity.	Miklasinska-Majdanik et al.,2018 Jamal et al., 2018 Kumar et al., 2019
Coumarins and Furano-coumarins	 Umbelliferone R_1= R2=H Daphnetin R_1=OH, R_2=H Esculetin R_1=H, R_2=OH	DNA gyrase inhibition Type III secretion system Genes HrpG and PrhA	Khameneh et al., 2019 Kumar et al., 2019 Jamal et al., 2018

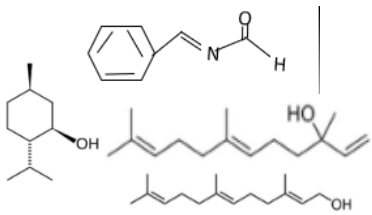
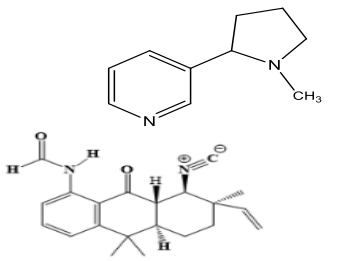
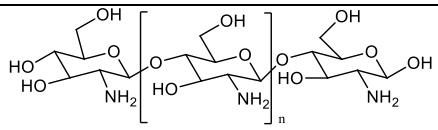
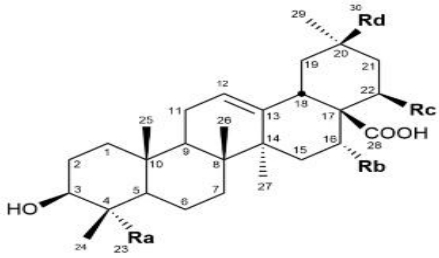
Terpenes geranyl farnesol, Thymol, Eugenol, Carvacol, Menthol, geraniol, ursolic acid, cinnamaldehyde, squalene		bacterial membrane disturbance -Efflux pum inhibition	Khameneth et al.,2019; Catteau et al., 2018
N- containing Compounds **Alkaloids** i.e. Reserpine, Barberine, Timodine Lysergol Quinine, Capsaicin, serpentine		Destruction of the bacterial cellular membrane -ATP synthase inhibition -Efflux pump inhibition -Protein and DNA synthesis inhibition -Cellular división inhibition	Kumar et al.,2019 Khalifa et al,, 2018
Chitosan		Inhibition of biosynthesis of bacterial cell Wall	Abd El-Hack et al.,2020
Cyanogenic-glycoside i.e. saponin *Quillaja saponaria*		Desestabilization of the celular membrane. -Adjuvant	Pacholak et al., 2018; Deise Flecke et al., 2019 Reichert et al., 2019
-Pinipesin arenicin-3,from *Arenicolamarina* hemocyanin-derived cationic peptide from shrimp *Litopenaeus vannamei* Moronecidin from rockfish *Sebastiscus marmoratus*	V|A|E|A|R|Q|G|S|F|S|Y GFCWYVCVYRNGVRVCYRRCN VNFLLHKIYGNIRYS MRFITLFLVLSMVVLMAEPGEA	-Efflux pum inhibition of Protein synthesis Induces membrane permeability	Ladram and Nichols., 2016 Kang et al., 2017 Chaparro-Aguirre et al., 2019 Neshani et al., 2019 Bo et al., 2019 Rončević T, et al., 2019 Jiao et al., 2019

Table 1: Targets and mechanism of action of the natural anti-infective agents.

Natural bactericidal agents

The chemical structure of natural anti-infective agents with bactericidal properties are characterized as phenolic compounds (flavonoids, non flavonoids, triterpenes, thymol, butyric and cinnamic acid). These compounds can damage and irreversibly alter the bacterial cell membrane, affecting thus, membrane permeability and polarization, thus, interfering with flux activity. Polyphenols constitute a promising weapon against infections in nosocomial settings when *Staphylococcus. aureus* strains are present [21,23,36], due to their chemical structure, these compounds are able to interact with the membrane bilayer of bacteria [34]. Tannins, which is one of the most representative phenolic compounds subclassified into condensed tannins (parmathocyanidins or catechins) and hydrolysable tannins (gallotannins and ellagitannins) can penetrate and interact with lipid bilayers and can cause membrane fusión, a process that results in leakage of intramembranous materials and aggregation [34].

