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Original Research Article

Targeting *Staphylococcus aureus* from Wounds Infections: Experimental Evaluation of Clove Extract and In Silico Docking of Eugenol

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Abstract: ***Background:*** The rapid emergence and spread of multidrug-resistant *Staphylococcus aureus* (MRSA), especially strains isolated from wounds, represent a major clinical challenge worldwide. This study aims to elucidate the molecular basis of eugenol's activity by investigating its binding interactions with key therapeutic targets in *S. aureus,* also evaluating the antibacterial effect of Clove extract on pathogenic *S. aureus*. ***Methods****:* Swabs from wound infection were taken and cultured on Blood, MacConkey and mannitol agar, then incubated in 37 C for 24 hours. The obtained S. aureus isolates then used for plant extract antibacterial tests. The ethanolic extract of Clove plant (*Syzygium aromaticum*) by mean of three concentrations (25, 50 and 75 mg/dl) were used to evaluate their antibacterial efficiency against *Staphylococcus aureus* that isolated from wound infection. By using the molecular docking, the molecular basis of eugenol's activity were elucidate by investigating its binding interactions with key therapeutic targets in *S. aureus*: Penicillin-binding protein 2a (PBP2a, PDB ID: 1VQQ), Beta-lactamase (PDB ID: 6WGR), Dihydrofolate reductase (DHFR, PDB ID: 6PR6), and the CHAP domain of lysin L1 (PDB ID: 11CF). ***Results****:* The results showed that large inhibition diameter (27 mm) was observed surrounding the high concentration of plant extract (75 mg/dL), followed by 50 mg/dl with (23mm), and 20 mg/dl (18mm). The negative control didn't showed any zone of inhibition. The molecular docking study revealed moderate to good binding affinities of eugenol with the selected *S. aureus* proteins, with Vina scores ranging from -5.5 to -6.2 kcal/mol. Eugenol exhibited the strongest binding at -6.2 kcal/mol ***Conclusion****:* The high concentration of Clove extract exhibits good efficiency against *S. aureus*. Molecular docking supports these findings by investigating favorable interaction between Eugenol and bacterial essential protein. These revealed that Clove plant with their bioactive compounds has a potential role as antibacterial agents against pathogenic bacteria.

Keywords: *Syzygium aromaticum, Staphylococcus aureus,* Beta-lactamase, molecular docking.

1. INTRODUCTION

In the field of biomedicine, wounds are a crucial entry point for the body's healing process, but they also provide a serious risk of microbial invasion. Wounds are prone to infection, whether they are acute (caused by trauma or surgery) or chronic (related to conditions like diabetes), which makes the natural healing process difficult and time-consuming. This may result in non-functioning scar tissue, delayed healing, or even systemic sepsis (Linz *et al.,* 2023; Taylor & Unakal, 2023).

As the most prevalent bacteria in wounds and lesions to the skin, Staphylococcus aureus stands out as one of the most significant pathogens in this context. Cytotoxic, cell wall-associated proteins, and the ability to form a biofilm that protects it from the immune system and antibiotics are just a few of the many virulence characteristics of this organism (Tong *et al.,* 2015). This bacterium can target acute infections in the pores and skin and smooth tissues, which can undoubtedly result in fatal diseases such as bacterial disease, osteomyelitis, or toxic surprise syndrome c. Aureus is one of the leading causes of bacterial mortality, accounting for approximately 1.1 million contamination-related deaths worldwide

in 2019 (Ikuta *et al.,* 2022). Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA) spores, which transform the wound into a common treatment-resistant environment, inhabit the wound healing system, enhance scientific values, and increase the morbidity and mortality of increase the chances (Almuhayawi *et al.,* 2023). These problems make it important to find safe, green natural treatment options that can fight infections without encouraging microbial resistance.

