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Review Article

# Different Mechanisms Of Biofilm Formation And Regulation By Staphylococcus Aureus And The New Approaches To Combat It

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## Abstract

Staphylococcus aureus can readily form biofilm which enhances the drug-resistance, resulting in life-threatening infections involving different organs. Also the formation of biofilm can protect bacteria from being attacked by the host immune system and antibiotics and thus bacteria can be persistent against external challenges. Biofilm formation occurs due to a series of developmental events including bacterial adhesion, aggregation, biofilm maturation, and dispersion, which are controlled by multiple regulatory systems and lead to hospital acquired infections. Rapidly increasing research and development outcomes on natural products targeting S. aureus biofilm formation and/or regulation led to an emergent application of active phytochemicals and combinations. This review aimed at providing an in-depth understanding of biofilm formation and regulation mechanisms for S. aureus, outlining the most important antibiofilm strategies and potential targets of natural products, and summarizing the latest progress in combating S. aureus biofilm with plant-derived natural products. These findings provided further evidence for novel antibiofilm drugs research and clinical therapies

## Aim of the work

The objectives of this article are to focus on the formation and regulation of biofilm by Staphylococcus aureus bacteria and the recent approaches of treatment by natural compounds.

**Keyword:** Biofilm, Staphylococcus aureus, Accessory gene regulator genes.
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**List of abbreviations**

*S. aureus: Staphylococcus aureus*
PIA: *Polysaccharide intercellular adhesin*
Agr: Accessory gene regulator
Bap: Biofilm-associated protein
CWA: Cell wall-anchored
EPS: Extracellular polymeric substance
Fib: Fibrinogen-binding protein
PSMs: Phenol-soluble modulins
QS: Quorum sensing system
Sas: *S. aureus* surface protein
TCSs: Two-component signal transduction systems
*Ica*: Intercellular adhesin
Eos: Essential oils

## Introduction

Staphylococcus aureus is a drug- resistant bacteria that generates infections in skin and soft tissues and advances to catastrophic illnesses such as endocarditis, osteomyelitis, pneumonia, and others. One of the main aspects that leads to the development of drug-resistance in S. aureus is its capacity to create biofilms.[1] Microbes in a biofilm may have a survival advantage of up to 1500 times, making them more resistant to certain strains of bacteria. [2]

In an effort to find alternatives to conventional antibiotics that are safe for both humans and the environment, research into natural compounds as potential biocompatible antibiofilm agents has been substantial. Examples include plant-based essential oils, flavonoids, [3] and, phenolic acids [4] may include terpenoids [5] shown antagonistic and disruptive effects on biofilms via a variety of pathways, including as lowering adhesin levels, degrading biofilm matrix, and preventing bacterial communication. [6]

### Formation and properties of S. aureus biofilm

There are four distinct phases of biofilm development: attachment and adhesion, aggregation including biofilm formation, biofilm maturation, biofilm dispersion, biofilm synthesis of extracellular matrix, and bacterial proliferation by cell detachment. [7]

The planktonic cells of Staphylococcus aureus adhere to either living or nonliving surfaces and form a complex coating of host macromolecules such proteins during the early adhesion or gathering phase.[8] When bacteria infect tissues, they may bind to one another and form aggregates in abnormally thick mucus (such as cystic fibrosis) or on damaged host tissues (like bones and heart valves). [9], and the skin around long-term wounds). [10]

The Over the course of their proliferation, linked or aggregated cells produce an extracellular polymeric material (EPS). the maturation and proliferation stage. This substance is crucial for building three-dimensional biofilm scaffolds. [11] Biofilm cells are mechanically stabilized by endoplasmic reticulum (EPS) molecules, which include polysaccharides, nucleic acids, proteins, and lipids; as a result, the EPS molecules control the cells' life circumstances. [12]

