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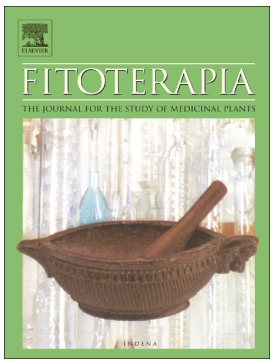

Anacardic acid as a promising natural antimicrobial agent: Mechanisms of action, biofilm inhibition, and advances in nano-encapsulation for enhanced therapeutic efficacy

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Anacardic acid as a promising natural antimicrobial agent: Mechanisms of action, biofilm inhibition, and advances in nano-encapsulation for enhanced therapeutic efficacy

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Abstract

Anacardic acid (AA), a phenolic lipid primarily found in *Anacardium occidentale* and related plants, exhibits promising antimicrobial activity, notably against biofilm-associated infections. AA disrupts bacterial membranes, inhibits vital metabolic pathways, and interferes with quorum sensing, making it effective against both planktonic and biofilm-forming bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, and methicillin-resistant *S. aureus* (MRSA). Its distinctive chemical structure, particularly the length and degree of unsaturation of its alkyl side chains, plays a critical role in antimicrobial potency. Higher unsaturation enhances membrane disruption and antibacterial effects, while structural modifications, including branching and derivatisation, improve bioavailability and efficacy. Despite its potent action, AA has limited solubility and stability; thus, advances in nano-encapsulation techniques and complexation with carriers like hydroxypropyl-β-cyclodextrin have improved its clinical potential. AA and its analogues not only disrupt biofilm architecture but also suppress bacterial metabolism and inhibit extracellular polymeric substance (EPS) production, key factors in biofilm resilience.

AA-based coatings and formulations offer promising applications in biomedical devices to prevent biofilm formation and combat antimicrobial resistance. AA effectively inhibits biofilm development. Its ability to eradicate mature biofilms is weaker, emphasizing the need for combination therapies or enhanced delivery systems. AA represents a multifunctional, natural antimicrobial candidate with considerable scope for further development to address persistent biofilm-related infections and reduce the global threat of antimicrobial resistance.

Keywords: Anacardic acid, antimicrobial, biofilm inhibition, alkyl side chain, nano-encapsulation, quorum sensing

1. Introduction

Microorganisms are known to be responsible for the development of various diseases in the world, specifically infectious [1]. In 2024, an estimated 7.7 million people died from bacteria infections, with an additional 1.3 million deaths attributed to antibiotic resistance [2]. In clinical implementation, nearly all principal types of antibiotics have exhibited resistance. Bacteria resistance

to vancomycin, macrolides, quinolones, and beta-lactam antibiotics is rising as a significant global health issue [3]. The vancomycin family of antibiotics, which was formerly the final line of defense against infectious diseases, is currently facing serious challenges due to a rise in resistance [4]. According to 2020 EARS-Net data, over 35,000 individuals die annually in the European Union/European Economic Area (EU/EEA) due to antimicrobial-resistant diseases [5]. A significant contributor to this escalating antimicrobial resistance is the formation of biofilms, complex microbial communities encased in a self-produced polymeric matrix, which renders bacteria up to 1,000 times more resistant to antibiotics and immune responses than their planktonic counterparts, leading to persistent and recurrent infections that current therapies often fail to resolve [6]. This condition has prompted scientists to develop novel strategies for eliminating germs that are becoming more resistant to current antibiotics.

Natural products have received significant attention as potential sources for novel pharmacological medicines. *Anacardium occidentale* L. is a plant with medicinal properties utilised for the treatment of dermatological diseases, tooth pain, pneumonia, and diarrhoea. [7], [8]. *A. occidentale* L. is a traditional medicine to treat infectious diarrhea in West Africa. Another plant, *Amphipterygium adstringens* produces anacardic acid (AA) for the prevention of microbial infections [9], [10]. Moreover, French Polynesians often utilise *Syzygium malaccense* leaves to cure cystitis and other infectious diseases [11].

AA is a combination of multiple chemical substances that are structurally linked, all of which are made up of aliphatic chains with varying degrees of unsaturation in place of salicylic acid [12]. AA contains a salicylic acid core substituted with 15-carbon side chains, either saturated (C15:0) or unsaturated, with one (C15:1), two (C15:2), or three (C15:3) double bonds [13].

Several studies have examined issues concerning AA, although none have specifically addressed the characterization of a particular molecule. Phenolic chemicals, such as AA, possess the capability to hinder fungal and bacterial development. Previous reviews focused for harmful microorganisms such as *Streptococcus mutans* and *Staphylococcus aureus* [14], the antioxidant, anticancer, and anti-inflammatory properties of AA and its semi-synthetic derivatives have been examined [15], [16]. The existing reviews are still limited to descriptions of the growing environment and cultivation of Anacardium species, along with examinations of phytochemical and nutritional composition, including studies on active metabolites in general [17], [18].

This review explores the distinctive chemical structure of anacardic acid and its impact on antimicrobial efficacy, particularly its ability to disrupt bacterial membranes and inhibit key metabolic pathways. Discussion emphasizes recent advances in synthetic derivatives and nano-encapsulation technologies that significantly enhance the stability, bioavailability, and antibiofilm activity of anacardic acid. Furthermore, the utilization of anacardic acid formulations in biomedical

applications such as catheter coatings, dental adhesives, and wound dressings underscores their potential to address persistent biofilm-related infections and combat antimicrobial resistance. The safety profile and multifunctional mechanisms of anacardic acid and its analogs make them promising candidates for novel therapeutic development against challenging microbial biofilm infections. This review is expected to contribute scientifically to the development of alternative antimicrobial agents using natural materials.

2. Materials and methods

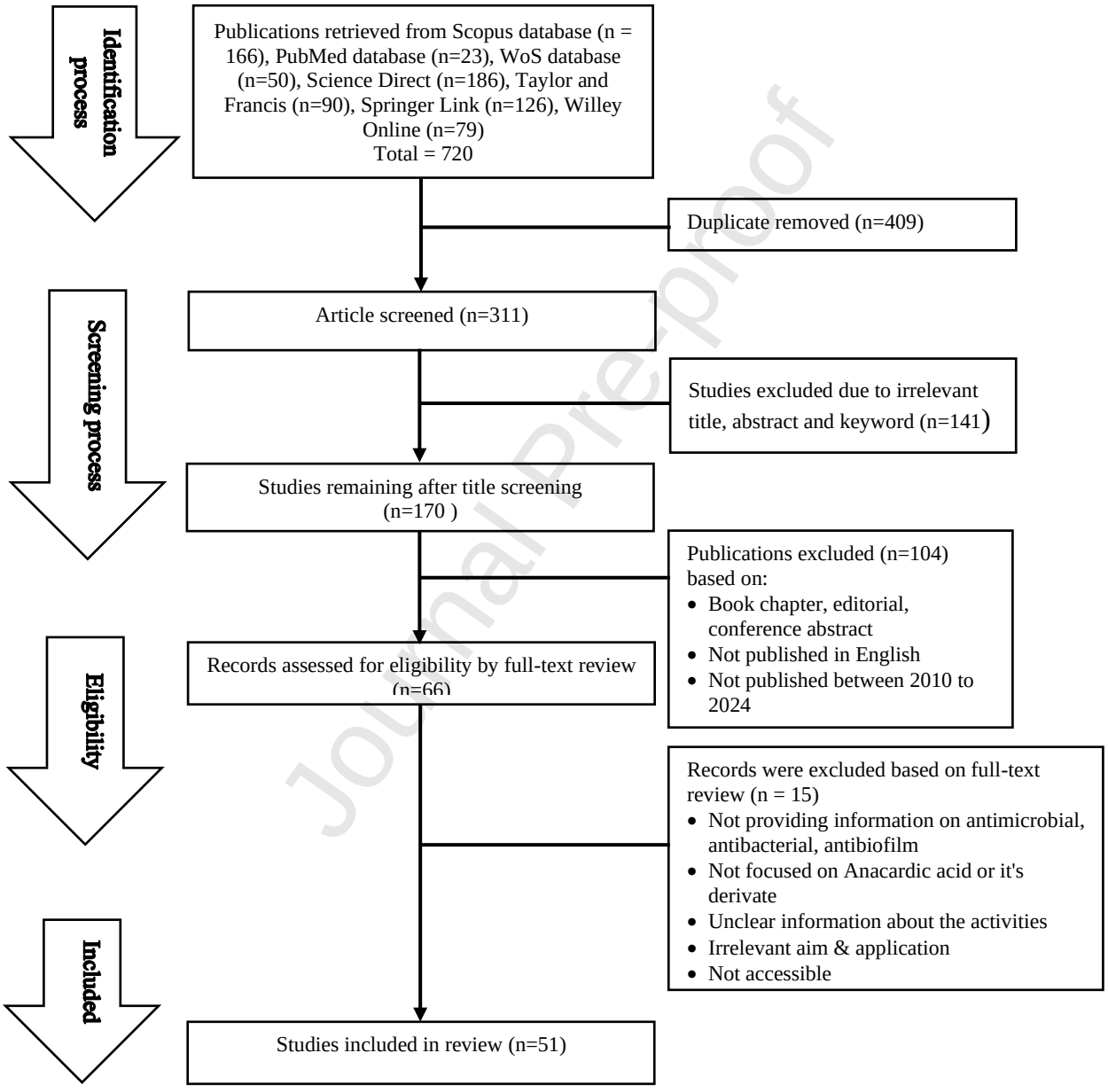

Fig.1. The literature selection process flowchart.

The references for this review are based on databases from Scopus, PubMed, Web of Science (WoS), SpringerLink, Taylor & Francis, ScienceDirect, and Wiley Online. Keywords as descriptors for literature or article searches related to AA and antimicrobials using TITLE-ABS-KEY ("anacardic

acid") AND TITLE-ABS-KEY ("antibacterial"); TITLE-ABS-KEY ("anacardic acid") AND TITLE-ABS-KEY ("antimicrobial"); TITLE-ABS-KEY ("anacardic acid") AND TITLE-ABS-KEY ("antimicrobial" OR "antibacterial"). The articles obtained from the initial search were further limited in terms of publication years from 2010 to 2024 (15 years). Articles written in English were selected. Restrictions are applied to facilitate the updating of information relevant to current conditions in a universal language while ensuring clarity of understanding (Fig.1). Significant scientific contributions are obtained from the selection of journals. Proceedings (conference papers), book chapters, books, newspapers, letters, and editorials were excluded from the dataset. Articles containing redundant content and those with only one keyword are not included in the dataset. Manual searches are conducted if the obtained data is insufficient [19]. Following data collection, each selected article was systematically evaluated for methodological quality to ensure the reliability and reproducibility of the findings.

Bias analysis for in vitro studies was conducted in accordance with the Methodological Guide for Comparative Effectiveness. The assessment includes six items (Table 1): clearly stated objectives are considered item 1, accurate experimental design is item 2, sample identification and evaluation are item 3, comparison and reproducibility are item 4, other biases are item 5, and adequate statistical analysis is item 6 [20]. Item 1 is scored zero if the article's objectives are not consistent with the research, one if the objectives are unclear, and two if the objectives are clearly reported. Item 2 (experimental design) is scored zero if the experimental design is not explained, one if there is an unclear experimental design, and two if the experimental design is reported in detail and accurately. Item 3 (sample identification and evaluation) is scored zero if anacardic acid, its analogs, and derivatives are present but not reported; 1 if anacardic acid, its analogs, and derivatives are present and reported with the MIC or zone of inhibition used; and two if anacardic acid, its analogs, and derivatives, including mechanism, MIC, zone of inhibition, and IC_{50}, are reported in detail. Item 4 (comparison and repeatability) is scored zero if non-standardised antibacterial, antimicrobial, and antibiofilm activity testing methods are required, one if the testing methods are less complex but have low reproducibility, and two if the testing methods can be easily practised in a standard laboratory with consistent results. Item 5 (other bias) is scored zero if the abstract, methods, and conclusions are poorly described or not described, one if they are too brief, and two if they are adequately described. Item 6 (statistical analysis) is scored zero if not done, one if partially done, and two if done in detail (e.g., standard deviation). The total score is determined by risk category: 0–6 for high risk, 7–9 for moderate risk, and 10–12 for low risk. Overall bias risk analysis shows high, moderate to low bias risk across various parameters. Articles with high bias risk (11.8%) were still used because they contained information related to plants that produce anacardic acid compounds. However, 88.24% of the studies were relevant and closely related to the current topic.

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Table 1. In-vitro study bias analysis on AA-related articles.

No.	Reference	Score						Total Score	Risk
		Item 1	Item 2	Item 3	Item 4	Item 5	Item 6		
1.	Stasiuk, et.al (2010)	2	1	1	0	1	0	5	H
2.	Watanabe, et.al (2010)	2	2	2	2	2	2	12	L
3.	Nallamilli, et al (2011)	2	2	1	2	2	0	9	M
4.	Rivero-Cruz (2011)	2	2	2	2	1	0	9	M
5.	Silva, et.al (2011)	2	2	0	0	1	0	5	H
6.	Vempati, et.al (2011)	2	2	1	2	2	0	9	M
7.	Reddy, et.al (2011)	2	2	1	2	2	0	9	M
8.	Yuliana, et.al (2012)	2	2	1	2	2	0	9	M
9.	Castillo-Juarez, et.al (2013)	2	2	2	2	1	2	11	L
10.	Mamidyala, et.al (2013)	2	1	1	2	1	0	7	M
11.	Murata, et.al (2013)	2	2	2	2	1	0	9	M
12.	Sharma, et.al (2013)	2	2	2	2	2	2	12	L
13.	Vempati, et.al (2013)	2	2	1	2	2	0	9	M
14.	Hamad, et.al (2015)	2	1	2	2	1	0	8	M
15.	Hollands, et.al (2015)	2	2	2	2	1	0	9	M
16.	Muzaffar, et.al (2015)	2	2	2	2	2	2	12	L
17.	Rodriguez-Garcia, et.al (2015)	2	2	1	2	2	2	11	L
18.	Rambabu, et.al (2016)	2	2	1	2	2	0	9	M
19.	Oh, et.al (2017)	2	2	2	2	2	2	12	L
20.	Salleh, et.al (2017)	2	1	1	0	1	0	5	H
21.	Baptista, et.al (2018)	2	2	2	0	2	0	8	M
22.	Prakash, et.al (2018)	2	2	2	2	1	0	9	M
23.	Sajeevan, et.al (2018)	2	2	2	2	2	2	12	L
24.	Anjum, et.al (2019)	2	2	2	2	2	2	12	L
25.	Filho, et.al (2019)	2	2	2	2	2	2	12	L
26.	Gomez, et.al (2019)	2	2	2	2	1	1	10	L
27.	Salehi, et.al (2019)	2	0	1	0	1	0	4	H
28.	Hurtado, et.al (2019)	2	1	1	1	1	0	6	H
29.	Anjum, et.al (2020)	2	2	2	2	2	2	12	L
30.	de Araujo, et.al (2020)	2	2	0	2	2	1	9	M
31.	Rodrigues-Costa, et.al (2020)	2	2	2	2	1	0	9	M
32.	Khatib, et.al (2020)	2	2	2	2	2	0	10	L
33.	Lima, et.al (2020)	2	2	1	2	2	2	12	L
34.	Saedtler, et.al (2020)	2	2	2	2	1	2	11	L
35.	Salehi, et.al (2020)	2	1	1	1	1	0	6	H
36.	de Araujo, et.al (2021)	2	2	2	2	2	1	11	L
37.	Silveira, et.al (2021)	2	2	2	2	2	2	12	L
38.	Sousa, et.al (2021)	2	2	2	2	2	2	12	L

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39.	Di Pierro, et.al (2021)	2	2	1	2	1	2	10	L
40.	Sotelo-Barrera, et.al (2022)	2	1	2	1	2	0	8	M
41.	dos Santos, et.al (2022)	2	2	1	2	2	2	11	L
42.	Oke, et.al (2022)	2	2	1	2	2	0	9	M
43.	Souza, et.al (2022)	2	2	2	2	2	2	12	L
44.	Quenon, et.al (2022)	2	2	1	2	2	0	9	M
45.	Oliveira, et.al (2022)	2	2	2	2	2	2	12	L
46.	Dame-Teixeira, et.al (2022)	2	2	1	2	2	2	11	L
47.	Campos, et.al (2023)	2	2	2	2	2	0	10	L
48.	Khatana, et.al (2023)	2	2	1	2	2	0	9	M
49.	Oliveira, et.al (2023)	2	2	2	2	2	0	10	L
50.	de Souza, et.al (2024)	2	2	0	2	2	2	10	L
51.	Mendes, et.al (2024)	2	2	1	2	2	2	11	L

3.AA physical-chemical properties

3.1. The basic structure, natural occurrence, and physicochemical behavior.

AA is the main ingredient in natural CNSL. The first pure form of AA (6[8'(Z)-pentadecyl] salicylic acid) was found in the cashew apple *A. occidentale* (Anacardiaceae). A pentadecyl group replaces the salicylic acid chain at the 6-position to form AA, a hydroxybenzoic acid. In nature, AA is composed of salicylic acid groups that have been substituted for long-chain alkyls with 15–17 carbons (Table 2) [21].

AA is usually found as a mixture of -ene (Fig.2) [22]. Salicylic acid and long-chain non-isoprenoid side groups, in the form of C15-alkyls with C15:1, C15:2, or C15:3 at positions C-8, C-10, and C-14, respectively, make up the mixture of AA-ene [23]. Two distinct characteristics of AA are the aliphatic and aromatic chains. The degree of unsaturation in the side chain influences the biological activity. In general, lower unsaturation may be more effective in antifungal activities. C15:1 form is the most effective against fungi showing that the molecule linearity is essential for antifungal action. This implies that increasing the side chain's number of double bonds typically enhances its antibacterial effectiveness [24].

