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# *In vitro* antibacterial activity and molecular docking studies of root extract of glycyrrhiza glabra against bovine mastitis pathogens

**Ravikumar C, Ranjith D, Pawan Kumar KN, Ranjith J, Prakash N and Shridhar NB**

**Abstract**
The present study was conducted to assess the antibacterial activity of aqueous and methanolic root extracts of *Glycyrrhiza glabra* against *Staphylococcus aureus*, *Streptococcus agalactiae* and *E*-coli and *in silico* antibacterial activity against Glucoseamine-6-phosphate synthase (GlcN-6-P). The extracts prepared using soxhlet apparatus, antibacterial activity was conducted using disc diffusion method followed by MIC and MBC and the molecular docking studies was performed using ArgusLab 4.0.1. The phytochemical screening revealed the presence of alkaloids, glycosides, saponins, tannins, steroids and flavonoids. The zone of inhibition ranges from 6-14 mm and 12 -17 mm against pathogenic *S. aureus*, *St. agalactiae* and *E. coli* in both aqueous and methanolic extract respectively. The methanolic extract showed lower MIC and MBC as compared to acqueous extract. Among the sixteen bioactive molecules of *Glycyrrhiza glabra* roots screened for *in silico* anti-bacterial activity against Glucosamine 6 phosphate synthase enzyme, isoliquiritin showed excellent inhibitory action followed by shinflavanone and Licochalcone A respectively. In conclusion, Glycyrrhiza glabra root extract (acqueous and methanolic) has potentantimicrobial activity and can be used as raw materials for herbal therapy for bovine mastitis infection.

**Keywords:** Glycyrrhiza glabra, *S aureus*, *St. agalactiae*, *E coli*, ArgusLab, Isoliquiritin

## Introduction
Bovine mastitis, an infection-contagious disease and continues to be among costliest disease to the dairy cattle with annual economic loss attributing to about $35 billion worldwide annually with significant impact on animal production and welfare (Radostits *et al*., 2000) [1]. Several pathogens are attributed for the cause viz. *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* respectively (Mubarack *et al*., 2011, Monecke, 2007, Nemeth *et al*., 1994) [2, 3, 4]. The development of drug resistance of pathogens and with high cost of treatment and related side effects have drawn the pursuit of the researchers and general population towards traditional medicine and ethnomedicinal plants for potential discovery of compounds (Hassan *et al*., 2014, Annapoorani, 2007) [5, 6]. A multifarious plants has been traditionally used for the therapy of mastitis including *Allium sativum*, *Bunium persicum*, *Oryza sativa* and *Triticum aestivum* etc (Amber *et al*., 2017) [7]. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Shaikh *et al*, 1994) [8]. Plants produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Samie *et al.*, 2010) [9] *Glycyrrhiza glabra* (Liquorice) is belongs to Family: Fabaceae, commonly known as Yashti-madhuh or Mulhati. The roots are widely used for medicinal purposes, which are red or lemon colored with yellowish and pale inside (wealth of India, 1985) [10]. Traditionally it has been used for anti-inflammatory, antiulcer, expectorant, antimicrobial, antioxidant and anxiolytic activities (Sharma *et al*, 2013) [11]. The roots are potentially used as antimicrobial and anxiolytic agent (Ambawade *et al*., 2001) [12]. The pharmacological activity is due to the presence of triterpenes like glycyrrhizin, glycyrrhetinic acid and liquirtic acid and flavonoids like liquirtin and formononetin (Farag *et al*., 2012) [13].
The computational methodology, involving molecular docking analysis could be an easy gate-way for searching effective drugs of natural origin against diseases (Mohapatra *et al*., 2015) [14]. Structure based drug design relies on knowledge of other molecules that bind tothe biological target of interest, lately the scientific focus has turned on the application of virtual dockingmethods which are less intensive on labour, time and cost (Gamarro *et al*., 1995, Krieger *et al*., 2000, Mackey *et al*., 2004 and Suganya & Radha Mahendran, 2016) [15, 16, 17, 18].

Bacterial proteins are the ultimate target to inhibit their growth sincethese are the executors of many cellular functions. The key enzymeL-glutamine: D-fructose-6phosphate amidotransferase, known underthe trivial name of glucosamine-6-phosphate synthase is responsible for the synthesis of glucosamine-6-phosphate (GlcN-6-P) from D-fructose-6-phosphate and L-glutamine. This is the keyenzyme in the pathway leading to the formation of UDP-N-acetylglucosamine (UDP-GlcNAc), the major intermediate in thebiosynthesis of all aminosugar containing macromolecules both inprokaryotic (Bates and Pasternak 1965; Imada *et al*. 1977) [19, 20] andeukaryotic cells (Cabib *et al*. 1982; Winzler and Bekesi 1967) [21, 22]. Inbacteria, this enzyme is concerned to build peptidoglycan ofbacterial cell wall. This is due to the fact that glucosamine-6-phosphate synthase (GlcN-6-P synthase) has been exploited as atarget molecule for the authentication of antibacterial drug. Inaddition, inhibition of this bacterial life sustaining enzyme has someimportant implications for therapy (Chmara *et al*. 1984) [23]. It has beenreported that even a short-time inactivation of GlcN-6-P synthasewas lethal to the pathogenic microorganisms by inducingmorphological changes, agglutination and lysis. (Bates *et al*. 1966; Chmara and Borowski 1986; Milewski *et al*. 1986) [24, 25, 26]. Therefore the present study was undertaken to evaluate the antimicrobial activity of *Glycyrrhiza glabra* root extract (methanolic and acqueous) against major pathogens like *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* isolated from the clinical cases of bovine mastitis and *In silico* molecular docking of small molecules present in *Glycyrrhiza glabra* viz... Glucoliquiritin apioside, Shinflavanone, Shinpterocarpin, Prenyllicoflavone A, 1-methoxyphaseollidin, Liquiritin, Isoliquiritin, Glycyrrhizin, Liquiritigenin, Isoliquiritigenin, Liquiritin apioside, Glycyrrhetic acid, Licochalcone A, Licochalcone E, Glabridin, Hispaglabridin A against glucosamine 6 phosphate synthase enzyme.