Other natural compounds with antimicrobial properties besides the triterpene *Quillaja saponaria* (QS), are the dimeric quinolizidine sesquiterpene thioalkaloide (i.e. 6,6'dihydrothiobiparidine) which inhibited DNA topoisomerase IV or DNA gyrases [18,40-42], exerting also exert a synergistic effect either with drugs against multi-resistant strains of *S. aureus* (MRSA) or with vancomycin against resistant bacteria of the genus *Streptococcus* [27,28]. The polyamine squalamine acts by disturbing bacterial membrane integrity leading to ion efflux in gram-positive bacteria, and membrane disruption in Gram-negative bacteria [29] The benzo phenanthridine alkaloid as Sanguinarine, obtained from the root of *S. canadensis I*., acts through the release of autolytic enzymes, destroy tissues when applied to the skin [42]. The lignan meso-dihydroguaiaretic acid (MDGA) also isolated from *Larrea tridentate*, showed activity against methicillin-resistant *S. aureus* [42] (Table 1).

In the last years, antimicrobial peptides (AMPs) and bacteriocins (bAMPs) obtained either from bacteria or animals are a promising therapeutic alternative due to their low-resistant induction and greater bactericidal than bacteriostatic ability [43,44]. (Cationic peptides (+2 - 11 amino acids, or even of +34 amino acids (a.a.), which can directly interact with the membranes of microorganisms) can easily cause leaky membranes due to their amphipathic nature and secondary structure. AMPs can trigger the host innate immune responses or hemolytic molecules [44] (e.g. colistin, melittin, indolicidin, risin, CAMA, defensins, protegrins, magainins) [44,45] implying that these small peptides might be considered as potential safe natural antibiotics and able to act against Gram-positive or Gram-negative bacteria [46,47].

The so-called bacteriocins, gene-encoded, natural antibiotics, ubiquitous, small antimicrobial peptides (AMPs) produced by bacteria [43,44]. They often act toward species related to the producer with very high potency (at pico- to nanomolar concentration) and specificity. Different modes of actions or mechanisms of killing by bacteriocins includes, the destruction of target cells by pore formation and/or inhibition of cell-wall/nucleic acid/protein synthesis [43,44]. They have enormous potential in controlling antibiotic-resistant pathogens [47]. Due to this mode of killing mechanisms, it is thought to be less likely to induce resistance. Therefore, are being extensively evaluated as novel antimicrobial drugs as alternatives to conventional antibiotics. However, antimicrobial peptides have some disadvantageous that should be taken in account: Among them the high cost of production, the *in vivo* toxicity by the interaction with mammalian cells. Moreover, the bacteria can develop also evasion mechanism, i.e., changing membrane composition that can avoid the effective electrostatic interaction of AMPs with cell membrane [47]. Despite this, of relevance is the current status of the clinic investigation and commercial development of antimicrobial peptides that are in different phases of studies [47-49]. Interestingly, the novel antimicrobial mechanism of action has been described for marine peptides, such as anti-microtubule in the fungus *Aspergillus nidulans* [45,46]. Class I histone deacetylase inhibitors, a cyanobacterial secondary metabolite with a thioester enclosed [45,46] (Table 1).

