Int J Adv Life Sci Res. Volume 9(1) 82-95
https://doi.org/10.31632/ijalsr.2026.v09i01.006

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

In silico Identification of Potential Quorum-Sensing Inhibitors Against the AgrA of *Staphylococcus aureus*

A. Sowmiya[1], Indu Purushothaman[2], K.A. Selvam[3], K. Sangeetha[1*]

[1]*Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Thandalam, Chennai 602105, Tamil Nadu, India*

[2]*Department of Research, Meenakshi Academy of Higher Education and Research, Chennai 600078, Tamil Nadu, India*

[3]*Department of Microbiology, Thiruvalluvar University, Serkkadu, Vellore 632115, Tamil Nadu, India*

**Corresponding Author's Email: sangeethak.sse@saveetha.com*

Abstract

AgrA (Accessory gene regulator) plays a crucial role as a regulator in the agr quorum-sensing system by controlling the production of virulence factors, including exotoxins and enzymes involved in the pathogenesis of *Staphylococcus aureus*. The emergence of resistant strains highlights the urgent need for alternative therapeutic agents. Therefore, in this study, molecular docking of potential phytochemicals was performed against AgrA, followed by the evaluation of pharmacokinetic properties, allergenicity, and toxicity. Binding free energies were predicted using AutoDock Vina 1.5.6. Diosgenin, stigmasterol, hypericin, and betulin exhibited strong binding interactions with critical residues of AgrA, while colchicine, procyanidin B2, and quinine interacted with the transcriptional activation site of AgrA with high binding affinities, forming significant hydrogen bond interactions. The non-toxic, non-allergenic, and favorable pharmacokinetic properties of these compounds suggest that these phytochemicals could be developed into drugs for use in combination with antibiotics as adjuvants or synergists, pending further *in vitro* and *in vivo* evaluation.

Keywords: *AgrA gene, Plant-derived compounds, Quorum-sensing inhibitors, Staphylococcus aureus*

Introduction

Staphylococcus aureus is an opportunistic pathogen and a major public health burden, affecting millions of people worldwide. The spectrum of infections ranges from mild skin infections to severe and persistent conditions such as sepsis, infective endocarditis, and endogenous infections associated with significant morbidity and mortality (Tong *et al*., 2015). *S. aureus* produces toxic shock syndrome toxin-1 (TSST-1), a bacterial superantigen that activates $CD4^+$ T cells and induces the production of large quantities of cytokines, resulting in systemic toxic responses (Heyer *et al*., 2002; Wardenburg *et al.,* 2007).

The growing concern surrounding *S. aureus* infections is driven by increasing resistance to β-lactam antibiotics, including methicillin, as well as to daptomycin, linezolid, ceftaroline, trimethoprim, vancomycin, and mupirocin (Turner *et al*., 2019). Among these, methicillin-resistant *Staphylococcus aureus* (MRSA) represents a major contributor to healthcare-associated infections.

The increasing prevalence of resistant *strains* underscores the need to develop alternative therapeutic approaches. A quorum-sensing (QS)–based antivirulence strategy would be ideal, as the quorum-sensing system in *Staphylococcus aureus* regulates virulence, biofilm formation, colonization, and pathogenesis. This system is governed and modulated by the staphylococcal accessory regulator (Sar)

Received on :16th April 2025; Revised version received on :23rd December 2025; Accepted: 29th December 2025

and the accessory global regulator (Agr) cascades. The Agr regulator is upregulated upon reaching a specific cell density and plays a central role in controlling quorum sensing. The QS system utilizes oligopeptides as signaling molecules to regulate the production of secreted virulence factors. The agr locus consists of RNA II and RNA III transcripts driven by two distinct promoters and is further divided into four components: agrB, agrD, agrC, and agrA (Bronner, Monteil & Prévost, 2004).

In this context, targeting the conserved C-terminal region of the LytTR domain of AgrA in *Staphylococcus aureus* would be optimal, as it plays a critical role in DNA binding within the P2–P3 promoter region of the agr operon. When the extracellular concentration of autoinducing peptide (AIP) reaches a specific threshold, the agr system is activated. The P2 and P3 promoters, along with several other transcriptional targets, are activated when AgrC phosphorylates AgrA following AIP binding (Queck *et al*., 2008). The autoinducing peptide (AI) is an eight–amino acid peptide encoded by the agrD gene, and the accumulation of AIs to a threshold concentration is required for activation of the transcriptional regulator. This process subsequently triggers the expression of multiple genes responsible for the production of virulence factors.

Therefore, suppression of AgrA can effectively reduce virulence, rendering the pathogen more susceptible to both the host immune response and antibiotic treatment. Consequently, the development of agr antagonists through cross-inhibition of autoinducing peptides (AIs) could attenuate the virulence of staphylococcal pathogens. Accordingly, in this study, a library of phytochemical compounds was screened against the target AgrA gene, followed by evaluation of their pharmacokinetic, allergenicity, and toxicity properties.

Materials and Methods

Protein and Ligand retrieval

The structure of Agr A (PDB ID: 4G4K) was derived from the RCSB PDB (https://www.rcsb.org/). The PDB structure was modeled using the Swiss-Model tool (https://swissmodel.expasy.org/), and the three-dimensional structures of 100 Plant-derived compounds showing wound healing properties were derived from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The Open Babel software retrieved all compounds from PDB structures (O'Boyle *et al.,* 2011).

Pharmacokinetic Properties

The Swiss ADME tool was used to predict pharmacokinetic properties of the selected compounds (http://www.swissadme.ch/). Molecular weight, the number of hydrogen bond acceptors and donors, Lipophilicity (Log po/w using ILOGP), water solubility, Gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, and Skin permeation (Log kp) were analyzed. Additionally, the potential inhibition of CYP1A2 was assessed. Drug likeness was evaluated using Lipinski's rule of five, and the bioavailability score was determined (Daina *et al*., 2017).