## Materials and Methods

### Collection and preparation of extract

The roots of *Glycyrrhiza glabra* were collected from Biligiranganabetta, Chamrajanagar District, Karnataka, India and were washed thoroughly with running water and dried at 40°C and grounded into coarse powder for extract preparation. The powdered root material 50 gram was extracted with distilled water and methanol in Soxhlet apparatus up to 7 cycles and evaporated using rotary flask evaporator (Scientek, Mumbai). The dried powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4, 0.01M) and the final yield will be calculated. The extract will be filtered through membrane filter (pore size 0.45 µm) and stored in airtight vials at 4°C till further use for antimicrobial assay. The yield for acqueous extract is 9 gms and for methanolic extract is 8 gms.

### Phytochemical screening

Phytochemical screening of the plant material was performed following the method described by Ranjith and Maria, 2017. The extracts were tested for the following phytochemicals: alkaloids, glycosides, saponins, tannins, steroids and flavonoids.

### Test microorganisms

The pathogenic microorganisms were isolated from mastitis milk sample as per the standard procedure (Griffin *et al.*, 1977) [27]. Microorganisms were initially identified on the basis of colony morphology and odour on 5% blood agar as per Cruikshank (1962) [28] and later by gram staining and growth on selective media, later identified by standard biochemical kits (Hi Staph, Hi Strep and Hi E. coli identification kit HiMedia, Mumbai).

### Antimicrobial assay

The *Staphylococcus aureus*, *Streptococcus agalactiae* and *E. coli* organism were isolated from milk, 3-4 colonies were suspended in Nutrient broth. The organism was thoroughly mixed in solution; thereafter the turbidity of the inoculum was matched with 0.5 of the McFarland tube standards which was equivalent to $1.5 \times 10^8$ cfu/ml. A sterile swab was dipped in this solution and smeared over Mueller-Hinton (MH) agar plate and were dried for 15 minutes. Sterile blank discs were impregnated in 25 µl of the prepared herbal extracts (with concentration of 2.5 mg, 5mg, 7.5mg and 10mg respectively) and standard antibiotic disc of Gentamicin (10µg/disc) was used as positive control (HiMedia Company). The discs were then dried and placed on the plates. The diameter of the zone of inhibition was measured using millimeters scale. All the tests were done in triplicates to minimize the test error.

### Determination of minimum inhibitory concentration (MIC)

MIC used as a research tool to determine the *in-vitro* activity of new antimicrobials. Determination of MIC was carried out using the tube dilution method (Oyeleke *et al*, 2008). A series of two fold serial dilution of each extract ranging from 100 mg/ml to 0.78 mg/ml was made in Mueller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). 1000 µl of standard inoculum of the bacterial strains matched to 0.5 McFarland standards was seeded into each dilution. Two control tubes were maintained for each test batch, negative control tube containing extract and growth media without inoculum and positive control tube containing the growth medium and the inoculum. The tubes were incubated at 37°C for 24 hours and observed for turbidity. MIC was determined as the highest dilution of the extract that prevents visible growth of bacteria.

### Minimum bactericidal concentration (MBC)

MBC is the lowest concentration of antimicrobial that will prevent the growth of an organism after sub-culture on to antibiotic free media. Tube showing no growth during MIC determination was selected for MBC determination, a loop full from each tube was sub cultured on to Muller Hinton agar plates and incubated for further 24 hours at $37^0$ C. The least concentration, at which no growth was observed, was denoted as the MBC.

### Retrieval of Protein structure from Database

The crystal structure of enzyme Glucosamine 6 phosphate synthase (PDB ID 4VF5) obtained from RCSB Protein Data Bank (http://www.pdb.org) containing resolution about 2.9 A° respectively (Hetal *et al.*, 2013, Ranjith, 2019) [29, 30].

### Processing of target proteins

The water molecules present will disturb the binding nature of the compounds to the active site thereby reducing the efficiency of the compound against the target proteins. Thus, by using Argus lab, crystallographic water molecules and other unwanted ligands were cleaved and were removed from the protein. Crystallographic disorders and void atomic spaces were corrected to improve binding energy. Then, the protein

was subjected to energy minimization and on the final stage by using Swiss PDBviewer, addition of hydrogen atoms to the target protein molecule before docking was performed (Naganathan, 2016) [31] and geometric optimization was performed according to Hartree –Fock (HF) calculation method by ArgusLab 4.0.1 software.