In the diffusion stage, the biofilm becomes less stable due to cell aggregates; as a result, it spreads across the environment, attaches to other surfaces, and produces new infections. Dissected biofilms may spread to other tissues, either locally (as in the case of implant biofilms that cause osteomyelitis) or distantly (as in the case of detached biofilms that cause endocarditis). [13]

Biofilm cells can be classified categorized into four distinct metabolic states: (i) aerobic, is found in the outer layer of the film where oxygen and nutrients are abundant; (ii) fermentative, which is found in the inner layer of the film where oxygen and nutrients are scarce; (iii) dormant, which is found in the anoxic layer where growth is slow and metabolism is inactive; and (iv) dead. [14]

Bacteria may become less susceptible to antibiotics when dormant cells cause a decrease in intracellular adenosine triphosphate. Biofilms also have other gradients. One example is how S. aureus biofilms are formed. In the early stages, a vertical gradient of

viscoelasticity is set up, which helps the biofilms spread and removes bacteria that are weakly attached while keeping a layer of bacteria that are embedded in the biofilm structure. [15]

**Mechanism of S. aureus biofilm formation**

When it comes to antibiofilm strategies, the EPS matrix degradation is crucial. Degradation of self-produced adhesins, nucleic acids, and polysaccharides has been the primary mechanism for the removal of both mixed-population and single-species biofilms using the different agents that have been used so far. [16]

- **Polysaccharide intercellular adhesin (PIA) mechanism**

Most extracellular polysaccharide (EPS) components of Staphylococcus aureus biofilm are PIA, that is crucial to the development of the biofilm, colonization, immune evasion, and antibiotic resistance, among other things. Various regulatory mechanisms regulate PIA production, which is in turn influenced by environmental factors such anaerobiosis and glucose. [17] The variable expression of PIA in various staphylococcal strains may be explained by the fact that ica expression is regulated by a number of different genes and proteins. The methicillin-sensitive S. aureus strain is the most common one that forms a biofilm reliant on PIA. By deacetylating the polymer, PIA increases biofilm retention and drug resistance by creating a net positive charge that facilitates cell attachment and intercellular adhesion. Biochanin A, an isoflavone found in nature, may suppress the secretion of PIA and, after biofilms have developed, can be disintegrated by means of the fragmented EPS matrix. [18] In addition, by inhibiting the production at 50 μg/mL significantly inhibited the growth of S. aureus biofilms, thanks to icaA, a naturally occurring (+)-nootkatone.mL. [19]

- **Extracellular proteins mechanism**

During the first phases of biofilm formation, Staphylococcus aureus cells primarily invade host cells by surface attachment. [20] The vast array of surface proteins produced by S. aureus that are covalently bound to peptidoglycans are known as cell wall-anchored (CWA) proteins. The majority of CWA proteins are proteins that are involved in biofilm formation or associated with adhesion, such as protein A, fibronectin-binding proteins (FnBPs), clumping factors (ClfA, ClfB), serine-aspartate repeat family proteins (SdrC, SdrD, and SdrE), fibrinogen-binding protein (Fib), and S. aureus surface protein (SasG). [21]

They promote biofilm accu-mulation via facilitating the attachment of Staphylococcus aureus cells to host cells and extracellular phospholipids (EPS). Biofilm development may be inhibited by reducing the expression of genes that code for surface adhesion proteins (e.g., fnbpA and fnbpB). Certain natural chemicals found in Euphorbia humifusa, such as kaempferol, quercetin, and luteolin, play this role.). [20]

the cytoplasmic plexus. Disrupting SrtA expression may reduce the effects of acute infection and the formation of cell wall surface adhesins. Extensive research has been conducted on the possibility of several natural substances inhibiting SrtA. Take kaempferol as an example. It inhibits S. aureus biofilms from forming by lowering SrtA activity and genes related to CWA proteins. Similarly, it was discovered that chalcone, quercetin, and myricetin all effectively inhibited SrtA. [22]

