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Review

In Vitro and In Silico Studies of Antimicrobial Saponins: A Review

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Abstract: Antibiotics are important drugs for the treatment of microbial infections and related diseases. However, due to the abuse of antibiotics, drug resistance has become a serious and urgent problem. The development of new antibiotics is a crucial area of research, and natural products are one of the main sources of novel antibiotics. Among various potential natural antimicrobial products, saponins attracted much attention due to their excellent and broad-spectrum antimicrobial properties. Although there are several reviews on antibacterial saponins, this review is the first to highlight the potential antibacterial mechanisms of saponins from both experimental and molecular simulation perspectives to provide a comprehensive panorama of the field. This review presents the current progress in the development and repurposing of natural-product antibiotics. The focus is centered on antimicrobial saponins discovered in recent years as well as the synergistic effect of some saponins with traditional antibiotics. This review presents experimental and simulation studies in this field to provide a multiscale overview of the antimicrobial mechanisms of saponins and potential directions for future research.

Keywords: antibiotics; drug-resistance; natural products; saponins; glycosides; biomolecular interactions; molecular dynamics simulation

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1. Introduction

1.1. The Basics

Antibiotics are drugs that treat microbial infections by killing them or inhibiting their growth. They are also considered one of the most successful chemotherapy agents in the history of human medicine, traced back to more than 2000 years ago. China, Greece, and Egypt are known to have used moldy bread to treat wounds [1]. However, it was not until Alexander Fleming discovered penicillin in 1928 that the era of the large-scale discovery of natural-product antibiotics truly started [2]. This era reached the "Golden Age" between the 1940s to 1960s [3,4]. Since then, a large number of antibiotics derived from microorganisms have been discovered and applied clinically. For example, macrolides [5], tetracyclines [6], aminoglycosides [7], and chloramphenicol [8] were found in bacteria. Fungal microorganisms also provide humans with a large number of natural-product antibiotics, e.g., cephalosporins [4]. In addition, a variety of synthetic antibiotics have been developed, inspired by natural products such as quinolones or nitrofurans [9,10]. The use of antibiotics revolutionized medical treatments and lifestyles; before antibiotics, more than half of the deaths in the US during the 20th century were related to infectious diseases [11]. Antibiotics are also a reliable method to control infections after surgical operations, significantly reducing postoperative complications and extending the average human life expectancy by more than 10 years in just half a century after the application of antibiotics [4,12].

1.2. Challenge

The success of any therapeutic agent is limited by the development of drug resistance. Since the discovery of penicillin, antibiotics have been used as a "panacea" for various microbial-related diseases. With the abuse of these products, antimicrobial resistance has become an inescapable problem [13]. Pathogens such as bacteria develop the ability to survive antibiotic treatments, resulting in super-microorganisms [14]. These generally have the following characteristics: reduced membrane permeability towards antibiotics, the active export/removal of antibiotics, modified or protected drug targets, mechanisms to destroy or degrade the drug structure, and the bypassing of the antibiotic by making the original target redundant [15]. Initially developed antibiotics are not able to inhibit the growth of such super-microorganisms.

Although antimicrobial resistance emerged in the 1950s, it has experienced a significant acceleration in the last decade, presenting a serious challenge to antibiotic therapy and society in general [16,17]. In 2019, the number of deaths due to antimicrobial resistance worldwide reached 1.2 million, with expectations to reach 10 million in 2050 if no measures are taken to address the problem [18]. The socio-economic repercussions are also troubling and expected to cost low-income countries more than 5% of their gross domestic product by 2050, with more than 20 million people living in extreme poverty as a result [19]. The effects will also affect the developed world; a report showed that the cost of hospital care due to antibiotic resistance in the European Union had already reached £1.6 billion in 2012 [20]. Therefore, in addition to better regulations for the use of antibiotics, the development of novel antibiotics is a fast-growing research area.

1.3. Current Status

Natural-product antibiotics have achieved great clinical success and inspired the development of synthetic alternatives. Between the 1940s and 1970s, 55% of all antibiotics discovered came from bacteria from the *Actinomycetes* genus [21]. As of 2018, 28 of the 45 new antibiotic candidates in US clinical trials were natural products, compared to the 17 synthetic ones [22]. In the past, molecules with antimicrobial activity were mainly extracted and discovered from microbial metabolites in soil [23]. Scientists believe that novel metabolites will be discovered if the soil environment is sampled more extensively. Since 2000, the use of advanced screening technologies helped establish that the amount of metabolites produced by microorganisms was about ten times higher than previously recognized [24]. For example, *Actinomycetes* can produce about 30–50 secondary metabolites [12]. Given the abundant chemical diversity of natural products, it is generally believed that the best route for the development of new antibiotics lies in the discovery and repurposing of natural products [25]. Advances in high-throughput screening, bioinformatics, mass spectrometry, proteomics, transcriptomics, and metabolomics have greatly promoted the identification of novel antibiotics [26,27]. The application of these technologies accelerates the identification of new natural products from soil and previously underexplored areas [28–31]; for example, from new bacterial strains, algae, marine invertebrates, and some plants. Table 1 lists typical natural-product antibiotics from different sources.

Table 1. Typical natural-product antibiotics.