**Binding site detection**

The amino acids involved in active binding site will be detected by using a database, PDBsum which is a web based database providing a largely pictorial summary of the key information on each macromolecular structure deposited at protein data bank (PDB), providing summary with annotations and analysis of their key structural features. So, for each PDB entry there is a corresponding web page in PDBsum, accessible by the four character PDB identifier. PDBsum was used for the identification of most potent active site for binding and interaction of target protein and ligand (Laskowski., 2001) [32].

**Selection and retrieval of ligand structure**

The structures, SMILES, physical and chemical properties of the small molecules present in *Glycyrrhiza glabra*viz.. Glucoliquiritin apioside, Shinflavanone, Shinpterocarpin, Prenyllicoflavone A, 1-methoxyphaseollidin, Liquiritin, Isoliquiritin, Glycyrrhizin, Liquiritigenin, Isoliquiritigenin, Liquiritin apioside, Glycyrrhetic acid, Licochalcone A, Licochalcone E, Glabridin, Hispaglabridin Awere retrieved from pubchem and chemspider and saved in JSmol format for molecular docking studies (Ranjith, 2019) [30]

**Processing of ligand structure**

The structures were visualized in 3D in Chemspider database and were saved in JSmol format for Argus lab. The geometric optimization was performed using Argus Lab 4.0.1 software. Molecular Mechanics (MM) method UFF was used for refining initial geometries, using the "Clean Geometry" option in the ArgusLab. Hydrogens were added using "Add Hydrogens" option under edit column of ArgusLab 4.0.1.

**Molecular docking using Argus Lab 4.0.1**

All the computational docking studies were performed using ArgusLab 4.0.1, a computerized structure program, generally based on the quantum mechanics and is used to predict the potential energies, molecular structures; geometrical optimization of structure, vibrational frequencies of various atom coordinates, bond length and reactions pathway. Target proteins i.e. Glucosamine 6 phosphate synthase (PDB ID – 2VF5) were docked against the 16 ligands (obtained from the chemspider and pubchem) using Argus Lab 4.0.1 to find the reasonable binding geometries and explore protein ligand interactions. The docking was mainly targeted only on to the predicted active site. Simulations for docking were performed by selecting "Argus Dock" as the docking engine. The residues (in the receptor) that have been selected were defined to be a part of the binding site. A 0.4 Å spacing was used between the grid points and an comprehensive search was performed by enabling "High precision" option in Docking precision menu, "Dock" was chosen as the calculation type, "flexible" for the ligand and the A Score was used as the scoring function. The A Score function was generally used to calculate the binding energies of the resulting docked structures. All the compounds present in the data file were docked into the active site of antimicrobial target, using the same protocol. The molecular visualization of the ligand-protein interactions were analyzed by PyMOL software to examine the type of interactions. The docking poses saved for each compound were ranked according to their dock score function. The pose having the highest dock score was selected for further analysis (Ranjith, 2019) [30].

**Results**

**Table 1:** Phytochemical analysis of the roots of *Glycyrrhiza glabra* extract

| Phytoconstituents | Aqueous extract | Methanolic extract |
|---|---|---|
| Alkaloids | - | - |
| Glycosides | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Steroids | - | + |
| Flavonoids | + | + |

**Table 2:** Antimicrobial activity of *Glycyrrhiza glabra* showing zone of inhibition against microorganisms

| SL. No. | Solvent | Concentration (in mg) | *Staphylococcus aureus* (in mm) | *Streptococcus agalactiae* (in mm) | *E. coli* (in mm) |
|---|---|---|---|---|---|
| 1 | Aqueous | 2.5 | 6 | 7 | Negative |
| | | 5.0 | 10 | 10 | Negative |
| | | 7.5 | 11 | 11 | Negative |
| | | 10 | 12 | 13 | Negative |
| 2 | Methanol | 2.5 | 12 | 8 | 8 |
| | | 5.0 | 14 | 12 | 12 |
| | | 7.5 | 15 | 13 | 13 |
| | | 10 | 17 | 14 | 14 |
| 3 | Gentamicin | 10µg | 22 | 21 | 21 |

**Table 3:** General characteristics of the biomolecules of *Glycyrrhiza glabra* Linn

| Sl. No. | Compounds | Pub Chem ID | Mol. formula | Mol. Weight (g/mol) |
|---|---|---|---|---|
| 1 | Glucoliquiritin apioside | 74819335 | $C_{32}H_{40}O_{18}$ | 712.6 |
| 2 | Shinflavanone | 197678 | $C_{25}H_{26}O_4$ | 390.5 |
| 3 | Shinpterocarpin | 10336244 | $C_{20}H_{18}O_4$ | 322.4 |
| 4 | Prenyllicoflavone A | 11349817 | $C_{25}H_{26}O_4$ | 390.5 |
| 5 | 1-methoxy phaseollidin | 480873 | $C_{21}H_{22}O_5$ | 354.4 |
| 6 | Liquiritin | 503737 | $C_{21}H_{22}O_9$ | 418.4 |
| 7 | Isoliquiritin | 5318591 | $C_{21}H_{22}O_9$ | 418.4 |
| 8 | Glycyrrhizin | 14982 | $C_{42}H_{62}O_{16}$ | 822.9 |