- **Extracellular DNA (eDNA)mechanism**

A number of biological processes rely on eDNA, including as adhesion, gene transfer, and repair of DNA damage. [23] In addition, the biofilm of Pseudomonas aeruginosa may be protected against DNase enzymatic attacking by establishing a connection between the matrix and the microbes inside the biofilm by the use of eDNA cross-linking with lipoproteins that are linked to the membrane. Also, there was some indication that the holin-like protein CidA might potentially improve the release of eDNA when biofilms are growing. By decreasing cidA expression and interfering with the release of eDNA, Emodin—a derivative of polygonum cuspidatum and rheum palmatum—inhibited the development of S. aureus biofilms.[24]

- **Phenolsoluble modulins (PSMs)mechanism**

S. aureus and S. epidermidis, along with the majority of staphylococcal species, produce PSMs, which are short peptides characterized by an α-helical structure and surfactant-like characteristics.

S. aureus secretes the δ-toxin encoded by RNAIII in addition to four PSMα peptides, each roughly 20 amino acids long, and two PSMβ peptides, each around 40 amino acids long. It seems that PSMs play a role in S. aureus pathogenicity and may serve as inflammatory response stimulants. Thanks to their surfactant-like characteristics, PSMs support the formation of channels inside biofilms, which in turn allows S. aureus to access nutrients in the biofilm's deeper layers and encourages biofilm detachment, allowing free bacteria to thrive. Since PSMs are soluble in S. aureus, they may bind to amyloid fibers and help keep the biofilm in place. [25]

**Promising targets in the regulation of *S. Aureus* biofilm formation**

One aspect is herd behavior. of biofilm production. From adhesion to biofilm development and dissemination, many regulatory systems tightly govern each stage. [25]

- **Quorum sensing (QS) system**

Bacteria have an internal communication system called the system for quality assurance. A threshold is reached at which the synthesis of relevant genes is triggered by changes in the signal molecules. Through a complex web of signal transduction channels, it regulates biofilm formation, pathogenicity, migration, and sporulation. [26] Research on the QS system in Staphylococcus aureus has focused on the Agr and LuxS/autoinducer-2 (AI-2) systems. [26]

S. aureus has two promoters, P2 and P3, that activate different parts of the Agr system, which include RNAII and RNAIII, respectively. Essential for the creation, transport, and regulation of autoinducing peptides (AIPs), the AgrB, D, C, and A proteins are encoded in RNAII. The production and transportation of AgrD, the precursor of AIP, to the plasma membrane are facilitated by an AgrB-dependent mechanism. [27] After the AIP builds up, it activates the histidine kinase AgrC, which in turn induces a positive feedback loop on the P2 and P3 promoters. The process of signal transduction is then initiated, leading to autophosphorylation. The elevated AIP may also encourage biofilm depolymerization by increasing the secretion of extracellular protease. [28]

As a key Thus, RNAIII upregulates the production of exoproteins such haemolysins, toxins, and exoproteases and downregulates the production of surface adhesins like FnBPs and serine-aspartate repeat family proteins. Since activating the Agr system both inhibits biofilm production and disperses existing biofilms, it may be an appealing antibiofilm approach. Another flavonoid found in Caesalpinia sappan, Brazilin, has the ability to manipulate the Agr-related function and hence prevent biofilm development. The Agr system, on the other hand, may enhance virulence factors, which allows bacteria to quickly adapt to new environments. Therefore, it is very difficult to precisely manipulate the Agr system in a way that inhibits biofilm formation without enhancing virulence. Given that the autoinduction of all four Agr subgroups is crucial to the strategy's practical use, a cocktail of "clean" activators that stimulate one or more agr variations without significantly impacting the others might be a potential reagent. One possible method for suppressing biofilms is to activate AgrC, which creates a barrier to an intrasteric inhibitory docking contact. [29]