Name/Class	Natural Source	Clinical/Potential Application	Ref.
Penicyclones A–E	Deep-sea *Penicillium* species	*S. aureus*.	[32]
Tetronates lobophorins G	*Actinomycetes* strain	*B. subtilis*.	[33]
Hunanamycin A	Marine-derived *Bacillus hunanensis*	*Salmonella enterica*.	[34]
Curvulamine 15	*Curvularia* sp.	*Veillonella parvula*	[35]
Baulamycins A and B	*Streptomyces tempisquensis*	*S. aureus*, *B. anthracis*, *E. coli*.	[36]
Vermelhotin 19	Fungi	*M. tuberculosis*.	[37]
Viridicatumtoxins	*Paecilomyces* sp.	Ancomycin-resistant *Enterococci*.	[38]

In the process of discovering novel natural products, saponins (listed in Table 2) have attracted much attention due to their excellent and broad-spectrum antimicrobial properties. These are organic compounds widely distributed in the plant kingdom, though some are produced by marine invertebrates [39]. Saponins consist of various hydrophilic glycone side chains and hydrophobic aglycone backbones (see Figure 1). According to the type of aglycone, these molecules can be mainly divided into triterpenoid (30 carbons) or steroidal (27 carbons) [40]. Common glycone side chains include glucose, arabinose, gluconic acid, and galactose, among others [41]. These saccharides are linked to the aglycone via an ester or ether bond, which further contributes to the complexity of saponin molecules [42]. 2,3-Oxidosqualene, with 30 carbon atoms, is synthesized first via the linkage and oxidation reaction of six isoprene units, which is also the starting point for the biosynthesis of both triterpenoid and steroidal saponins [43,44]. Subsequently, the oxidosqualene is cyclized into a cyclic aglycone precursor compound via protonation and the epoxide ring opening [45,46]. After rearrangement, degradation, and additional modifications that include oxidation and glycosylation reactions, naturally derived saponins are synthesized [44]. These compounds can result in positive effects on plant growth and development as well as to protect the plant from microbes [47]. The biosynthesis, metabolism, and corresponding extraction of saponins have been described in published reviews in the past two decades and are not discussed in detail here [40,46,48,49].

Table 2. List of typical saponins with antimicrobial activity.

Name/Class	Natural Source	Target/Potential Application [1]	Ref.
Tigogenin saponins (Steroidal)	*Agave Americana* leaves	Fungi: *C. albicans* (5–10), *C. glabrata* (5–20), *C. krusei* (10–20), *C. neoformans* (0.63–1.25) [2].	[50–52]
Flabelliferin B (Steroidal)	*Borassus flabellifer* L. fruit	Bacteria: *E. Coli*, *S. aureus.* (None) [3] Fungi: *S. epidermidis*, *P. aeroginosa.* (None)	[53]
Dioscin (Steroidal)	*Dioscorea nipponica*	Fungi: *C. albicans* (22.5 ± 9.2), *C. parapsilosis* (11.3 ± 4.6), *T. beigelii* (11.3 ± 4.6), *M. furfur* (22.5 ± 9.2).	[54]
Fruticoside I (Steroidal)	*Cordyline fruticose* leaves	Bacteria: *E. faecalis* (128).	[55]
Sansevistatin 1 (Steroidal)	*Sansevieria ehrenbergii*	Fungi: *C. albicans* (2), *C. neoformans* (1–2).	[56]
Aginoside saponin (Steroidal)	*Allium nigrum* L.	Fungi: *C. gloeosporioides* (None), *F. verticillioides* (None), *B. squamosa* (None), *C. albicans* (47).	[57,58]
Persicosides A and B (Steroidal)	*Persian leek*	Fungi: *P. italicum*, *A. niger*, *T. harzianum.* (None)	[59]
chonglouoside SL-6 (Steroidal)	*Paris polyphylla* var. *yunnanensis*	Bacteria: *P. acnes* (3.9).	[60]
Quinoa saponin (Triterpenoid)	*Quinoa* husks	Bacteria: *P. gingivalis* (62.5), *C. perfringen* (31.3), *F. nucleatum* (31.3).	[61]
3β,19α,23,24–tetrahydroxyurs-12-en-28-oic acid and Ternifoliaoside A (Triterpenoid)	*Gardenia ternifolia Schumach.* & *Thonn* (*Rubiaceae*)	Bacteria: *P. aeruginosa* (12.5), *S. aureus* (25), *S. typhi* (12.5), *E. coli* (12.5).	[62]
Aridanin and Lotoidoside E (Triterpenoid)	*Paullinia pinnata*	Bacteria: *S. aureus* (1.56–6.25), *E. coli* (0.78–3.13), *P. smartii* (0.78–3.13).	[63]
Betulinic acid (Triterpenoid)	*Tovomita krukovii*	Fungi: *C. albicans* (16).	[64]
3-O-a-L-arabinopyranosyl-echinocystic acid (Triterpenoid)	*Cussonia bancoensis* bark	Fungi: *C. albicans* (12.5).	[65,66]

[1] The bacterial strains summarized here are the strains in the antimicrobial tests reported in the literature, and it does not mean that these saponins have antimicrobial activity only against these strains. [2] The numbers in the parentheses are MIC value ranges reported in the corresponding references, with the unit of μg/mL. [3] The "None" means that there is no MIC report in the corresponding reference.