Molecular Docking

Molecular docking of plant-derived ligands with the target protein AgrA was performed using AutoDock 1.5.6. The three-dimensional structures of the ligands were prepared in PDB format and converted to PDBQT format using AutoDock tools. The AgrA structure in PDB format was processed with AutoDock tools to remove water molecules, add polar hydrogens, assign Kollman charges, and convert it to PDBQT format. Blind docking was employed to explore all potential binding sites across the protein surface. A grid box was manually defined to encompass the entire protein, and the grid parameter file (gpf) was generated using AutoDock 1.5.6 to produce the required grid maps for docking. Subsequently, the docking parameter file (.dpf) was prepared using AutoDock tools, with the Lamarckian Genetic Algorithm selected as the search algorithm. Docking was carried out, and the resulting .dlg files were analyzed. The optimal binding conformation for each ligand was selected based on the lowest binding energy and the number of favorable interactions formed. The final protein–ligand complexes were visualized and analyzed for significant interactions using Discovery Studio Visualizer (Vivek-Ananth *et al*., 2020).

Allergenicity and Toxicity Prediction

Allergenicity prediction of the selected compounds was performed using the ChAlPred web server, which employs machine learning algorithms to identify potential allergenic compounds (https://webs.iiitd.edu.in/raghava/chalpred/) (Sharma *et al*., 2021). Toxicity prediction of the selected compounds was conducted using the ProTox 3.0 web server, which evaluates potential toxic effects based on chemical properties (https://tox.charite.de/) (Banerjee *et al*., 2024).

Results

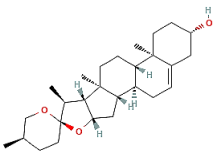
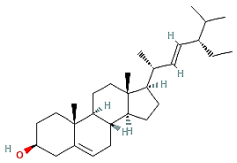
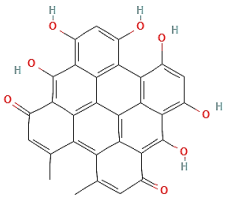
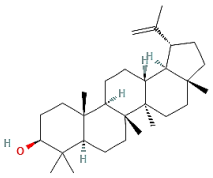
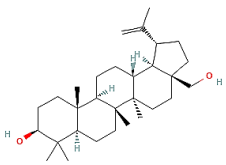
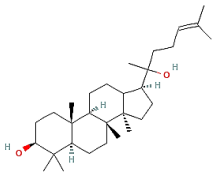
Molecular Docking

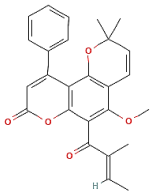
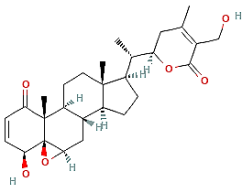
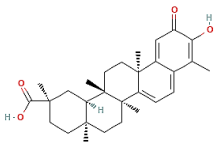
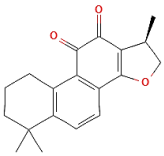
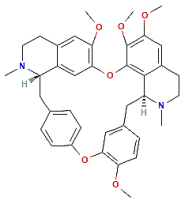
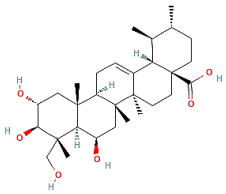
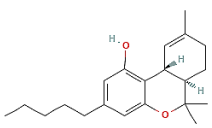
In this study, the binding energies of several plant-derived compounds were determined using AutoDock molecular docking analysis. Diosgenin exhibited the highest binding affinity (−10.94 kcal/mol), followed by stigmasterol (−10.32 kcal/mol). Other compounds with notable binding affinities included hypericin (−9.82 kcal/mol), lupeol (−9.71 kcal/mol), betulin (−9.66 kcal/mol), withaferin A (−9.60 kcal/mol), ginsenosides (−9.37 kcal/mol), callophyloide (−9.25 kcal/mol), celastrol (−9.06 kcal/mol), cryptotanshinone (−8.92 kcal/mol), tetrandrine (−8.79 kcal/mol), medacassic acid (−8.70 kcal/mol), tetrahydrocannabinol (−8.63 kcal/mol), calocedrin (−8.52 kcal/mol), taspine (−8.48 kcal/mol), oxymatrine (−8.48 kcal/mol), and carnosol (−8.40 kcal/mol). Significant binding affinities were also observed for catechin (−7.97 kcal/mol), apigenin (−7.96 kcal/mol), savinin (−7.94 kcal/mol), sanguinarine (−7.92 kcal/mol), naringenin (−7.91 kcal/mol), pinocembrin (−7.78 kcal/mol), and gallocatechin (−7.78 kcal/mol). Among the 100 compounds evaluated, these compounds demonstrated the highest binding affinities, suggesting that they may serve as promising candidates for further *in silico* analysis. The corresponding binding energy values of these compounds are presented in Table 1.