In this regard, clove extract (Syzygium aromaticum) shows promise as a remedy that strikes a compromise between conventional medicine and contemporary scientific data. Because of its analgesic, anti-inflammatory, and antibacterial qualities, clove has been utilised in traditional medicine for ages. According to recent research (Haro-González *et al.,* 2021; Kennewell *et al.,* 2019), it can facilitate wound healing by lowering the bacterial load and promoting granulation and collagen synthesis.

The primary active component of clove extract, eugenol, which usually makes up 70–90% of the essential oil, is what gives it its significance. Eugenol has a complex mode of action: it breaks down the integrity of bacterial cell membranes, allowing essential cellular contents to leak out; it prevents the synthesis of proteins and DNA; it lessens the formation of biofilms; and its anti-inflammatory and antioxidant qualities aid in the healing process (Xu *et al.,* 2016). Research has shown that it is quite effective against Streptococcus species. S.aureus is a perfect option for creating novel topical treatments for infected wounds since it contains resistant strains (Yadav *et al.,* 2015).

Eugenol, the primary bioactive phenylpropanoid found in clove essential oil (*Syzygium aromaticum*), constitutes 70–90% of the oil and is widely recognized as the main compound responsible for its potent antibacterial activity (Xu *et al.,* 2016; Li *et al.,* 2022). Previous studies have demonstrated that eugenol exerts significant inhibitory effects against *S. aureus* through multiple mechanisms, including disruption of cell membrane integrity, inhibition of biofilm formation, and suppression of virulence factors (Elbestawy *et al.,* 2023). This study aims to elucidate the molecular basis of eugenol's activity by investigating its binding interactions with key therapeutic targets in *S. aureus*: Penicillin-binding protein 2a (PBP2a, PDB ID: 1VQQ), Beta-lactamase (PDB ID: 6WGR), Dihydrofolate reductase (DHFR, PDB ID: 6PR6), and the CHAP domain of lysin L1 (PDB ID: 11CF). These proteins play critical roles in antibiotic resistance, cell wall synthesis, folate metabolism, and peptidoglycan hydrolysis.

2. MATERIALS AND METHODS

Isolation and identification of *S. aureus*

Swabs from wound infection were taken and cultured on Blood, MacConkey and mannitol agar, then incubated in 37 C for 24 hours. The obtained S. aureus isolates then used for plant extract antibacterial tests.

Preparation of plant extract

The S. aromaticum buds were detached, cleaned, and allowed to dry in the shade. The plant extract was made by soaking 800 g of dry plant powder in 2.5 L of 70% ethanol for 8–10 days. A sterile glass rod was used to agitate the mixture every 10 hours. It was run through Whatman filter paper no. 1 (Whatman Ltd., England) after extraction. After concentrating the ethanoic filtrate in a water bath at 40°C until a sticky semisolid mass was produced, it was kept at 4°C for further use. In order to create a stock solution with a concentration of 100 mg/ml, a known quantity of the dry extract was dissolved in dimethyl sulfoxide (DMSO) (Ahmad and Aqil, 2007).

Antimicrobial proprieties of Clove extract

Sterile swabs were used to disseminate the S. aureus inoculum (1.5 x108 CFU/ml) on agar plates. The agar plates containing the bacterial inoculum were divided into four 8 mm-sized wells using a sterile borer, and the lower part was sealed with a small amount of melted agar media. A well of infected plates was filled with 0.04 ml of various plant extract concentrations (25, 50, and 75 mg/dl). As a negative control, sterilised distilled water was added to a fourth well in place of the plant extract from Syzygium aromaticum. In order to allow the extracts to diffuse into the agar, the prepared plates were left at room temperature for 10 minutes. The plates were examined following a 24-hour incubation period at 37 C. An inhibitory zone encircling the well holding the plant extract demonstrated the presence of antibacterial action on the plates. Millimeters were used to measure and express the Diameter of Inhibition Zone (DIZ). The Ajobiewe *et al.,* (2022) approach was used to obtain the mean values of the diameter of inhibitory zones.