The unsaturated side chain of AA has significant activity against Gram-positive bacteria. C15:3 molecule with three unsaturated bonds, showed the highest activity against *S. mutans*, bacteria linked to dental caries. The efficacy against various germs may fluctuate depending on the level of unsaturation. C15:3 form showed greater efficacy against *S. mutans*, while C15:1 form had enhanced activity against *Cutibacterium acnes*, bacteria implicated in acne. The side chain morphology and dimensions are critical in the interaction with bacteria membranes and enzymes [25]. The degree of unsaturation correlates with increased membrane disruption. For instance, linolenic acid (C18:3) and linoleic acid (C18:2) cause significant changes in membrane morphology at higher concentrations compared to oleic acid (C18:1), which has fewer unsaturated bonds. The changes indicate that higher

unsaturation levels lead to more pronounced membrane destabilization [26]. More importantly, the amphipathic nature of the phenolic lipids is connected to the antibacterial effect. Aside from the lipophilic side chain, the significant attraction of CNSL for bilayers of lipids in bacteria membranes can be determined by the contact between the aromatic ring hydroxyl groups and phospholipids through hydrogen bonding [27].

Table 2. Information on plants producing AA, along with their molecular formulas and pharmacological activities.

No.	Plants	Plants parts	Bioactive compound	Molecular formula of AA	Pharmacological activity	Reference
1.	*A. occidentale* L.	Nut and fruit juice	AA	C_{15} aliphatic	Parasiticidal, antimicrobial, antioxidant, lipoxygenase inhibition, inhibition of NF-κB, antitumor.	[28]
2.	*A. adstringens* (Schltdl.) Standl.	Bark	AA mixture	C_{15} – C_{19} aliphatic	Antibiofilm	[29]
3.	*A. occidentale* L.	Cashew nut shell	CNSL (total AA, cardol and cardanol)	C15:1 C15:2 C15:3	Rumen fermentation toward enhanced propionate production and decreased acetate and butyrate production.	[30]
4.	*A. occidentale* L.	Cashew apple	AA mixture	C_{15} – C_{19} aliphatic	Antibacterial activity	[16]
5.	Synthesis compound	NA	AA derivate	C_8-C_{15} aliphatic	Antibacterial activity	[31]
6.	Synthesis compound	NA	AA derivate	C_8-C_{15} aliphatic	Antibacterial activity	[23]
7.	Synthesis compound	NA	AA derivate	C_8-C_{15} aliphatic	Antibacterial activity	[32]
8.	*Ginkgo biloba*	Ginkgo fruit	AA mixture	C13:0, C15:1, and C17:1	Enhance propionate production in rumen fermentation	[33]
9.	*A. occidentale* L.	Cashew nut shell	AA	A combination of hydroxypropyl-ß-cyclodextrin (HP-ß-CD) with AA (C15:3)	Antimicrobial and antibiofilm activity	[34]

Saturated AA, in the form of transparent, rod-shaped crystals, can be recrystallized into a white solid obtained from hydrogenated, unsaturated side chains. To ensure stability while maintaining biological activity, AA has to crystallise into a monoclinic system with powerful interactions between intramolecular hydrogen bonds. The monoclinic configuration promotes robust intramolecular hydrogen bonding interactions. Strong hydrogen bonds in the monoclinic system can alter AA stability at high temperatures and dissolution in water. More specifically, strong hydrogen

bonds make AA more biologically active, affecting the interaction with biological targets such as enzymes or receptors. The monoclinic structure also influences methods for isolating and purifying AA. The conditions needed for crystallization and purification may impact the yield and purity of the isolated compound due to the strong hydrogen bonding [15]. The carboxyl group in AA potentially forms internal hydrogen bonds after ionization, and may also act as an acceptor of hydrogen bonds. In addition, internal hydrogen bonding influences the molecule conformation and interactions with other molecules [35].

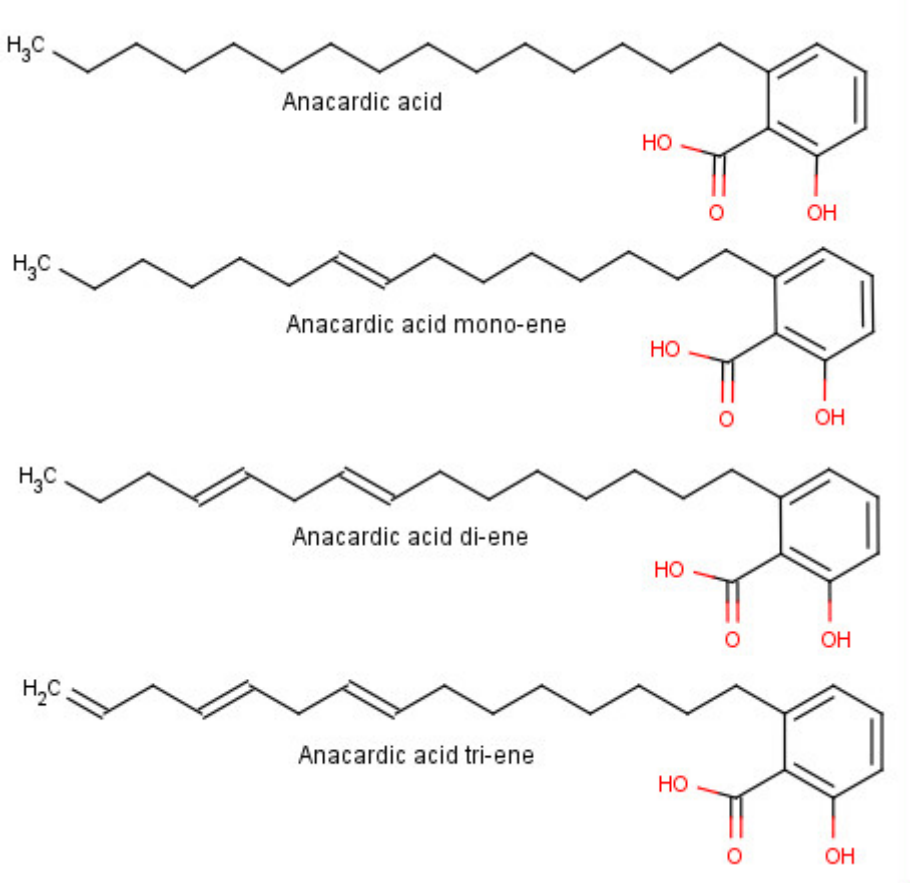

Fig.2. Components of natural AA mixture in cashew nut shell liquid.

The ability to penetrate bacteria membranes and disrupt the integrity is influenced by the alkyl side chain's unsaturation. Compounds with longer chains and more double bonds have higher antibacterial activity. This can be explained by the idea that adding unsaturation or branching into the hydrophobic group improves the solubility of surfactants in water. Bactericidal efficacy of AA $C_{15:3}$ against *S. aureus* is associated with the physical disruption of the membrane [12], [36].

Saturated AA destabilize microbial membranes by forming micelles that disrupt lipid bilayer integrity. AA has surfactant characteristics that harm bacteria cell membranes due to the hydrophobic alkyl side chains, allowing the molecules to combine with the lipid layer. The principal reaction to the antibacterial efficacy of AA is the biophysical alteration of the membrane (the biophysical disruption of bacterial cell membranes by AA involves the insertion of its hydrophobic alkyl chain into the lipid bilayer, leading to increased permeability and cell death) due to surfactant characteristics

[37]. The lipophilicity of the side chain, which makes it easier for the side chain to integrate into the lipid bilayer of bacteria membranes, contributes to the disruption. The amphiphilic configuration of AA also infiltrates the lipid bilayer and interferes with the electron transport chain and/or ATPase as a surfactant. AA inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in bacteria through a non-competitive mechanism. Given that the glycolysis pathway is essential for the survival of bacteria, the blockage might significantly impact viability. The structural properties of AA, which enables binding to GAPDH, underscore another aspect of antibacterial activity [38].

Cardol (a phenolic lipid distinct from AA) showed efficacy against the *MRSA* ATCC 33591 strain with a minimum bactericidal concentration (MBC) of 12.5 µg/mL (39.8 µM). A structurally related compound, 6[8'(Z), 11'(Z), 14-pentadecatrienyl] salicylic acid, [AA ($C_{15:3}$)], exhibited similar antibacterial activity, extracted from the identical source (cashew apple from *A. occidentale* L.) [39]. Cardanol, derived from the pyrolysis of the equivalent AA, showed slight antibacterial activity against *S. aureus*, even at concentrations reaching 100 µg/mL. CNSL, mostly composed of AA, cardanol, cardol, and 2-methyl cardol, safeguards the growing yeast *Saccharomyces cerevisiae* against damage from oxidization caused by hydrogen peroxide. AA may interfere with integral proteins inherent membrane-associated activity due to the status as a nonionic surfactant. The structural characteristics of cardols and AA show that enhancing antibacterial activity could be feasible by synthetic methods. Despite structural variations, cardol and AA molecules have comparable antibacterial properties, providing reassurance about the potential of these compounds [15]. The basic framework of AA may be modified to increase the antibacterial efficacy by selecting the length of the side chains ensuring the log P partition coefficient is maximized. AA shares an identical hydrophobic tail moiety, while the hydrophilic head region may not necessitate a particular structural characteristic to activate the function. It also possesses an identical hydrophobic tail to cardol, and the hydrophilic head may not necessitate particular structural characteristics to activate the function [40].

Structural modifications of AA offer a promising avenue for developing molecules with enhanced pharmacological activity, potentially representing a breakthrough in combating antimicrobial resistance [32]. The antibacterial activity of AA analogs featuring diverse side chains has been assessed, showing that the most effective action against *MRSA* occurs in analogs with branched and alicyclic side chains. The mechanism is further enhanced by the molecular characteristics of the side chain, which promote the generation of reactive oxygen species and neutrophil extracellular traps, suppress the formation of superoxide radicals, and show potent antimicrobial efficacy against a variety of microorganisms. AA interaction with bacteria cell membranes includes hydrophobic binding and selective targeting of biochemical pathways, including the VEGF signaling cascade and DNA damage mechanisms [41].

The derivative such as salicylic acid 6-(4′,8′-dimethylnonyl), exhibited potent antibacterial action against *S. mutans*, with a minimum inhibitory concentration (MIC) of 0.78 µg/mL. 2-(O-carboxymethyl)-6-[(8Z,11Z)-pentadeca-8,11,14-triene] benzoate and the disodium carboxylate salt showed extensive and impressive antibacterial efficacy against a wide range of bacteria, including Gram-positive (*S. aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) [42], [43]. The inhibition levels were comparable to those of the antibiotics erythromycin and amikacin. Merulinic acid C was found to be the most potent derivative of AA against antibiotic-resistant enterococci. It only attacks bacteria membrane, leaving peptidoglycan alone and causing cellular ATP to leak out quickly. Merulinic acid C also showed synergy with gentamicin, an aminoglycoside antibiotic, against *Enterococcus faecium*, suggesting that this compound may facilitate the creation of novel antibiotic combinations effective against drug-resistant organisms [44].

The formation of biofilms by microbes as a defense against antimicrobial compound attacks is substantially influenced by the phenolic structure, side chains, and modifications of antimicrobial compound. These side chains improve the anti-biofouling qualities of the final polyAA coating by facilitating cross-linking during polymerization. To create a stable polymer that successfully inhibits the formation of biofilms, cross-linking through these unsaturated alkyl chains is essential. The cross-linking improves the durability and efficacy of the resultant polyAA coating in mitigating biofilm growth on surfaces [45]. The existence of double bonds in the side chains augments antibacterial action, thereby influencing biofilm development. Optimal antibacterial efficacy is more significant at alkyl chain lengths of C_{10} and C_{12}, which also show enhanced synergistic effects in combination with methicillin against *MRSA*. The characteristics of the side chains enhance the prolonged release and efficacy of AA in inhibiting biofilm formation. Diverse side chain architectures, including branched or acyclic forms, significantly influence performance against biofilm-forming bacteria, with certain analogs showing enhanced activity relative to natural AA. The hydrophobic characteristics of AA enable the infiltration of the lipid bilayer, resulting in membrane instability and enhanced permeability. This disturbance may lead to cell lysis and mortality, thereby diminishing the quantity of viable bacteria capable of forming biofilms [46]. Ginkgo extract and the constituent AA caused significant sensitivity in *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, and *Clostridium aminophilum*. AA with a $C_{17:1}$ alkyl side chain was slightly less effective at inhibiting bacteria growth than the other two ($C_{13:0}$ and $C_{15:1}$) [33].

AA, due to the poor physicochemical stability and low solubility in water, the bioactivity and clinical relevance are limited. To render the drug appropriate for systemic administration, it is essential to augment the aqueous solubility, thereby improving bioavailability and pharmacological efficacy through the formation of an inclusion complex of AA (15:3) and hydroxypropyl-β-

cyclodextrin (HP-β-CD). An increase in the concentration of HP-β-CD was reported to cause the solubility of AA to rise [47]. This could be explained by the increasing amount of AA in the HP-β-CD cavity. The development of inclusion complexes is mostly driven by non-covalent interactions, with dipole-dipole interactions being particularly important in releasing high-energy water molecules from the HP-β-CD cavity into the solvent medium. As the concentration of HP-β-CD rises, more AA molecules enter the HP-β-CD cavity, leading to increased solubility [34].

The structure of AA has been modified to produce 2-(O-carboxymethyl)-6-[(8Z,11Z)-pentadeca-8,11,14-trienyl]-benzoic acid (AAd1) and the salt of disodium carboxylate (AAd2). The separation process used facilitated the straightforward extraction of AA. AA is a significant natural raw material due to the dual functionalities and the existence of an unsaturated alkyl chain, enabling the production of diverse "green" surfactants with varying surface and interfacial activity. The suggested synthesis method for producing AAd1 and AAd2 is efficient in terms of time and cost, yielding high outputs, and represents an environmentally friendly alternative for synthesizing surfactants from agricultural waste materials [37]. This process shows the diverse interfacial characteristics of AA derivatives as surfactant entities. The double-headed group surfactants work better than the single-headed. Anionic double-headed surfactants tend to show excellent performance and are characterized by low interfacial tension as well as high solubility across diverse salinities, showcasing significant resilience [48].

Phenolic lipids derived from AA were shown to have varied levels of bactericidal activity, depending on the length of the alkyl chains ranging from 14, 12, 10, 8, or 6 carbon atoms (Fig.3). Antibacterial effectiveness, cytotoxicity, mobility across epithelial monolayers, and physicochemical characteristics were assessed in comparison to standard antibiotics. However, no cytotoxic effects were observed in renal cell lines, hepatic, fibroblast, or colorectal. Treatment with C10 and C12 considerably prolonged the lives of *Galleria mellonella*-infected rats compared to a control group that did not receive treatment with MRSA or VRE (vancomycin-resistant Enterococci) [36].

AA compounds including C_{14}, C_{12}, C_{10}, C_8, and C_6 decrease water surface tension, with C_{14} yielding the lowest reported critical micelle concentration (CMC). The balance between lipophilicity and hydrophilicity plays a crucial role in permeability. The apparent permeability coefficient (Papp) is closely related to the length of the alkyl chain, impacting intestinal absorption. C_8 and C_6 had Papp values exceeding $(2-5) \times 10^{-6}$ cm/s, signifying complete human intestinal absorption. However, the evaluation of Papp values for lipophilic substances (C_{14}, C_{12}, and C_{10}) showed inadequate recovery rates. The suboptimal recovery rate is not attributable to nonspecific absorption since repeating the experiment in the absence of cells showed elevated recovery rates for all chemicals alongside a trend of diminishing recovery with an extended length of alkyl chain. All investigated compounds showed promising cytotoxicity results, and no IC_{50} values were recorded within the examined concentration

range. C_{14} and C_{12} at higher concentrations inhibited cell growth, showing the correlation between the cytotoxic properties of lipophilic compounds and the capacity to engage with the cell membrane [36], [49].

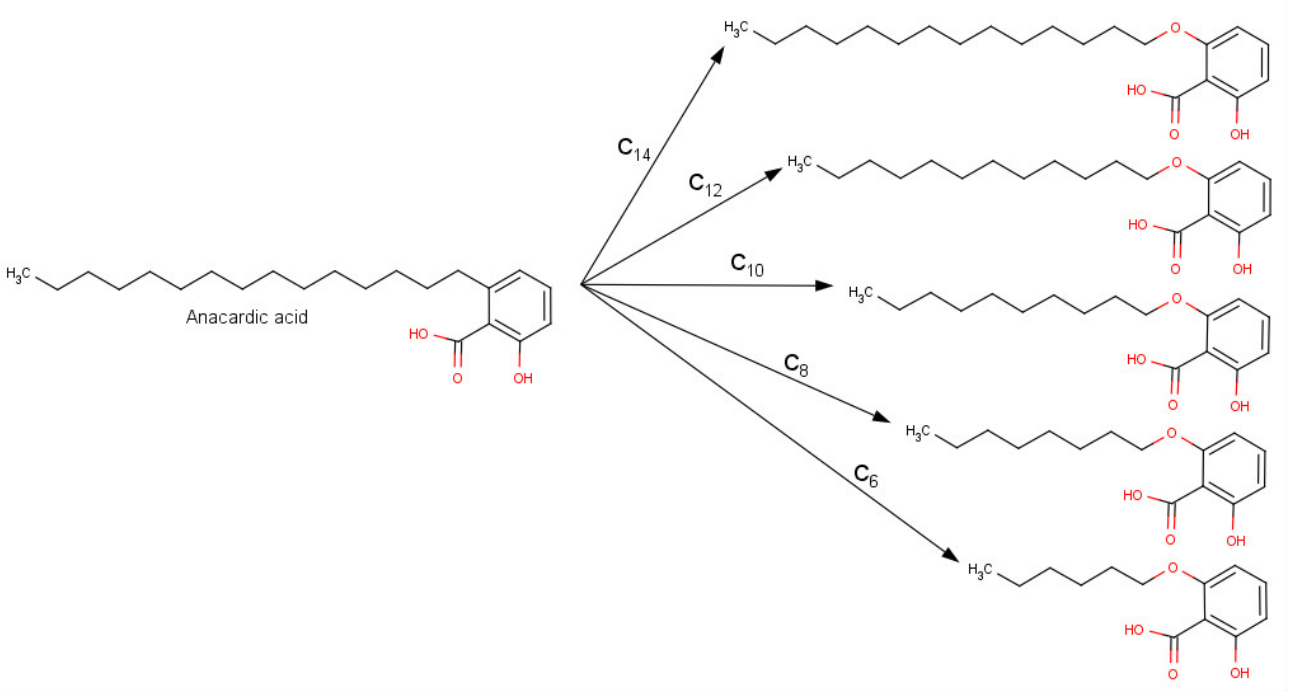

Fig.3. Alkyl chain length affects the bactericidal action of AA.