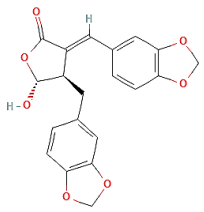
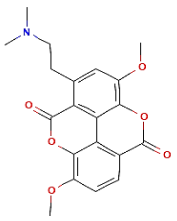
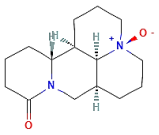
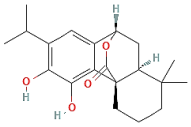
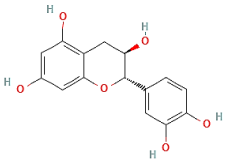
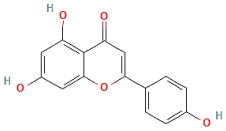
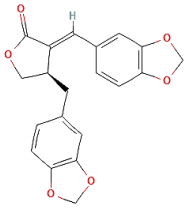
Hydrogen Bond Interactions

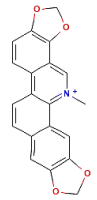
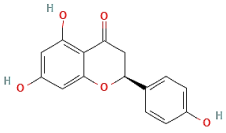
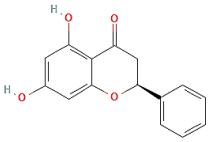
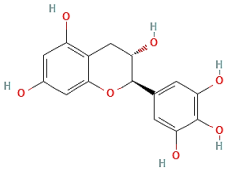
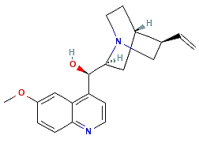
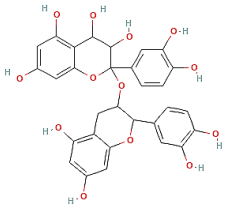
The phytochemicals exhibited characteristic hydrogen-bonding interactions with various amino acid residues, particularly within the LytTR domain of AgrA. Diosgenin formed hydrogen bonds with His227, Asp176, and Asn177, whereas stigmasterol interacted with Asp176. Hypericin formed hydrogen bonds with Asn185, Tyr183, and Thr142, while lupeol did not exhibit any hydrogen-bond interactions. Betulin established a hydrogen bond with Glu188, whereas callophyloide interacted with Tyr153 and His227. Withaferin A formed hydrogen bonds with Thr166, Ser165, Lys167, and His227, while celastrol interacted with Lys146 and Glu144. Cryptotanshinone and taspine both interacted with His227, whereas tetrandrine interacted with Lys146. Medacassic acid formed hydrogen bonds with Glu144 and Asn185, while tetrahydrocannabinol interacted with Asp148. Calocedrin formed hydrogen bonds with Lys167 and Asp158, whereas oxymatrine interacted with His227. Carnosol interacted with Asn185, Glu188, and Lys146, while catechin established hydrogen bonds with Glu144, Tyr183, Tyr156, and Asn185. These interactions highlight the binding capability of these plant-derived compounds through hydrogen bonding with key amino acid residues. The hydrogen-bond interactions of the compounds under investigation are illustrated in Figure 1(a–c) and summarized in Table 1.

Table 1: *Molecular Interaction and Binding Energy of the Compounds Interacting with the Agr A of Staphylococcus aureus*

Sl. No.	Compounds	Structure	Binding energy (Kcal/mol)	Conventional Hydrogen bonds
1	Diosgenin		-10.94	His 227 Asp 176 Asn 177
2	Stigmasterol		-10.32	Asp 176
3	Hypericin		-9.82	Asn 185 Tyr 183 Thr 142
4	Lupeol		-9.71	-
5	Betulin		-9.66	Glu 188
6	Ginsenosides		-9.37	-

7	Callophylloide		-9.25	Tyr 153 His 227
8	Withaferin A		-9.6	Thr 166 Ser 165 Lys 167 His 227
9	Celastrol		-9.06	Lys 146 Glu 144
10	Cryptotanshinone		-8.92	His 227
11	Tetrandrine		-8.79	Lys 146
12	Medecassic acid		-8.7	Glu 144 Asn 185
13	Tetrahydrocannabinol		-8.63	Asp 148

14	calocedrin		-8.52	Lys 167 Asp 158
15	Taspine		-8.48	His 227
16	Oxymatrine		-8.48	His 227
17	Carnosol		-8.4	Asn 185 Glu 188 Lys 146
18	Catechin		-7.97	Glu 144 Tyr 183 Tyr 156 Asn 185
19	Apigenin		-7.96	Glu 188 Asn 185 Tyr 183 Thr 142
20	Savinin		-7.94	Asn 185 Glu 144

21	Sanguinarine		-7.92	-
22	Naringenin		-7.91	Asn 185 Tyr 183 Lys 146 Thr 142
23	Pinocembrin		-7.78	Asn 185 Tyr 183 Lys 146
24	Gallocatechin		-7.78	Tyr 183 Asn 185 Glu 144 Tyr 156
25	Quinine		-7.97	Asn 177 Tyr 229
26	ProcyanadinB2		-6.88	Glu 181 Lys 216 Glu 217 Arg 218 Arg 178 Gln 179