Natural anti-infective agents and microbial resistance mechanisms

The world of nature anti-infective agents is immense, the microbial mechanism of resistance is as well. Inherent resistance mechanisms codify in the prokaryotic and eukaryotic cells that can remain silent or expressed under selection pressure. Other resistance mechanisms microbial genome arise as an adaptation mechanism to the environmental conditions [48,49]. In either case, bacteria are infinite endlessly to overcome the effects of antibiotics or drugs and develop critical resistance mechanisms [50]. One current challenging question is how natural anti-infective agents to overcome this. The hypothesis is that chemical properties could shape antimicrobial activity and microbial resistance mechanisms [39]. A potential cue is that anti-infective agents can inhibit or enhance the expression of genes involved in the enzymatic system for the biosynthesis of the structures in a prokaryotic cell. Typical examples of the microbial resistance mechanisms are the lipid barrier, the pumping systems, or efflux pumps (regulatory genes

that control multidrug resistance by enhanced system efflux pumps allow only specific molecules, or glycopeptides resistant (van gene cluster) [40]. The inhibition of the enzymatic process developed by the intracellular pathogens (*M. tuberculosis) i*s the proteasome. This system protects from the toxic effects of the products (NO_2) of innate cells (macrophages). Nitric oxide may combine with superoxide to generate peroxynitrite causing oxidative damage to *M. tuberculosis* [50].

By another hand, bacteria can survive and adapt either to the environmental conditions (physical, chemical factors, growth, nutrients competence) or within the host (pH, ions, microbial competence, immune system) [51]. In the remotion of the specific signal or stimulus, the bacteria revert to the original susceptible state. This type of resistance seems to result in gene expression regulation in response to environmental changes or epigenetic changes (i.e. changes in a bacterial population) [51].

Furthermore, antibiotic resistance can be transferred to a prokaryotic (resistant genes encoded in plasmid vectors) by genetic engineering (transformation) or microbial genetics techniques (conjugation, transduction). The acquired resistance refers to a susceptible bacterial population achieving resistant genes or a mechanism by mutation or transferring resistance genes from other bacteria. Usually, the transfer of these acquired genes is by horizontal transfer: transformation, conjugation, or transduction. An example is a bacterial resistance of *Neisseria gonorrhoeae* (Gram-positive; DNA gyrase and parC mutations) to ciprofloxacin [51]. Two of the most well-known mechanisms of bacterial resistance that lead to antibiotic destruction or modification are target alteration to expel antibiotics. Indeed, the antibiotic resistance mechanism resembles a worldwide natural process of microbial adaptation [51,52].

Bacteria are infinite endlessly using mechanisms to overcome the effects of antibiotics or drugs. Antimicrobial resistance to antibiotics generally develops by three other mechanisms: (a) Modifying the protein targets of antibiotics (modifying DNA enzymes for resistance to fluoroquinolones and Rifampin). (b) Production of the enzymatic systems involved in the degradation or modification of the structures of antibiotics renders them inactive, therefore, unsuccessful and ineffective (b-lactamases can hydrolyze the b-lactam). (c) The use of pumping systems to expel or remove antibiotic molecules to subtoxic concentration levels. To block or prevent antibiotic porins crossing in the outer membrane (OM), increasing OM selectivity, number of OM, or mutated OM [19,34,51]. Moreover, the production of antibiotic-destructing enzymes will also cause a lower affinity for antibacterial recognition. Mutations in a gene encoding a drug target lead to the activation of some enzymes [52,53], thus, decreasing the susceptibility of the bacteria to a specific drug. A well-described example of an antimicrobial resistance mechanism is the increased activity of enzymatic systems [52,53]. A clear example of this type of microbial resistance mechanism is the enzymatic system of the genus *Mycobacteria*. A mutation on the mycothiol enzymatic system renders mycobacteria unsusceptible to antibiotics [13,22,35,54].

One resistance mechanism that has become common and successful among clinical strains is biofilm formation. Biofilm acts like a matrix containing several molecular components (DNA released, proteins, and polysaccharides). One of the primary functions of biofilms is to decrease the penetration of antimicrobial agents [35]. *S. aureus* and *S.* mutants could capture positively charged molecules on extracellular polymeric biofilm and concentrate enzymes to inactivate antibiotics [42,54,55]. Thus, biofilm formation is a bacterial resistance and defense mechanism against several environmental stresses limiting bacterial survival. The microbial adaptation to the different environmental factors, physic (temperature) or chemicals (pH, ions, O2, antibiotics, nutrients), provide an opportunity for the induction of the virulence factors as biofilms, to induce mutations to overcome the host response [35,42,54,55].