The antimicrobial activity of AA is significantly influenced by the saturation level of their side chains. Saturated side chains are more effective in disrupting bacterial membranes and enhancing antibiotic efficacy, while unsaturated side chains, particularly those with higher degrees of unsaturation, exhibit stronger antioxidant and enzyme inhibitory activities but lower antibacterial efficacy (except against *S. mutans*). Both saturated and unsaturated side chains of AA interact with microbial cell membranes to exert antimicrobial effects. Saturated side chains primarily disrupt the membrane through surfactant properties, while unsaturated side chains enhance membrane fluidity and permeability, leading to more effective antimicrobial action. The degree of unsaturation plays a crucial role in determining the extent of membrane disruption, overall antimicrobial efficacy and biofilm inhibition.

3.2. Impact of side chain modifications on AA action against biofilms

Changes influence the ability of AA to rupture bacterial membranes in the degree of unsaturation (C15:1, C15:2, C15:3) and chain length (Table 3). Increased membrane permeability, cell lysis, and reduction in biofilm mass can result from longer chains with more double bonds integrating into lipid bilayers. The effective surface cross-linking through unsaturated alkyl side chains in polyanacrylic acid coatings reduces biofilm formation more effectively than other methods. These break down surface-associated biofilms and allow for long-lasting antibiofouling coatings [50].

AA analogues featuring saturated alkyl side chains (e.g., C15:0) demonstrate an improved capacity to break bacterial membranes within biofilms directly. Their surfactant characteristics infiltrate and disrupt biofilm architecture, resulting in cell lysis. The disturbance is predominantly physical, leading to a loss of membrane integrity and inhibiting bacterial adherence to surfaces. These analogues are especially efficacious in scenarios requiring swift biofilm separation or the inhibition of early colonization [51].

Based on the impact of long alkyl chains (Section 3.1), the combination of structural properties makes AA a powerful tool for destroying the physical structure of biofilms and the integrity of bacterial membranes. However, to effectively target established biofilms, improved delivery methods, such as nanoparticle formulations, may be necessary [52].

Table 3. Mechanistic effect of AA side chain modifications

Mechanism	Structural features	Example of enhanced effect	Reference
Surface disruption	Long/C15:3 side chains	Increased permeability, cell lysis	[53]
Metabolic inhibition	Branching/cyclization	Enzyme inhibition, metabolic suppression	[54]
Signal interference	Amphipathic balance	QS blocking, reduced biofilm maturation	[55]

3.3.Metabolic inhibition

The interaction of AA analogues with bacterial enzymes (e.g., lipases, oxidases) or metabolic regulators can be altered by side chain modifications, which can affect their ability to inhibit processes crucial for the maintenance and development of biofilms. Certain analogues influence the metabolic activity of *S. aureus* and *S. mutans* biofilms, correlating with antibacterial efficacy and metabolic downregulation [56].

Higher unsaturated AA derivatives, such as C15:3, help suppress the metabolism of bacteria embedded in biofilms. Increased unsaturation makes membranes more fluid and permeable, which disrupts metabolic processes and hinders the biofilm's ability to transport nutrients and generate energy. The growth and viability of established biofilms, particularly those that are resistant to conventional antibiotics, can be compromised by this metabolic disruption [52].

AA derivatives disrupt the biofilm's extracellular polymeric substance (EPS) and adhesion processes, compromising the biofilm's structural integrity and hindering bacterial communication and nutrient transport. This disturbance modifies bacterial metabolism by compromising protective biofilm activities and nutrient exchange. Customised AA derivatives as coatings enhance delivery and contact with bacterial cells in biofilms, increasing antimicrobial effectiveness [57].

3.4. Signal interference (quorum sensing)

AA and certain analogues, which exhibit structural diversity through branching, acyclic, or aromatic groups, can disrupt quorum sensing (QS), therefore inhibiting the signals essential for biofilm maturation, virulence, and the development of resistance. Modifications in the side chain,

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particularly those that improve amphipathic equilibrium, can augment AA's capacity to inhibit the QS circuit, disrupt synchronised biofilm activity (e.g., interfere with the synthesis or reception of autoinducer signals, such as acyl homoserine lactones), and increase bacterial susceptibility to treatment [58].

The evidence for the inhibition of QS mediated by AA predominantly derives from experiments involving combinations of AA extracted from the plant *Amphipterygium adstringens*. AA have the potential to substantially inhibit QS-regulated virulence factors in bacteria such as *Chromobacterium violaceum* and *P. aeruginosa*, without compromising bacterial viability. The potential suggests that they have a targeted anti-QS effect rather than bactericidal activity, as evidenced by these studies. Mixtures of AA suppressed more than 90% of violacein pigment formation in *C. violaceum*, a traditional QS reporter system, at sub-inhibitory doses. This indicates disruption of QS signalling pathways associated with the production of virulence factors. AA suppressed QS-controlled virulence determinants, including pyocyanin (86% inhibition), rhamnolipid (91%), and elastase activity (75%), in a dose-dependent manner, all of which are key factors in biofilm formation and pathogenicity. The inhibition occurs without killing or slowing bacterial growth, indicating QS-specific modulation rather than general antibacterial activity [10], [59].

The ability of AA combinations to inhibit critical virulence factors in multi-species or resistant biofilms indicates their potential effectiveness in infiltrating biofilm matrices and disrupting QS signalling networks that regulate biofilm development and antimicrobial resistance. Focussing on conserved QS pathways across several species to diminish pathogenicity and biofilm durability, potentially minimising selective pressure for resistance development because of the non-lethal mechanism of action. Modifications of side chains in QS molecules (like acyl-homoserine lactones) strongly influence receptor binding affinity and inhibitory potency. For example, extending or substituting the acyl side chain can increase stability, specificity, and biofilm penetration. In similar way, side chain substitutions or functional group additions in AA could enhance binding to QS receptors, thereby optimising activity, especially against complex, multi-species, or resistant biofilms. Phenolic compounds related to AA have been reported to disrupt QS via enzyme inhibition and chemical interactions that interfere with autoinducer synthesis or signalling, suggesting that carefully engineered derivatives might amplify these effects [60].

4. In silico study

In silico studies showed that standard precision (SP) and extra precision (XP) docking of AA with 1Y54 showed antibacterial activity against *Enterococcus spp* more effectively than conventional antibiotics (Streptomycin and Tetracycline), as evidenced by docking scores and binding affinities

[61]. The three viral protein targets selected include P DB: 6Y2E (SARS-CoV-2), 1AT3 (Herpes), and 2VSM (Nipah). To assess the action of bioactive AA molecules, four bacteria protein targets were chosen including P DB: 2VF5 (*E. coli*), 2VEG (*S. pneumoniae*), 1JIJ (*S. aureus*), and 1KZN (*E. coli*). The composition, action, and capacity of these substances to interact with the identified protein targets for the remediation of several diseases have been considered in line with the capacity to impede the progression of microorganisms. Several of the chosen molecules underwent 100 ns long MD simulations to evaluate the effectiveness of active derivatives against bacteria and viruses compared to regularly used pharmaceuticals [62]. The phenolic groups and alkyl chains of AA derivatives are more likely to bind with microbe targets, potentially explaining the antibacterial efficacy. AA derivatives have shown promise as active therapeutic components targeting microbial proteins. However, further experimental studies are crucial for the clinical validation of the pharmacological properties of AA derivatives [63].

Several active drug-like compounds have been screened out from the group of 17 AA derivatives through in-silico docking studies. The extensive microbiological applicability of AA derivatives has also been shown through systematic docking with several bacteria including PDB: 2VEG, 1JIJ, 1KZN, and 2VF5 as well as viruses namely PDB: 6Y2E, 1AT3, and 2VSM. Although the compounds have shown significant efficacy against 2019-CoV, Nipah, and *S. aureus* compared to commercial pharmaceuticals such as cefalexin, methicillin, umifenovir, ribavirin, and hydroxychloroquine, the highest activity was found against gram-negative *E. coli* and the human herpes virus. Among the candidates, AA 75, 16, 25, and 33 were identified as the primary candidates for comprehensive computational, experimental, and clinical evaluation. Biomaterials with slight toxicity and favorable bioactivity possess significant potential for future medicinal uses [64].

5. Mechanisms of antimicrobial and antibiofilm action

Various mechanisms influence the AA ability to inhibit or eradicate bacteria and fungi (Table 4). AA can inhibit biofilm formation or damage mature biofilms of microbes. Bacteria cell membranes have been observed to be physically disrupted due to the primary surface agent action of AA [30]. AA can enter the lipid bilayers of bacteria membrane, where it disrupts the membrane, obstructs the activity of NADH oxidase, and inhibits the electron transport network [65].

The primary mechanism of AA antibacterial activity is by causing physical rupture of bacteria cell membrane. It also functions by penetrating the lipid bilayers of the cytoplasmic membrane and interfering with energy-converting systems such as ATPase and the electron transport system (ETS) [15]. Due to the ability to influence the extracytoplasmic area, AA also prevents bacteria resistance by bypassing cellular pump-based resistance mechanisms.

AA functions as a histone acetyltransferase inhibitor. This inhibition is significant in preventing biofilm formation by *C. tropicalis*. The interaction of AA with the histone acetyltransferase Rtt109 is particularly important, as it downregulates the expression of drug-resistance genes (CDR1 and MDR1), thereby reducing biofilm formation [66]. AA's side chain allows it to interact with various signal transduction pathways. This interaction can modify the pathways involved in biofilm formation, such as QS and two-component signalling systems. These pathways are crucial for the regulation of biofilm formation in response to environmental signals [67]. The presence of unsaturated alkyl side chains in AA plays a significant role in its anti-biofouling properties. Specifically, the cross-linking of poly-AA coatings through unsaturated alkyl side chains has been shown to reduce biofilm formation on surfaces, particularly against Gram-positive bacteria. This suggests that unsaturated side chains enhance the biofilm inhibitory effect of AA [68]. The antibacterial activity of AA with different alkyl chain lengths, including saturated chains, was found to be a parabolic function of their lipophilicity. The activity maximized with alkyl chain lengths of C10 and C12, indicating that the length and saturation of the side chains influence the effectiveness of AA in inhibiting biofilm formation, their surfactant properties can aid in biofilm destruction [46].

AA has demonstrated the capacity to impede the proliferation and adhesion of multiple bacteria associated with dental biofilms, including *S. mutans* and *L. casei*. This inhibition is dose-dependent and effective at relatively low concentrations [69]. AA can enhance the impact of other bioactive substances, such as quercetin. Evidence suggests that this synergy effectively inhibits biofilm-associated genes and pathways, preventing biofilm development [66].

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Table 4. Antimicrobial and antibiofilm activity of AA along with the mechanism and microbes tested.

No.	Compound/Material Type	Specific Compound/Growth Phase	Microorganism (Strain)	MIC (µg/mL)	MBC (µg/mL)	Inhibition Zone (mm)	Mechanism	Activity Type	Reference
1.	AA extract (mixture)		*Eubacterium ruminantium*	6.25	-	-	Surfactant/membrane disruption	Antibacterial	[30]
			Streptococcus bovis	25	-	-			
			Lactobacillus ruminis	50	-	-			
2.	AA extract (mixture)		*Bacillus subtilis*	3.13	-	-	Not specified	Antibacterial and antifungal	[16]
			Staphylococcus aureus	6.25	-	-			
			Brevibacterium ammoniagenes	3.13	-	-			
			Streptococcus mutans	1.56	-	-			
			Escherichia coli	>1600	-	-			
			Pseudomonas aeruginosa	>1600	-	-			
			Propionibacterium acnes	1.56	-	-			
			Helicobacter pylori	10	-	-			
			Saccharomyces cerevisiae	>1600	-	-			
			Candida utilis	>1600	-	-			
			Rhizopus stolonifer	>1600	-	-			
			Mucor mucedo	400	-	-			
			Penicillium chrysogenum	200	-	-			
			Aspergillus niger	>1600	-	-			
3.	Synthetic AA derivatives	5a, 5b, 5c, 5e, 5f, 5j, 5k, 5m, 5n, 5q	*Staphylococcus aureus*	-	-	19–22	Not specified	Antibacterial	[31]
		5a, 5f, 5k, 5o, 5p, 5r, 5s	*Streptococcus pyogenes*	-	-	19–22			
		5i, 5l	*Pseudomonas aeruginosa*	-	-	19–22			
		5d, 5e, 5m, 5o	*Escherichia coli*	-	-	19–22			
4.	Synthetic AA derivatives	6q	*Staphylococcus aureus*	-	-	18	Not specified	Antibacterial	[23]
		6a, 6d, 6g, 6i, 6l	*Escherichia coli*	-	-	15			
5.	Hexane extract (AA mixture)		*Pseudomonas aeruginosa*	200–500	-	-	QS inhibition	Antibiofilm	[29]
6.	Synthetic AA derivatives	8f	*Staphylococcus aureus*	-	-	19	Not specified	Antibacterial	[32]
		8g, 8h	*Streptococcus pyogenes*	-	-	19			
		8a, 8g, 8h, 8k, 8l	*Pseudomonas aeruginosa*	-	-	21–22			
		8k, 8l	*Escherichia coli*	-	-	21–22			
7.	Synthetic AA derivative		*MRSA*	64	-	-	Not specified	Antibacterial	[21]
8.	*S. jambos* leaf	Ethanol extract	*Propionibacterium acnes*	31.3	-	-	Membrane deformation	Antibacterial	[70]
		AA analog		7.9	-	-			

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9.	Synthetic AA derivatives	16 a, 16b	*Escherichia coli*	-	-	15	Not specified	Antibacterial	[22]
10.	AA extract (mixture)		*MRSA*	6.85	-	-	NET induction	Antibacterial	[71]
			Streptococcus pyogenes	1.71	-	-			
			Streptococcus agalactiae	1.71	-	-			
11.	AA extract (mixture)	Conidial germination	*Magnaporthe oryzae*	25.69	-	-	Apoptosis-like induction	Antifungal	[72]
		Inhibits mycelial growth		102.74	-	-			
		Induces apoptosis-like phenotype		1.71	-	-			
12.	Synthetic AA derivatives	9c, 9e, 9g, 9h , 9j	*Staphylococcus aureus*	-	-	9–12	Not specified	Antibacterial	[73]
		9d, 9e, 9h, 9i, 9j	*Candida albicans*	-	-	12–26	Not specified	Antifungal	
13.	AA (C13:0, C15:1, C17:1)		*Peptostreptococcus anaerobius*	1.56–6.25	-	-	Surfactant/membrane disruption	Selective antibacterial	[33]
			Clostridium sticklandii	6.25–12.5	-	-			
			Clostridium aminophilum	12.5	-	-			
14.	AA solution		*Staphylococcus aureus*	20–2500	-	-	Membrane disruption	Antibiofilm	[65]
15.	HP-β-cyclodextrin–AA inclusion complex		*Staphylococcus aureus*	0.78	-	-	Membrane disruption, QS inhibition	Antbacterial/Antibiofilm	[34]
16.	Chitosan-Alginate-AA nanoparticles		*Candida spp.*	625	312	-	Membrane disruption	Fungistatic/fungicidal	[27]
			Trychophyton rubrum	625	312	-			
17.	AA; AA-SLNPs; AA-SLNPs-CH; AA-SLNPs-CH-DNase		*Staphylococcus aureus*	0.78–1.56	-	-	eDNA degradation, membrane disruption	Antibacterial/Antibiofilm	[74]
18.	AA, Merulinic acid (AA derivative)		*Enterococcus faecium*	16	-	-	Membrane disruption	Antibacterial	[44]
19.	AAd2 (disodium salt)		*Staphylococcus aureus*	-	-	24	Surfactant	Antibacterial	[37]
20.	AA nanoparticles		*Streptococcus mutans*	0.36	0.36	-	Not specified	Antibiofilm	[14]
21.	C10 & C12 AA derivatives		*Staphylococcus aureus*	17–34	-	-	Surfactant	Antibacterial	[36]
22.	AA-loaded zein nanoparticles		*Pseudomonas aeruginosa*	3.12	-	-	Membrane disruption, enzyme inhibition	Antibacterial	[75]
			Staphylococcus aureus	0.05	0.2	-			
			Candida rugosa	1.17	2.34	-			
			Candida parapsilosis, Candida albicans	2.34	4.69	-			
			Candida glabrata, Candida jardinii, Candida tropicalis, Candida auris	4.69	4.69	-			

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23.	AA extract (mixture)	*Streptococcus salivarius K12*	0.5	-	-	Not specified	Antibacterial	[76]
		Streptococcus salivarius M18	0.5	-	-			
		Streptococcus pyogenes	0.25	-	-			
		Streptococcus pneumoniae	0.125	-	-			
		Moraxella catarrhalis	0.03	-	-			
		Haemophilus influenzae	512	-	-			
24.	AA-Co; AA-Cu complexes	*Penicillium citrinum*	-	-	30 ± 7.07 (Co); 15 ± 2.83 (Cu)	Not specified	Antifungal	[61]
		Aspergillus fumigatus, Aspergillus niger	-	-	15 ± 0 (Co); 11.5 ± 3.54 (Cu)			
25.	AA or ginkgolic acids (various)	*Staphylococcus aureus*	18.75–75	-	-	Not specified	Antibacterial	[11]
		Streptococcus pyogenes	2.34–18.75	-	-			
		Pseudomonas aeruginosa	150	-	-			
26.	AA extract (mixture)	*Streptococcus mutans*	6.25	6.25	-	Surfactant	Antibacterial/Antibiofilm	[77]
		Streptococcus mitis	6.25	6.25	-			
		Streptococcus sanguinis	3.12	6.25	-			
		Streptococcus sobrinus	3.12	3.12	-			
		Streptococcus salivarius	6.25	6.25	-			
		Lactobacillus casei	6.25	6.25	-			
27.	LDT11 (AA derivative)	*Streptococcus mutans*	>5	>5	-	Not specified	Antibacterial/Antibiofilm	[13]
		Veillonella parvula	5	5	-			