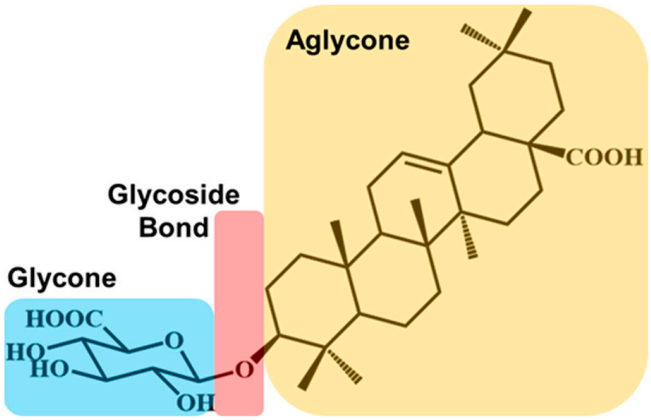

Figure 1. Basic chemical structure of saponins (This structure was made based on [41]).

This review introduces the study and characterization of antimicrobial saponins derived from natural products. First, recent studies on the activity of antimicrobial saponins from in vitro experiments are presented. Then, molecular simulations aimed to characterize the antimicrobial mechanism of these molecules are discussed. Finally, the main conclusions, challenges, and a research outlook on this field are summarized.

2. Experimental Studies

Various biotechniques have been used to identify saponins with antimicrobial activity. These studies reported molecular structures, biochemical information (including solubility, toxicity, or target information), and antimicrobial testing (e.g., minimum inhibitory concentration, MIC). Both steroidal and triterpenoid saponins have been found to have antimicrobial activity. Table 2 shows the typical antimicrobial saponins discovered in recent years.

Steroidal saponins first attracted the attention of researchers as a potential antifungal drug [67]. For example, Yang et al. tested the antifungal activity of steroidal saponins extracted from *Agave Americana* leaves against the opportunistic pathogens *C. albicans*, *C. glabrata*, *C. krusei*, *C. neoformans*, and *A. fumigatus* [52]. They found 10 saponin structures that were active against these fungi. Additionally, Qin et al. extracted 24 steroidal saponins from the stems and leaves of *Paris polyphylla* var. *yunnanensis* and found that 11 of them had moderate or significant inhibitory effects on *P. acnes* [60]. On the other hand, triterpenoid saponins have also shown antibacterial and antifungal activity, especially those found in quinoa crops [68]. For example, oleanane-type saponins from *Paullinia pinnata* showed antibacterial effects on *S. aureus*, *E. coli*, and *P. smartii* [61]. Additionally, the 3-O-a-L-arabinopyranosyl-echinocystic acid found in the stem bark of *Cussonia bancoensis* shows antifungal activity against *C. albicans* [65]. Betulinic acid extracted from *Tovomita krukovii* can also inhibit the aspartic protease secreted by *C. albicans*, which is one of the important virulence factors of Candida infection [64].

An interesting finding is that the antibacterial or antifungal activity of saponins depends on the carbohydrate groups attached to their aglycone (Figure 2). Qin et al. found that Chonglouoside SL-6, a saponin containing a trisaccharide moiety at the C-1 position, had the lowest MIC for *P. acnes* (3.9 μg/mL) compared with other saponins [60]. MIC is generally considered as the most basic measurement index for the effectiveness of antimicrobial agents, and the lower the MIC, the better the effect on microbials [69]. Among the 10 active C-27 steroidal saponins extracted from several monocotyledonous plants by Yang et al., four molecules with more carbohydrate groups showed stronger activity against *C. neoformans* and *A. fumigatus* [52]. However, this does not imply that saponins with more carbohydrate groups attached to the backbone will have stronger antimicrobial activity. Some studies have shown that hydrophobic saponins more easily bind to microbial cell membranes and thus show stronger antimicrobial activity [61,70]. For example, San Martín et al. obtained more hydrophobic saponin derivatives from quinoa via alkali treat-

ment; these molecules showed an obvious increase in the inhibitory effect on the mycelial growth of *B. cinereal* [70]. In contrast, saponins from non-alkali-treated quinoa had little effect against the fungus. The aglycone skeletons of saponins also show an impact on antimicrobial activity. Sadeghi et al. extracted various new steroidal saponins from *Persian leek*, which can be further classified into spirostanol, furostanol, and cholestanol based on the chemical structure of aglycone [59]. This study found that two spirostanol saponins were more active against the tested fungi than other steroidal ones.