| | | | | |
|---|---|---|---|---|
| 9 | Liquiritigenin | 114829 | $C_{15}H_{12}O_4$ | 256.25 |
| 10 | Isoliquiritigenin | 638278 | $C_{15}H_{12}O_4$ | 256.25 |
| 11 | Liquiritin apioside | 10076238 | $C_{26}H_{30}O_{13}$ | 550.5 |
| 12 | Glycyrrhetic acid | 10114 | $C_{30}H_{46}O_4$ | 470.7 |
| 13 | Licochalcone A | 5318998 | $C_{21}H_{22}O_4$ | 338.4 |
| 14 | Licochalcone E | 46209991 | $C_{21}H_{22}O_4$ | 338.4 |
| 15 | Glabridin | 124052 | $C_{20}H_{20}O_4$ | 324.4 |
| 16 | Hispaglabridin A | 442774 | $C_{25}H_{28}O_4$ | 392.5 |

**Table 4:** Canonical SMILES of the biomolecules of *Glycyrrhiza glabra* Linn

| Sl. No. | Compounds | Canonical SMILES |
|---|---|---|
| 1 | Glucoliquiritin apioside | C1C(OC2=C(C1=O)C=CC(=C2)OC3C(C(C(C(O3)CO)O)O)O)C4=CC=C(C=C4)OC5C(C(C(C(O5)CO)O)O)OC6C(C(CO6)(CO)O)O |
| 2 | Shinflavanone | CC(=CCC1=C(C=CC(=C1)C2CC(=O)C3=C(O2)C4=C(C=C3)OC(C=C4)(C)C)O)C |
| 3 | Shinpterocarpin | CC1(C=CC2=C(O1)C=CC3=C2OCC4C3OC5=C4C=CC(=C5)O)C |
| 4 | Prenyllicoflavone A | CC(=CCC1=CC2=C(C=C1O)OC(=CC2=O)C3=CC(=C(C=C3)O)CC=C(C)C)C |
| 5 | 1-methoxy phaseollidin | CC(=CCC1=C(C=CC2=C1OC3C2COC4=C3C(=CC(=C4)O)OC)O)C |
| 6 | Liquiritin | C1C(OC2=C(C1=O)C=CC(=C2)O)C3=CC=C(C=C3)OC4C(C(C(C(O4)CO)O)O)O)O |
| 7 | Isoliquiritin | C1=CC(=CC=C1C=CC(=O)C2=C(C=C(C=C2)O)O)OC3C(C(C(C(O3)CO)O)O)O)O |
| 8 | Glycyrrhizin | CC1(C2CCC3(C(C2(CCC1OC4C(C(C(C(O4)C(=O)O)O)O)O)OC5C(C(C(C(O5)C(=O)O)O)O)O)C)C(=O)C=C6C3(CCC7(C6CC(CC7)(C)C(=O)O)C)C)C)C |
| 9 | Liquiritigenin | C1C(OC2=C(C1=O)C=CC(=C2)O)C3=CC=C(C=C3)O |
| 10 | Isoliquiritigenin | C1=CC(=CC=C1C=CC(=O)C2=C(C=C(C=C2)O)O)O |
| 11 | Liquiritin apioside | C1C(OC2=C(C1=O)C=CC(=C2)O)C3=CC=C(C=C3)OC4C(C(C(C(O4)CO)O)O)OC5C(C(CO5)(CO)O)O |
| 12 | Glycyrrhetic acid | CC1(C2CCC3(C(C2(CCC1O)C)C(=O)C=C4C3(CCC5(C4CC(CC5)(C)C(=O)O)C)C)C)C |
| 13 | Licochalcone A | CC(C)(C=C)C1=C(C=C(C(=C1)C=CC(=O)C2=CC=C(C=C2)O)OC)O |
| 14 | Licochalcone E | CC(C1=C(C=C(C(=C1)C=CC(=O)C2=CC=C(C=C2)O)OC)O)C(=C)C |
| 15 | Glabridin | CC1(C=CC2=C(O1)C=CC3=C2OC[C@H](C3)C4=C(C=C(C=C4)O)O)C |
| 16 | Hispaglabridin A | CC(=CCC1=C(C=CC(=C1O)C2CC3=C(C4=C(C=C3)OC(C=C4)(C)C)OC2)O)C |