The S-ribosylhomocysteine lyase (LuxS)/AI-2 system regulates the PIA-dependent biofilm development by impacting the transcriptional regulation of the intercellular adhesin (ica) gene. The synthesis of PIA is specifically carried out by proteins IcaA/D/B/C.. IcaA and IcaD work together to produce UDP-N-acetylglucosamine, which is then transported out of the body by means of IcaC. Then, IcaB improves adhesion by controlling PIA's partial deacetylation, which increases positive charge. [30] LuxS has the ability to reduce PIA-dependent biofilm formation, increase icaR expression, and inhibit rbf expression, all of which contribute to AI-2 biosynthesis. The monoterpenoid glycoside paeoniflorin, which is derived from plants in the family Paeoniaceae, including peonies, has the ability to decrease the virulence and biofilm development that is regulated by the luxS/AI-2 system. [30]

- **Two-component signal transduction systems** (TCSs)

Sensory histidine kinase (HK) detects environmental cues, The expression of genes that are down-

stream targets is controlled by response regulators (RRs), and it quickly rises. the adaptive survivability of bacteria. These two components make up transmembrane signal transduction systems (TCSs). [31] Not only the Agr system, but also the YycFG and SaeRS systems of Staphylococcus aureus have become new targets in the fight against biofilms. [32] One of the two-component regulators that contribute to S. aureus biofilm formation and bacterial pathogenicity is YycFG, which is also called VicRK or WalRK. It was shown that YycF could directly control the anticipated promoter areas several biofilm-involved genes, including sarA and icaA. The antisense yycG RNA (ASyycG) approach successfully reduced biofilm buildup by repressing the transcription of these genes. [32] For S. aureus, YycFG regulates biofilm formation and cell wall metabolism via autolysin production. The results suggest that To control S. aureus infections, inhibiting YycFG could lessen the bacteria's ability to build biofilms and cause disease. Rhodomyrtone, an isolated compound from Rhodomyrtus tomentosa, suggests that it may suppress the formation of methicillin-resistant Staphylococcus aureus. of secreted proteins such exoenzymes and antigenic proteins by interfering with the YycFG system. [25]

- **SarA family proteins**

One group of proteins that control how Staphylococcus aureus forms biofilms is the SarA family. This group mostly consists of SarA, Rot, and MgrA. By directly increasing exoprotein expression and by interacting with the Agr system, SarA may prevent the production of extracellular proteases during biofilm growth. Increased transcription of the ica operon and synthesis of the PIA precursor are two ways in which SarA, in its role as a transcriptional activator, encourages biofilm formation. Rot controls the expression of genes involved in biofilm formation and S. aureus pathogenicity. It does this via positively regulating ClfB, SdrC, and SarS, which in turn increases the surface protein level and decreases the extracellular enzyme level. One possible regulator of Rot synthesis in S. aureus is RNAIII, which inhibits its translation. The negative regulator MgrA stops biofilm formation by preventing the production of adhesins. [33] (+)-Nootka tone treatment resulted in down-regulation of the sarA gene expression, which in turn reduced PIA production and biofilm formation.. [19]

- **Alternative sigma factor σB (SigB)**

As a biofilm facilitator, SigB also aids in the inhibition of biofilm dispersion. Indirectly, SigB controls biofilm formation by controlling other regulatory mechanisms, such as RNAIII and SaeRS TCS suppression and sarA expression upregulation in response to relevant circumstances. [34] Several naturally occurring chemicals have the potential to modulate SigB and thereby limit. biofilm formation. As an example, researchers have shown that Ginkgo biloba exocarp extract downregulates the MRSA biofilm-associated factors sarA and sigB. [35] S. aureus icaA, icaD, sarA, agrA, and sigB gene expression may be suppressed by Scrophularia ningpoensis honey. [36]

**Emerging natural products-based therapeutics against *S. aureus* biofilm**

Since the mid-20th century, when antibiotics were at their peak, natural compounds have been potent treatments against infections. New antibiofilm compounds upon a variety of natural components, including phytochemicals and plant extracts, are anticipated to inspire novel tactics to fight biofilm, as combinatorial techniques have produced beneficial medications.[37]

