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INTERPRETATION OF MOLECULAR INTERACTIONS ORIGINATED FROM BACTERICIDAL ACTIVITY OF GLYCYRRHIZIC ACID AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FRESH RAW MINCED BEEF

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Abstract
S. aureus is considered as one of the most popular bacterial agents those are causing foodborne diseases in humans. Additionally, this bacterial can be the cause of food poisoning through production of enterotoxins. For the sake of evaluation of the anti-bacterial activity that possessed by the glycyrrhizic acid we have determined the values of MIC and inhibition zone. The value of MIC obtained was 5000(µg/ml), while the inhibition zone value obtained was 12 mm. On top of that, the glycyrrhizic acid's anti-bacterial action has been examined by molecular docking studies to show how it is delivered through bacterial cell. Moreover, with the aim of understanding the mode of action of glycyrrhizic acid with msrA, mecA, blaZ gene expression protein end products from the molecular level, we have docked glycyrrhizic acid to the active site of different proteins. Glycyrrhizic acid has presented a compact pattern that binds to the active pocket of the protein. For msrA the 2D and 3D binding conformation of glycyrrhizic acid in the active site of msrA it make 1 hydrogen bond with Phe95 (B), while mecA 2D and 3D binding conformation of glycyrrhizic acid in the active site of mecA showed 1 hydrogen bond with Gln 59(c) and, lastly, blaZ 2D and 3D binding conformation of glycyrrhizic acid revealed 1 hydrogen bond with Ser 216 (A), 1 hydrogen bond with Ser 235 (A) ,and 2 hydrogen bonds with Arg 244 (A). .Another detailed analysis showed the hydrogen bonds with the amino acid residues. These interactions permit glycyrrhizic acid to achieve formation of a stable complex with the proteins.

Keywords: Molecular docking, *Staphylococcus aureus*, Glycyrrhizic acid, minced beef

1. Introduction
S. aureus is considered as one of the most popular food pathogens. It has been the cause of many of diseases ranging from skin infections to diseases such as pneumonia and septicemia. And, when it comes to the food processing industry we are facing a very crucial problem which is the ability of some *S. aureus* strains to produce heat stable enterotoxins that cause staphylococcal food poisoning (SFP), which referred as one of the most major causes of gastroenteritis in the world [1]. In considering with the other diseases those are caused by *S.aureus, S.aureus* has been reported to be the causative agent for variety of diseases, such as complicated cases of necrotizing pneumonia, endocarditis, osteomyelitis, staphylococcal scalded skin syndrome and toxic shock syndrome [2]. *S.*

aureus in Individuals those are carrying them in their nasopharynx due to infection are the main source for spreading of staphylococci to other people and to contaminate food. Furthermore, when it comes to the pathogenesis process of *S.aureus* to an individual, *S.aureus* can release many extracellular enzymes like proteases, including serine-, cysteine- and metalloproteases. *S.aureus* proteases have the ability to interact with the host immune system and aid in completion of the infection process. *S.aureus* lipases are also considered virulence factors and responsible for contribution to the colonization and invasion processes of the bacteria especially in skin infections [3]. A certain number of plant extracts has been known to possess antibacterial effects. For example, polyphenols, which are categorized under the umbrella of two groups: flavonoids (e.g., catechin, flavonol, and tannin) and non-flavonoids (e.g., phenolic acid, neolignan), both have been gone through many studies [4, 5, 6, 7]. For example, it has been known that tannins from tea leave have antibacterial activity against *S. aureus* and *Escherichia coli* [8, 9]. Liquorice is a herbaceous perennial exists in the Mediterranean region, south Russia, central Asia, northern China and America. In the past, this plant has been used as an Empirical therapy because of its antibacterial, anti-viral, anti-inflammatory and calming effects, which are mediated through the major component in Liquorice that is called glycyrrhizic acid. The glycyrrhizic acid is consists of two moieties, one of them is glucouronic acid and the other one is 18β-glycyrrhetinic acid which is considered a metabolite that produced through the hydrolysis process of glycyrrhizic acid. In fact, 18β-glycyrrhetinic acid is one of the most remarkable components in Liquorice extract. Previous studies have displayed that 18β-glycyrrhetinic acid has many characteristics, including the ability of being anti-inflammatory, anti-allergic, and anti-viral activities [10, 11]. 18β-Glycyrrhetinic acid has also been confirmed in many researches to has antibacterial activity against some bacterial species, but the mechanism of action behind this activity remains obscure [12, 13]. Molecular docking [14] is a kind of structure-based drug design method that mimics the interaction between receptors and their ligands. Furthermore, it has the ability to expect the binding mode and affinity. Nowadays, this technology [15] has been tremendously used in drug design research field. By using a huge database to aid in selection of the potential pharmacophores is not only convenient for researchers to purchase, synthesize and complete follow-up pharmacological tests, but also greatly increase the efficiency and lower the research expenditure. Moreover, the emergence of the reverse molecular docking technology has resulted in significant improvement in the drug target predictive capacity and the comprehension of the related molecular mechanism for drug design. Molecular docking has been classified into two main types; the first one is the rigid docking which is used in docking between macromolecules and can involve only the change of the position for the component, not the length or the angles of the bond. The second one is the flexible docking which is more precise in results than rigid docking and it has the advantage of changing the confirmation of both the receptor and ligands [16].
The aim of this study is to present information that is provided through molecular docking studies about binding mode and affinity of 18β-glycyrrhetinic acid towards gene expression products of msrA, mecA&blaZ genes in *S. aureus* which describes the anti-bacterial effect of it.