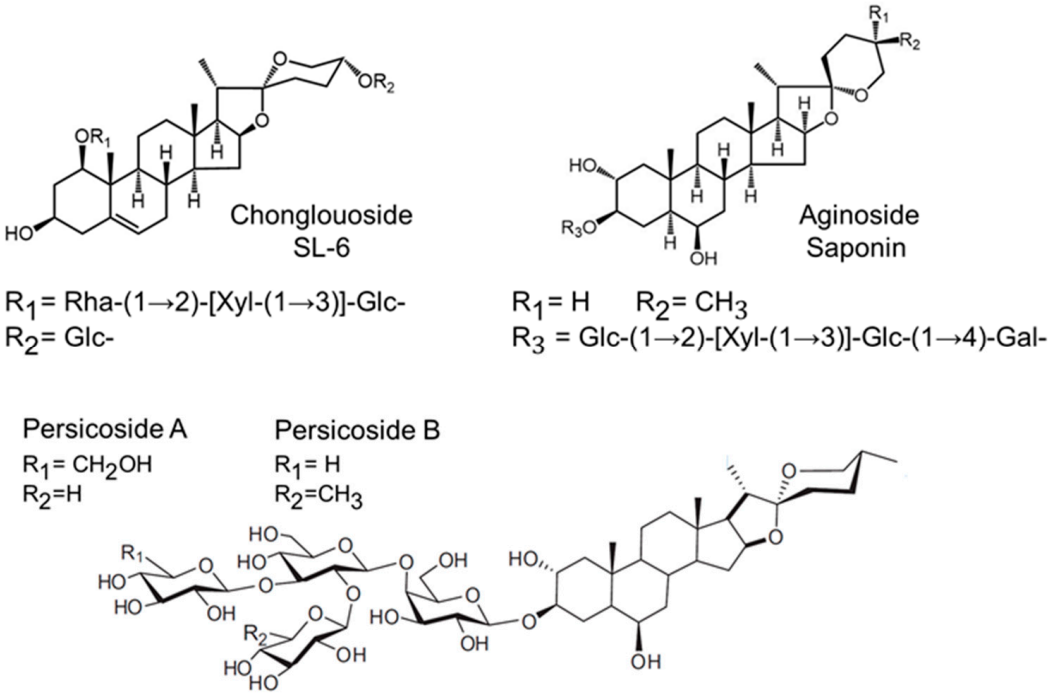

Figure 2. Typical saponin structures with stronger antimicrobial activity (Reprinted from the original figures in [60] for Chonglouoside, Copyright (2012), with permission from Elsevier, [58] for Aginoside, Copyright (2013), with permission from Elsevier, and [59] for Persicoside saponins, Copyright (2013), with permission from Elsevier).

In the studies of bioactive saponins, some found that their antimicrobial properties may be related to their interaction with the cell membranes [61,71]. Experiments showed that the interaction of saponins with microbial membranes can change the membrane surface morphology and even destroy its integrity. For example, Choudhary et al. observed the cell morphologies of control and treated cells using a scanning electron microscope (SEM) [72]. As shown in Figure 3A, compared with the smooth surface of control cells, the cells treated with safflower seed saponins (triterpenoids) showed a rough appearance with much debris, pits, and gaps. Sun et al. treated *F. nucleatum* cells with quinoa saponin extract ATS-80; complete cells could not be observed under MIC, and the cell membrane was completely disintegrated [61]. In vitro model membrane systems were also used to characterize the interaction mechanism of saponins with cell membranes. Orczyk et al. employed Langmuir monolayers of phospholipids to determine the effect of digitonin, a steroidal saponin, on lipid organization [73]. The results showed that digitonin inserted easily into the hydrophobic lipid tails' region, which significantly increased the bilayer surface pressure. However, especially for DPPE, DMPE, and DPPS, the inserted digitonin-kept lipid tails in a disordered state inhibited the transition to the liquid-condensed phase caused by the high surface pressure. This phenomenon is similar to the previously reported behavior of antimicrobial peptides penetrating negatively charged PG lipid monolayers [67].