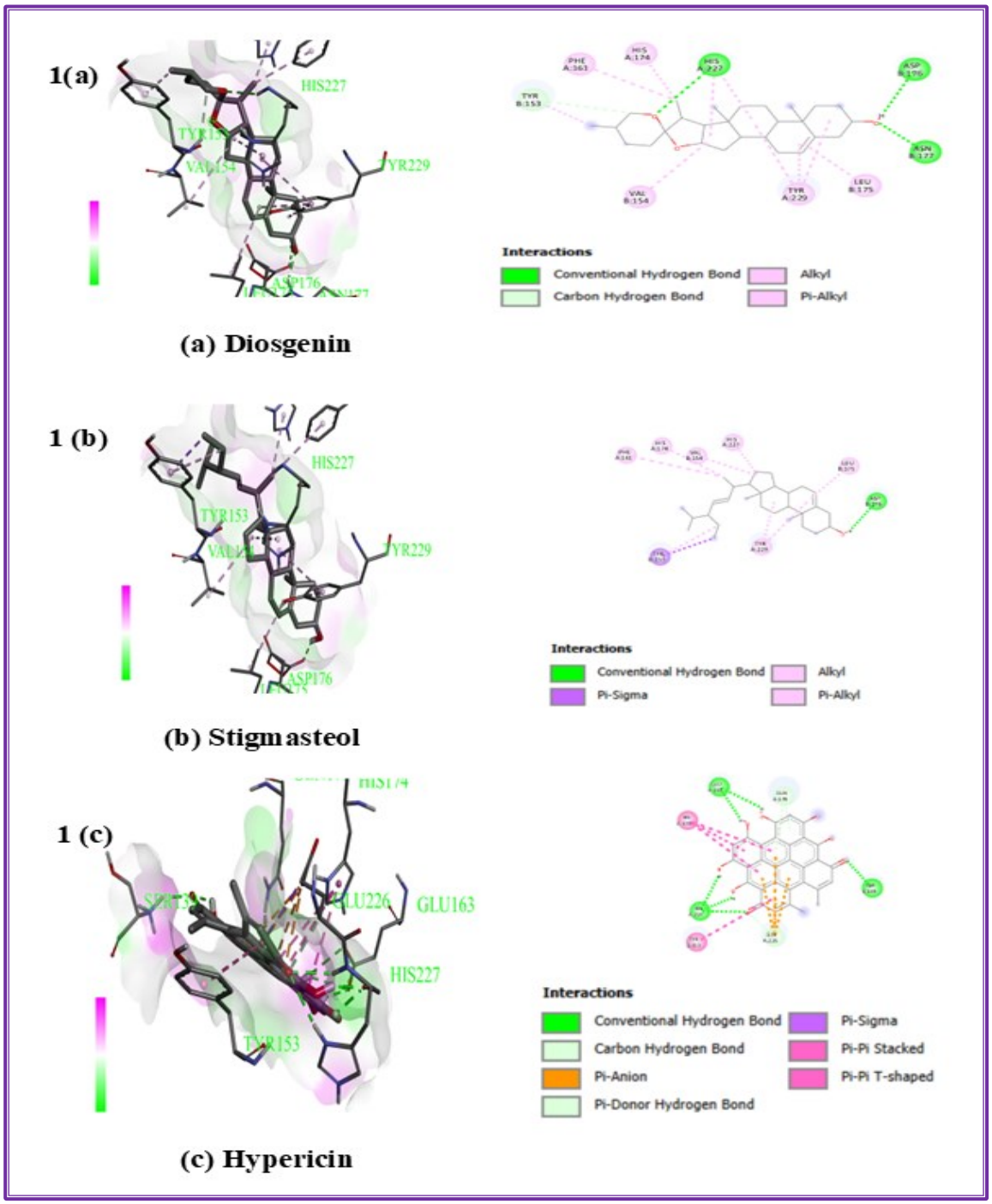


Figure 1: *Molecular Interactions between AgrA and Selected Phytocompounds: (a) Diosgenin Forming Hydrogen Bonds with His227, Asp176, and Asn177; (b) Stigmasterol Showing a Single Hydrogen Bond with Asp176; (c) Hypericin Interacting with Asn185, Tyr183, and Thr142*

Pharmacokinetic Properties

Diosgenin has a Molecular weight of 414.62 g/mol, featuring 3 hydrogen bond acceptors and a single hydrogen bond donor. It possesses a log Po/w value of 4.49, suggesting moderate lipophilicity and solubility in water. It demonstrated significant gastrointestinal (GI) absorption and can cross the blood-brain barrier (BBB), yet it does not inhibit CYP1A2. Its skin permeation coefficient (Log Kp) is measured to be -4.80 cm/s. Diosgenin adheres to Lipinski's rule of five with a single infraction (MLOGP > 4.15) and possesses a bioavailability rating of 0.55.

Stigmasterol possesses has a molecular weight of 4.12.69 g/mol, featuring 1 hydrogen bond acceptor and 1 hydrogen bond donor. It has a log Po/w value of 5.08, which increased its lipophilicity. It is slightly soluble in water but exhibits low gastrointestinal absorption and does not pass through the blood-brain barrier. It does not block CYP1A2, and its Log kp measures -2.74 cm/s. Stigmasterol violated Lipinski's rule as MLOGP exceeds 4.15 and possesses a bioavailability score of 0.55. The pharmacokinetic characteristics of the other compounds are presented in Table 2.