- **Plant extracts**

**Essential oils (EOs*)***

There is encouraging therapeutic evidence that essential oils, which are volatile compounds extracted from medicinal plants, may inhibit the growth of microbial biofilms. [38]

Essential oils extracted from Croton species, including C. blanchetianus and C. conduplicatus, have the potential to inhibit the production of biofilms and reduce the prevalence of preformed biofilms of MSSA and MRSA strains. [39] Possible biofilm-eradicating agents include tannins, free steroids, alkaloids, flavonoids, and saponins; these chemicals dissolve cell walls, break up clumped colonies, prevent nourishment from being restocked, and break down the structure of established biofilms. [40] Sharifi et al. conducted more research into the antibiofilm mechanism of cuminum cyminum

essential oil (CcEO) against multidrug-resistant Staphylococcus aureus. At sub-MIC dosages (0.625-1.25 μL/mL), CcEO reduced hld and ica expression by 3.13- and 2.33-fold, respectively, in relation to QS inhibitory potential.). [41] Also, at a concentration of 1/8 MIC (6 μg/mL) for Origanum vulgare essential oil and 1/4 MIC for terpinene-4-ol within this EO, the anti-attachment effect against S. aureus was shown. Use of essential oils (EOs) to reduce bacterial adhesion is one efficient method for avoiding biofilm formation, according to the research. A 10.36-05.05% reduction in mature biofilms was seen with O. vulgare EO at dosages ranging from MIC to 4 MIC, whereas a 62.28%-70.97% reduction was observed with terpinene-4-ol.%. [42]

**Other plant extracts**

The inclusion of chlorogenic, quercetin, and rutin in hydroethanolic plant extracts may explain why their ability to significantly reduce *S. aureus* biofilm development has been shown. [43] Just like amoxicillin, the methanolic extract of Capsicum annuum showed a 53.8% inhibition rate against S. aureus biofilm at 64 μg/mL. This is because it contains a diverse array of phytochemicals. [44] Extracts from Aphanamixis polystachya and Melia azedarach were shown to inhibit and eradicate biofilm formation by methicillin-resistant *Staphylococcus aureus* (MRSA) at concentrations below the lethal threshold. Limonoids, oxygenated triterpenoids, and phenolics are components of these biofilms. [45]

- **Phytochemicals**

**Flavonoids:**

Polyacetylenes, phenolics, terpenoids, alkaloids, polypeptides, and lectins are some of the novel natural compounds that may inhibit biofilm formation. [46] Among the many important classes of phenolics, flavonoids have gained widespread recognition as potent antibacterial agents. They do this by interfering with a variety of microbial processes, including pathogenicity, cytoplasmic membrane function, inhibition of nucleic acid production, alteration of membrane permeability, cell attachment and biofilm development, energy metabolism, and porin on the cell membrane. Medicinal plants, fruits, and vegetables often include flavonoids including kaempferol, quercetin, and luteolin. Scientific studies have demonstrated that quercetin hinders the growth of biofilms and breaks up existing ones. This is achieved by drastically decreasing the production of elastase, protease, and pyocyanin in Pseudomonas aeruginosa, violacein in Chromobacterium violaceum, and extracellular proteolytic soluble protein (EPS) in Yersinia enterocolitica. Studies in S. aureus found that luteolin, quercetin, and kaempferol, when administered at dosages between 8 and 128 μg/mL, decreased the expression levels of genes associated with biofilm. Combinations of kaempferol, quercetin, and luteolin have a synergistic effect that makes them more effective against biofilms. [47]

**Naphthoquinones:**

Naphthoquinones and their derivatives are highly sought-after antimicrobials because of the wide range of biological activities they exhibit and the remarkable structural diversity among them. [48] 1At a dosage of 10 μg/mL, 1,4-NQ was discovered to decrease microbial enhance mobility and inhibit S. aureus biofilm formation by 55%. Furthermore, 1,4-NQ increased cellular ROS production, which could impact biofilm formation. [49]