Molecular Docking

The crystal structures of the target proteins were retrieved from the RCSB Protein Data Bank: PBP2a (PDB ID: 1VQQ), Beta-lactamase (PDB ID: 6WGR), Dihydrofolate reductase (PDB ID: 6PR6), and the CHAP domain of lysin L1 (PDB ID: 11CF). The use of Discovery Studio Visualizer v21.1 was prepared to remove water molecules, meaningless ions, and pre-existing ligands from the protein system, with the addition of hydrogen atoms and the assignment of Gasteiger partial charges. The grid box parameters for each protein were defined as follows:

For Beta-lactamase (PDB ID: 6WGR, Pocket C1): center coordinates (19, 29, 43) Å, grid box size 35 × 34 × 30 Å ;For Dihydrofolate reductase (DHFR) (PDB ID: 6PR6, Pocket C1): center coordinates (0, 32, -8) Å, grid box size 30 × 18 × 18 Å ;For PBP2a (PDB ID: 1VQQ, Pocket C1): center coordinates (8, 38, 31) Å, grid box size 35 × 29 × 32 Å ;For CHAP domain of lysin L1 (PDB ID: 11CF, Pocket C1): center coordinates (22, 5, -45) Å, grid box size 24 × 18 × 32 Å. The 3-dimensional shape of eugenol was converted to SDF format downloaded from PubChem and the force was minimized using the MMFF94 pressure field. The molecular docking simulations were performed by myself specifically on eugenol, as it is by far the primary bioactive component (70–90%) in the essential oil of cloves and S. in general. (Xu *et al.,* 2016; Li *et al.,* 2022; Elbestawy *et al.,* 2023). This focused approach enables a precise understanding of the molecular recognition mechanisms underlying the observed inhibitory effect of clove extract on wound-isolated *S. aureus* strains. Binding pockets were identified using CB-Dock2, focusing on the highest Vina scores. Molecular docking was carried out using AutoDock Vina within the CB-Dock2 platform (exhaustiveness = 8). The best binding pose for each protein was selected based on binding energy and the number and type of non-covalent interactions. All interaction analyses and visualizations were performed using Discovery Studio Visualizer v21.1.

3. RESULTS

3.1Antibacterial effect of Clove extract

Three different concentrations of Clove extract were used as antibacterial agents against S. aureus with distilled water as negative control. The results in figure (1) showed that large inhibition diameter (27 mm) was observed surrounding the high concentration of plant extract (75 mg/dL), followed by 50 mg/dl with (23mm), and 20 mg/dl (18mm). The negative control didn't showed any zone of inhibition.

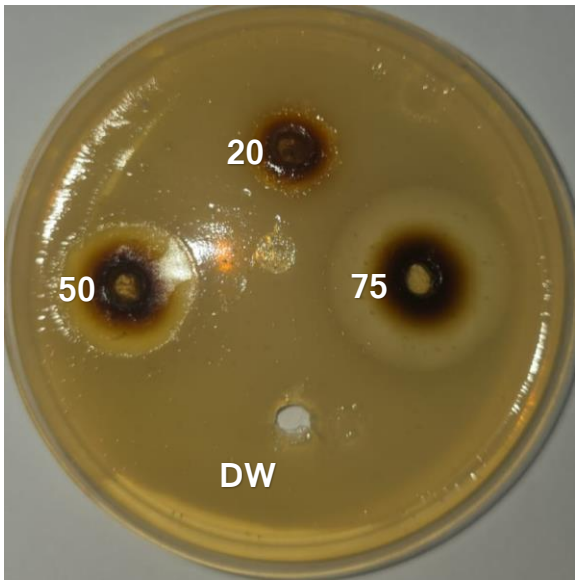

Figure 1: Antibacterial effect of clove extract against S. aureus on Muller Hinton agar Molecular docking

The molecular docking study revealed moderate to good binding affinities of eugenol with the selected *S. aureus* proteins, with Vina scores ranging from -5.5 to -6.2 kcal/mol (Table 1). Detailed two- and three-dimensional interaction diagrams are shown in Figures 2–5.