**Table 5:** Physical and Chemical properties of small molecules of *Glycyrrhiza glabra* Linn

| Sl. No. | Compounds | Physical and Chemical properties | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | XLogP3-AA | HBDC | HBAC | RBC | TPSA ($A^{02}$) | HAC | CBUC |
| 1 | Glucoliquiritin apioside | -2.6 | 10 | 18 | 10 | 284 | 50 | 1 |
| 2 | Shinflavanone | 5.4 | 1 | 4 | 3 | 55.8 | 29 | 1 |
| 3 | Shinpterocarpin | 3.6 | 1 | 4 | 0 | 47.9 | 24 | 1 |
| 4 | Prenyllicoflavone A | 6.3 | 2 | 4 | 5 | 66.8 | 29 | 1 |
| 5 | 1-methoxyphaseollidin | 4.2 | 2 | 5 | 3 | 68.2 | 26 | 1 |
| 6 | Liquiritin | 0.4 | 5 | 9 | 4 | 146 | 30 | 1 |
| 7 | Isoliquiritin | 1.7 | 6 | 9 | 6 | 157 | 30 | 1 |
| 8 | Glycyrrhizin | 3.7 | 8 | 16 | 7 | 267 | 58 | 1 |
| 9 | Liquiritigenin | 2.2 | 2 | 4 | 1 | 66.8 | 19 | 1 |
| 10 | Isoliquiritigenin | 3.2 | 3 | 4 | 3 | 77.8 | 19 | 1 |
| 11 | Liquiritin apioside | -0.8 | 7 | 13 | 7 | 2.5 | 39 | 1 |
| 12 | Glycyrrhetic acid | 6.4 | 2 | 4 | 1 | 74.6 | 34 | 1 |
| 13 | Licochalcone A | 4.9 | 2 | 4 | 6 | 66.8 | 25 | 1 |
| 14 | Licochalcone E | 5 | 2 | 4 | 6 | 66.8 | 24 | 1 |
| 15 | Glabridin | 3.9 | 2 | 4 | 1 | 58.9 | 24 | 1 |
| 16 | Hispaglabridin A | 5.8 | 2 | 4 | 3 | 58.9 | 29 | 1 |

HBDC- Hydrogen Bond Donor Count, HBAC- Hydrogen Bond Acceptor Count, RBC- Rotatable Bond Count, TPSA – Topological Polar Surface Area, HAC – Heavy Atom Count, CBUC- Covalently Bonded Unit Count