Table 2: *Pharmacokinetic Properties of Plant-Derived Compounds*

Sl. No	Plant Compounds	Molecular Weight (g/mol)	Num. H-bond Acceptors	Num. H-bond Donors	Lipophilicity	Water Solubility	GI Absorption	BBB Permeant	CYP 1A2 Inhibitor	Log Kp (cm/s)	Lipinski	Bioavailability
1	Diosgenin	414.62	3	1	4.49	Moderately soluble	High	Yes	No	-4.80	1	0.55
2	Stigmasterol	412.69	1	1	5.08	Moderately soluble	Low	No	No	-2.74	1	0.55
3	Hypericin	504.44	8	6	3.10	Poorly soluble	Low	No	No	-5.32	2	0.17
4	Lupeol	426.72	1	1	4.72	Poorly soluble	Low	No	No	-1.90	1	0.55
5	Betulin	442.72	2	2	4.47	Poorly soluble	Low	No	No	-3.12	1	0.55
6	Ginsenosides	444.73	2	2	5.01	Poorly soluble	Low	No	No	-2.95	1	0.55
7	Callophyloide	416.47	5	0	3.68	Poorly soluble	High	No	No	-5.39	0	0.55
8	Withaferin A	470.60	6	2	3.24	Soluble	High	No	No	-6.45	0	0.55
9	Celastrol	450.61	4	2	3.17	Moderately soluble	Low	No	No	-4.83	1	0.85
10	Cryptotanshinone	296.36	3	0	2.81	Moderately soluble	High	Yes	Yes	-5.41	0	0.85
11	Tetrandrine	622.75	8	0	5.23	Insoluble	High	No	No	-5.37	1	0.55
12	Medecassic Acid	504.70	6	5	3.04	Soluble	High	No	No	-6.28	1	0.56
13	Tetrahydrocannabinol	314.46	2	1	4.15	Moderately soluble	High	Yes	No	-3.27	1	0.55
14	Calocedrin	368.34	7	1	0.00	Moderately soluble	High	No	No	-6.35	0	0.55
15	Taspine	369.37	7	0	3.10	Poorly soluble	High	No	Yes	-6.59	0	0.55
16	Oxymatrine	264.36	2	0	1.71	Soluble	High	Yes	No	-7.12	0	0.55
17	Carnosol	330.42	4	2	2.93	Moderately soluble	High	Yes	No	-5.21	0	0.55
18	Catechin	290.27	6	5	1.33	Soluble	High	No	No	-7.82	0	0.55
19	Apigenin	270.24	5	3	1.89	Moderately soluble	High	No	Yes	-5.80	0	0.55
20	Savinin	352.34	6	0	3.14	Moderately soluble	High	Yes	Yes	-5.88	0	0.55
21	Sanguinarine	332.33	4	0	0.04	Poorly soluble	High	Yes	Yes	-5.17	0	0.55
22	Naringenin	272.25	5	3	1.75	Soluble	High	No	Yes	-6.17	0	0.55
23	Pinocembrin	256.25	4	2	2.11	Soluble	High	Yes	Yes	-5.82	0	0.55
24	Gallocatechin	306.27	7	6	1.47	Soluble	High	No	No	-8.17	1	0.55
25	Quinine	324.42	4	1	3.36	Moderately soluble	High	Yes	No	-6.23	0	0.55
26	Procyanidin B2	578.52	12	10	1.35	Soluble	Low	No	No	-8.15	3	0.17

Allergenicity and Toxicity Prediction

The evaluation of allergenicity and toxicity showed that all examined plant compounds were non-allergenic and non-toxic. Diosgenin (0.17), Stigmasterol (0.23), Hypericin (0.19), Lupeol (0.25), and Betulin (0.22) were recognized as non-allergenic substances with no toxic effects, classified within toxicity classes 4 to 6. In the same manner, Ginsenosides (0.19), Callophyloide (0.24), and Withaferin A (0.29) displayed non-allergenic characteristics as non-toxic in class 3. Celastrol (0.27), Cryptotanshinone (0.09), Tetrandrine (0.24), Medacassic acid (0.24), and Tetrahydrocannabinol (0.18) similarly showed non-allergenic and non-toxic properties. Moreover, Calocedrin (0.25), Taspine (0.28), Oxymatrine (0.20), Carnosol (0.21), Catechin (0.21), and Apigenin (0.26) were found to be non-toxic and non-allergenic, with their toxicity classifications between 4 and 6. Additionally, Savinin (0.15), Sanguinarine (0.21), Naringenin (0.17), Pinocembrin (0.13), and Gallocatechin (0.21) exhibited a similar pattern, validating their non-allergenic and non-toxic characteristics. The toxicity and allergenicity profiles of the investigated plant compounds are shown in Table 3.

Table 3: *Allergenicity and Toxicity Assessment of Plant-Derived Compounds*

S. No	Plant compounds	Allergenicity	Score	Toxicity	Class
1	Diosgenin	Non-allergen	0.17	Non-toxic	6
2	Stigmasterol	Non-allergen	0.23	Non-toxic	4
3	Hypericin	Non-allergen	0.19	Non-toxic	4
4	Lupeol	Non-allergen	0.25	Non-toxic	4
5	Betulin	Non-allergen	0.22	Non-toxic	4
6	Ginsenosides	Non-allergen	0.19	Non-toxic	5
7	Callophyloide	Non-allergen	0.24	Non-toxic	5
8	Withaferin A	Non-allergen	0.29	Non-toxic	3
9	Celastrol	Non-allergen	0.27	Non-toxic	4
10	Cryptotanshinone	Non-allergen	0.09	Non-toxic	6
11	Tetrandrine	Non-allergen	0.24	Non-toxic	4
12	Medecassic acid	Non-allergen	0.24	Non-toxic	4
13	Tetrahydrocannabinol	Non-allergen	0.18	Non-toxic	4
14	calocedrin	Non-allergen	0.25	Non-toxic	4
15	Taspine	Non-allergen	0.28	Non-toxic	4
16	Oxymatrine	Non-allergen	0.2	Non-toxic	4
17	Carnosol	Non-allergen	0.21	Non-toxic	4
18	Catechin	Non-allergen	0.21	Non-toxic	6
19	Apigenin	Non-allergen	0.26	Non-toxic	5
20	Savinin	Non-allergen	0.15	Non-toxic	4
21	Sanguinarine	Non-allergen	0.21	Non-toxic	4
22	Naringenin	Non-allergen	0.17	Non-toxic	4
23	Pinocembrin	Non-allergen	0.13	Non-toxic	4
24	Gallocatechin	Non-allergen	0.21	Non-toxic	6
25	Quinine	Non-allergen	0.18	Toxic	3
26	ProcyanadinB2	Non-allergen	0.17	Non-toxic	5