Of relevance is that the chemical structure of the anti-infective agents such as of phenolic compounds, terpenes can decrease slims layer production of Gram-positive bacteria [8,19,34,22]. Another microbial resistance mechanism against antimicrobial peptides would be the reduction in cell permeability (downregulation of genes and proteins involved in the cell wall and external membrane formation) and the increase in the expression of the efflux pumps [19,34].

The potential of the anti-infective agents to overcome microbial resistance mechanisms

Anti-infective agents may be acting as direct or indirect resistance breakers [55]. As forward breakers of microbial resistance, they could be acting as 1) Enzymes inhibitors to prevent antimicrobial agent degradation; 2) Efflux inhibitors to allow the antimicrobial to reach the target; 3) Outer membrane (OM) permeabilizers that allow the antimicrobial agent entry by destabilizing the bacterial cell; and siderophore-antimicrobial combination agents that allow these compounds transported through the specific OM. As indirect breakers of microbial resistance are through a mechanism of gene silencing (antisense oligomers or by using the microbial genetic scissors, or the system of microbial defense, so-called "clustered regularly interspaced short palindromic repeats-Caspase nine) (CRISPR-Cas9) m to inhibit expression of resistance factors, reversing antimicrobial resistance [56]. Three different strategies are available to fight against microbial resistance mechanisms (WHO). First, assays to understand the critical microbial resistance

mechanism, be inherent, adaptative. Second, To get the maximum benefit with the combination of the immense resources of natural anti-infective products, in terms of chemical and modes of action [57,58]. Third, synergy among natural compounds to control drug resistance because the combination will target more than one site of action, increasing the bioavailability or modifying the resistance mechanism induction [52,53,57,58].

Fourth, to curb MDR bacteria, AMPs targeted immunomodulation of the host response. AMPs' detergent-like properties (membrane disruption) along with their role in innate immune response induction [6,59-61] keep bacteria with no chance to mutate, express resistance genes, or prepare their defense [59-61]. In each of this alternatives, the anti-infective agent's diversity should be taken in consideration [8,10,12, 54,59].

Table 2

Concluding Remarks and Perspectives

In the present review, we aimed to pinpoint basic aspects of the natural anti-infective agents that make them a feasible frontline strategy against microbial resistance mechanism development certainly an old and endless biological process that goes hand in hand with the diversity of the natural anti-infective agents. To further insight into the understanding of the mode of action of the pathogens (bacterial or viruses), and the unlimited, microbial resistance mechanism, it is necessary the use of the most updated technology. With the advancements in the last years of the omics technologies and the application of the biotechnological issues, becomes more feasible and available the improvement of pool of natural anti-infective agents without spending a lot of time in extraction methodologies. This will allow us to approach more straightforward the properties of the anti-infective agents, the crosstalk that is achieved at the surface of the microbial, which remains a challenge in alternative medicine, pharmacology and phytotherapy, among others.

One of the perspectives that deserves further investigation on medicinal plants is that the properties and design of innovative anti-infective agents should not be based, not only on their MIC values but on the integration of the *in vitro* and *in vivo* studies. A second perspective is to acknowledge advances and to profit from the innovative technologies, e.g. nanotechnology, that will allow developing nano carriers which can increase drug bioavailability and decrease treatment duration or involve new ways to increase effective anti-microbial concentration within the bacterial cell and therefore, of a hope for more effective treatments to overcome the increasing emergence of the multidrug resistance strains.

Conflicts of Interest

The authors declare no conflict of interest/The author declare that they have no competing interests.

Availability of Data and Material

This review was based on search and data from Pubmed database without limitation to 2021.

Code Availability

No applicable.

Author Contributions

G.G.G.M. content design, drafting the manuscript. G.G.G.M. J.M. F.H. conceptualization, drafting figures, tables. A.R.M. revising manuscript content. All authors have read and agreed to the published version of the manuscript.

Ethics Approval

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Consent to Participate

The author don't require consent to participate.

Consent for Publication

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