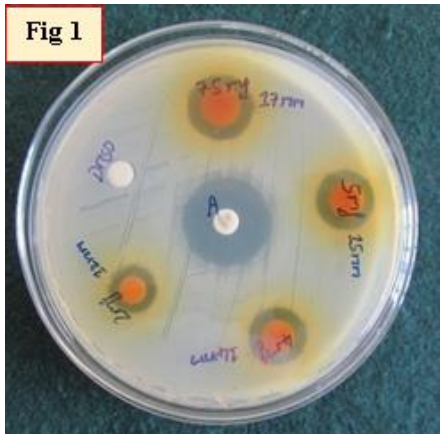


**Fig 1:** ZOI, Methanolic extract of S aureus,

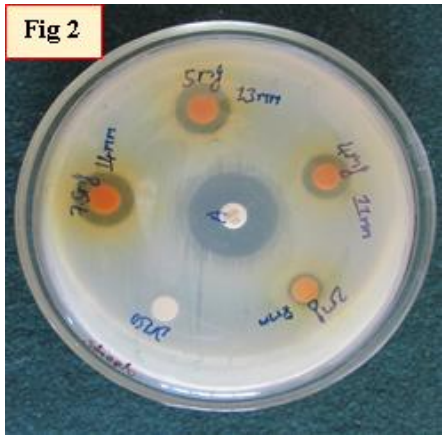


**Fig 2:** ZOI, methanolic extract of St. agalactiae

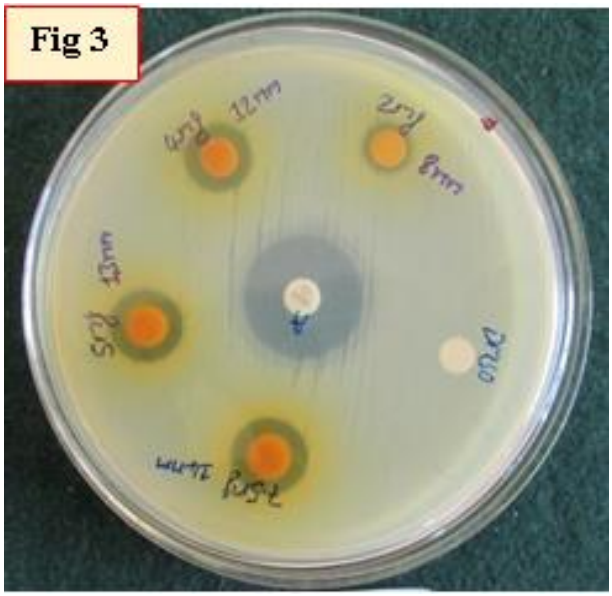


**Fig 3:** ZOI, methanolic extract of E coli,

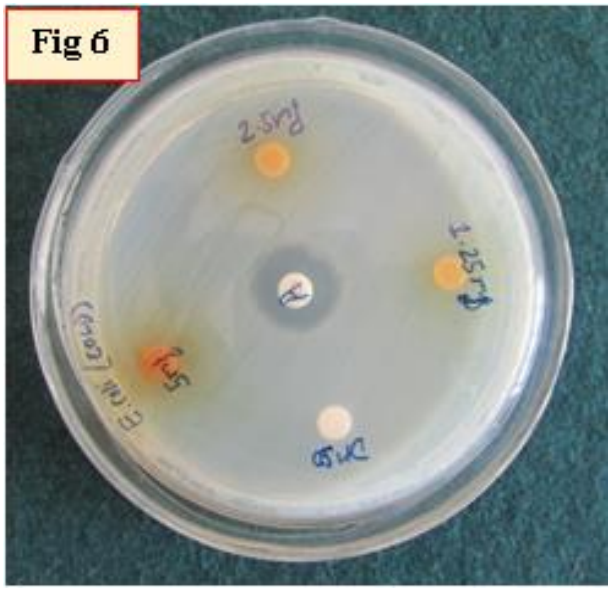


**Fig 6:** ZOI, acqueous extract of E coli. ZOI – Zone Of Inhibition

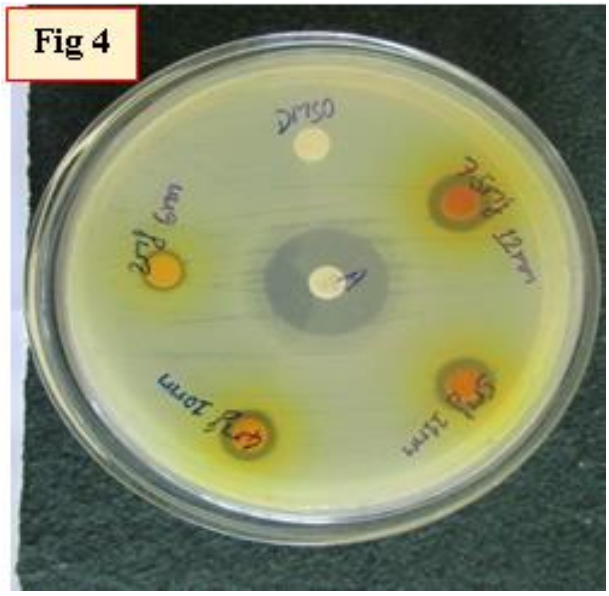


**Fig 4:** ZOI, acqueous extract of S aureus,

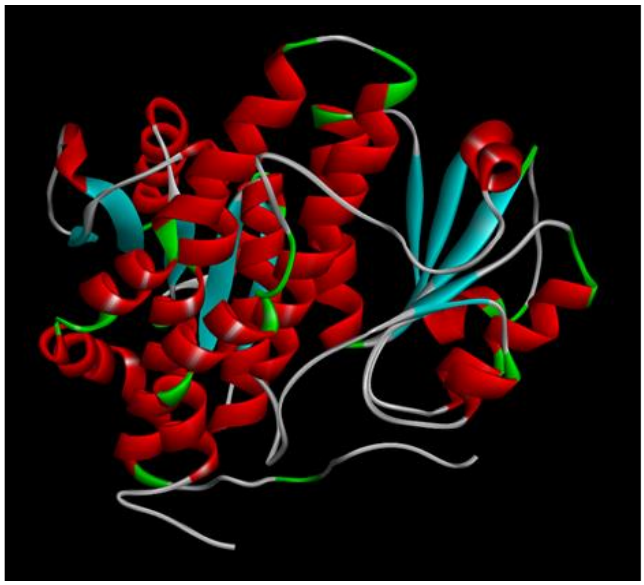

**Fig 7:** Crystal structure of Glucosamine 6 Phosphate synthase (PDB ID – 2VE5)

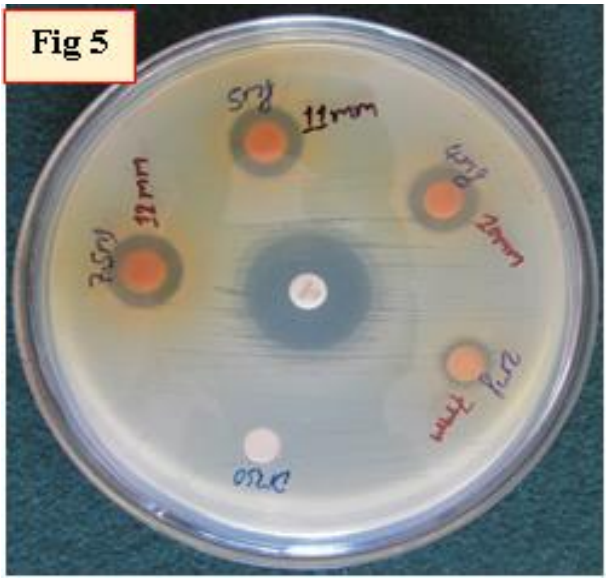


**Fig 5:** ZOI, acqueous extract of St agalactiae,

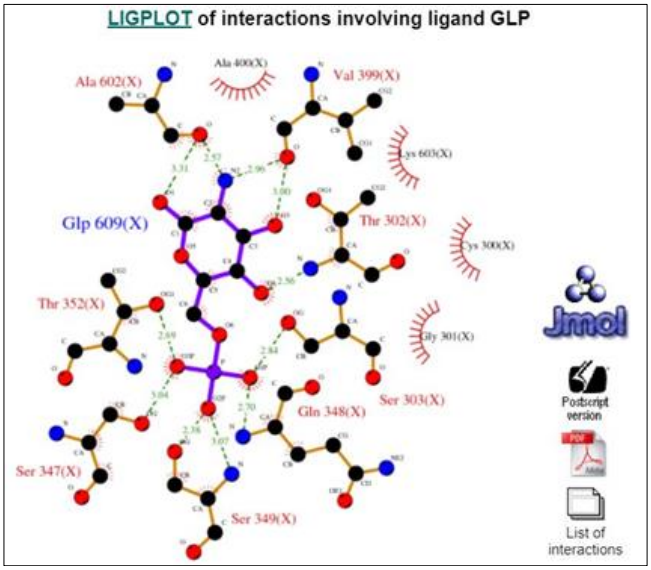


**Fig 8:** Active site residues of Glucosamine 6 phosphate synthase

**Table 6:** Active site residue of Glucosamine 6 phosphate synthase

| Sl. No. | Protein module | Contacts | Active site residues |
|---|---|---|---|
| 1 | Glucosamine 6 Phosphate Synthase PDB ID – 4VF5 | Non bonded contacts | ALA602, ALA 400, CYS300, GLY301, GLN348, LYS603, SER303, SER347, SER349, SER401, THR352, THR302, VAL399. |
| 2 | Glucosamine 6 Phosphate Synthase PDB ID – 4VF5 | Hydrogen bonds | ALA602, GLN348, SER303, SER347, SER349, SER349, THR302, THR352, VAL399. |