Discussion

The agr quorum-sensing gene represents an optimal drug target for inhibiting *Staphylococcus aureus*, as its deletion leads to reduced virulence and plays a critical role in the early stages of abscess formation (Abdelnour *et al*., 1993; Booth *et al*., 1995; Das *et al*., 2016; Gajdács & Spengler, 2019; Wright III *et al*., 2005). By regulating toxin production and key pathogenic processes in *S. aureus*, the Agr quorum-sensing (QS) system represents a viable target for non-antibiotic therapeutic interventions (Yamaguchi *et al*., 2024). Accordingly, in this study, the AgrA gene of *Staphylococcus aureus* was targeted using phytocompounds through an in-silico approach to inhibit quorum sensing and regulate biofilm formation. The compounds evaluated interacted with the AgrA LytTR domain (Asp137 to Ile238) by forming two or three hydrogen bonds. These interactions involved residues ranging from Glu141 to Ile238 in the A chain or from Ser139 to Ile238 in the B chain. Notably, none of the compounds interacted with residues involved in conserved salt bridge formations, including Glu141–Arg195, Asp157–His208, Asp193–Arg195, Asp157–Arg195, and His174–Glu226. Additionally, the apo AgrA protein adopted a

β–β–β sandwich fold, consistent with previous observations in the DNA-bound state. Overall, this study presents a novel strategy for quorum-sensing inhibition and biofilm control by targeting the conserved C-terminal LytTR domain of AgrA, providing a mechanistic foundation for the rational design of antivirulence therapeutics.

This research strongly supports targeting the conserved C-terminal region of the LytTR domain of AgrA in *Staphylococcus aureus*, as this region is essential for binding to the P2–P3 promoter region of the agr operon, which regulates quorum sensing and virulence factor expression (Nicod *et al*., 2014; Patel & Rawat, 2023). Notably, the C-terminal loop of AgrA contains a hydrophobic cleft capable of accommodating small-molecule fragments, and binding at this site has been shown to inhibit the DNA-binding activity of the protein (Manu *et al*., 2024). Colchicine, Procyanadin B2, and quinine interacted with the transcriptional activation site residues Leu171 and Glu181, as well as with the highly conserved amino acid residue Tyr229, respectively. Additionally, artemisinin exhibited a promising binding profile through interaction with His227, while catechin interacted with residues such as Glu144, Tyr183, and Asn185. These hydrogen-bond interactions with key residues are believed to inhibit virulence factor production and biofilm formation by disrupting the conformational changes required for AgrA activation. Importantly, compounds including epicatechin, resveratrol, and quercetin also interacted with critical residues such as Glu144, Asn185, and Tyr183.

Berberine inhibits biofilm formation in methicillin-resistant *Staphylococcus aureus* by modulating the expression of the quorum-sensing genes agrA, agrB, agrC, and agrD, which subsequently alters the production of extracellular proteins and virulence factors (Zhou *et al*., 2025; Xia *et al*., 2022). Notably, molecular docking analyses revealed that neither cineole nor berberine exhibited strong binding affinities toward key residues within the AgrA active site, suggesting that their inhibitory effects are likely mediated through indirect mechanisms or upstream regulatory pathways rather than direct interaction with AgrA. Consequently, the quorum-sensing inhibitors identified in this study may be used as adjuvants to existing antibiotic therapies (Qader *et al*., 2025; Ramasamy *et al*., 2023). Although colchicine exhibited interactions with the target AgrA, its potential negative effects on the clinical manifestation of staphylococcal infections—by altering interactions between *S. aureus* and osteoblasts—raise concerns regarding its direct therapeutic use. Therefore, further *in vitro* and *in vivo* investigations are required (Baysal *et al*., 2019). Larch bark procyanidin was shown to inhibit bacterial growth at a minimum inhibitory concentration of 1.75 mg/mL and to bind within the DNA major groove, further supporting our findings regarding its interaction with the transcriptional activation site (Li *et al*., 2017). Similarly, artemisinin and quinine derivatives have demonstrated substantial antibacterial activity against Staphylococcus aureus. However, the remaining compounds evaluated in this study have not yet been explored for antibacterial activity specifically targeting the agr gene. Therefore, further investigation of these compounds may be promising, as it could contribute to reducing antimicrobial resistance and mitigating staphylococcal pathogenesis.

Previously identified compounds that inhibit agr-mediated quorum sensing include Savinin (a virulence inhibitor of *S. aureus*), its analogues (*S. aureus* virulence inhibitors), Staquorsin, and a new analog, triazoloquinazoline derivative (Gordon, Williams, & Chan, 2013; Sully *et al.*, 2014). Savinin has been shown to bind AgrC, thereby preventing the activation of AgrA, and also to interact directly with AgrA at a downstream stage of the quorum-sensing pathway. This dual interaction disrupts agr operon activity and suppresses virulence gene expression in *S. aureus* (Cui & Kim, 2024; Otto, 2023). Staquorsin, which contains a phthalazine nucleus, was identified as a suitable substitute for the quinazoline ring of savinin and demonstrated promising *in vitro* Agr activity. Similarly, the l-isomer of 3-oxo-C12-HSL 1 inhibited agr with an IC_{50} value of 22 ± 6 μM, whereas the d-isomer 2 was approximately twofold less active, with an IC_{50} of 37 ± 9 μM (Murray *et al*., 2014). Bumetanide interacted with the conserved amino acid Tyr229 of AgrA, exhibiting 70% AgrA inhibition at 0.1 μM, while also suppressing the expression of other virulence genes (Touati *et al*., 2025).

All plant-derived compounds subjected to *in silico* analysis were found to be non-toxic and non-allergenic. The limited intestinal absorption of several compounds identified in this study, including

chrysin and luteolin, may limit their effectiveness as oral medications. However, their bioavailability and clinical applicability could be enhanced through the use of absorption enhancers or alternative routes of administration, such as parenteral delivery. In addition, compounds such as Epilobium and catechin exhibit favorable safety profiles as potential therapeutic agents due to their non-hepatotoxic nature and lack of interaction with CYP2D6. Similarly, the lead compounds with higher binding energies demonstrated desirable pharmacokinetic properties.