5a = Methyl-2-methoxy-6-(8-(pyrrolidin-1-yl)-octyl) benzoate
5b = Methyl-2-methoxy-6-(8-(piperidin-1-yl)-octyl) benzoate
5c = 2-Methoxy-6-(8-morpholinooctyl) benzoate
5d = Methyl-2-methoxy-6-(8-thiomorpholinooctyl) benzoate
5e = Methyl-2-(8-(cyclohexylamino)-octyl)-6-methoxybenzoate
5f = 1-(8-(3-methoxycarbonyl)-phenyl)-octyl)-piperidine-2-carboxylic acid
5i = Methyl-2-methoxy-6-(8-(piperazin-1-yl)-octyl) benzoate
5j = Methyl-2-methoxy-6-(8-(4-methylpiperazin-1-yl)-octyl) benzoate
5k = Methyl-2-(8-(4-(2-hydroxyethyl)-piperazin-1-yl)-octyl)-6-methoxybenzoate
5l = Tert-butyl 4-(8-(3-methoxy-2-(methoxycarbonyl)-phenyl)-octyl)-piperazine-1-carboxylate
5m = Methyl-2-(8-(4-benzylpiperazin-1-yl)-octyl)-6-methoxybenzoate
5n = Tert-butyl 4-(8-(3-methoxy-2-(methoxycarbonyl)-phenyl) octyl)-1,4-diazepane-1—carboxylate
5o = Methyl-2-(8-(1H-pyrazol-1-yl)-octyl)-6-methoxybenzoate
5p = Methyl-2-(8-(1H-imidazol-1-yl)-octyl)-6-methoxybenzoate
5q = Methyl-2-(8-(1H-1,2,4-triazol-1-yl)-octyl)-6-methoxybenzoate
5r = 2-(8-(2-chloro-6-methoxypyridin-3-ylamino)-octyl)-6-methoxybenzoate
5s = 2-(8-(3-methoxy-2-(methoxycarbonyl)-phenyl)-octyl)-1,1,3,3-tetramethylisoindoline-5-carboxylic acid
6a = 2-(2-Methoxy-6-pentadecyl-benzylamino)-benzoic acid
6d = 2-(2-Methoxy-6-pentadecyl-benzylamino)-6-methyl -benzoic acid
6g = 2-Bromo-5-fluoro-N-(2-Methoxy-6-pentadecylbenzyl)-aniline
6i = 3-(2-Methoxy-6-pentadecyl-benzylamino)-pyrazine -2-carboxylic acid

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6l = Benzyl-furan-2-ylmethyl-(2-Methoxy-6-pentadecyl-benzyl amine
6q = (2-Methoxy-6-pentadecyl-benzyl) pyrimidin-2-ylamine
8a = N-(2-methoxy-6-pentadecylbenzyl)-methanesulfonamide
8f = 2,5-dichloro-N-(2-methoxy-6-pentadecylbenzyl)-benzenesulfonamide
8g = N-(2-methoxy-6-pentadecylbenzyl)-1-phenyl-methanesulfonamide
8h = N-(2-methoxy-6-pentadecylbenzyl)-naphthal-ene-2-sulfonamide
8k = 1-(5-fluoropyridin-2-yl)-N-(2-methoxy-6-pentadecylbenzyl) -1H-pyrazole-4-sulfonamide
8l = 1-(4-fluorophenyl)-N-(2-methoxy-6-penta-decylbenzyl) -1H-pyrazole-4-sulfonamide

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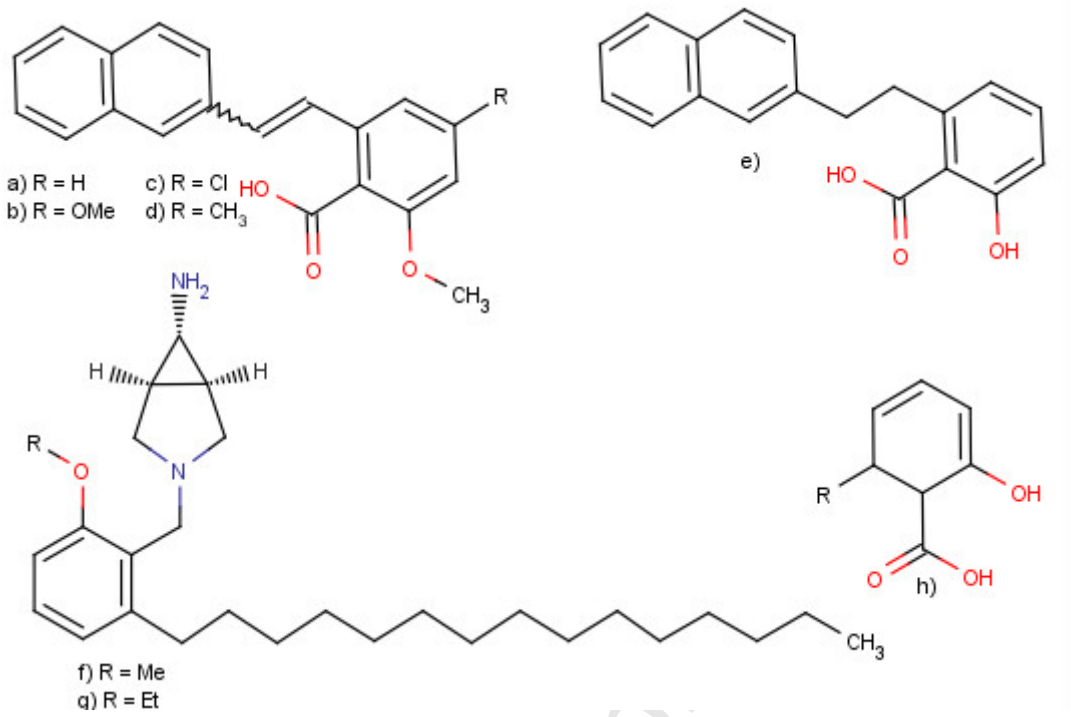

Fig.4. Numerous potential derivatives and analogs of AA.

AA compounds and their derivatives inhibit biofilm formation and destroy mature biofilms through a combination of antibacterial activity, surface modification, polyphenolic coatings, nanoparticle delivery systems, and direct disruption of the biofilm matrix. These multifaceted mechanisms make AA a potent agent against biofilm-associated infections. AA and its derivatives show strong antibacterial properties, which contribute to biofilm inhibition. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for AA range from 3.12 to 25 µg/mL against oral bacteria [77]. Antibacterial activity is crucial in preventing the initial formation of biofilms. AA, a versatile compound, can be incorporated into a variety of materials, including dental adhesives and catheter surfaces. Once modified, these surfaces demonstrate improved resistance to biofilm development and bacterial colonization. AA-impregnated silicon catheters have been found to significantly suppress *S. aureus* colonization and biofilm development. AA, during enzymatic polymerization, produces polyphenolic coatings that proficiently impede biofilm formation on surfaces. These coatings, capable of inhibiting both Gram-positive and Gram-negative bacteria, are crucial in suppressing biofilm formation [65].

AA-loaded nanoparticles have been developed to enhance the delivery and efficacy of AA. The nanoparticles demonstrate sustained release and higher antimicrobial efficacy, leading to significant reductions in biofilm thickness and biomass [18, 53]. The incorporation of DNase in anacardic acid-loaded SLNPs helps degrade extracellular DNA (e-DNA), a critical component of the biofilm matrix (e-DNA is crucial for initial adhesion and structural integrity). This degradation facilitates the breakdown of the biofilm structure, making bacteria more susceptible to antimicrobial

agents [74]. AA may suppress genes associated with exopolysaccharide production, reduce matrix cohesiveness, and enhance sensitivity to environmental exposures or antimicrobials. AA functions as a QS inhibitor (QSI), particularly in Gram-negative bacteria. Disrupting QS has an indirect effect of reducing EPS synthesis because QS controls the development and maintenance of biofilms [78].

The following describes some analogs, anacardic acid derivatives and their modifications as antimicrobials and antibiofilms. Methyl ether compound 5c, containing a carbon-carbon double bond and a free carboxylic acid (Fig.4), showed an antibacterial activity of 64 µg/mL against *MRSA*. Other derivatives with a carbon-carbon double bond binder, a free carboxylic acid, and a phenolic group, had 16–64 µg/mL activity against Gram-positive bacteria. In contrast, compounds with a carbon-carbon single bond linker, free carboxylic acid, and phenolic group showed 16-128 µg/mL activity against Gram-positive bacteria [21]. AA C_{10} and C_{12} derivatives showed strong antibacterial activity and good safety profiles. Inhibition of biofilm formation in *S. aureus* was noted at subtherapeutic doses of C_{10}. The pKa values suggest that the compound will become ionized in the blood (pH 7.4) or the intestine (pH 6.8). The ionization reaction causes the compound to become amphiphilic, making it easier to interact with biological barriers [79].

Compounds C_{10} and C_{12} have excellent antibacterial activity comparable to the natural counterparts, AA (C_{15}) and ginkgolic acid (C_{13}). The inclusion of ether functionality did not influence activity against Staphylococci strains, but activity against Enterococci and Streptococcus strains was relatively reduced. In contrast to antibiotics which only work against certain strains and resistance traits, C_{10} and specifically C_{12} showed extensive antibacterial efficacy comparable to octenidine. Both compounds have been subjected to additional evaluation in an infection model using *G. mellonella*, a system in which pathogen pathogenicity is assessed relative to mice. The results are in line with the previously determined MIC values for amoxicillin, ciprofloxacin, vancomycin, and linezolid against *E. faecium* UL602570. A key observation regarding octenidine was that, despite having superior antibacterial effectiveness compared to C_{10} and C_{12} in vitro, this superiority did not translate into improved survival in vivo. Octenidine, C_{10}, and C_{12} all have similar amphipathic properties, which may reduce the availability of the compounds throughout the body due to high cellular uptake and reduced presence at the site of infection [36].

AAd_1 and AAd_2 were tested against Gram-negative (*K. pneumoniae*, *E. coli*, *P. aeruginosa*) and Gram-positive (*S. aureus*, *E. faecalis*) bacteria strains. AAd_2 showed superior action against both bacteria species. At the used concentration (10 ppm), AAd_2 showed a broad microbiological spectrum, with an inhibitory zone of 24 mm, which is relatively close to the 32 mm of the reference antibiotic. *S. aureus* was the most susceptible bacteria to the effects of these compounds. The harmful effects of these compounds were greatly increased when AA was modified to protect the phenolic and carboxyl groups. The length of the alkyl chain significantly influenced the effectiveness of

various surfactant types against bacteria, reaching between 10 and 14 carbon atoms. The amino acids used to make these derivatives include 15 carbon atoms in alkyl chains that have different amounts of unsaturation [37].

Analysis of the methanol extract from *S. malaccense* leaves showed that AA had the highest efficacy against *S. pyogenes* [11]. Bacteriological assays showed a reduction in activity between $C_{15:0}$ and $C_{17:0}$ compounds, while an increase was observed between $C_{15:0}$ and $C_{15:1}$, as well as among $C_{17:0}$, $C_{17:1}$, $C_{17:2}$, and $C_{17:3}$. The level of unsaturation was linked to increased antibacterial activity, with the most unsaturation compound showing the highest efficacy against Gram-positive bacteria. The efficacy of compound $C_{17:1}$ was also analogous to that of $C_{19:1}$ (CMI = 18.75 g/mL). A specific chain length and degree of unsaturation are optimal for enhancing the ability to inhibit *S. pyogenes* bacteria. Comparable observations were also observed for other Gram-positive bacteria (*S. aureus*) [80].

AA-IC and single AA were efficient against S. aureus. The antibacterial activity of AA was preserved during the complexation process with HP-β-CD, as evidenced by the identical MIC value of 0.78 μg/ml for both free AA and AA-IC against planktonic *S. aureus*. Only the free AA released from the inclusion complex is accessible for activity [34].

At maximum concentration, both free AA and AA-IC caused over 80% reduction in biofilm formation. At concentrations below the MIC, antibiofilm activity of both showed no significant difference. However, at concentrations exceeding the MIC, AA-IC showed exceptional biofilm dispersal potential, indicating a significant difference between the activities of free and complexed AA. This difference can be attributed to the fact that at concentrations below the MIC value, AA has superior aqueous solubility, showing biofilm dispersal capability comparable to the complexed version [75]. Furthermore, the management of biofilms necessitates a substantial dosage of the drug. The efficacy at elevated concentrations is limited, as the insoluble portion cannot penetrate biofilms [34].

AA complexes were synthesized with hydrated metal (II) ions of cobalt and copper in a 1:1 ratio, using a modified method. The antifungal efficacy of these complexes was evaluated against five fungi species. Although AA had minimal antifungal action, the complexes showed significant outcomes [14]. The complex of cobalt (AA-Co) showed antifungal activity against three of the five tested fungi, with the most significant effect observed against *P. citrinum* (30±7.07 mm), surpassing the efficacy of the reference standard medication clotrimazole (12.5±0.71 mm). The AA compound of copper (AA-Cu) had antifungal activity against all investigated fungi, except for *Aspergillus flavus*, showing the highest efficacy against *P. citrinum* (15±2.83 mm) [61].

Enterococci (*E. faecalis* and *E. faecium*), two of the most prevalent gram-positive nosocomial infections globally, are innately resistant to several antibiotics. Both are a prime target for AA as bactericidal agent. Merulinic acid (AA derivative) and gentamicin work synergistically to treat

nosocomial infections. The presence of cellular abnormalities in Gram-positive cells treated with merulinic acid, as shown by fluorescence microscopy, suggests that acid affects bacteria membrane. Given the hydrophobic nature of the C-chain side of acid and the extremely rapid effects on PI permeability and ATP leakage, merulinic acid mechanism of action is most likely direct interaction with the membrane bilayer and structural disruption. Merulinic acid alters liposomes permeability and the hemolytic effects, suggesting potential toxicity toward eukaryotic cells [44].

Ginkgolic acids may inhibit *P. aeruginosa* pathogenicity by eliminating pyocyanin pigment synthesis. The optimal antibacterial activity of these salicylic derivatives against Gram-positive bacteria depends on the length of the aliphatic chain and the quantity or position of unsaturations. Therefore, the aliphatic chain helps to explain how the plant extract kills microbes [11].

The inhibitory action of AA occurs through uncompetitive and non-competitive pathways. Enzymatic, structural, and computational chemistry approaches work together to reveal the inhibitory mechanism. This mechanism presents the opportunity to create more potent and efficient GAPDH-targeted inhibitors for antimicrobial treatment [81]. AA suppresses GAPDH in a non-competitive manner with respect to both G3P and NADH substrates. AA is also a strong inhibitor of GAPDH, exhibiting essentially identical low micromolar Ki values for both substrates. The non-competitive inhibitory method enables AA to bind to the enzyme-substrate complex as well as the unbound enzyme, likely at a compartment on the surface less influenced by catalytic or substrate binding activity [82]. The blocking mode and Ki values of AA were the same for SpGAPDH and AvGAPDH, implying a shared-binding pocket. However, CpGAPDH remained uninhibited by AA at the studied concentrations despite the sequence and structural resemblance to SpGAPDH and AvGAPDH. AA presumably interacts with a preexisting pocket on the enzyme surface that is available in both the unbound and the Michaelis complex, showing non-competitive inhibition with a single K [83]. AA effectively inhibits microbial growth at sub-millimolar doses. Antibacterial susceptibility testing reported that AA showed superior activity compared to curcumin. All four *S. pyogenes* strains demonstrated susceptibility to low amounts of AA (0.5–2 mg/ml, 1.4–5.7 mM), with a MIC of 1.4 mM (0.5 mg/ml). AA also had MIC values of 5 mM against *S. pyogenes* and *S. agalactiae*, effectively inhibiting growth at concentrations near the established Ki. Low micromolar quantities of AA show that GAPDH may be the pertinent physiological target for antimicrobial effects [38]. AA inhibits glycerol-3-phosphate dehydrogenase, which stops bacteria from synthesizing lipids, and functions as a surfactant, physically disrupting the membrane. It also upsets ATPase and the electron transport chain, thereby inhibiting the respiratory chain [84].

Alginate and chitosan are among the most prevalent polysaccharides used in nanoparticle manufacturing [85]. Concerning the in vitro antifungal efficacy of AA in both the free and encapsulated states, the compounds showed no significant activity against strains of *Candida spp*. (*C.*

albicans LABMIC 0105, *C. albicans* LABMIC 0407, *C. tropicalis* LABMIC 0110, and *C. tropicalis* LABMIC 0111), except for AA, which showed effectiveness in inhibiting the microbial growth of *C. albicans* LABMIC 0105 [68]. For all four strains of dermatophyte including *T. rubrum* LABMIC 5906, *T. rubrum* LABMIC 6205, *T. rubrum* LABMIC 6212, and *T. rubrum* LABMIC 6753, the results showed MIC values of 625 µg/mL and MFC values of 312 µg/mL. AA may have suppressed the growth of the microorganisms in a dose-dependent manner by acting on the fungal cell membrane, as showed by the fungistatic (MIC) and fungicidal (MFC) effects against dermatophyte strains. *T. rubrum* is a fungi that causes dermatophytosis in human and animal skin infections, indicating a strong affinity for keratinized tissue. Encapsulating AA reduced the MIC and MFC values, signifying enhanced antifungal activity, as lower concentrations were required to achieve equivalent effects compared to non-encapsulated samples [27].