**Table 7:** Docking interactions of Glucosamine 6 phosphate synthase (PDB ID -2VF5) with small molecules of Glycyrrhiza glabra Linn using ArgusLab 4.0.1.

| Sl. No. | Drug / Ligands | Maximum number of poses | Number of ligand torsions | Precision | Search points | Total Grid points | Best ligand pose energy (kcal/mol) |
|---|---|---|---|---|---|---|---|
| 1 | Glucoliquiritin apioside | 150 | 4 | Regular | 12745 | 17476 | -8.23 |
| 2 | Shinflavanone | 150 | 4 | Regular | 12700 | 17476 | -10.84 |
| 3 | Shinpterocarpin | 150 | 1 | Regular | 7502 | 17476 | -8.96 |
| 4 | Prenyllicoflavone A | 150 | 3 | Regular | 78051 | 17476 | -8.69 |
| 5 | 1-methoxyphaseollidin | 150 | 5 | Regular | 9262 | 17476 | -8.97 |
| 6 | Liquiritin | 150 | 8 | Regular | 12816 | 17476 | -8.07 |
| 7 | Isoliquiritin | 150 | 12 | Regular | 12837 | 17476 | -10.85 |
| 8 | Glycyrrhizin | 150 | 12 | Regular | 5511 | 17476 | - |
| 9 | Liquiritigenin | 150 | 3 | Regular | 11761 | 17476 | -9.09 |
| 10 | Isoliquiritigenin | 150 | 6 | Regular | 12733 | 17476 | -9.09 |
| 11 | Liquiritin apioside | 150 | 14 | Regular | 11779 | 17476 | -9.48 |
| 12 | Glycyrrhetic acid | 150 | 2 | Regular | 5498 | 17476 | -9.12 |
| 13 | Licochalcone A | 150 | 8 | Regular | 12612 | 17476 | -10.69 |
| 14 | Licochalcone E | 150 | 8 | Regular | 12611 | 17476 | -9.67 |
| 15 | Glabridin | 150 | 3 | Regular | 12728 | 17476 | -9.04 |
| 16 | Hispaglabridin A | 150 | 5 | Regular | 12537 | 17476 | -8.87 |