2. Material and methods

2.1. *In vitro* studies

2.1.1. Isolation and identification of staphylococci

A total of 120 samples of fresh raw minced beef were gathered between January and April 2023 from 16 grocery stores. Stores were visited only once, and raw minced beef samples were selected mainly from national brand products, where available. Samples were transferred to laboratory and were kept at 4 °C until the time of processing; the samples were processed within 6 h from being purchased. Staphylococci were isolated on Baird-Parker Agar (Oxoid CM275; Oxoid, Basingstoke, UK) with Egg Yolk Tellurite Emulsion (Oxoid SR 0054) and typical colonies were subcultured.Pure cultures were kept in 20 % (v/v) glycerolstocks at -80 °C. Staphylococci were identified initially by Gram staining technique which forms the basis for the selection of biochemical tests. Catalase, coagulase

biochemical tests (Oxoid DrySpot Staphytect Plus Test) were further done to identify staphylococci [17].

2.1.2. The anti-bacterial effect of 18β-glycyrrhetinic acid against S. aureus isolates

The inhibition zone assay was carried out by the well diffusion method to evaluate the anti-bacterial action of glycyrrhizic acid [18].The inoculum suspension was prepared from colonies those have grown overnight on agar plate at 37°C and inoculated into Mueller-Hinton broth using a sterile swab that was immersed in the suspension to inoculate Mueller-Hinton agar plates with the bacteria. Glycyrrhizic acid was dissolved in dimethyl sulfoxide (DMSO) with different concentrations (10, 5, 2.5 … mg/ml) to obtain MIC value, while the inhibition zone was measured around each well after 24 h at 37°C.

2.2. *In silico* studies via molecular docking

In the sake of investigation of the binding mode between different bacterial proteins msrA, mecA and blaZ from *S.aureus* and glycyrrhizic acid at the molecular level. Proteins with PDB ID (Table 2) were selected, the chemical structure of glycyrrhizic acid was retrieved from drugbank database (DrugBank Accession Number: DB13751). Autodock Vina 1.5.6 [19] was used to generate all protein structures for docking and was used to explore the molecular interaction of ligands and proteins, the water molecules were removed and the macromolecules were converted to Autodock PDQBT format where Gasteiger charges were added to each atom, non-polar hydrogens merged followed by re-distribution of atomic partial charges and determination of atom types. The ligands were loaded to Auto Dock Tools 1.5.6, which then automatically computed Gasteiger charges and mapped the atom types. The root and torsion number were determined and subsequently the ligands were converted to Autodock PDBQT format. The exhaustiveness parameter was left at its default value and the grid size was set to 20 20 20 with a spacing of 1. The binding site was created using the ligand's core from the PDB structure. Additionally, 3D binding interactions were shown using the Pymol and 2D structures were generated using Ligplot

Table 2. Proteins involved in molecular docking studies with their PDB codes.