Since AA is released from solid lipid nanoparticles (SLNPs) in a controlled manner, *S. aureus* is only exposed to a small amount, causing the MIC value of AA-SLNPs to be higher than that of free AA. AA-SLNPs-CH also showed infiltration into the biofilm, indicating the capacity to adhere to both the biofilm and bacteria cells within the extracellular matrix [86]. Chitosan also facilitates SLNPs entry into the biofilm matrix and speeds up the diffusion of analytes. AA and AA-encapsulated SLNPs both impede biofilm development. However, the efficacy of AA-encapsulated SLNPs is comparatively diminished relative to free AA. The encapsulation of AA in SLNPs resulting in reduced free drug concentration and diminished antibacterial efficacy [75].

The absence of substantial inhibition or increased effect was observed in the DNase used alone or incorporated into the SLNPs. This activity may be because DNase acts on developed biofilms, while the drug directly affects planktonic bacteria. In the instance of mature biofilm, DNase alone failed to show substantial antibiofilm efficacy, potentially due to the scattered bacteria cells reconstituting the biofilm before eradication. SLNPs with DNase (AA-SLNPs-CH-DNase) as well as AA and DNase both had higher biofilm release activity, showing that DNase works better in combination. This process may be ascribed to the degradation of e-DNA within the biofilm matrix by DNase, resulting in increased mobilization of SLNPs. Antibiofilm effect of AA-SLNPs-CH-DNase is the strongest because the positively charged SLNPs containing AA and DNase can enhance the ability to penetrate and kill bacteria cells [74].

AA-loaded nanoparticles have stronger bacteriostatic than bactericidal action in the planktonic form [14]. However, when AA was loaded into zein-based nanoparticles, the antifungal activity was increased with MIC values against *C. albicans*, *C. rugosa*, and *C. parapsilosis* reducing by 50% compared to the AA solution [61]. The efficacy of AA in inhibiting *S. aureus* (25 µg/ml) and *MRSA* (6.25 µg/ml) by blocking the molecular structures of $C_{15:0}$ and $C_{15:3}$. However, the effectiveness is limited against Gram-negative bacteria, as shown by the weak ability to kill *P.*

aeruginosa. When loaded into zein nanoparticles, an inhibitory effect was recorded at 3.12 μg/ml suggesting enhanced pharmacological activity due to the incorporation into the biopolymer nanocarrier. The formation of the zein-AA complex facilitated exposure of the pharmacophore groups, thereby increasing AA antibacterial ability, as the unsaturation in the aliphatic groups plays a role in the bioactivity. The stronger aromatic bond between AA and the zein nanostructure, made the pharmacophore groups in the aliphatic chain more exposed, thereby increasing the antibacterial effect. Additionally, both zein nanoparticles and CHX gluconate at a concentration of 0.12% completely inhibited the cariogenic biofilm of *S. mutans* [75].

AA in *A. occidentale* extract is recognized for the ability to inhibit the proliferation of oral bacteria biofilms. AA inhibits bacteria's first attachment to the dental surface, a crucial phase in biofilm development. It also decreases bacterial cell wall synthesis and QS, obstructing the formation of a stable biofilm matrix [87]. AA also inhibits the production of EPS, which are essential for the structural integrity and stability of biofilms [88]. The composition of EPS in biofilms of *S. aureus* and *S. mutans* differed, resulting in a varied effect of AA nanoparticles on these two bacteria. EPS is crucial for the entrapment of microbial cells and offers three-dimensional protection that restricts diffusion, safeguards against antibacterial and accommodates extremely acidogenic or aciduric microbial cells. Nano-encapsulated AA at minimal dosages inhibited the formation of *S. mutans* biofilms for 5 days following a single 2-minute application. However, it was ineffective in eradicating biofilms that had already developed during the period [14].

AA showed inhibitory and bactericidal effects at low concentrations against bacteria linked to tooth caries including *S. mutans, S. mitis, S. sanguinis, S. sobrinus, S. salivarius*, and *L. casei*. The antibacterial action is primarily attributed to the chemical composition which includes an amphipathic molecular structure consisting of the aromatic nucleus, various functional groups, and the acyclic side chain [89]. Consequently, the surfactant properties of phenolic lipids physically compromise bacteria cell membrane, resulting in the efflux of critical intracellular constituents and, ultimately, cell death. AA has superior efficacy in certain biological activities relative to other constituents of CNSL, attributable to the presence of carboxyl groups in the chemical structure [90], [91]. The combination of cardol, AA, and CNSL in adhesive formulations has shown promising results in preventing the growth of biofilms comprising two different species. Dental adhesives containing these ingredients had physicochemical characteristics equivalent to those of the control group. AA was shown to raise elastic modulus, while cardol was found to cause a reduction. CNSL did not have any effect on elastic modulus [77].

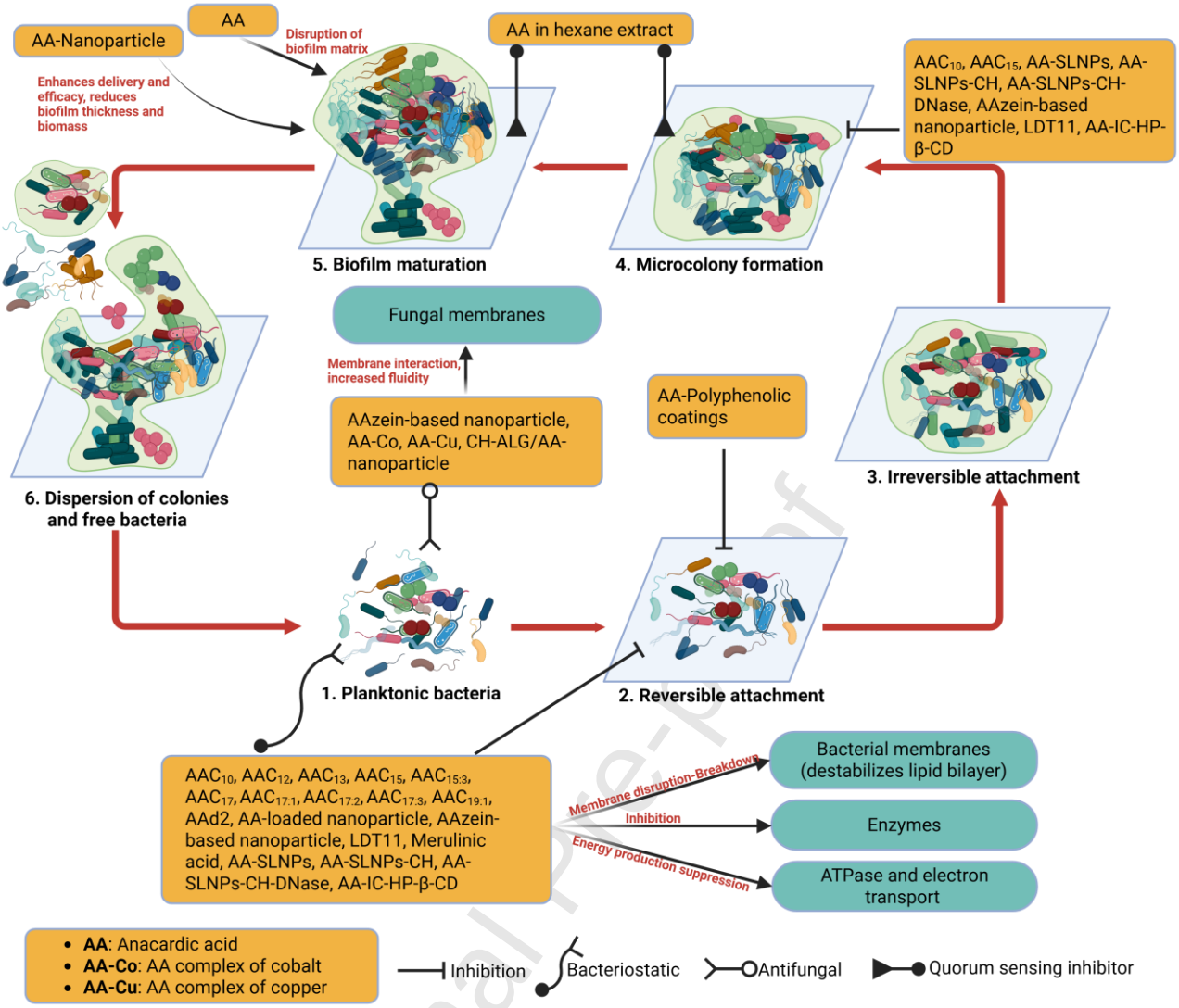

Fig.5. Depiction of AA's antibacterial and antibiofilm action. Microbes exist as free-floating (planktonic) cells prior to any surface adhesion. Microorganisms adhere loosely to a surface. However, this connection remains reversible. Microbes adhere tightly to the surface, dedicating themselves to biofilm development. Adherent microbes multiply and create tiny aggregates (microcolonies). Microcolonies develop and evolve into intricate, organized biofilms. Some microbes detach from the biofilm, going back to the planktonic state and moving into new surfaces [92]. Created with BioRender.com, June 18, 2025.

Dental adhesives can be treated with AA to prevent the development of CFU cariogenic biofilm. AA has been shown to inhibit the metabolic pathways of cariogenic bacteria, thereby reducing their acidogenicity. This reduction in acid production helps maintain a higher pH in the oral environment, preventing the demineralization of tooth enamel. By targeting the enzymes involved in acid production, such as enolase in the glycolytic pathway, anacardic acid effectively reduces the overall acid output of the biofilm [93]. The antibacterial activity of AA is shown by the ability to prevent living cells from multiplying and dividing into mature cells. This polyphenol can eliminate any lingering germs in dental cavities and stop further caries from developing around resin composite restoration edges when incorporated into adhesive formulations. AA dissolves easily in tooth hydrophobic monomers, which have a limited ability to form polymer structures. Since non-

aggregating substances are more uniformly distributed inside the resin matrix, low-concentration AA can have a potent antibacterial action while preserving the mechanical qualities of the adhesive [77].

Universal dental adhesive systems that use AA as a component of CNSL can inhibit biofilm formation without compromising the material dental qualities. AA was incorporated into zein nanoparticles to combat the *S. mutans* biofilm. However, the delivery into mature biofilms did not influence the viability of encapsulated bacteria (Fig.5). AA compounds influenced early biofilm development and reduced biomass. Furthermore, pre-formed biofilms showed a dose-dependent correlation in the assessment of bacteria viability [34]. The AA derivative (LDT11) showed substantial antibacterial effectiveness against mature biofilms at elevated doses.

Suppression of matrix metalloproteinases (MMPs) at an AA dose of 34.4 μg/mL reduced dentin erosion in vitro. These compounds show activity against bacteria collagenases, with LDT11 obtained from AA indicating stronger activity than LDT409 derived from cardanol. Novel strategies based on protease inhibitors to preserve the organic matrix may improve and optimize treatments for root and dentin caries. The over expression of genes encoding U32 proteases in certain bacteria species has been recognized. Bacteria collagenolytic activity in root caries biofilms is potentially related to collagen degradation functions during root caries development. The reduction in bacteria collagenase activity suggests a potential role for these compounds in managing dentin or root caries. [13].

5.1. Biological properties of AA relevant to natural antimicrobial action

AA demonstrates significant inhibition of *S. mutans* biofilm formation (Table 5), especially when delivered via zein nanoparticles. However, its effect on mature biofilms is much weaker than on forming biofilms. AA can promote biofilm porosity and void formation, but cranberry extracts and traditional agents like chlorhexidine often show greater effectiveness for pre-formed biofilm removal. Cranberry compounds have been shown to disrupt established biofilms more effectively than anacardic acid in some direct comparative studies, especially at higher concentrations and longer exposure times [94]. Essential oils (e.g. citral, eugenol, thymol) interfere with quorum sensing and disrupt extracellular polymeric substances, thereby preventing adhesion and facilitating biofilm detachment [57]. Phytochemical substances and peptides, including curcumin, resveratrol, and antimicrobial peptides, disrupt biofilm matrices and demonstrate broad-spectrum activity, frequently targeting both Gram-positive and Gram-negative biofilms [54].

Cellular uptake is influenced by AA amphiphilicity. Once formulated in nanocarriers (e.g., zein nanoparticles), cellular delivery into biofilms and tissues is enhanced. However, very little direct comparative uptake data with other natural antimicrobials is available, and most natural agents still face challenges with efficient tissue and cell penetration. The delivery of hydrophobic phytochemical

compounds (e.g., curcumin) often benefits from encapsulation in delivery systems, like liposomes or nanoparticles, to improve bioavailability and cellular uptake [95].

Table 5. Natural agents for biofilm disruption

Compound	Biofilm Inhibition (Forming)	Disruption (Mature)	Key Mechanism	Reference
Anacardic acid	Strong	Weak (vs. mature biofilm)	Enzyme inhibition, matrix interference	[94]
Cranberry extract	Strong	Strong	Matrix disruption, QS interference	[94]
Essential oils	Strong	Moderate to strong	Membrane and matrix disruption, blocking QS signals	[57]
Peptides/curcumin	Strong	Moderate	Membrane disruption and increased permeability, oxidative pathways	[54]

AA is known to inhibit certain inflammatory enzymes, like histone acetyltransferases, implying anti-inflammatory and immune-modulating effects. However, such activity appears less direct or broadly studied compared to leading botanical immunomodulators like curcumin or ginseng. In summary, AA's amphiphilic properties enable potent activity against forming biofilms and facilitate delivery via nanoparticles. However, it is less effective than other natural agents for disrupting established biofilms or robustly modulating immune responses. Its unique chemical structure allows for selective antimicrobial targeting and modest immunomodulation, complementing rather than replacing better-studied natural antimicrobials.

5.2. Mechanisms AA disrupts mature biofilms in chronic infections

Nanoformulations of AA, such as solid lipid nanoparticles (SLNs) coated with chitosan and DNase, can drastically decrease the biomass and thickness of established *S. aureus* biofilms. The extracellular DNA (eDNA) structures that stabilise mature biofilms are broken down by these formulations, which increases the acid's penetration and antibacterial activity. The direct application of surfaces impregnated with AA, such as catheters, effectively inhibits *S. aureus* biofilm development and demonstrates efficacy in in vivo studies. AA exhibit strong anti-quorum-sensing activity in *P. aeruginosa*, impeding the signalling processes needed for biofilm maintenance and virulence. While the anti-biofilm effect is more pronounced in the initial stages, nanoencapsulated forms improve the inhibition of established biofilms but with slightly less efficacy than against *S. aureus* [96].

Nanoencapsulated AA shows efficacy in reducing the viability of *C. albicans* within biofilm structures, demonstrating potential against mature fungal biofilms. The compound can induce morphological changes in *C. albicans*, such as cell shrinkage, which disrupts biofilm integrity. Notably, the antibiofilm effect is often more potent during biofilm formation than against fully mature biofilms, which are generally more resistant. Encapsulation strategies (e.g., nanoparticles) help overcome this resistance by improving drug delivery and retention within the biofilm matrix [97].

5.3. Anacardic acid and host immune responses, modulation of the oral microbiome, and control of gut biofilms

Antagonising bacteria and facilitating immune cell penetration into tissues are two ways AA might improve innate immune capabilities. One way it does this is by increasing the creation of neutrophil extracellular traps (NETs). This suggests a role for AA not only as an antimicrobial but also as an immune-modulating agent that can stimulate host defences against biofilm-associated infections. It has known inhibition of enzymes such as histone acetyltransferases, also implying anti-inflammatory action, which may balance immune activation to avoid tissue damage [98].

AA demonstrates significant antimicrobial activity, particularly against Gram-positive oral bacteria like *S. mutans*, a key cariogenic species. However, it is critical to note that eliminating one species does not suffice to prevent oral disease due to the functional redundancy and resilience of the oral microbial community. AA's impact includes modulating gene expression related to collagenases and metal-binding proteins in oral biofilms, potentially reducing tissue damage and virulence. This contributes to shifting the oral microbiome towards a less pathogenic state rather than completely eradicating bacteria, thus supporting homeostasis within the microbial ecosystem [56].

While direct data on AA effects on gut biofilms remain sparse, its bioavailability and immune-modulating activities suggest potential influence on gut microbial communities. The ability to induce NETs and modulate immune responses implies an indirect effect on controlling gut biofilms by supporting immune surveillance and limiting microbial overgrowth, which could be relevant in preventing dysbiosis-associated conditions [99].

5.4. Application or engineering advances

Silicon catheter tubes impregnated with AA have shown promising antibiofilm activity, specifically against *S. aureus*. These AA-impregnated catheters inhibit bacterial attachment and biofilm formation on their surfaces. Sustained release of anacardic acid from these catheters for up to 4 days, maintaining antimicrobial efficacy. The AA coatings exhibited no significant hemolytic activity and were cytocompatible with fibroblast cell lines, indicating safety for clinical use. In vivo antibiofilm activity was validated using an intraperitoneal catheter-associated medaka fish infection model, showing a significant reduction in bacterial colonisation. Challenges remain in improving impregnation efficiency and extending the duration of antimicrobial release. Chemical conjugation of AA to catheter surfaces is suggested as a strategy to provide long-lasting antibiofilm effects without relying solely on drug release. These findings collectively suggest potential clinical utility in preventing catheter-related infections, a major concern in healthcare settings [100].

AA has been successfully encapsulated into solid lipid nanoparticles (SLNPs) coated with chitosan and DNase. Chitosan provides a positive charge, enhancing adherence to negatively charged biofilm components, while DNase degrades extracellular DNA in the biofilm matrix. This multifunctional nanoparticle formulation exhibits sustained drug release and significant antibiofilm and antimicrobial activity against *S. aureus*, including reduction of biofilm thickness and biomass.