Table 1: Binding affinities of Eugenol with target proteins from *S. aureus* using CB-Dock 2.

Protein	PDB ID	Binding Affinity (kcal/mol)
Beta-lactamase	6WGR	-6.2
DHFR	6PR6	-5.9
CHAP domain (Lysin L1)	11CF	-6.0
PBP2a	1VQQ	-5.5

3.1. Interaction Results with *S. aureus* Proteins

3.1.1 .Beta-lactamase (PDB ID: 6WGR)

Eugenol exhibited the strongest binding at -6.2 kcal/mol (Figure 2). The complex is prominently stabilized by a dual-hydrogen-bonding network, where the functional oxygen atoms of eugenol form sharp conventional hydrogen bonds with the residues SER C:121 and SER C:63, driving the hydrophilic anchoring of the molecule. The hydrophobic framework of the ligand is accommodated through targeted alkyl and pi-alkyl interactions involving the residues TYR C:96 and ILE C:230, which wrap around the allyl and aromatic groups of eugenols. Furthermore, the overall binding topology

is structurally mediated by weak yet collaborative van der Waals forces with an extensive surrounding amino acid microenvironment comprised of ALA C:229, GLN C:228, GLY C:227, ARG C:235, SER C:226, LYS C:225, ASN C:123, and LYS C:66.

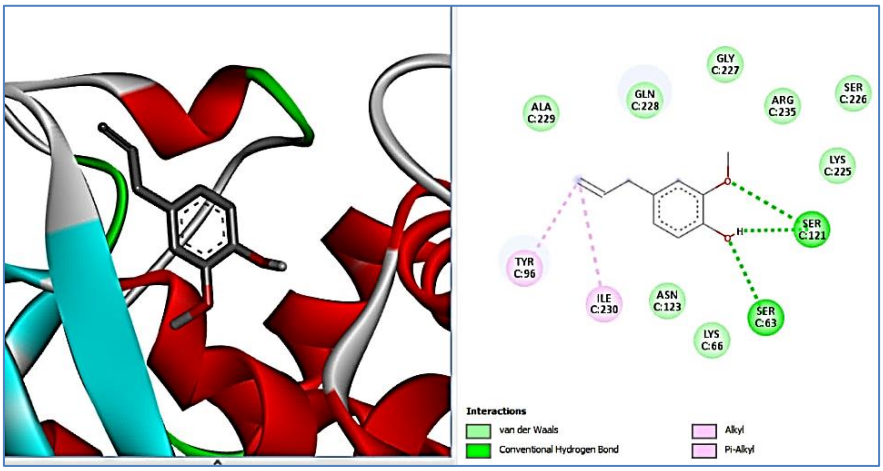

Figure 2: Binding mode of eugenol with beta-lactamase (PDB ID: 6WGR). (A) Three-dimensional representation of the docking pose; (B) Two-dimensional interaction diagram showing key protein-ligand contacts

3.1.2 .Dihydrofolate Reductase (DHFR) (PDB ID: 6PR6)

Eugenol showed a good binding affinity of -5.9 kcal/mol (Figure 3). The ligand is fastened into the active pocket via two conventional hydrogen bonds directed toward the residues LEU A:85 and THR A:1. A high degree of electronic stability is introduced via a distinctive pi-sigma interaction between the aromatic core of eugenol and the residue LEU A:2, complemented by an amide-pi stacked interaction with the same residue (LEU A:2), which highlights the intense pocket confinement. Hydrophobic binding pockets are further engaged through alkyl and pi-alkyl linkages with TYR A:83 and VAL A:89. This precise orientation is energetically locked by a series of van der Waals contacts with the surrounding shell residues, namely ILE A:82, GLY A:87, PRO A:86, ASP A:106, LYS A:104, and VAL A:105.