Protein	PDB code
msrA	4w8c
mecA	3JTP
blaZ	1blc

3. Results

3.1. Isolation and identification of *S.aureus*

After completing the isolation process of *S.aureus* on Baird-Parker Agar with Egg Yolk Tellurite Emulsion followed by subculturing of typical colonies, the number of isolates was obtained (Table 3).

Table 3. Number of *S.aureus* identified and isolated from fresh raw minced beef samples

Total number of samples	Number of *S. aureus* isolated
120	31

3.2. Evaluation of anti-bacterial activity of glycyrrhizic acid against *S.aureus* isolates

In regard to measurement of the anti-bacterial effect of glycyrrhizic acid (Table 4) and (Figure 1) reveal the susceptibility of *S.aureus* in terms of inhibition zone and MIC values when exposed to glycyrrhizic acid.

Table 4. The inhibition zone and MIC values of glycyrrhizic acid against *S.aureus* isolates from raw minced beef samples.

Tested organism	Inhibition zone(mm)	MIC(µg/ml)
S.aureus	12	5000

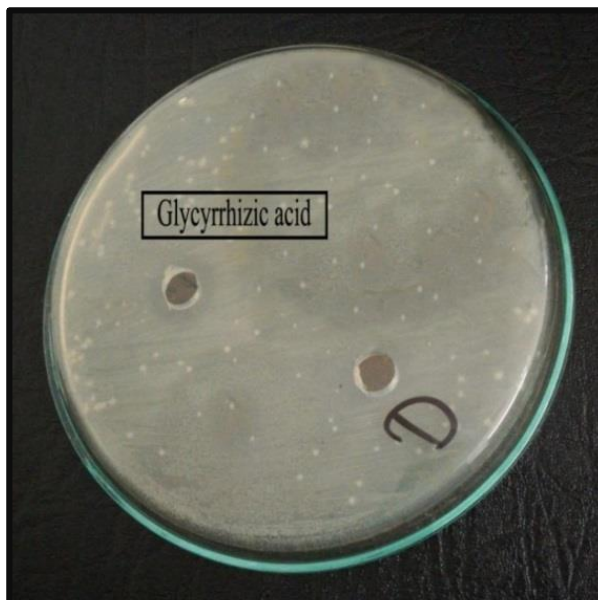

Figure 1. Mean zone of inhibition of glycyrrhizic acid measured in mm, the test has been done by agar well diffusion method.

3.3. Molecular Docking

3.3.1. Docking with msrA protein

The binding conformation of glycyrrhizic acid in the active site of msrA shows formation of 1 hydrogen bond with Phe95 (B).The red stand for the large hydrophobic region consisting of amino acid residues. Further detailed analysis showed the hydrogen bonds with the amino acid residues (Table 5).

Table 5. The docking results between glycyrrhizic acid and msrA protein

Affinity(kcal/mol)	Hydrophobic contact		Hydrogen bond		Weak hydrogen bond	
1. -4.1 **2.** -4	**Ligand atom**	**Receptor**	**Ligand atom**	**Receptor**	**Ligand atom**	**Receptor**
	C20	E55(B)CB	O4	C16(B)SG	C39	F95(B)O
	C25	I57(B)CG2	O15	I57(B)O		
	C19	F75(B)CE1	O12	Y68(B)OH		
	C17	M91(B)CB	O13	Y68(B)OH		
	C26	F95(B)CE1	O8	L71(B)O		
			O2	M91(B)O		
			O9	F95(B)N		
			O13	Y68(B)OH		
			O4	C16(B)SG		