The formulation was stable, non-toxic to human keratinocytes, and represents a significant engineering advance for treating biofilm-mediated infections with improved penetration and biofilm disruption capabilities. Such nanoparticle systems could be adapted as coatings for wound dressings or implant surfaces to enhance localised delivery and antibiofilm efficacy [101].

AA-loaded zein nanoparticles have been explored for oral applications against *S. mutans*, a key pathogen in dental plaque and caries. These nanoparticle formulations showed strong inhibitory effects on biofilm formation and bactericidal activity against planktonic bacteria. However, they had limited activity against established mature biofilms, indicating potential for preventive but not curative use in oral biofilm management. This suggests AA-containing nanoformulations could be incorporated in dental coatings or rinses targeting early biofilm formation stages [56].

The demonstrated antimicrobial and antibiofilm efficacy combined with biocompatibility supports progression toward translational development. Challenges to commercial application include ensuring sustained and controlled release, long-term stability, mechanical durability of coatings, and comprehensive in vivo safety evaluation. Chemical conjugation techniques, nanoparticle engineering, and multifunctional coating architectures are active areas of development aimed at optimising clinical outcomes. The data open new avenues for plant-derived compounds like anacardic acid to serve as natural, effective antimicrobials and antibiofilm agents in medical device coatings, wound dressings, and dental materials [102].

5.5. Clinical relevance

AA enhances wound healing by encouraging fibroblast migration, particularly at concentrations that are non-toxic to normal fibroblasts. The combination action of antimicrobial/antibiofilm properties and pro-healing activity indicates its potential application for chronic, biofilm-infected wounds. The incidence of *S. aureus* biofilm infections associated with catheters or implants is significantly decreased by coating them with polymers containing AA. The antibiofilm activity is prolonged by sustained release from impregnated materials, which makes it pertinent to infections acquired in hospitals through indwelling devices [100].

Oxidative stress and biofilm formation by pathogens like *P. aeruginosa* are critical in chronic pulmonary infections, including cystic fibrosis. AA's antioxidant and anti-inflammatory activity helps attenuate lung tissue damage caused by infection-induced oxidative stress. Although specific in vivo pulmonary biofilm disruption by AA requires further clinical study, in vitro evidence and its biofilm inhibitory mechanisms suggest a promising role as an adjunct in inhalational therapies for CF patients [103]. While AA alone has limited ability to disrupt well-established biofilms compared to classic agents like chlorhexidine, nanoencapsulation and combination with enzymes (e.g., DNase) markedly improve its biofilm dispersal properties, especially for *S. aureus* and *C. albicans*. The compound's synergistic wound healing effects and anti-inflammatory benefits further support its emerging clinical

relevance in managing chronic, biofilm-related infections [104]. AA surfactants encapsulated in functionalized nanoparticles overcome biofilm-specific resistance by physically disrupting bacterial membranes (including persister cells), enzymatically degrading the EPS matrix, enhancing penetration through electrostatic interactions, and impairing quorum sensing-regulated defences. This multifactorial attack dismantles the protective biofilm barriers and eradicates bacterial communities responsible for chronic infections [105].

6. AA action on biofilm-forming microorganisms

Biofilms are pivotal in the persistence and severity of chronic infectious diseases. They create protective conditions for microorganisms, enabling them to deflect immune responses and resist antimicrobial therapies [106]. Biofilms consist of microbial cells encapsulated within an EPS matrix. This matrix serves as a physical barrier that restricts drug penetration and protects germs from immune cells, complicating infection eradication [107]. The presence of resistant subpopulations, changes in metabolic states (slow growth or dormancy), and less drug penetration all contribute to the increased antibiotic resistance shown by bacteria within biofilms. Bacterial resistance requires extended or combined antibiotic treatments and frequent surgical procedures [108].

Biofilms are complex colonies of microbes, the most common of which are bacteria but which can also include fungi. Common biofilm-forming bacteria implicated in human illnesses are Streptococcus species, *S. aureus*, *Staphylococcus epidermidis*, *E. coli*, and *P. aeruginosa* [109]. A single species of bacteria formed the biofilm community on a surface. These biofilms display unique physical and mechanical characteristics, including viscoelasticity and cellular arrangement, which differ substantially among species. Single-species biofilms are significant in particular diseases and industrial biofilm applications; however, they may exhibit reduced complexity in interspecies interactions [110].

Biofilms often represent various bacterial species that interact possibly synergistically or competitively (Fig.6), accordingly augmenting biofilm mass, metabolic activity, antimicrobial resistance, and structural complexity [111]. Natural environments, such as the mouth, intestines, chronic wounds, and medical devices, are rich in multispecies biofilms [112]. Several bacterial species (and occasionally other microbes like fungi) coexisting in a biofilm community are known as multispecies biofilms [56]. These biofilms exhibit augmented biomass, elevated cell counts, enhanced metabolic activity, and increased antimicrobial tolerance relative to single-species biofilms attributable to interspecies interactions [113]. Species interactions involve synergy, collaboration, competition, and antagonism, which affect biofilm form, function, and resilience. Multispecies biofilms exhibit intricate spatial structures with distinct roles for various bacteria, establishing microenvironments within the biofilm [114].

Microorganisms that create multispecies biofilms are *P. aeruginosa* and *E. faecalis*. These establish multispecies biofilms characterized by increased matrix production and pathogenicity. *Veillonella parvula* aggregates with *Streptococcus gordonii*, enhancing biofilm complexity. *Candida albicans* can engage in multispecies biofilms with bacteria, particularly in oral and medical device-related biofilms, enhancing resistance and pathogenicity [115].

6.1. Streptococcus species

Streptococcus bacteria, a genus of Gram-positive, spherical bacteria, are known for their ability to form biofilms. This ability varies significantly across species, strains and environmental conditions, influencing their pathogenicity in infections ranging from dental caries to life-threatening systemic diseases [116]. Streptococcus species form biofilms through a multi-stage process similar to other bacteria but with distinct mechanisms and matrix compositions. Biofilm development involves three universal stages: initiation (attachment), maturation (microcolony development), and dispersal (detachment of cells). However, streptococcal biofilms exhibit unique characteristics [117].

Biofilm formation in Streptococcus species is orchestrated by a diverse set of proteins, including surface adhesins, glucosyltransferases, glucan-binding proteins, regulatory systems, and matrix-modifying enzymes. These proteins represent potential targets for therapeutic intervention to prevent or disrupt biofilm-associated infections [118]. The matrix composition of Streptococcus is dominated by e-DNA and proteins, with limited polysaccharides. e-DNA provides structural integrity and facilitates genetic exchange [119]. Proteases (SpeB) can influence biofilm formation and dissemination by degrading matrix components. SpeB is strictly controlled in *Streptococcus pyogenes* to maintain the stability and spread of biofilms [120].

MSCRAMMs are exposed to the surface and facilitate adhesion to constituents of the host extracellular matrix, including collagen, fibronectin, and salivary glycoproteins. Attachment relies on surface proteins like MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) and pili, which bind to host extracellular matrix components (e.g., fibronectin, collagen). PI-1, PI-2a, and PI-2b are examples of pilus islands that encode pili, which are crucial for early adhesion and biofilm formation, particularly in *Streptococcus agalactiae* and similar species [118].

Some strains (e.g., *S. pyogenes* serotypes M6/M14) adhere directly to abiotic surfaces, while others (e.g., M2/M18) require protein-coated surfaces [56]. The M protein, expressed by the emm gene, is essential for biofilm development and immune evasion in *S. pyogenes* [121]. Streptococcus, particularly *S. pyogenes*, has significant pathogenic implications; biofilms facilitate necrotizing fasciitis by generating endoplasmic reticulum stress in host cells. *S. mutans* biofilms go through surfaces (e.g., tooth roots) by proliferation-induced forces rather than through active movement [118]. SspA/B proteins facilitate adhesion to host salivary glycoproteins, promoting the early colonisation as well as *S. mutans* biofilm production [122]. *S. pyogenes* and *S. agalactiae* utilize protein/e-DNA

matrices for tissue attachment, whereas oral streptococci such as *S. gordonii* and *S. mutans* employ polysaccharides for tooth plaque formation [123]. This diversity highlights the necessity for customized strategies in addressing streptococcal biofilm-related illnesses [124].

Glucosyltransferases (GtfB, GtfC, and GtfD) are enzymes that convert sucrose into extracellular polysaccharides, or glucans, which are necessary for bacterial adherence and the biofilm's structural matrix [125]. The synthesis of glucans occurs during the early (initiation) phase of biofilm formation, while Gtfs are responsible for maintaining the growth of biofilms. GbpA, GbpC, and GbpD are secreted glucan-binding proteins that assist in the preservation of biofilm structure by attaching bacterial cells to glucan polymers during the maturation phase of biofilm formation [126]. Glucan-Binding Proteins (GbpA, GbpB, GbpC, GbpD) associate with glucans and contribute to cell aggregation and biofilm integrity. GbpC is an important cell wall-anchored protein essential for biofilm structure. GbpB provides a significant role in cell wall metabolism and bacterial proliferation, hence indirectly facilitating biofilm formation [127]. The bacterial surface glucan receptor GbpC is linked to the cell wall and plays a crucial role in aggregation and the formation of biofilm biomass [128].

Transcriptional regulator proteins regulate the expression of genes essential for biofilm growth. The two-component systems that sense environmental inputs and alter gene expression include a sensor kinase and a response regulator. These systems detect environmental signals and modulate the expression of genes related to biofilms, including those responsible for adhesins and exopolysaccharide production (e.g., ComCDE, CovRS, CsrRS) [118]. Competence-stimulating peptide (CSP) is a QS peptide that modulates gene expression in relation to cell density, hence affecting biofilm formation and bacteriocin synthesis [127]. Biofilm Regulatory Protein A (BrpA) is involved in cell wall metabolism, chain length, acid tolerance, and biofilm formation, particularly in *S. mutans* and *S. agalactiae* [129]. Depending on the availability of nutrients, CodY regulates biofilm development and stress responses on a global scale. Proteins and regulatory systems involved in the biofilm formation process encompass the initial adhesion stage, early colony growth, and subsequent biofilm development and maturation [130]. DUF2207 family proteins (SMU.243) are additional notable proteins that are involved in the formation of biofilms and stress tolerance in *S. mutans* [131]. Mutanofactin is a secondary metabolite that enhances the hydrophobicity of the cell surface, thereby facilitating biofilm formation and adhesion [132].

6.2. Staphylococcus species

Staphylococcus bacteria, specifically *S. aureus* and *S. epidermidis*, form biofilms through a complex, multi-stage developmental process that enables them to adhere to surfaces, survive hostile environments, and resist antibiotics [133]. The biofilm formation process can be described in detailed stages with underlying molecular mechanisms. The biofilm matrix contains polysaccharides like

polysaccharide intercellular adhesin (PIA), proteins (e.g., biofilm-associated protein (Bap), fibronectin-binding proteins (FnBPs)), e-DNA, and teichoic acids [111].

Initial attachment involves the reversible and then irreversible adherence of planktonic (free-floating) staphylococcal cells to biotic (e.g., host tissue) or abiotic (e.g., medical devices, plastics) surfaces [134]. Attachment is mediated by hydrophobic and electrostatic interactions with key bacterial surface components such as wall teichoic acids (WTAs), FnBPs, surface proteins like SdrC and SasG, and Bap. Autolytic enzymes (AtlA in *S. aureus* and AtlE in *S. epidermidis*) contribute by modifying surface hydrophobicity and releasing extracellular DNA (e-DNA), which acts as an initial adhesive component in *S. epidermidis* but less so in *S. aureus*. e-DNA plays a significant role in *S. epidermidis* attachment, as DNase treatment inhibits biofilm formation, while in *S. aureus* early attachment is less affected by DNase, indicating species-specific differences [111].

After attachment, proliferating cells create an EPS matrix, primarily composed of proteins, e-DNA, and polysaccharides [135]. The EPS matrix acts as a scaffold, stabilizing the biofilm structure and facilitating microcolony formation. PIA, synthesized by the icaADBC operon, is a key exopolysaccharide in *S. aureus* and *S. epidermidis* biofilms, promoting cell-cell adhesion. Accumulation-associated proteins such as Aap in *S. epidermidis* and SasG in *S. aureus* also mediate intercellular adhesion during this phase [136]. Six to eleven hours after initial attachment, a subset of cells begins to leave the biofilm, marking a recently reported stage in *S. aureus* biofilm development. This process is believed to be governed by metabolic heterogeneity within the biofilm, perhaps supporting biofilm remodelling and adaptability to environmental changes [137].

The composition and density of the EPS matrix augment, hence enhancing the protection of implanted bacteria. Enzymatic breakdown of the EPS matrix releases single cells or clusters to colonize other places, a process called dispersal [138]. This process is triggered by environmental cues such as nutrient depletion or waste product accumulation. This phase is crucial for the dissemination of infection, particularly in clinical environments with indwelling medical devices [56].

The significance of eDNA during attachment, the function and shape of phenolsoluble modulins (PSMs), and the regulatory processes governing biofilm development are among the key differences between *S. aureus* and *S. epidermidis*, despite the fact that both bacteria use polysaccharides, eDNA, and proteins to produce biofilms [139]. Furthermore, methicillin resistance affects biofilm gene expression and processes in *S. aureus*, resulting in alternate biofilm formation pathways not observed in *S. epidermidis* [54].

Protein/DNA matrices and mecA-associated PIA repression are characteristics of MRSA biofilms that make them more resilient to treatment than other staphylococcal species and PIA-dependent methicillin-susceptible *S. aureus* (MSSA). Enzymatic matrix disruption is one example of

a biofilm-targeted therapy required for optimal management of this condition [111]. Antibiotic diffusion is more efficiently inhibited by the thick protein/DNA matrix of MRSA biofilms than by PIA-dependent matrices. The action of cationic antibiotics like vancomycin is rendered ineffective when bound to e-DNA. PIA-based biofilms (MSSA/*S. epidermidis*), on the other hand, have lower physical barriers, which means antibiotics can get through them more easily [140].

6.3. *Pseudomonas aeruginosa*

P. aeruginosa is a motile, rod-shaped, Gram-negative bacteria distinguished by a solitary polar flagellum. Nitrate serves as a terminal electron acceptor, allowing it to develop facultatively anaerobically in addition to aerobically [141]. *P. aeruginosa* forms a biofilm, which is a well-organized bacterial colony set within an extracellular polymeric substance (EPS) matrix. This matrix predominantly comprises three principal exopolysaccharides—Psl, Pel, and alginate—alongside extracellular DNA (eDNA), proteins, and lipids, collectively constituting approximately 90% of the biofilm biomass [142]. *P. aeruginosa* infections are infamously hard to treat because this matrix protects bacteria from environmental stressors, antibiotics, and immune clearance, in addition to providing structural integrity and adhesion [143].

Released through cell lysis, eDNA functions as a structural component that promotes biofilm cohesion and cell adhesion [105]. Additionally, in situations where nutrients are scarce, it provides nutrients. Proteins, such as adhesins CdrA and lectins (LecA, LecB), facilitate bacterial aggregation, surface adhesion, and matrix stabilization. Lipids contribute to the hydrophobic characteristics and structural complexity of the biofilm [144]. The EPS matrix serves as a defensive barrier against drugs and host immunological responses, facilitating *P. aeruginosa*'s persistence in chronic infections. The matrix promotes QS, or cell-to-cell communication, which synchronizes the formation of virulence factors and biofilms. The structural complexities of the biofilm enable *P. aeruginosa* to endure in various environments, including medical devices and host tissues [145]. *P. aeruginosa* biofilms induce chronic, challenging-to-treat illnesses, including cystic fibrosis lung infections, persistent wounds and burns, device-related infections, chronic sinusitis, and osteomyelitis. The biofilm lifestyle provides increased antibiotic resistance and immunological evasion, rendering these infections chronic and a significant clinical problem in healthcare environments [146].

6.4. *Escherichia coli*

The formation of *E. coli* biofilms is a regulated, multi-faceted process that encompasses motility, adhesion organelles (such as flagella, fimbriae, and curli), extracellular matrix synthesis, and surface features like lipopolysaccharides (LPS) and conjugative pili. These features facilitate the bacteria's attachment, colonization, and persistence on diverse surfaces [147]. Biofilm development differs among *E. coli* strains and environmental settings, with clinical isolates frequently exhibiting increased biofilm production associated with antibiotic resistance. Research demonstrates that

effective biofilm producers are more prevalent in controlled settings than in wild isolates, underscoring the ecological and methodological aspects that affect biofilm development [148].

Biofilm development initiates with reversible attachment, during which motile *E. coli* cells utilize flagella to navigate towards and temporarily adhere to surfaces. Flagella assist in overcoming repulsive forces and enabling initial contact, with their synthesis regulated by the flhDC operon. Reflecting a dynamic regulation of motility during early biofilm development, some cells express flagella constantly while others inhibit them at this point [149]. Fimbriae (Type 1 pili), encoded by the fimAICDFGH operon, possess a rod-like morphology with the FimH protein at the terminus, facilitating the binding of mannose residues to eukaryotic cells, extracellular polymeric substances (EPS), and abiotic surfaces, hence enhancing adhesion [150]. Curli fimbriae are amyloid fibres encoded by the csgBAC and csgDEFG operons. Curli facilitate cell aggregation and adherence to surfaces, becoming a significant component of the biofilm biomass [57].

Organelles like fimbriae and curli fibres aid bacteria in reinforcing their adherence to surfaces after initial attachment. The transcriptional regulator CsgD controls the synthesis of curli fibres and cellulose, and cyclic-di-GMP (c-di-GMP) signalling is crucial for regulating the synthesis of these extracellular matrix elements necessary for biofilm maturation [147]. Conjugative pili, like plasmid-encoded F-pili, support cell-to-cell connections and stabilize the biofilm architecture, which aids in the production of biofilms in addition to these structures. Biofilm resilience and genetic interchange are both enhanced by these pili, which also allow attachment to abiotic surfaces and horizontal gene transfer inside the biofilm [151].