This study lacks experimental validation, and the evaluation of phytocompounds could be expanded to include a broader range of bioactive molecules. Compounds exhibiting weaker binding affinities could also be explored against alternative molecular targets. Additionally, the screened phytochemicals with promising activity may serve as lead scaffolds for structure-based drug design and optimization, allowing improvements in binding affinity, selectivity, and pharmacokinetic properties through chemical modification and molecular dynamics simulations. Therefore, further investigations are required to compare the efficacy of newly identified compounds with known AgrA inhibitors, such as savinin, staquorsin, and bumetanide, in order to establish structure–activity relationships and identify potential synergistic mechanisms.

Overall, the in-silico findings suggest that these plant-derived compounds have strong potential as inhibitors of the agr quorum-sensing system in *Staphylococcus aureus*, pending further *in vitro* and *in vivo* validation, either as adjuvants or synergistic agents.

Conclusion

The in-silico results indicate that plant-derived compounds have significant potential as inhibitors of the AgrA-mediated quorum-sensing system in *Staphylococcus aureus*; however, as an inherent limitation of computational screening, these findings require experimental validation through *in vitro* and *in vivo* assays to confirm binding efficacy and biological activity. The identified compounds, which exhibit strong binding interactions with critical residues involved in AgrA activation, offer a promising strategy to inhibit pathogenic protein production and biofilm formation. Furthermore, these compounds may enhance the efficacy of existing antibiotics when used in combination as adjuvants or synergistic agents.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgement

The authors acknowledge the SIMATS, Engineering, for providing the necessary infrastructure, facilities, and support to carry out this study.

References

Abdelnour, A., Arvidson, S., Bremell, T., Ryden, C., & Tarkowski, A. (1993). The accessory gene regulator (agr) controls Staphylococcus aureus virulence in a murine arthritis model. *Infection and Immunity, 61*(9), 3879-3885. https://doi.org/10.1128/iai.61.9.3879-3885.1993

Banerjee, P., Kemmler, E., Dunkel, M., & Preissner, R. (2024). ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research, 52*(W1), W513-W520. https://doi.org/10.1093/nar/gkae303

Baysal, N. B., Emecen, A. N., Arslan, F., & Vahaboğlu, H. (2019). Metisilin Dirençli Staphylococcus aureus Septik Artriti Olgu Sunumu: Kolşisinin Olası Etkisi [Case Report of Methicillin-Resistant Staphylococcus aureus Septic Arthritis: Possible Effects of Colchicine]. *Mediterranean Journal of Infection Microbes and Antimicrobials, 8*(1), 12. https://doi.org/10.4274/mjima.galenos.2019.2019.12

Booth, M. C., Atkuri, R. V., Nanda, S. K., Iandolo, J. J., & Gilmore, M. S. (1995). Accessory gene regulator controls Staphylococcus aureus virulence in endophthalmitis. *Investigative Ophthalmology & Visual Science, 36*(9), 1828-1836. https://iovs.arvojournals.org/article.aspx?articleid=2180197

Bronner, S., Monteil, H., & Prévost, G. (2004). Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. *FEMS Microbiology Reviews, 28*(2), 183-200. https://doi.org/10.1016/j.femsre.2003.09.003

Cui, S., & Kim, E. (2024). Quorum sensing and antibiotic resistance in polymicrobial infections. *Communicative & Integrative Biology, 17*(1), 2415598. https://doi.org/10.1080/19420889.2024.2415598

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*(1), 42717. https://doi.org/10.1038/srep42717

Das, S., Dasgupta, A., & Chopra, S. (2016). Drug repurposing: a new front in the war against Staphylococcus aureus. *Future Microbiology, 11*(8), 1091-1099. https://doi.org/10.2217/fmb-2016-0021

Gajdács, M., & Spengler, G. (2019). The role of drug repurposing in the development of novel antimicrobial drugs: Non-antibiotic pharmacological agents as quorum sensing-inhibitors. *Antibiotics, 8*(4), 270. https://doi.org/10.3390/antibiotics8040270

Gordon, C. P., Williams, P., & Chan, W. C. (2013). Attenuating Staphylococcus aureus virulence gene regulation: a medicinal chemistry perspective. *Journal of Medicinal Chemistry, 56*(4), 1389-1404. https://doi.org/10.1021/jm3014635

Heyer, G., Saba, S., Adamo, R., Rush, W., Soong, G., Cheung, A., & Prince, A. (2002). Staphylococcus aureus agr and sarA functions are required for invasive infection but not inflammatory responses in the lung. *Infection and Immunity, 70*(1), 127-133. https://doi.org/10.1128/iai.70.1.127-133.2002

Kar, A., Mukherjee, S. K., & Hossain, S. T. (2025). Quorum sensing mediated attenuation of biofilm formation and virulence traits in Staphylococcus aureus by trigonelline. *Microbial Pathogenesis, 205,* 107731. https://doi.org/10.1016/j.micpath.2025.107731

Li, X., He, C., Song, L., Li, T., Cui, S., Zhang, L., & Jia, Y. (2017). Antimicrobial activity and mechanism of Larch bark procyanidins against Staphylococcus aureus. *Acta Biochimica et Biophysica Sinica, 49*(12), 1058-1066. https://doi.org/10.1093/abbs/gmx112

Manu, P., Nketia, P. B., Osei-Poku, P., & Kwarteng, A. (2024). Computational mutagenesis and inhibition of Staphylococcus aureus AgrA LytTR domain using phenazine scaffolds: Insight from a biophysical study. *BioMed Research International, 2024*(1), 8843954. https://doi.org/10.1155/2024/8843954