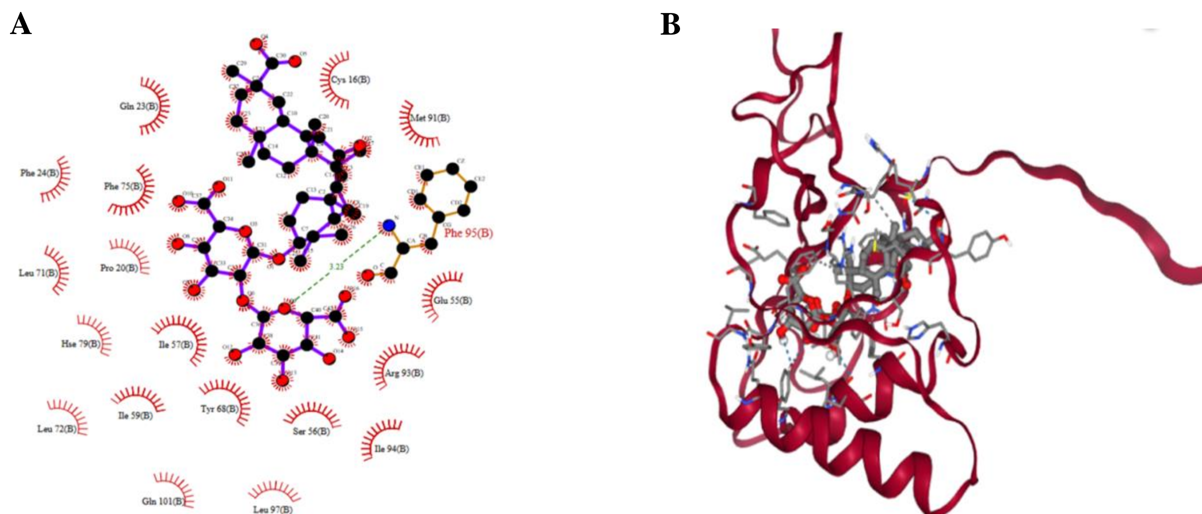

Figure 2. The binding conformation of glycyrrhizic acid with the active site of msrA. **(A).** The 2D binding conformation. **(B).** The 3D binding conformation.

3.3.2. Docking with mecA protein

The binding conformation of glycyrrhizic acid in the active site of mecA is displaying that one hydrogen bond was made with Gln 59(c), and one Hydrogen bond with Lys 94(c).The red stand for the large hydrophobic region consisting of amino acid residues. Additional details about bonds formation with the amino acid residues were obtained (Table 6).

Table 6. The docking results between glycyrrhizic acid and mecA protein

Affinity(kcal/mol)	**Hydrophobic contact**		**Hydrogen bond**		**Weak hydrogen bond**	
1. -7.8 **2.** -7.7	**Ligand atom**	**Receptor**	**Ligand atom**	**Receptor**	**Ligand atom**	**Receptor**
	C17 C26 C21	E91(C)CB E91(C)CG K95(C)CB	O12 O11 O15 O7 O10 O14 O15 O1	N27(C)O N27(C)O S31(C)OG E55(C)OE2 Q59(C)NE2 Q59(C)NE2 S31(C)OG K94(C)NZ	______	______