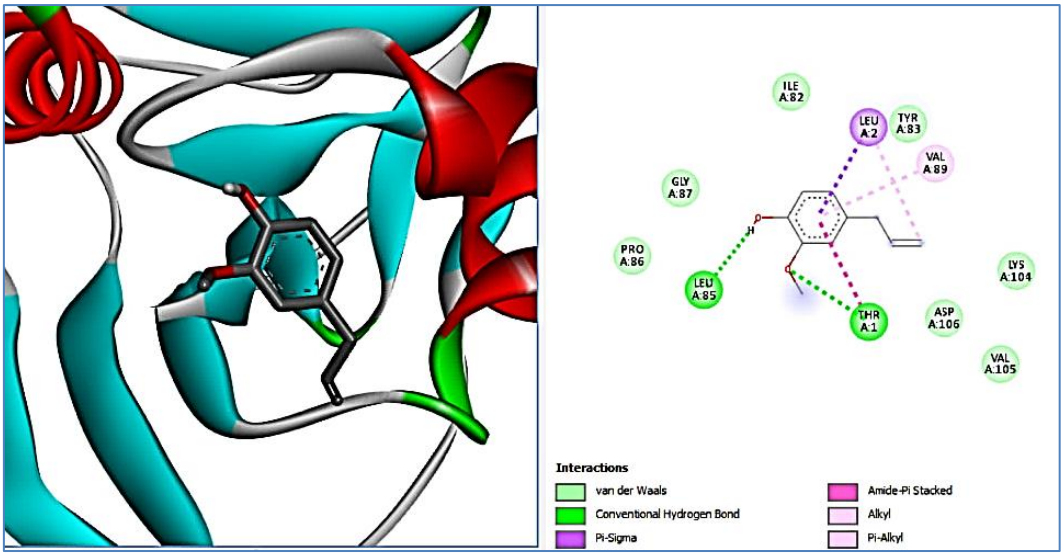

Figure 3: Binding mode of eugenol with dihydrofolate reductase (DHFR) (PDB ID: 6PR6). (A) Three-dimensional representation of the docking pose; (B) Two-dimensional interaction diagram showing key protein-ligand contacts

3.1.3 .PBP2a (PDB ID: 1VQQ)

Eugenol displayed a binding affinity of -5.5 kcal/mol (Figure 4). The binding profile of eugenol within the active site of the 1VQQ complex presents a dynamic balance of favorable and unfavorable energetic contributors. The favorable binding affinity (-5.5 kcal/mol) is heavily sustained by the formation of conventional hydrogen bonds with the binding pocket residues LYS A:604 and GLN A:521. The hydrophobic allyl and phenyl rings engage in a cluster of alkyl and pi-alkyl interactions with TYR A:344, LYS A:634, ILE A:614, and LYS A:604 (which exhibits a dual hydrogen-bonding/hydrophobic role). Notably, the docking topography reveals an unfavorable acceptor-acceptor interaction with the residue GLU A:602 due to electrostatic repulsion between proximate electronegative atoms, indicating a localized

conformational strain. Despite this, the complex maintains steric equilibrium via supportive van der Waals forces acting across the surrounding residues ASN A:632, THR A:399, SER A:400, ALA A:601, and LEU A:603.