One 1,4-NQ analogue, menadione, proved successful in inhibiting the growth of many MRSA strains, according to research by Mone et al. This occurred because it may raise levels of reactive oxygen species (ROS), limit biofilm formation (>90% at MICs ranging from 64 to 256 μg/mL), and eliminate preexisting biofilms (>85% at 1024 μg/mL). [50] At SKN, a 1,4-NQ derivative found in the root of Lithospermum erythrorhizon, was shown to inhibit the biofilm formation of clinical MRSA strains at concentrations lower than the minimum inhibitory concentration (15.6 μg/mL). [51]

**Other natural compounds:**

Antibiofilm activity against *Staphylococcus aureus* have been observed in natural substances such as sesquiterpenes, aromatic acids, and alkaloids. An alkaloid called sinomenine, for instance, has the ability to dramatically increase agrA expression while decreasing icaA level.[52] The biofilm activity of Staphylococcus aureus, which targets the Agr and SarA systems, is inhibited by aromatic acids like 3-HBA. At doses ranging from 1 to 4 mg/mL,

the sesquiterpene nerolidol inhibited S. aureus biofilm by more than 70%. [50]

- **Combinatorial approaches**

**Combination of different compounds:**

Finding good combinations provide an alternative for infection therapy, which is important since there are few therapeutic alternatives for *S. aureus*. As an example, metal complexes based on curcumin not only have substantial biological activity, but they also increase curcumin's bioavailability. Curcumin inhibited biofilm the oxovanadium compound of curcumin, however, at a concentration of 100 μM (55.6%), due to S. aureus had an even stronger effect (82%). This difference may be due to the combined effects of multiple mechanisms, such as the inhibition of alkaline phosphatase and the antibacterial mechanism. [53]

Combinational terpenes such as Additionally, the antibiofilm activities of linalool, (-)-trans-caryophyllene, (S)-cis-verbenol, (S)-(-)-limonene, and (R)- (+)-limonene were investigated. As the results shown, no terpene combination affected bacterial growth while simultaneously reducing biofilm development by over 50%. At 500 μg/mL, the most efficient inhibitory effect was seen with (-)-trans-caryophyllene and linalool, with an 88% success rate. Reasons for this include an increase in the expression of genes involved in capsular polysaccharide formation (cap5B and cap5C) and a decrease in genes involved in cell adhesion and the QS system. By using several pathways, the combinations are anticipated to provide amplified effects, ultimately defeating S. aureus's resistance. [54]

**Combination of plant extract and antibiotic:**

It is feasible to combine antibiotics with plant extract. way to increase effectiveness, as natural product treatment or medicines alone may not be enough to fight drug-resistant bacterial illnesses. Using a combination of ampicillin and an artocarpin-rich extract from Artocarpus heterophyllus, Bazmi et al. showed that MRSA's membrane permeability was changed, resulting in the release of intracellular components. Additionally, at their minimum inhibitory concentrations (MICs), the components that were tested showed a significant reduction of biofilm formation (62.7–76.6 percent). This suggests that these components could work together to enhance antibacterial effects at doses below the inhibitory threshold (1/2-1/16 MIC) in combination cocktails that work synergistically to increase the chemicals' biofilm penetration. [55] The combination antibiofilm capability of Polyalthia longifolia leaf extracts and penicillin was shown in a study against a clinical MRSA strain, where the former compound synergistically acted while the latter substantially reduced biofilm formation. [56]

**Combination of the natural compound and antimicrobial peptide:**

At a minimal concentration of 50 μg/mL, the ethanolic Glycyrrhiza glabra extract successfully eliminated S. aureus biofilms via glabridin. The antimicrobial peptide ε-poly-L-lysine and glabridin, when combined, increased the biofilm removal activities, making them more effective and extensive. The reason the synergistic effect was thought to occur was because ε-poly-L-lysine, in biofilms, makes microbial cell membranes more permeable. The result would be an upregulation of glabridin intracellular transport, which would raise ROS levels and damage DNA, lipids, and cell structure via oxidative stress. [57]