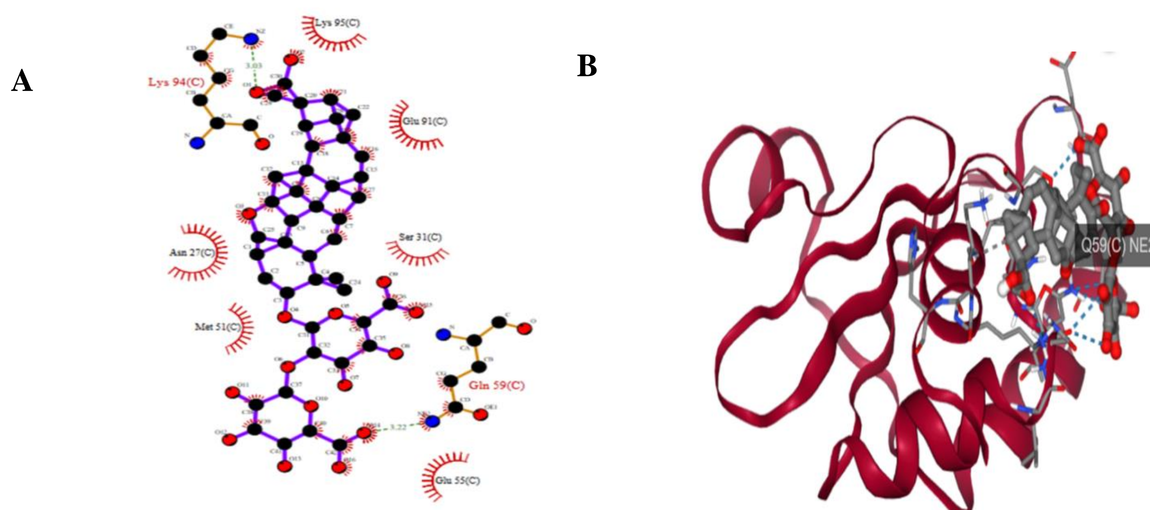

Figure 3. The binding conformation of glycyrrhizic acid with the active site of mecA **(A).** The 2D binding conformation. **(B).** The 3D binding conformation.

3.3.3. Docking with blaZ protein

The binding conformation of glycyrrhizic acid in the active site of blaZ reveals one hydrogen bond with Ser 216 (A), one hydrogen bond with Ser 235 (A) and two hydrogen bond with Arg 244 (A). The red stand for the large hydrophobic region consisting of amino acid residues. More information about bonds which were formed with the amino acid residues was gained (Table 7).

Table 7. The docking results between glycyrrhizic acid and blaZ protein

Affinity(kcal/mol)	**Hydrophobic contact**		**Hydrogen bond**		**Weak hydrogen bond**	
1. -9.7 **2.** -9	**Ligand atom**	**Receptor**	**Ligand atom**	**Receptor**	**Ligand atom**	**Receptor**
	C25	Y105(A)CB	O10	T128(A)O	C38	S216(A)O
	C14	Y105(A)CE1	O13	S216(A)O	O4	I239(A)CA
	C20	Y105(A)CE2	O10	S216(A)OG		
	C26	P107(A)CG	O13	S235(A)OG		
	C21	Q237(A)CB	O12	S235(A)OG		
			O5	A238(A)O		
			O5	I239(A)O		
			O4	I239(A)O		
			O6	S216(A)OG		
			O13	S235(A)OG		
			O13	R244(A)NH1		
			O13	R244(A)NH2		

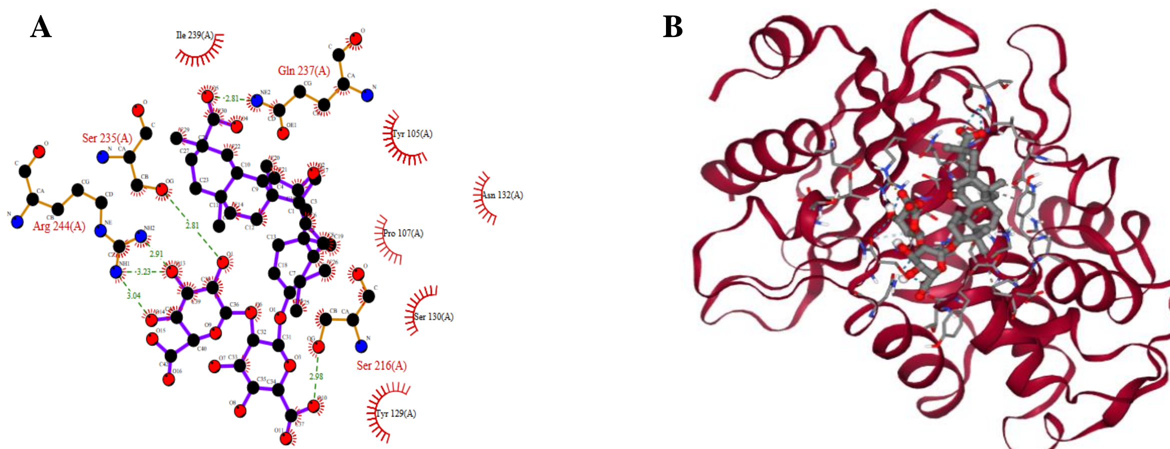

Figure 4. The binding conformation of glycyrrhizic acid with the active site of blaZ. **(A).** The 2D binding conformation. **(B).** The 3D binding conformation.