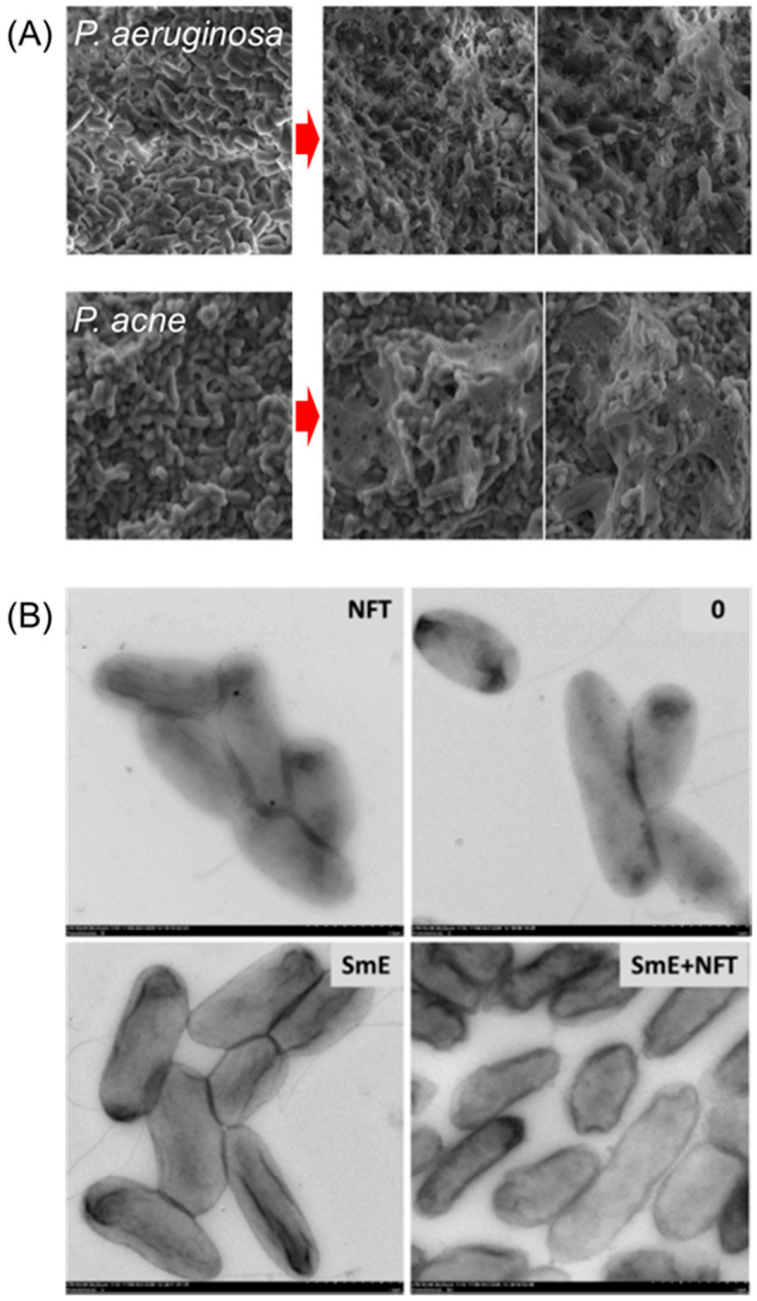

Figure 3. Sample figures from experimental studies on the impact of saponins on bacteria. (**A**) SEM micrograph images of *P. aeruginosa* and *P. acne* control (without treatment) and after treatment with isolated saponins (Reproduced with permission from *Springer Nature* [72]). (**B**) Transmission electron microscopic images of *P. aeruginosa*; 0—untreated cells (control sample), SmE—cells exposed to 100 mg/L of saponin extract, NFT—cells exposed to 5 mg/L of nitrofurantoin, SmE + NFT—cells exposed to both saponin extract and nitrofurantoin (Reprint from the original figure in [74], Copyright (2022), with permission from Elsevier).

In addition to their antimicrobial function, saponins can synergize with other antibiotics to enhance their activity. This synergy between known antibiotics and natural products is also considered to be one of the important ways to overcome antimicrobial resistance. Smulek et al. found that *Sapindus mukorossi* saponins can enhance the antibacterial activity of nitrofurantoin (NFT) [74]. NFT is a broad-spectrum antibiotic commonly used to treat urinary tract infections [75,76]. The cell toxicity test on *P. aeruginosa* strains showed that in the presence of NFT drugs alone (5 mg/L), the bacterial cell viability decreased by about

35%. However, its antibacterial ability was significantly enhanced when saponin extracts were introduced. The test results indicated that when 100 mg/L of saponin was used, the cell viability decreased by more than 75%. They observed the shape and morphology of individual bacterial cells via TEM and AFM. From Figure 3B, NFT alone has no significant effect on cell morphology, but when saponin extract is introduced, wrinkles and ripples appear on the cell surface.

A. baumannii is an important pathogen that causes infections such as bacteremia, pneumonia, and meningitis. Unfortunately, this strain is resistant to almost all conventional antibiotics [77]. Shin et al. studied the synergistic effect of oleanolic acid (OA) on multiple antibiotics against *A. baumannii* [78]. The microdilution checkerboard method is one of the traditional methods for the measurement of antibiotic synergy; it tests two antimicrobics in double serial dilutions, where the concentration of each drug is tested both alone and in combination [79]. Using this approach, OA was found to significantly reduce the MIC of aminoglycoside antibiotics. For example, if gentamicin was used alone, the MIC for *A. baumannii ATCC17978* was 16 μg/mL, compared to 4 μg/mL in the presence of OA. Microarray and quantitative reverse transcription-PCR analysis indicated that OA regulates bacterial ATP synthesis and the genes related to cell membrane permeability, which ultimately changes the intake of aminoglycoside antibiotics and exerts a synergistic antibacterial effect.

Antimicrobial assays showed that the MICs of saponins in combination with antibiotics against *E. coli*, *S. flexneri*, *S. aureus*, *C. parapsilosis*, *C. albicans*, and *C. neoformans* were all much lower than the MICs of conventional antibiotics alone. For example, saponins from *Melanthera elliptica* could enhance the antibacterial activity of Vancomycin and Fluconazole [80]. Additionally, Ye et al. reported that the camelliagenin derived from the defatted seeds of *Camellia oleifera* can be used to treat infections caused by amoxicillin-resistant *E. coli* and erythromycin-resistant *S. aureus* [81]. Their antibacterial tests indicated that the MICs of amoxicillin and camelliagenin saponin for amoxicillin-resistant *E. coli* were 72.6 $\pm$ 7.9 and 50.2 $\pm$ 5.7 μg/mL, respectively. However, the MIC of a 5:1 saponin–amoxicillin mixture was reduced to 23.4 $\pm$ 5.6 μg/mL. This study reported that the synergistic effect of camelliagenin saponin was related to its inhibition of bacterial biofilm. Generally, bacterial biofilm is a key factor in antibiotic resistance. Some studies have shown that the formation of bacterial biofilm is related to mannitol dehydrogenase (MDH) and extracellular DNA (eDNA). Ye et al. found that both the activity of MDH and the content of eDNA in saponin-treated biofilm decreased, suggesting the inhibition of bacterial biofilm by saponins. Together, these studies indicate that, in addition to the direct identification and development of saponins as novel antibiotics, their synergistic effect with known antibiotics may bring new options for the treatment of drug-resistant bacteria [82–84].