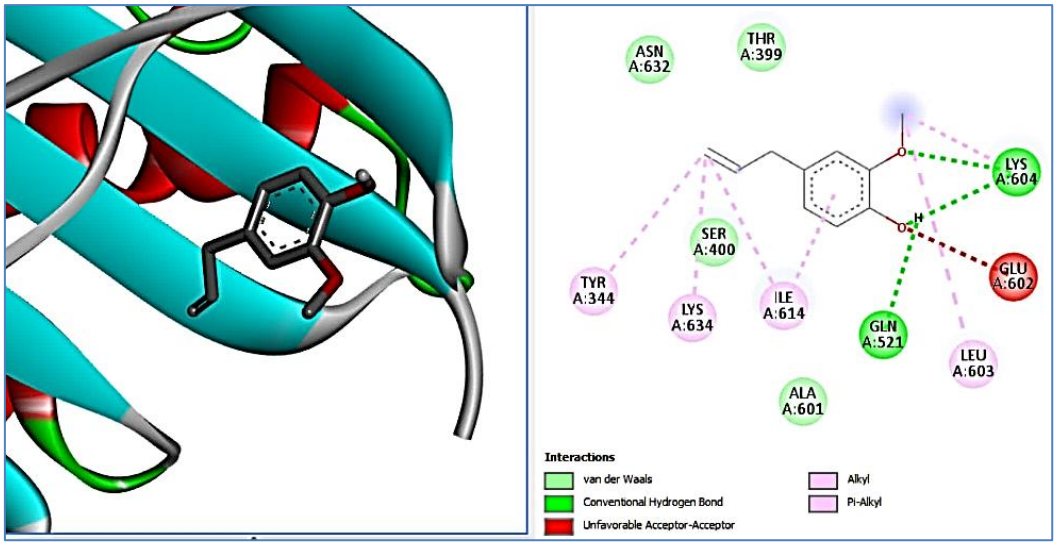

Figure 4: Binding mode of eugenol with penicillin-binding protein 2a (PBP2a) (PDB ID: 1VQQ). (A) Three-dimensional representation of the docking pose; (B) Two-dimensional interaction diagram showing key protein-ligand contacts

3.1.4 .CHAP Domain of Lysin L1 (PDB ID: 11CF)

Eugenol recorded a binding affinity of -6.0 kcal/mol (Figure 5). The ligand established a crucial conventional hydrogen bond with the residue GLY D:153, anchoring the hydroxyl/methoxy core within the catalytic site. Stability was further enhanced by prominent hydrophobic interactions, featuring a pi-pi T-shaped stacking interaction between the aromatic benzene ring of eugenol and the residue HIS C:136. Additionally, the aliphatic side chains and aromatic moiety of the ligand participated in multiple hydrophobic contacts, specifically alkyl and pi-alkyl interactions with TYR C:124, LYS D:120, PRO C:123, and ILE C:134. This binding conformation was tightly supported by a dense matrix of van der Waals forces with neighboring residues, including TYR D:124, ILE C:207, SER D:152, PRO D:123, and VAL D:150, which collectively optimize the structural complementarity and thermodynamic favorability of the complex.