Outer membrane proteins, including Antigen 43 (Ag43, encoded by the flu gene), AidA, and TibA, facilitate robust cell-to-cell adhesion and autoaggregation, hence enhancing microcolony development and irreversible attachment. Ag43 is crucial for cellular aggregation and the production of mixed biofilms. These proteins specifically interact with bacterial cell surfaces to facilitate aggregation and adherence to both living and non-living surfaces [152]. Lipopolysaccharides (LPS) present on the bacterial surface also affect biofilm development. Changes in LPS expression regulate biofilm formation by varying the exposure of adhesins and requiring an intact LPS core for adherence to surfaces [153].

The mature biofilm comprises bacterial cells encased in a self-synthesized extracellular matrix mostly consisting of curli fibres, cellulose, and poly-N-acetylglucosamine (PGA), which safeguards the community and enhances antibiotic resistance and persistence [147]. The primary exopolysaccharides in *E. coli* biofilms consist of poly-β-1,6-N-acetyl-D-glucosamine (PGA), cellulose, and colanic acid. PGA is synthesised by proteins encoded inside the pgaABCD operon. PGA acts as an adhesin, reinforcing the biofilm by facilitating cell-to-cell adhesion and surface

attachment [152]. The synthesis of these polysaccharides is governed by the second messenger cyclic-di-GMP (c-di-GMP), which stimulates EPS production and biofilm development [154].

Biofilms spread when environmental factors (such as temperature fluctuations or nutrient depletion) become unfavourable. The biofilm matrix deteriorates, and bacteria return to planktonic (free-swimming) states. Regulation of gene expression and enzymatic degradation of matrix components are the processes that are involved in dispersion; however, the specific proteins involved are less detailed in the sources provided [105].

6.5. *Candida sp.*

C. albicans biofilms significantly contribute to infections linked to permanent medical devices (e.g., catheters, prosthetics) and exhibit notable resistance to antifungal therapies attributed to their protective extracellular matrix and diverse cellular composition [155]. Pathogenic *C. albicans* biofilms are formed by a series of steps, including yeast adherence, filamentous growth, extracellular matrix development, and cell dispersion. These steps culminate in communities that are resistant to drugs and very resilient [156]. Intercellular communication through QS and environmental variables like pH are among the complicated molecular pathways that control biofilm formation and pathogenicity. The yeast-to-hyphal transition, for instance, is inhibited by an acidic pH, but filamentation is promoted by a neutral to alkaline pH, which influences the creation of biofilm [157].

The preliminary phase entails the adhesion of Candida yeast cells to a substrate, resulting in the formation of a foundational layer of cells. The adherence phase may require 60 to 90 minutes. The cell surface protein Hyphal Wall Protein 1 (Hwp1) is crucial for *C. albicans* to interact with and attach to host cells [158]. Agglutinin-like sequence 3 (Als3) is essential for the attachment of *C. albicans* to host cells and plays a role in biofilm development. Cells start to multiply and develop filaments (hyphae and pseudohyphae) after adhesion. This phase entails cellular organisation and may persist for 12 to 30 hours in *C. albicans* [159]. Extracellular polymeric substances (EPS) surround a multi-layered network of polymorphic cells (yeast, hyphal, and pseudohyphal cells) as they develop into an intricate structure. This matrix offers protection and organisation. Mature biofilms often develop within 24 hours, although they may require 38 to 72 hours to mature [160].

Candida sp. exhibit significant variations in biofilm formation, impacting their pathogenicity and treatment resistance. The most complicated biofilms are formed by *C. albicans*, which have a substantial extracellular matrix (ECM), whereas *C. parapsilosis* relies on blastospore clusters that have a small amount of matrix [161]. The only species that consistently forms real hyphae is *C. albicans*; other species produce pseudohyphae or structures that are yeast-only. *C. parapsilosis* demonstrates elevated metabolic activity despite fluctuating biomass in comparison to *C. albicans* and *C. tropicalis*. This interspecies variance underscores the importance of understanding and addressing the differences in biofilm formation, necessitating customized antifungal approaches

aimed at biofilm-specific weaknesses [162]. *C. albicans* exhibited a resistance pattern to fluconazole within 6 hours, manifesting as a biofilm. The density of the extracellular matrix and hyphae enhances resistance to fluconazole. Non-albicans species exhibited resistance, but at a diminished rate, attributable to a less intricate extracellular matrix [163].

6.6. Polimicrobes

Polymicrobial biofilms are organized communities in which various microbial species typically bacteria and occasionally fungi attach to surfaces and one another, encased inside a self-generated extracellular matrix [164]. Their creation entails dynamic interactions that augment microbial survival, pathogenicity, and resilience to environmental stressors and antimicrobials. Direct interactions between microbes, also known as coaggregation, allow the first species to operate as a scaffold, attracting secondary colonists. Secondary species can facilitate further microbial adhesion by binding to molecules on the biofilm surface or forming aggregates [165].

Microbes interact via chemical signals (QS) to coordinate biofilm growth, virulence factor production, and resistance mechanisms [166]. The infection or biofilm can spread to other areas when cells or aggregates separate and colonize them. *S. mitis* and *S. oralis* are the first colonizers in oral cavity biofilms [167]. Secondary colonizers include Actinomyces, Gemella, Neisseria, and Veillonella [168], whereas late colonizers, typically anaerobes, consist of Porphyromonas, Fusobacterium, Prevotella, and Capnocytophaga. *S. mutans* frequently establish biofilms in conjunction with *C. albicans*, which can augment the metabolic processes and biofilm development by *S. mutans* [169].

Symbiotic connections are prevalent in many polymicrobial biofilms, where species gain from one another's presence. This collaboration improves biofilm development, adherence, proliferation, and defence against antimicrobial agents. For instance, *C. albicans* and *S. aureus* generate biofilms exhibiting enhanced biomass and resilience relative to single-species biofilms [170].

Polymicrobial biofilms significantly contribute to antibiotic treatment failure and the persistence of chronic infections. This impact arises from several interrelated mechanisms that make these biofilms far more resistant to antibiotics (sometimes 10 to 100 times) compared to single-species biofilms or planktonic (free-floating) bacteria [171]. This resistance is due to both genetic and phenotypic mechanisms, including the presence of persister cells (dormant, metabolically inactive cells highly tolerant to antibiotics) and the physical barrier created by the biofilm matrix, which limits antibiotic penetration [172]. Within polymicrobial communities, bacteria can exchange antibiotic resistance genes through mechanisms like conjugation and transformation. For example, methicillin-resistant *S. aureus* (MRSA) can acquire additional resistance genes from neighbouring species, further enhancing its survival [173].

Some bacteria within the biofilm produce enzymes (e.g., β-lactamases) that degrade antibiotics. These enzymes can protect not only the producing bacteria but also neighbouring, otherwise susceptible species, effectively shielding the entire community [170]. Microbes within a polymicrobial biofilm communicate via metabolites and QS molecules, which can induce changes in gene expression that reduce the susceptibility to antibiotics. Antibiotic efficacy can be reduced, for example, by metabolites that affect membrane characteristics or hinder the electron transport chain [174].

The extracellular matrix of polymicrobial biofilms is often more complex and robust, forming a physical barrier that prevents the diffusion of antibiotics and shields the deeper layers of cells from their exposure [171]. The interactions among several bacterial species inside a biofilm facilitate the genesis and maintenance of chronic illnesses. These communities exhibit resilience, capable of adapting to host immune responses and medicinal interventions, hence complicating the eradication of diseases [105].

Biofilm heterogeneity exhibits considerable variance across distinct anatomical sites, including the oral cavity, dermis, implanted medical devices, and gastrointestinal tract, with this diversity influenced by disparities in biofilm structure, matrix composition, and microbial ecology. These disparities influence the response of biofilms to antimicrobial drugs, host immunology, and environmental stressors. Compared to simpler, less dense biofilms on skin, thicker, multispecies biofilms with intricate matrices that are present on medical devices and in the intestines are more difficult to eradicate. The particular combination of architecture, matrix chemistry, and microbial species determines biofilm resilience, virulence, and persistence at each body site [175].

Biofilm heterogeneity among body sites is defined by differences in structural complexity, extracellular matrix composition, and microbial community ecology. Oral, cutaneous, device-associated, and gastrointestinal biofilms exhibit substantial differences, with multispecies biofilms in the gastrointestinal tract and medical devices demonstrating notably intricate topologies and resistance mechanisms attributed to varied matrix components and microbial interactions. Comprehending these distinctions is crucial for developing customised therapy approaches [176].

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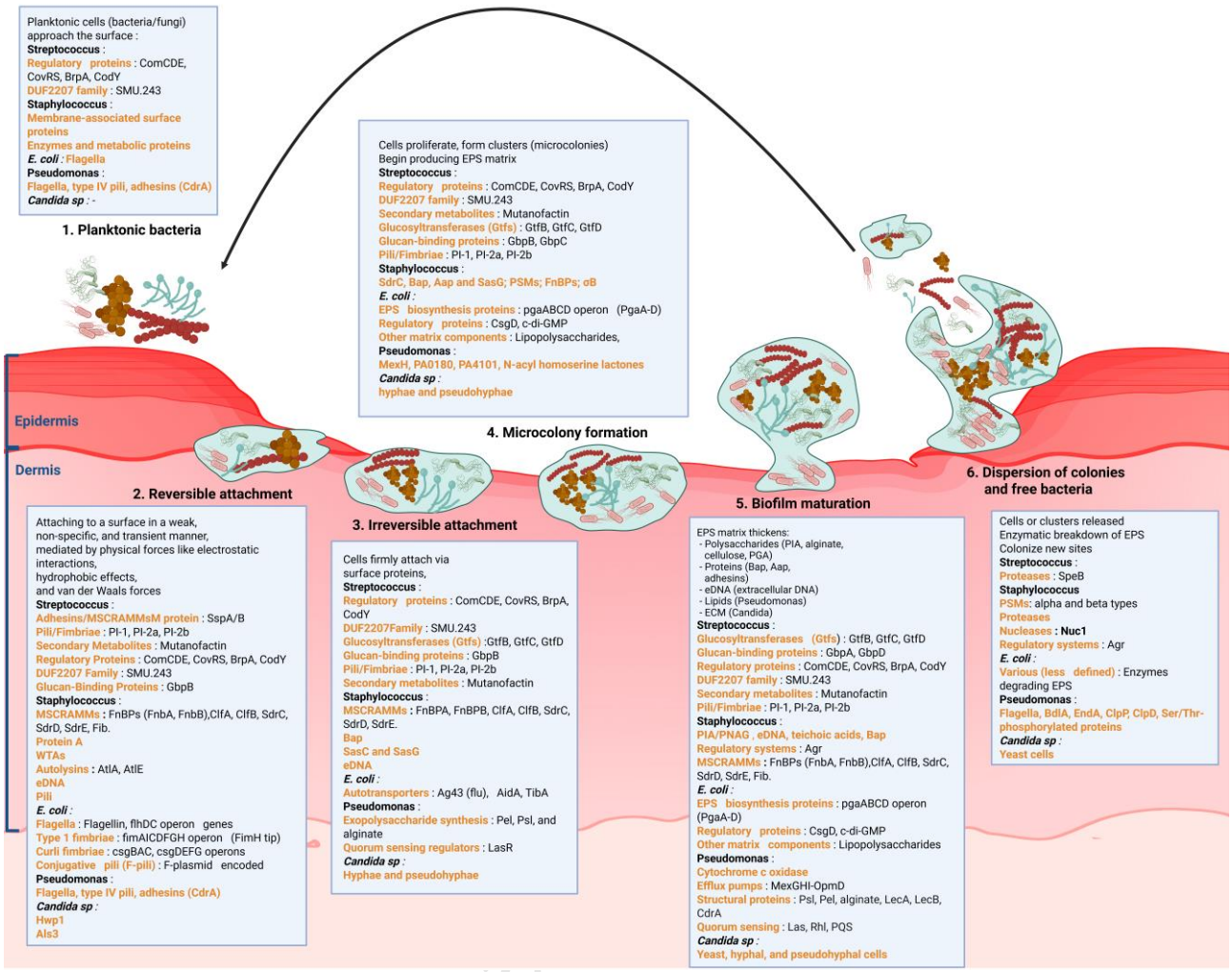

Fig.6. Proteins responsible for the formation of biofilms in *Streptococcus sp.*, *Staphylococcus sp.*, *E.coli*, *P.aeruginosa*, and *C. albicans* at each stage of the process. Created with BioRender.com, July 8, 2025

7. AA in nanoparticle form

7.1. Overview and rationale

The stability of AA is enhanced by nanoparticles, enabling greater property preservation over time. Encapsulation within nanoparticles shields the molecule from deterioration by external elements such as light and oxygen [27]. Nanoparticles can be engineered to deliver the content at controlled rates, which is advantageous for prolonged therapeutic outcomes. Chitosan-alginate nanoparticles have a prolonged release profile, potentially extending the efficacy against target infections (biofilms and resistant bacteria strains) [177]. AA-loaded nanoparticles significantly suppress bacteria biofilms, including those produced by *S. mutans*. Nanoparticles enhance the solubility of AA and promote infiltration into biofilms, rendering it more efficacious than the unencapsulated version [178]. The biocompatibility and targeting to particular tissues or cells are both within the realm of possibility. Zein nanoparticles infused with AA had a negative zeta potential, which may improve compatibility with negatively charged biological tissues and minimize adverse

interactions during topical treatments (Fig.7). This attribute is essential for formulating targeted treatments that reduce adverse effects while enhancing therapeutic advantages. Due to the antifungal qualities, AA nanoparticles present opportunities for many applications beyond pharmaceuticals, such as food preservation and agricultural usage [75].

7.2. Nanoparticle materials and preparation methods

Zein has physicochemical characteristics that make it easier to create nanocarriers [179]. Due to the safety as an excipient, natural abundance, accessibility, cost-effectiveness, and delayed digestion, nanoparticles derived from zein polymer appear attractive for oral administration. Zein nanoparticles can be produced using nanoprecipitation and antisolvent precipitation [180]. PH-driven and electrospraying are alternative methods for synthesizing nanoparticles to deliver bioactive chemicals. The initial method is a straightforward, efficient, environmentally friendly, and economical procedure that does not require elevated temperatures or organic solvents. Monodisperse nanoparticles with scalability, small size, and high encapsulation efficiency may be produced using antisolvent precipitation [181].

High energy levels are required for homogenization when using the solvent emulsification-evaporation method for amphiphilic, hydrophilic, and hydrophobic polymers [182], [183]. Zein nanoparticles have been used to encapsulate molecules, medications, and bioactives with antibiofilm, antibacterial, antioxidant, anti-inflammatory, and anticancer benefits [184], [185]. The characteristics of zein nanoparticles are determined by the composition including the ability to resist stomach acid, stick to mucus, move across the intestinal epithelial barrier, and maintain shape [186], [187]. The production methods affect nanoparticle size, surface charge, production efficiency, and waste generation. The ability to stick together, the use of different polymers or binders, the surface modification of nanoparticles, size, and surface charge are all important factors for the use of dispersions [188].

Encapsulating cardol (CD) and AA in chitosan (CH)-alginate (ALG) NPs requires a two-step process namely gelification and exterior coating. The surfactant dosage and the nature of the encapsulated substance significantly influence the properties of nanoparticles. A minimal surfactant dosage is necessary to achieve increased production and enhanced loading. More importantly, the negative zeta potential readings showed that the particles outer layer was made up of negatively charged ALG chains. When the interface becomes saturated, the interfacial boundary ruptures and the release medium quickly increases. Microbiological investigations reported that CH-ALG-CD showed comparable mild activity to free CD relative to the standard, indicating efficacy in the encapsulation system [27]

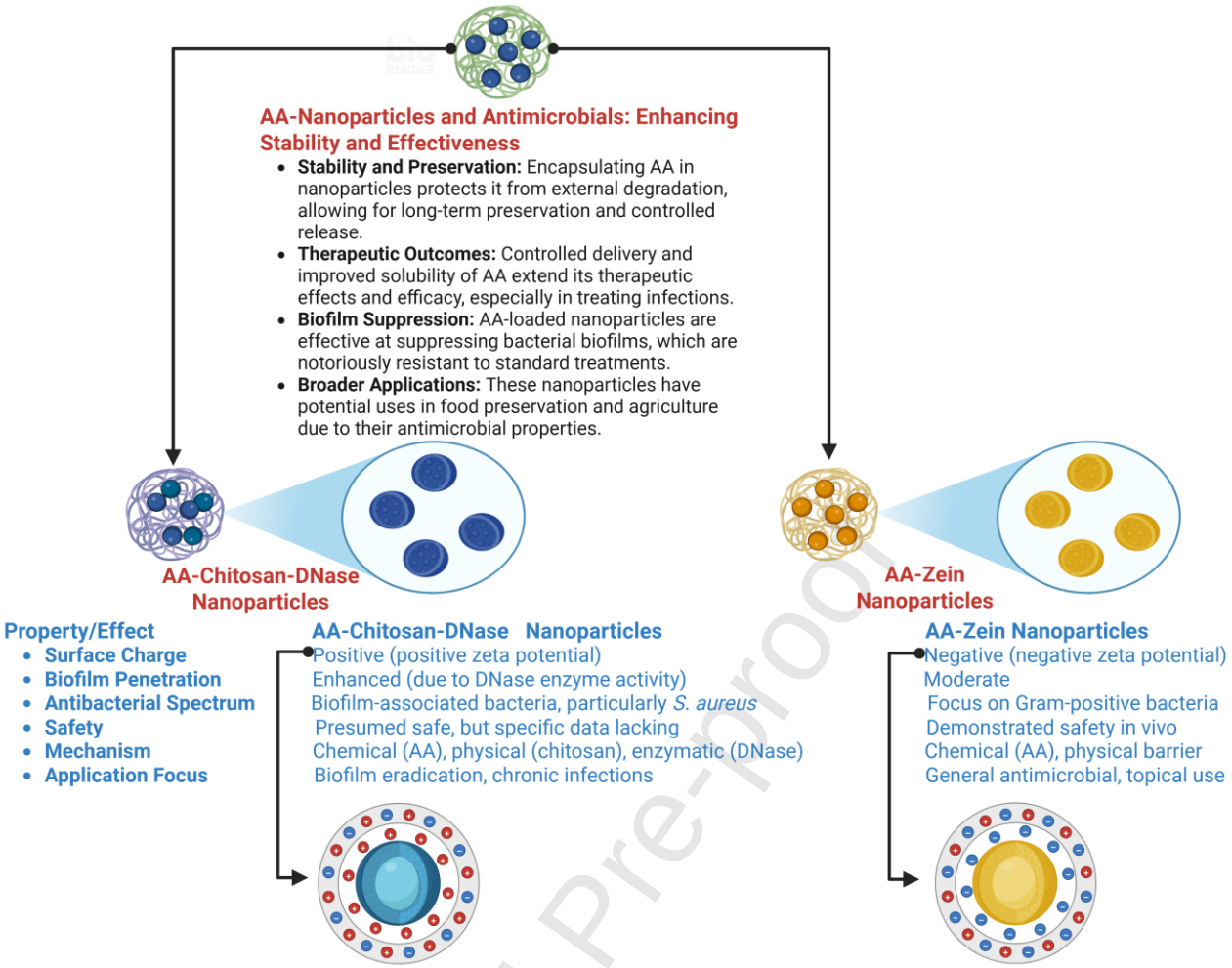

Fig.7. AA-containing organic nanoparticle subtypes. The figure illustrates the differences between the two nanoparticle systems, illustrating their internal structure, key characteristics, and application focus. Blue (left): AA-Chitosan-DNase—refined for penetrating and eradicating biofilms, particularly in persistent infections. Yellow (right): AA-Zein—appropriate for broad antibacterial and topical applications, emphasizing Gram-positive bacteria [178]. Created with BioRender.com, June 16, 2025.