Despite excellent antimicrobial properties identified for saponins, cytotoxicity limits their biomedical potential [39,85,86]. Particularly, saponins are known for their hemolytic effects specially at the saponin–membrane interface [86–88]. Several studies found hemoglobin release when using saponins to treat red blood cells, including both triterpenoid and steroidal saponins [86,89–91]. Additionally, Baumann et al. use transmission electron microscopy to observe the destruction of lipid bilayers and the emergence of multilamellar buds in erythrocytes treated by saponins [87,89]. Such a hemolytic effect was found to be related to the chemical structure of saponins [91,92]. For example, Takechi et al. found that steroid saponins induced faster hemolysis in a study that compared 75 triterpenoid and steroid saponins [93]. Vo et al. found that the polar regions on sapogenins significantly enhanced hemolysis [91]. Finally, Savarino et al. characterized the hemolytic effect of saponins extracted from *Holothuria scabra*'s viscera. Their findings show that hemolysis is nearly eliminated by the desulfurization of glycones during the assay [89]. These new insights can help to reduce saponin cytotoxicity when leveraging its antimicrobial properties.

3. Computational Studies

With the increase in computational power, molecular modeling plays a key role in drug discovery and development [94,95]. Since the first computational simulations by Metropolis et al., there have been various well-established methods for the study of biomolecular systems [96]. These techniques are suitable for describing the motion and interaction of biomolecules and can increase the understanding of macromolecular observables. Among common methods, molecular docking is a powerful technique for studying protein–ligand interactions and predicting their binding modes [97,98]. Meanwhile molecular dynamic (MD) simulations model biological processes by predicting the motion of atoms in a system to predict the thermodynamic, physical, and dynamic properties [99]. In the study of saponins, both modeling approaches have provided important insights into antimicrobial molecular mechanisms. The basic principles of each approach are briefly described below, followed by the main contributions to saponin research in recent years.

3.1. Molecular Docking Studies

In the process of drug development, molecular docking can predict the potential structures of a drug–protein complex based on the stability of its configuration by calculating the free binding energy [100,101]. Molecular docking simulations are relatively simple and require only modest computational resources [102–104]. The first step is posing; the receptor and ligand structures are positioned in several initial configurations using a stochastic or systematic algorithm [105,106]. Then, scoring takes place in which all binding modes obtained in the previous stage are evaluated using a scoring function to determine the most likely conformations. Commonly used scoring functions include force-field-based, empirical, and knowledge-based functions [107]. The results predict which ligands may be more favorable to interact with a given protein and the most probable binding mode. In drug discovery, these results can help narrow down the number of molecules that are promising drug candidates [108].

A variety of studies have shown that saponins exert antimicrobial activity by acting on specific proteins. Molecular docking can provide atomic-level details of the interaction between saponins and protein receptors and rank them according to their corresponding binding free energy [109,110]. This is an important supplement to understanding antimicrobial molecular mechanisms. As discussed earlier, Ye et al. found that camelliagenin saponin can reduce the activity of mannitol dehydrogenase (MDH) and the content of extracellular DNA (eDNA), which are both key components in bacterial biofilm formation [81]. To further examine these interactions, they simulated saponins using molecular docking and showed that saponins can bind to MDH and eDNA easily (see sample diagrams in Figure 4). The calculated average binding energies were -86.94 ± 1.99 and -105.01 ± 1.19 kcal/mol, respectively, which indicated that camelliagenin can spontaneously bind to both MDH and eDNA and modulate their function. In addition, Wei et al. examined the synergistic antibacterial activity of Sapindoside A and B against *M. luteus* [111]. The experimental data indicated the antibacterial activity of this combination was achieved by attacking the cell membrane proteins. To further explore their molecular mechanism, molecular docking was employed to simulate the binding modes of Sapindoside A and B with membrane protein PBP 2 (PDB ID: 3UN7). The results showed that saponin and PBP 2 form a stable complex via the hydrogen bonds between saponin sugar groups and specific amino acids, illustrating the importance of sugar chains for antibacterial activity.

3.2. MD Simulation Studies

MD simulations predict the trajectory of atoms in a system based on the forces exerted on them [112]. In a nutshell, given the positions of all atoms in the system of interest, MD simulation predicts the motion of particles by solving Newton's equations of motion and a function that models the potential of interaction across all atom types [113]. The thermodynamic, physical, and dynamic properties of the system can be computed from the resulting trajectories to predict the biomolecular mechanisms and functions that help

in the interpretation of experimental phenomena. To examine the antimicrobial activity of saponins, MD simulations can reproduce the interaction dynamics between saponins and different targets. The ability to simulate larger systems enables the study of saponin–protein, saponin–saponin, and saponin–membrane interactions [114,115]. The mechanism of membrane disruption by antimicrobials is of particular interest; MD simulations can contribute immensely to modeling such interactions.

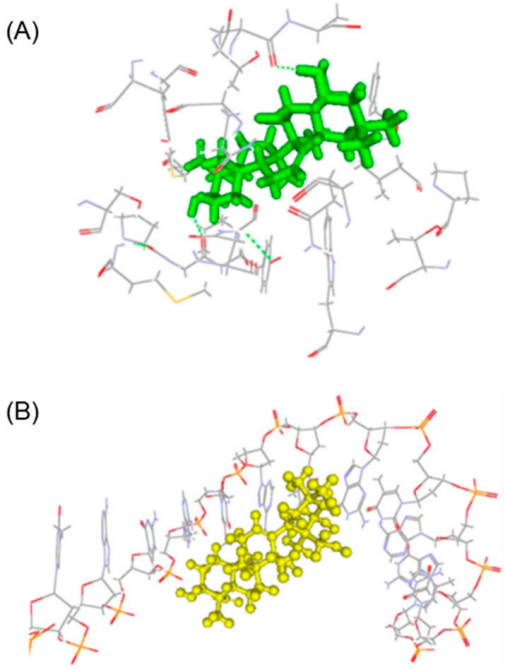

Figure 4. Molecular docking simulation on interactions of camelliagenin saponin with (**A**) MDH and (**B**) eDNA (Adapted with permission from the original figure in [81]. Copyright (2015), Ye et al.).