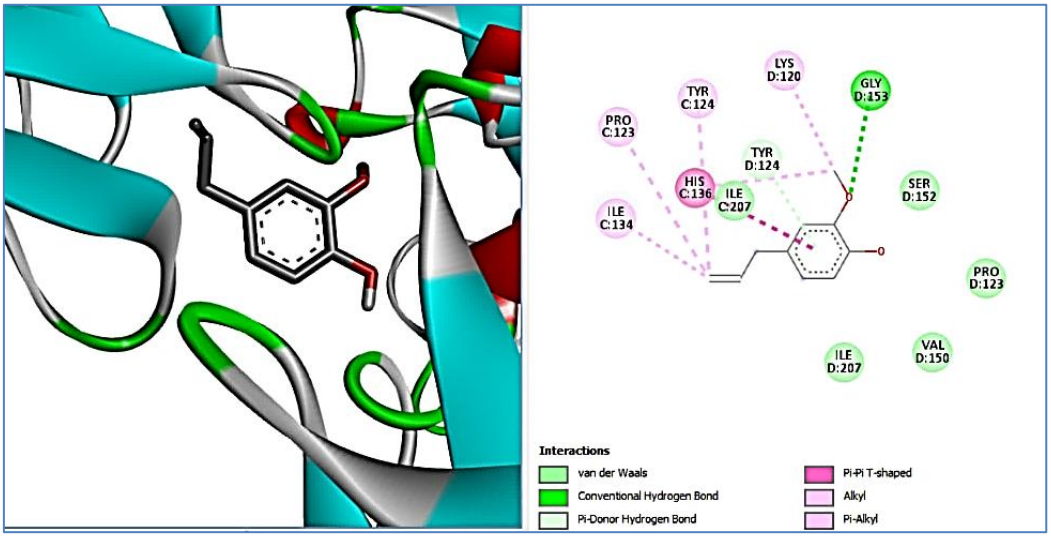

Figure 5: Binding mode of eugenol with the CHAP domain of lysin L1 (PDB ID: 11CF). (A) Three-dimensional representation of the docking pose; (B) Two-dimensional interaction diagram showing key protein-ligand contacts

4. DISCUSSION

One of the most important and effective natural antibacterial plants is clove bud (Syzygium aromaticum). Clove oil's primary constituent is eugenol (Tariq *et al.,* 2025). The primary volatile component of *S. aromaticum*, clove oil, exhibits a variety of benefits in terms of antibacterial activity (Pandey *et al.,* 2022).

The present study noted that *S. aureus* was found to be the most sensitive Gram-positive organisms to the tested clove plant extract. *S. aureus* can be significantly inhibited by eugenol (Jafri & Ahmad, 2021). Eugenol can also destroy the biofilm integrity and reduce the adhesion of methicillin-resistant *S. aureus* (MRSA) (El-Far *et al.,* 2021).

In a study by Pulikottil and Nath, S. (2015), clove oil and eugenol demonstrated strong antivirulence and antibacterial action against strains of Staphylococcoal and S. mutans. It is clear that eugenol and other pure chemicals were more efficient against bacterial infections than essential oils (El Atki *et al.,* 2019). The antimicrobial properties of clove oil, eugenol, and S. aromaticum plant extract have been thoroughly studied by Atanasova-Pancevska *et al.,* (2017). Eugenol exhibits strong antibacterial action against MRSA clinical strains that create biofilms and effectively eliminates established biofilms, according to a study by Buru *et al.,* (2022). It reduced the expression of genes linked to biofilms. Both the US Food and Drug Administration and the European Union have approved eugenol as a safe food preservative. Eugenol may therefore be utilised to treat MRSA biofilm infections.

The molecular docking results demonstrate that eugenol exhibits moderate to good binding affinities toward multiple essential and resistance-related proteins in *Staphylococcus aureus*, with Vina scores ranging from -5.5 to -6.2 kcal/mol. The strongest binding was observed with Beta-lactamase (6WGR, -6.2 kcal/mol), followed by the CHAP domain and DHFR. These findings are consistent with previous reports highlighting eugenol as the major active component responsible for the antibacterial activity of clove extract (Xu *et al.,* 2016; Li *et al.,* 2022; Elbestawy *et al.,* 2023) .The interaction with PBP2a is particularly significant, as this protein is the primary mediator of β-lactam resistance in MRSA. The observed hydrogen bonds and hydrophobic contacts may interfere with peptidoglycan cross-linking, potentially restoring susceptibility to β-lactam antibiotics. Binding to Beta-lactamase suggests eugenol may protect β-lactam antibiotics from enzymatic hydrolysis. Furthermore, the interaction with DHFR indicates possible disruption of folate metabolism, while binding to the CHAP domain highlights effects on cell wall remodeling .These multi-target interactions provide a molecular explanation for the potent antibacterial and anti-biofilm activities of eugenol and clove extract against *S. aureus* (Elbestawy *et al.,* 2023). Although molecular docking has limitations (e.g., lack of protein flexibility and solvent effects), the results strongly support further experimental validation through enzyme inhibition assays and synergy studies.

5. CONCLUSION

The high concentration of Clove extract exhibits good efficiency against *S. aureus*. Molecular docking supports these findings by investigating favorable interaction between Eugenol and bacterial essential protein. These revealed that Clove plant with their bioactive compounds has a potential role as antibacterial agents against pathogenic bacteria.

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