7.3. Physicochemical characterization and bioactivity

Nanoparticles salicylic acid groups were responsible for the sufficient physical-chemical characteristics, which included a particle size below 500 nm. AA-loaded nanoparticles showed a size reduction of nearly 50% and remained stable after dilution. Conversely, the zeta potential decreased the valueand did not alter the charge, suggesting a more stable system. Nanoparticle characterization using TEM corroborated the particle size measurements acquired through DLS, which ranged from 300 to 490 nm for zein nanoparticles with dosage AA and blank. Both systems had spherical and non-aggregated properties. Signal assignment analysis confirmed that nanoparticles were composed solely of the purified source material. Nanoparticles smaller than 400 nm were prepared, and remained the same size with negative zeta potential throughout the process. The major molecules were C15:1 and C15:2 aliphatic chain compounds, with no observable contaminants or degradation products [189].

. The SLNPs were synthesized through the homogenization process and subsequently assessed for particle size, polydispersity index, zeta potential, and entrapment efficiency. The SLNPs

showed prolonged release for 24 hours and remarkable stability at ambient temperatures for 3 months. The kinetic analysis also indicated an optimal profile, best represented by a zero-order model, likely with two stages because active principles migrate to the CH-ALG interface [27] .

7.4. Antibiofilm efficacy and spectrum

Biofilm-associated bacteria infections are crucial in the advancement of infectious diseases, due to the rise of antibiotic resistance. To address this difficulty, formulated AA was encapsulated in solid lipid nanoparticles (SLNPs) and subsequently coated with chitosan and DNase (AA-SLNPs-CH-DNase). The DNase coating was proposed to destroy the e-DNA, while chitosan was applied to produce positively charged solid lipid nanoparticles with enhanced adherence to biofilms. The formulated SLNPs showed non-toxicity towards human immortalized keratinocyte (HaCaT) cells while demonstrating significantly enhanced antibacterial activity against *S. aureus*. The remarkable impact of the formulated SLNPs on minimum biofilm inhibition concentration (MBIC) and minimum biofilm eradication concentration (MBEC) further validated the efficacy of the suggested formulation method. The created SLNPs showed a notable reduction in biofilm thickness and biomass compared to the control group, as confirmed by confocal laser scanning microscopy ($p < 0.05$). The results collectively show the improved effectiveness of the formulated method in addressing biofilm-mediated antibiotic resistance [74].

Nano-encapsulated AA has shown efficacy in inhibiting dental biofilm development. The diminutive diameter of nanoparticles not only improves mechanical properties but also antibacterial efficacy, attributed to the elevated surface-area-to-volume ratio that causes damage to the cell membrane. A small amount of AA enclosed in nanoparticles inhibited the growth of *S. mutans* biofilm for five days after a single two-minute treatment. Neither zein nanoparticles containing AA (9.375 μg/mL) nor 0.12% CHX gluconate were able to kill the biofilm that formed over 5 days [14].

Recent tests in drug delivery systems have shown the potential of dimethylglyoxime as a stabilizing agent. The preparation helped stabilize the particles for a long period and encapsulated AA. The aromatic configuration contributes to the enhancement of antimicrobial response, which is linked to the arrangement of the pharmacophore. Nanoencapsulation also made AA effective against *P. aeruginosa* and *C. auris*. This formulation doubled the compound effectiveness at killing *S. aureus* and showed minimal fungistatic and fungicidal concentrations against the tested *Candida spp*. Furthermore, AA-loaded nanoparticles caused a more pronounced reduction in cell viability within *S. aureus* and *C. albicans* biofilms compared to AA in solution and CHX digluconate, indicating an inhibitory level comparable to that of amphotericin B. Regarding *P. aeruginosa* biofilm, nanoparticle indicated efficacy comparable to AA in solution, and both showed greater activity than CHX gluconate. Moreover, the inhibitory, bactericidal/fungicidal, and antibiofilm effects of AA

nanoparticles required only minimal concentrations to kill *S. aureus*, *P. aeruginosa*, and *Candida spp*. suggesting the potential as a new antimicrobial agent [75].

AA nanoparticles primarily inhibit biofilm formation, likely through disrupting cell signalling and gene expression crucial for early biofilm development, especially genes tied to quorum sensing, adhesion, and EPS production. However, direct evidence mapping the gene-level effects of AA nanoparticles is still needed, and future studies with comprehensive omics approaches are expected to clarify these molecular interactions and cement their clinical potential.

7.5. Stimuli-responsive and advanced nanoplatforms

Progressing AA-loaded nanoplatforms that respond dynamically to local biofilm cues such as pH, oxidative stress, or enzymatic triggers involves designing smart, stimulus-responsive delivery systems that release AA upon specific environmental signals associated with biofilms. Enzyme-responsive nanocapsules, cross-linked AA nanocapsules synthesised via interfacial polymerisation with enzyme-cleavable linkages (e.g., ester bonds cleaved by lipase), allow targeted AA release in response to enzymes present in the biofilm microenvironment [190]. For example, nanocapsules with lipase-sensitive ester linkages degrade specifically when lipase is present, releasing AA and possibly co-encapsulated agents locally, maximising activity and minimising off-target effects. This strategy is promising given that lipases are commonly secreted by bacteria within biofilms, thus serving as a direct enzymatic trigger for release [191].

Biofilms often exhibit acidic microenvironments. Designing AA nanoplatforms with pH-sensitive materials that destabilize or swell in acidic conditions could trigger AA release selectively within biofilms. Functionalizing AA-loaded nanoparticles with acid-labile bonds or coatings responsive to low pH can improve delivery specificity and biofilm penetration. Elevated levels of reactive oxygen species (ROS), which are present in biofilms, may serve as a signal for redox-sensitive nanocarriers. Incorporating disulfide bonds or other ROS-cleavable linkages in the AA nanoplatform could allow on-site AA release upon encountering oxidative stress. This approach exploits the pathological oxidative environment typical of infected tissues and biofilms [192].

Combining enzyme-, pH-, and redox-sensitivity in one system can increase release precision. For instance, AA nanocapsules with enzyme-cleavable shells and pH-responsive cores can provide sequential or synergistic responses to multiple biofilm cues. Inverse miniemulsion-synthesised polyurea AA nanocapsules exemplify this, employing polymer shells sensitive to enzymatic degradation, enabling co-delivery of AA with other active agents at infection sites [193].

Coating AA nanoparticles with polymers responsive to biofilm extracellular matrix components (e.g., DNase, chitosan) can disrupt the biofilm structure and enhance stimulus response efficiency. Targeting moieties responsive to biofilm-specific signals (e.g., lectins recognising polysaccharides) might further improve nanoplatform localisation for triggered release. Employing

fluorescence probes or HPLC-based quantification to monitor AA release under simulated biofilm conditions (presence of enzymes, acidic pH) is crucial for optimising the stimulus-responsiveness of nanoplatforms [194].

8. Toxicological aspect

The secure application of AA in a variety of therapeutic and environmental contexts is contingent upon toxicity. In general, in-depth toxicity evaluations ensure efficient use of AA without endangering people or the environment. These evaluations assist in identifying safe dosage levels and comprehending possible side effects. However, completely understanding the safety profile and optimizing therapeutic potential requires additional investigation [195].

The AA analog from the ethanol extract of *S. jambos* leaves (Fig.4h) showed cytotoxic activity against U937 human macrophage cells, with an EC_{50} value of 57.8 μg/mL [70]. In vitro, hemolysis tests and cytocompatibility tests, as well as in vivo using a medaka fish model, were used to confirm that the AA-impregnated catheter did not have any toxicity. The medaka fish did not show any unusual behavior, weight loss, or mortality [65].

Toxicology and genotoxicity in vivo were assessed for zein nanoparticles containing 9.375 μg/mL of AA. The long-term effects on mice given 2.25 and 112.5 μg/kg of nano-encapsulated AA orally for a week. The relative weights of the liver, kidney, and spleen remained unchanged. The group that received the greatest dose had an increase in relative weights in the stomach, lungs, and heart. Histological abnormalities were also observed in this group. The results showed that both doses sustained the micronucleus frequency within the normative range, and the mice at the higher dose had a slight weight loss, potentially elucidating the relative changes in organ weight. Both blank and AA-loaded zein nanoparticles showed nontoxicity upon repeated administration over 7 days, as no significant histopathological alterations or genotoxic effects were detected. AA formulations may serve as an antibacterial option for the prevention and treatment of oral infections in short-term applications [189].

AA does not cause cancer or changes in mouse models, and the lowest dose that can be given quickly is above 2,000 mg/kg [44]. AA-loaded zein nanoparticles did not significantly alter target organs at greater dosages in mice, presenting a relatively low toxicity profile under particular circumstances. Macroscopic examination of organs showed alteration of anatomical features including texture, size, and color [189]. Long-term studies show that zein nanoparticles loaded with AA demonstrated slight systemic toxicity after 30 days of administration. The impact on oral tissues was comparable to or less severe than that of chlorhexidine (CHX)-treated groups, suggesting great potential as a safe topical application for oral health [195].

The toxic effects of AA must be considered since it can greatly impact the health and integrity of oral epithelial cells. Barrier function and maintaining the balance of the oral mucosa are so

important. AA primarily exerts toxic effects by inhibiting histone acetyltransferases (HATs), which are essential for regulating gene expression and cellular function. The inhibition may lead to modified cellular responses, such as apoptosis and heightened vulnerability to further cytotoxic compounds [196]. These changes potentially hinder normal cell turnover and repair in oral epithelial cells, increasing vulnerability to processes that can cause cancer [197]. The multifunctional cytotoxic characteristics suggest that AA can reach oral epithelial cells, potentially causing cellular damage and promoting pathological alterations [198]. The cytotoxic effects on oral epithelial cells are determined by the concentration and exposure time. Elevated levels of AA have been shown to induce substantial cell death, potentially undermining the integrity of the oral mucosa [195]. Further investigation into the safety profile of AA is necessary due to the potential to upset cellular homeostasis and promote inflammation or carcinogenesis in the oral cavity, particularly in long-term usage [199]. In relation to the viability results, LDT11 was deemed non-toxic at a concentration of 50 μg/mL. LDT11 has minimal cytotoxicity when tested against macrophages, with viability exceeding 80% at dosages below 50 μM [13].

9. Conclusion and future prospect

AA is a promising natural antimicrobial agent with notable efficacy against a variety of bacteria and fungi, particularly in disrupting biofilms, which are often resistant to conventional antibiotics. Its antimicrobial action is primarily due to the disruption of bacterial membranes, inhibition of key metabolic pathways (like NADH oxidase and electron transport chain), and interference with QS. The chemical structure of AA, particularly its alkyl side chains, increases membrane fluidity and permeability, thereby enhancing its antibacterial and antibiofilm effects. Structural modifications, such as branching or cyclisation of side chains, often improve efficacy and could enhance ROS generation and enzyme inhibition. Derivatives with optimised lipophilicity (log P) show better bioavailability and potency. AA demonstrates particular potency against Gram-positive bacteria, including *S. aureus, S. mutans*, and MRSA strains, and also exhibits antibiofilm activity. However, its effect on mature biofilms is weaker than on forming biofilms. Advances in delivery methods, such as nano-encapsulation (e.g., zein nanoparticles, cyclodextrin inclusion complexes), significantly improve AA's sustained activity, making it a better candidate for both systemic and topical use. Compared to other natural antimicrobials, such as cranberry extracts and essential oils, AA exhibits excellent inhibition of biofilm formation but modest disruption of established biofilms, suggesting that synergy or combination therapies might be beneficial. AA's relatively low cytotoxicity at effective doses supports its potential for clinical application.

Structural optimisation and synthesis of AA derivatives should be a major focus to enhance antimicrobial and antibiofilm efficacy, targeting improved bioavailability, broader spectrum activity, including Gram-negative bacteria, enhanced stability and targeted delivery, greater disruption of

mature biofilms. Development of poly-AA coatings and surface modifiers leveraging AA's ability to cross-link through unsaturated side chains for durable antibiofouling materials, with applications in medical device coatings (catheters, implants) to prevent biofilm-related infections. Nanoformulations and encapsulation technologies that can improve penetration into mature biofilms, sustain release, and minimise toxicity require further exploration for clinical translation. Elucidation of precise molecular mechanisms, especially the interaction of AA with quorum sensing pathways and bacterial metabolic networks, to design derivatives targeting biofilm resilience and antibiotic resistance. Clinical studies and in vivo evaluations using relevant infection models, especially chronic biofilm-associated infections, to validate AA's therapeutic potential and safety profile. Exploration of combination therapies pairing AA with other antimicrobials or biofilm disruptors (e.g., peptides, essential oils) to leverage synergistic effects against complex polymicrobial biofilms. Investigations into biosourcing and sustainable production of AA from plants and agricultural waste, facilitating scalable and cost-effective manufacture.

Statement of Contribution by CRediT Authors

Arsa Wahyu Nugrahani: Writing - preliminary draft, formal analysis, administration of data. Triana Hertiani: Review and editing, software, supervision. Tetiana Haniastuti: Writing - review and editing, data curation, software, investigation, conceptualization. Khadijah Zai: Conceptualization writing, review, and editing, formal analysis.

Commitment to ethical standards

Inapplicable.

Competing statements of interests

The authors confirm that the work reported in this paper is not influenced by any competing financial interests or personal relationships.

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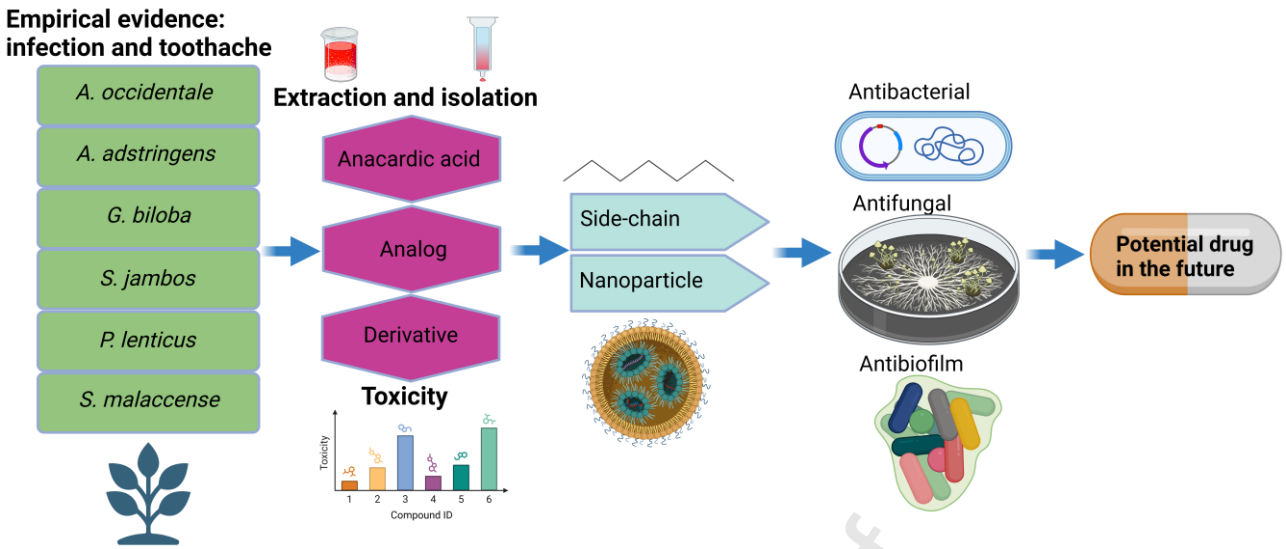
Graphical abstract



Highlights

- Chemical structure and length of alkyl side chains influence AA's antibacterial and antibiofilm efficacy.
- AA disrupts biofilms by physically damaging bacterial membranes and inhibiting quorum sensing.
- Nano-encapsulation advances improve AA's solubility, stability, bioavailability, and penetration ability.
- AA shows promise in biomedical devices to prevent biofilm formation and combat infections.
- Further research on AA derivatives, optimized delivery systems, combined therapies, and safety assessments is crucial.

Journal Pre-proof