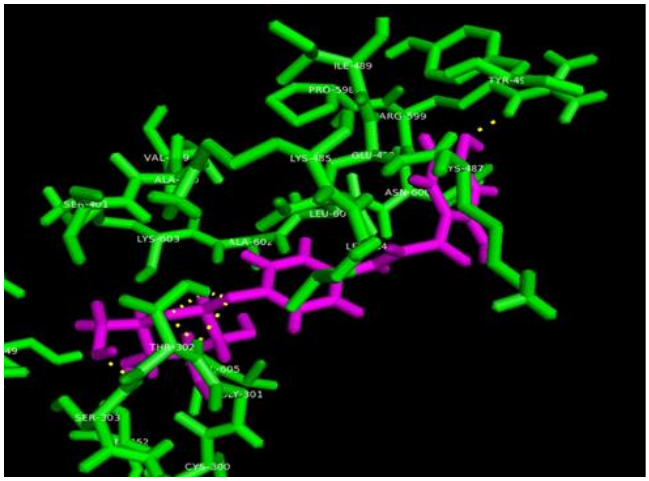

**Fig 9:** Docking interactions of Glucosamine 6 phosphate synthase with Isoliquiritin

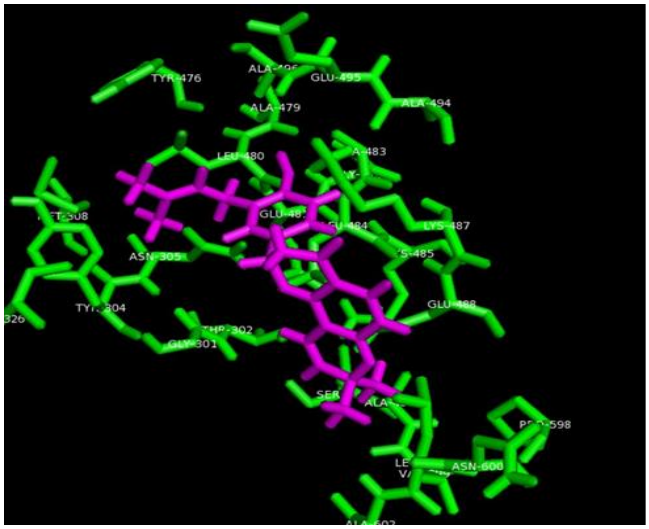

**Fig 10:** Docking interactions of Glucosamine 6 phosphate synthase with Shinflavanone

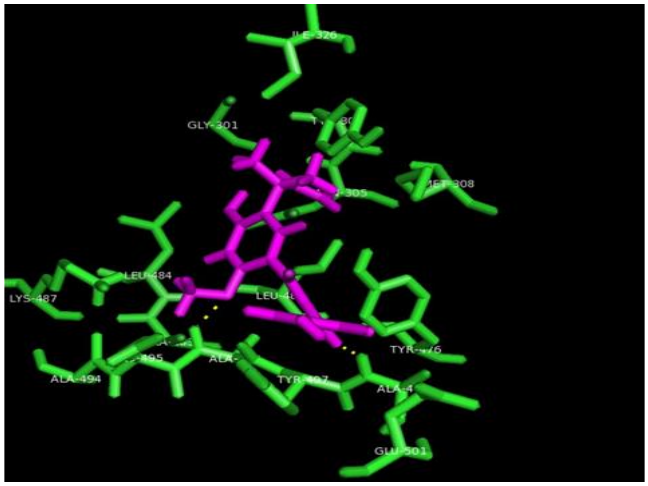

**Fig 11:** Docking interactions of Glucosamine 6 phosphate synthase with Licochalcone A

## Discussion

Mastitis is the inflammatory changes in the mammary parenchyma and affecting milk and milk quality (Viguier, 2009) [33]. For the dairy industry mastitis continues to be recognized as a most serious disease problem. The present study was to evaluate the antibacterial activity of aqueous and methanolic root extracts of *Glycyrrhiza glabra* against major mastitis causing organisms and molecular docking studies to identify the potent biomolecule in the plant with antibacterial activity. In the present study we have recorded the zone of inhibition of the *Glycyrrhiza glabra* extract which ranged between 6 mm to 14 mm and 12 mm to17 mm against pathogenic *Staph aureus, Streptococcus agalactiae and E. coli* in both acqueous and methanolic extract respectively. The Gentamicin (10µg) used as the standard for the Antibiotic sensitivity testing. The root extract of the *Glycyrrhiza glabra* showed more antimastitis activity towards *S. aureus*, compared to *St. agalactiae* and *E. coli.* The phytochemical analysis revealed presence of glycosides, saponins tannins and

flavonoids, the antibacterial activity of the extract of the licorice could be due the presence of these phytoconstituents (Ayyappa *et al.*, 2009) [34]. Licorice root contains triterpenoid saponins (Williamson, 2003) [35]. Soulef *et al.*, (2014) [36] reported glycoside of *Glycyrrhiza glabra* has a very large and diverse antibacterial activity. High flavonoid content has also been reported to exhibit antibacterial activity (Rauha, 2000) [37]. Tannins act as antimicrobial agents by preventing the development of microorganism by precipitating microbial proteins (Jain, 2011) [38].

The findings of this present study agreed with earlier studies for its antibacterial activity (Mahto *et al.*, 2014) [39]. The MIC and MBC (Minimum bactericidal concentration) value of methanolic extract was 3.125 mg/ml for *S. aureus*, 1.56 mg/ml for *St. agalactiae* and 12.5 mg/ml. Whereas the aqueous extract having higher MIC and MBC values i.e. 6.25 mg/ml for *S. aureus*, 3.125 mg/ml for *St. agalactiae* and the result was negative for E. coli (Syed *et al*., 2013) [40]

Antimicrobial properties of *G. glabra* were tested using Agar well diffusion method and Agar disc diffusion method. Streptomycin was used as standard drug with significant activity values, that is, 23 mm against *E. coli*, 36 mm *against S. epidermidi*s, 34 mm against *S. aureus* and 26 mm against *B. subtilis.* Analysis of data showed that the crude extract of G. glabra in dichloromethane exhibited superior activity against *E. coli* and *S. epidermidis* (Syed *et al.*, 2013, Amber *et al*., 2017) [40, 41].

Among the sixteen small molecules screened for antibacterial activity using ArgusLab 4.0.1, isoliquiritin emerged to be highly potent biomolecule followed by shinflavanone and Licochalcone A respectively.

## Conclusion

The present study aimed to evaluate the *invitro*antibacterial activity of aqueous and methanolic root extracts of *Glycyrrhiza glabra* against *Staphylococcus aureus, Streptococcus agalactiae and E-coli* the major pathogens isolated from the cases of clinical bovine mastitis and also in silico molecular docking studies of the potent biomolecules of Glycyrrhiza glabra against anti-bacterial target glucosamine 6 phosphate synthase. The roots of the plants showed excellent anti-bacterial activity proved by disc diffusion method and their MIC and MBC values. Further, isoliquiritin, shinflavanone and Licochalcone A emerged to be most potent small molecule possessing antibacterial activity. These research pave the way for further isolation and characterization of biomolecules of Glycyrrhiza glabra to develop as marketed product.

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