Murray, E. J., Crowley, R. C., Truman, A., Clarke, S. R., Cottam, J. A., Jadhav, G. P., ... & Williams, P. (2014). Targeting Staphylococcus aureus quorum sensing with nonpeptidic small molecule inhibitors. *Journal of Medicinal Chemistry, 57*(6), 2813-2819. https://doi.org/10.1021/jm500215s

Nicod, S. S., Weinzierl, R. O., Burchell, L., Escalera-Maurer, A., James, E. H., & Wigneshweraraj, S. (2014). Systematic mutational analysis of the LytTR DNA binding domain of Staphylococcus aureus virulence gene transcription factor AgrA. *Nucleic Acids Research, 42*(20), 12523-12536. https://doi.org/10.1093/nar/gku1015

Otto, M. (2023). Critical assessment of the prospects of quorum-quenching therapy for Staphylococcus aureus infection. *International Journal of Molecular Sciences, 24*(4), 4025. https://doi.org/10.3390/ijms24044025

Patel, H., & Rawat, S. (2023). A genetic regulatory see-saw of biofilm and virulence in MRSA pathogenesis. *Frontiers in Microbiology, 14,* 1204428. https://doi.org/10.3389/fmicb.2023.1204428

Qader, T. A., Ali, M. R., & Alsakini, A. H. (2025). Genotyping and siRNA-Mediated quorum sensing inhibition in Staphylococcus aureus: Exploring new approaches for treating persistent infections. *Journal of Bioscience and Applied Research, 11*(1), 196-213. https://doi.org/10.21608/jbaar.2025.419043

Queck, S. Y., Jameson-Lee, M., Villaruz, A. E., Bach, T. H. L., Khan, B. A., Sturdevant, D. E., ... & Otto, M. (2008). RNAIII-independent target gene control by the agr quorum-sensing system: insight into the evolution of virulence regulation in Staphylococcus aureus. *Molecular Cell, 32*(1), 150-158. https://doi.org/doi:10.1016/j.molcel.2008.08.005

Ramasamy, M., Vetrivel, A., Venugopal, S., & Murugesan, R. (2023). Identification of inhibitors for Agr quorum sensing system of Staphylococcus aureus by machine learning, pharmacophore modeling, and molecular dynamics approaches. *Journal of Molecular Modeling, 29*(8), 258. https://doi.org/10.1007/s00894-023-05647-9

Sharma, N., Patiyal, S., Dhall, A., Devi, N. L., & Raghava, G. P. (2021). ChAlPred: a web server for prediction of allergenicity of chemical compounds. *Computers in Biology and Medicine, 136*, 104746. https://doi.org/10.1016/j.compbiomed.2021.104746

Sully, E. K., Malachowa, N., Elmore, B. O., Alexander, S. M., Femling, J. K., Gray, B. M., ... & Gresham, H. D. (2014). Selective chemical inhibition of agr quorum sensing in Staphylococcus aureus promotes host defense with minimal impact on resistance. *PLoS Pathogens, 10*(6), e1004174. https://doi.org/10.1371/journal.ppat.1004174

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews, 28*(3), 603-661. https://doi.org/10.1128/cmr.00134-14

Touati, A., Ibrahim, N. A., & Idres, T. (2025). Disarming Staphylococcus aureus: Review of Strategies Combating This Resilient Pathogen by Targeting Its Virulence. *Pathogens, 14*(4), 386. https://doi.org/10.3390/pathogens14040386

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., ... & Fowler Jr, V. G. (2019). Methicillin-resistant Staphylococcus aureus: An overview of basic and clinical research. *Nature Reviews Microbiology, 17*(4), 203-218. https://doi.org/10.1038/s41579-018-0147-4

Vivek-Ananth, R. P., Rana, A., Rajan, N., Biswal, H. S., & Samal, A. (2020). *In silico* identification of potential natural product inhibitors of human proteases key to SARS-CoV-2 infection. *Molecules, 25*(17), 3822. https://doi.org/10.3390/molecules25173822

Wardenburg, J. B., Patel, R. J., & Schneewind, O. (2007). Surface proteins and exotoxins are required for the pathogenesis of Staphylococcus aureus pneumonia. *Infection and Immunity, 75*(2), 1040-1044. https://doi.org/10.1128/iai.01313-06

Wright III, J. S., Jin, R., & Novick, R. P. (2005). Transient interference with staphylococcal quorum sensing blocks abscess formation. *Proceedings of the National Academy of Sciences, 102*(5), 1691-1696. https://doi.org/10.1073/pnas.0407661102

Xia, S., Ma, L., Wang, G., Yang, J., Zhang, M., Wang, X., ... & Xie, M. (2022). *In vitro* antimicrobial activity and the mechanism of berberine against methicillin-resistant Staphylococcus aureus isolated from bloodstream infection patients. *Infection and Drug Resistance, 15,* 1933-1944. https://doi.org/10.2147/IDR.S357077

Yamaguchi, J., Manome, T., Hara, Y., Yamazaki, Y., Nakamura, Y., Ishibashi, M., & Takaya, A. (2024). Physalin H, physalin B, and isophysalin B suppress the quorum-sensing function of Staphylococcus aureus by binding to AgrA. *Frontiers in Pharmacology, 15*, 1365815. https://doi.org/10.3389/fphar.2024.1365815

Zhou, K., Shi, M., Chen, R., Zhang, Y., Sheng, Y., Tong, C., ... & Shou, D. (2025). Natural phytochemical-based strategies for antibiofilm applications. *Chinese Medicine, 20*(1), 96. https://doi.org/10.1186/s13020-025-01147-5