Most antibiotics need to cross cell membranes to reach their target, especially in Gram-negative bacteria [116]. Some bacteria with antimicrobial resistance reduce membrane permeability [15]. Modeling this process at the atomic level can aid in the discovery and development of saponin-related antibiotics. However, the energy barriers and timescale of the process make it challenging to sample with brute-force MD [117,118]. Enhanced sampling techniques have been developed to enable the modeling of such slow biological processes. In the study of active saponins, the most commonly used techniques are umbrella sampling (US) and metadynamics (MetaD) [119,120]. These techniques enable enough sampling of slow- and high-energy processes by directly applying forces (in US) or introducing external bias energy (in MetaD) on a specific reaction coordinate.

Saponin is a typical amphipathic molecule with hydrophilic and hydrophobic groups; it easily forms clusters in water, which may have an impact on the interaction with its targets. Zelikman et al. simulated the aggregation behavior of glycyrrhizic acid (GA) in water [121]. The results showed that GA easily forms tightly packed dimers that can rotate around the triterpenoid groups, allowing water molecules around it to induce the random motion of the saponins' sugar groups. In contrast, Kim et al. simulated the aggregation behavior of GA in a hydrophobic environment, mimicking the condition of the bilayer core [122]. Their results indicated that GA can form dimers or trimers in the heptane solvent, but these are unstable and form or dissociate easily.

Other simulation studies found that saponins prefer to bind to cholesterol in the membrane core due to the structural similarity between aglycone and sterol rings [123–125]. Lin et al. used MD simulations to explore the interaction of dioscin, a monosaccharide saponin, with lipids [126]. The results show that in a non-polar environment, the head-to-head configuration of dioscin with cholesterol is much more stable than in water, and that increased dioscin in the bilayer induces high curvature, possibly leading to membrane

rupture. Claereboudt et al. studied Frondoside A, a holothuroid saponin, and found it can promote the formation of cholesterol domains in the membrane, thereby altering its permeability [127]. In all the above studies, the membrane models used mimic the lipid diversity of eukaryotic cells. A recent study focused on the interaction of four triterpenoid saponins with the POPE/POPG/DPPG bilayer, which is a typical bacterial membrane model [128]. The study found that the sugar groups attached to the aglycone affect the orientation and relative location of saponins in the membrane (See Figure 5A). In addition, hydrophilic saponins tend to localize closer to the membrane surface, which is consistent with the sugar-modulated antimicrobial activity of triterpenoid saponins found in experiments [61,70]. This last simulation study also suggested saponins may be actively involved in the lateral resorting of lipids and the modulation of the local membrane structure.