Discussion

S. aureus is considered as one of the main bacterial agents those are causing foodborne diseases in humans. This microorganism can be the causative agent of food poisoning by its output of enterotoxins [20]. Previous studies have outlined that the humans are common asymptomatic carriers of enterotoxigenic *S. aureus* in nose, throat, and skin. Even, the workers in the food industry can be an important source of food contamination. The feature of formation biofilms permits *S. aureus* to survive in harsh environments such as food industry surfaces [21], and this increase the probability for the recurrence of food contamination. Adhesion and biofilm formation are crucial virulence factors in *S. aureus* since they help to colonize the food environments [22].Actually, biofilms those found on food processing surfaces improve the refractoriness to disinfectants, thereby increasing the risk of cross-contamination of food. Nowadays, it is obvious that the continued use of currently available antibiotics against *S. aureus* ramps up the risk of completely exhausting their efficacies [23]. As a replacement, there has been an increased desire in finding nontraditional treatment protocols; consisting of natural compounds those are efficacious against bacterial infections [23, 24]. In our

research we focused on making molecular docking studies on glycyrrhizic acid to interpret its anti-bacterial effect and show how this effect is mediated through studying its binding mode and affinity towards gene expression protein end products of some genes of *S. aureus*, such as msrA, mecA and blaZ genes. As a first step in our research we have done isolation and identification processes to *S. aureus* strains those are found on raw minced beef samples and those processes were quite similar when comparing them to the other former studies [25, 26]. Isolation has been done on Baird-Parker Agar (Oxoid CM275; Oxoid, Basingstoke, UK) with Egg Yolk Tellurite Emulsion (Oxoid SR 0054), while identification was completed through gram staining technique and biochemical tests consisting of catalase and coagulase tests. A further step was taken to evaluate the anti-bacterial activity of glycyrrhizic acid through measurement of MIC and inhibition zone values (table 3). The glycyrrhizic acid is in fact, a compound that gained a high interest from the scientific community cause of its wide range of uses which it provides as mentioned before in a variety of researches[27].In regard to the goal of our research which is investigation of anti-bacterial action of glycyrrhizic acid through molecular docking studies, we have evaluated the binding affinity and the hydrogen bonds with amino acids residues between different bacterial proteins msrA (Table 5)(Figure 2), mecA(Table 6)(Figure 3) and blaZ(Table 7)(Figure 4) for S.aureus and Glycyrrhizic acid at the molecular level. Molecular docking studies to those genes are very crucial and of medical importance as it experiment new routes to overcome the resistance of bacteria for the common Antibiotics treatment protocols.
If we considering about the genes those we are studying, they are of high importance to *S. aureus* as it was noted several times before in former researches that msrA specifies resistance to macrolides by ABC-transporter-mediated efflux, mec gene encodes for PBP2A transpeptidase which contributes to cell wall synthesis and permits peptidoglycan crosslinking in the presence of β-lactam and The blaZ gene that encodes for β-lactamase, which results of hydrolyses for penicillin-rings [28, 29, 30,31,32].

Conclusion

In this work, we have investigated the anti-bacterial activity of glycyrrhizic acid through determination of MIC and inhibition zone values. The value of MIC was 5000(μg/ml), while the inhibition zone value was 12 mm. Moreover, the glycyrrhizic acid's anti-bacterial action have further undergone through molecular docking studies to show how it is mediated through bacterial cell. To elucidate the mode of action of glycyrrhizic acid with msrA, mecA, blaZ gene expression protein end products from the molecular level, we have docked glycyrrhizic acid to the active pocket of different proteins. Glycyrrhizic acid has presented a compact pattern that binds to the active pocket of the protein. Further detailed analysis revealed the hydrogen bonds with the amino acid residues. These interactions allow glycyrrhizic acid to form a stable complex with the proteins.

Data availability statement:

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author

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