**Combination of the natural compound and photodynamic therapy:**

As a nonantibiotic microbicidal approach, photodynamic therapy utilizing blue light has been extensively studied for the disinfection of various bacteria, including MRSA. [58] According to Lu et al., a combination of blue light and carvacrol effectively destroyed a wide range of bacteria, including those in plankton, biofilms, and periplasms. The in vitro MRSA biofilm was found to be thinner (from 32.4 μm to 1.7 μm) and the infections caused by it were either fully or partially healed in a study on full-thickness third-degree burn wounds in mice when carvacrol at a concentration of 0.2 mg/mL was used in combination with blue light at 450 nm and 75 J/cm2. Mechanics-wise, what followed showed that photolysis or photosensitization of various photoreactive substrates to carvacrol resulted in potent cytotoxic ROS. [59]

**Combination based on natural compound and nanoparticle:**

Many researches have investigated the antibacterial and antibiofilm properties of nanoparticles (NPs), as well as the possibility of resistance, due to the fast growth of NPs in drug delivery systems. [(60)]

At a concentration of 512 μg/mL, cerium oxide (CeO2) nanoparticles synthesized from Pometia pinnata aqueous leaf extract reduced the S. aureus biofilm by 73%. [(61)] At The development of S. aureus biofilms was suppressed by the 10% Zr/Sn-dual doped sample of the Zr/Sn-dual doped CeO2 NPs at 512 μg/mL, but no antibiofilm effect was seen at lower concentrations of the dual doped NPs. [(61)]

Research on the phyto-fabrication of silver nanoparticles (AgNPs) using Gmelina arborea (GA) extract found that at 1000 μg/mL, the biofilm suppression by aqueous GA leaf extract was 46%. One novel distinguishing feature was the increased antibiofilm activity of GA-AgNPs when put on hydrogel as GA-AgNPs-PF127 (59%), which was already higher than GA-extract's effectiveness. The acidic-basic affinity of Ag+ for sulfur-containing proteins and phosphorous moieties of DNA may be used to enhance bactericidal activity. It is also possible that AgNPs' ability to disrupt bacterial cells is due to their atomicity. [(62)]

**Conclusion and recommendations**

The primary goals of research into antibiofilm agents Phytochemicals, plant extracts, and other naturally occurring substances may disrupt the QS system-targeting regulatory network of biofilm growth, impede biofilm formation, or break down mature biofilm that targets PIA, eDNA, and proteins. When it comes to fighting off long-term bacterial infections, these medicines are very promising. On top of that, natural antibiofilm chemicals are more reliable structurally and functionally than conventional antibacterial treatments, and they may prevent initial bacterial adherence and/or downregulate the expression of genes relevant to biofilms. Some possible new approaches to combating biofilms include phytochemicals, which may block the expression of genes involved in biofilm formation or regulation, alkaloids, which modify PIA and PSM more, terpenes, which target QS, aromatic acids, which interfere with AgrA and SarA, and an anti-adhesion technique. An enhanced approach to treating illnesses linked to biofilms may be the result of future study in this field. In order to screen possible targets for antibiofilm medicines, this preview is very helpful, and the active compounds/combinations shown here demonstrate promise in combating *S. aureus* infections. More study into the underlying processes is required, since most studies have been observational and have only partly addressed the mechanisms of action for certain natural compounds. In order to prevent the evolution of resistance, nanomaterials are constantly being refined to physically damage bacterial cell membranes and biofilm matrix. In addition, most data on the effectiveness of natural compounds against *S. aureus* biofilms comes from in vitro studies; hence, it is critical to create in vivo models that may mimic the biofilm in real disorders. More synergistic activities arising from naturally occurring substances with antibiofilm property are expected to be used by future antibiofilm drugs.

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