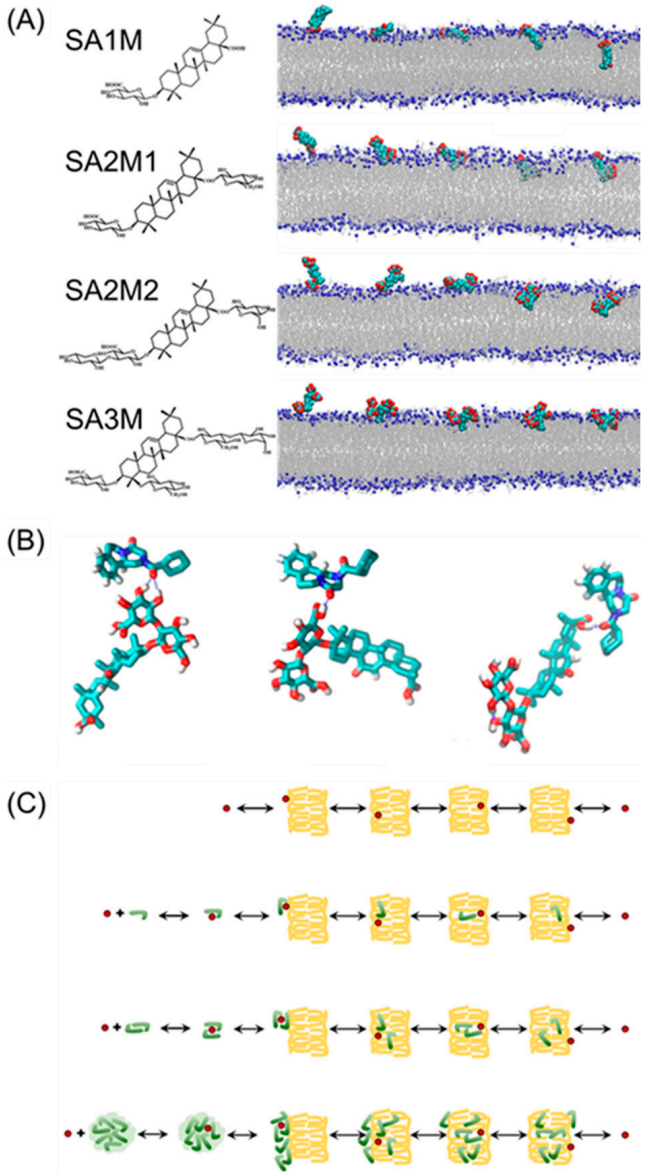

Figure 5. (**A**) The chemical structures and binding processes of four triterpenoid saponins with different glycone side chains (Adapted with permission from the figure in [128]. Copyright (2023), American Chemical Society). (**B**) Three typical configurations of PZQ and GA connected by hydrogen bonds. (**C**) Several proposed permeation processes of PZQ in the absence and presence of GA, where red balls represent PZQ molecules and green columns represent GA molecules ((**B**,**C**) are adapted with permission from original figures in [122]. Copyright (2019), American Chemical Society).

Finally, enhanced sampling techniques have also contributed to the study of the membrane permeation mechanism of saponin molecules. Praziquantel (PZQ) is a commonly used anthelmintic drug which is often used to treat various diseases caused by trematode infection [129]. The availability of orally administered PZQ is often very low due to its low solubility and membrane permeability. Kim et al. studied its membrane permeation in the presence of glycyrrhizic acid (GA) [122]. Parallel artificial membrane permeation assays showed that the membrane permeation of PZQ significantly increased in the presence of GA compared with the control group. Umbrella sampling was used to model this permeation process, revealing that GA forms hydrogen bonds with PZQ and reduced the energy barrier for permeation through the bilayer. Additionally, GA reduces the resistance of the local membrane surface to the PZQ insertion by rearranging the orientation of lipid headgroups and increasing PZQ permeability (see Figure 5B,C).

It should be noted that current MD simulations need further refinement. First, most studies on saponin–lipid interactions use pure PC lipid bilayers, which are the most abundant lipid species found in eukaryotic cells. In the future, studies should include lipids present in bacterial membranes, such as phosphatidylglycerol (PG) lipids. Second, bacterial cell membranes are usually asymmetrical, with different inner and outer membrane compositions, yet current bilayer simulations use only symmetric bilayer models [130]. The use of asymmetric membrane models can allow the characterization of antimicrobial drugs in a more realistic setting in both the lipid diversity and mechanical environment of the membrane. Enhanced sampling techniques have been used successfully to simulate the permeation of some natural products across bilayers. However, reaction coordinates (RC) for these simulations are generally selected based intuitively; for example, the distance between a drug molecule and the lipid bilayer centroid [131,132]. Such intuitive RCs are often too simple and cannot accurately describe the main motions during the permeation process. This is particularly true as the drug molecules of interest gain chemical complexity, like the addition of glycones to the backbone of saponin molecules. The selection of efficient RCs is far from trivial, using dimensionality reduction techniques like the principal component analysis, and more sophisticated machine learning approaches could speed up the design of smarter RCs to better describe drug permeation and the aggregation processes [133].

4. Conclusions

Antimicrobial resistance is a serious problem to human health and has strong socioeconomic repercussions. As presented in this review, saponins have attracted much attention because of their excellent ability to inhibit the growth of multiple bacteria or fungi, in addition to their abundance in the plant kingdom. Saponins can have a triterpenoid or steroidal skeleton and a wide range of carbohydrate groups attached to it. Several studies have shown that the antibacterial activity of saponins is directly related to their interaction with cell membranes. Experiments confirmed that saponins can indeed change the morphology of cell membranes and even destroy their integrity. Furthermore, saponins can synergistically enhance the antimicrobial activity of traditional antibiotics by increasing their permeability or by inhibiting biofilm synthesis—both of which are key aspects in the development of novel therapies to overcome antibiotic resistance.

In addition to experimental work, molecular modeling has increasingly become an important tool for drug discovery. In the field of antibiotics development, molecular docking and MD simulations are generally used to predict the antimicrobial mechanisms of various compounds. Simulations successfully provided molecular-level explanations for the interaction between saponins and microbial membranes as well as protein targets, which could lay the foundation for saponins to become feasible commercial drugs. It should be acknowledged that, although many saponin molecules with antibiotic efficacy have been identified, their antimicrobial mechanisms need to be further characterized. For example, there are many molecular simulation reports on the interaction between saponins and simple lipid bilayer models for eukaryotic cells. In these models, PC lipids are commonly used to mimic the membrane environment, which is not the main lipid species found in

bacterial membranes. The asymmetry of bacterial membrane composition should be further considered to better characterize the effect of saponins on the mechanical and structural properties of bacterial cell membranes.

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Abbreviations

The following abbreviations are used in this manuscript:

AFM	Atomic force microscopy
DPPE	1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine
DMPE	1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine
DPPS	1,2-Dipalmitoyl-sn-glycero-3-phosphoserine
eDNA	Extracellular DNA
GA	Glycyrrhizic acid
MEH	Mannitol dehydrogenase
MetaD	Metadynamics
MIC	minimum inhibitory concentration
MD	Molecular dynamic
NFT	Nitrofurantoin
OA	Oleanolic acid
PC	Phosphatidylcholine
PZQ	Praziquantel
RC	Reaction coordinate
TSM	Transmission electron microscopy
US	Umbrella sampling

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