International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Molecular Docking Studies of Tribulus Terrestris Phytochemicals Against Bovine Mastitis Caused by Methicillin-Resistant Staphylococcus Aureus

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ABSTRACT

Bovine mastitis caused by methicillin-resistant Staphylococcus aureus is among the diseases capable of transferring from animal to human. It often leads to the development of antibiotic-resistant genes in both humans and animals. It is marked by the failure of the active-serine site of the blaZ gene in association with mecA to hydrolyze antibiotics which will inhibit the production of the beta-lactamase enzyme. This research aims to assess the interactions and binding affinities of Tribulus terrestris phytochemicals and important bacterial proteins involved in bovine mastitis. The research process begins with the identification of important bacterial proteins responsible for bovine mastitis and then the preparation of protein and ligand structures. Consequently, docking was performed, and interactions between protein-ligand were analyzed and visualized. Four phytoconstituents of Tribulus Terrestris (2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine, gitogenin, tigogenin, and hecogenin) were chosen, and molecular docking simulations revealed all the four compounds exhibit good binding affinities. The docking scores of the ligands with blaZ are as follows; 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine (-7.9), gitogenin (-8.9), tigogenin (-8.9) and hecogenin (-9.0). Also, the docking scores of the ligands with mecA are as follows; 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine (-9.4), gitogenin (-6.4), tigogenin (-6.5) and hecogenin (-6.8). Based on the predicted ADMET values using the Lipinski and Veber rule, compounds with potentially good activities were identified. The results suggest that only tigogenin is likely to exhibit antibacterial activity by binding with the active-serine site of blaZ.

Keywords: Antibiotic resistance, BlaZ, MecA, Phytochemical constituents, Molecular docking, Protein, Ligands, Mastitis

Introduction

Dairy farm production is one of the world's most prolific food supply methods which provides milk for human consumption and other products produced by processing of milk (Garcia *et al.*, 2019). Mastitis is a major disease affecting dairy animal production worldwide which affects the quality and quantity of milk produced by the animals (Ali *et al.*, 2021). It accounts for almost 80% of diagnoses (Bradley,

2002). The contamination of milk due to high somatic cell count (SCC), toxins, and antibiotic residues reduces the quality and quantity of the milk and alters its nutritional value which will be harmful to consumers (Harjanti *et al.,* 2018). It is caused by many bacteria but the most prominent is *Staphylococcus aureus*, the methicillin-resistant strain of the bacteria leads to the development of mastitis which is primarily known as the inflammation of the udder (Pascu *et al.,* 2022). One of *Staphylococcus aureus*'s unique characteristics is its capacity to quickly become resistant to almost any antibiotic medication that is introduced into clinical practice (Pantosti *et al.,* 2007). The development of the mecA gene, which codes for the novel protein PBP2a, a member of an enzyme family essential for the construction of the bacterial cell wall, is the cause of methicillin resistance. According to Pantosti *et al.* (2007), PBP2a confers resistance to methicillin and other beta-lactam antibiotics while having a very low affinity for β-lactam antibiotics.

According to Chhatre *et al.* (2014), a preliminary phytochemical analysis of *Tribulus terrestris* showed the presence of flavonoids, alkaloids, glycosides, tannins, and saponins, and the study reveals that phytochemical constituent of *Tribulus terrestris* varies from one geographical location to another. Flavonoids and saponins were reported to have a wide diverse pharmacological activity (Bouabdallah *et al.,* 2024).

Patel *et al.* (2021), reported that the saponin extract of *Tribulus terrestris* possesses active compounds having anti-cancer properties, and specifically nuatigenin saponin can be considered as an important therapeutic drug for human breast cancer. It was reported by Parimala et al., (2021) that *Tribulus terrestris* leaves extract was proven to have potential antibacterial activity against certain disease pathogens including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhii*, and *Klebsialla pneumoniae.* Another finding reported by (Pandey, 2015) in her research of preliminary screening of phytochemicals and anti-bacterial potential activity of *Tribulus terrestris* reveals that the plant has the potential to inhibit bacterial growth.

A sort of computer modeling of complexes created when two or more molecules comprising of the target molecule and a ligand interact, is called molecular docking (Muhammed and Aki-Yalsin, 2022). By considering the binding characteristics of both the target and ligand molecules involved, it forecasts the three-dimensional configuration of adducts (Kamal and Chakrabarti, 2023). This research aims to assess the interactions and binding affinities of *Tribulus terrestris* phytochemicals and important bacterial proteins involved in bovine mastitis.

Methods

Software and Hardware

Various resources and tools were utilized in this research, including the Pubchem database, swissadme for compound analysis and property assessment (Kar and Leszczynski, 2020), and way2drug for biological activity and toxicity analysis. Protein structure data were retrieved from RCSB protein structure data bank. For molecular docking studies, we applied open babel, pymol, and auto dock vina (Eberhardt *et al.,* 2021; Troth and Olson, 2010), and for protein visualization and analysis we used Pymol and Biovia Discovery Studio.

Molecular Docking

Protein structures

As a first step, the crystal structure of beta-lactamase (PDB ID: 1GHI) and mecA (PDB ID: 5M19) was

selected for the study. The 3D structures were downloaded (Chen and Herzberg, 2001) and (Mahasenan *et al.*, 2017) and prepared using pymol. The preparation includes the removal of the previously attached ligands, removal of water molecules, the addition of hydrogen, the addition of Kollman charge running the file, and then downloading the final protein file.

Ligand structures

The 3D structures of tribulus phytoconstituents were retrieved from the Pubchem database in SDF format and then converted to PDB format using open babel software. The ligands were then prepared using autodock vina. In the final step, the docking simulation was performed using autodock vina 4.6.2. The grid dimension for blaZ in grid settings was 94x68x80 in x,y, and z dimensions with x,y, and z center dimensions as 6.725x-15.1x-19.311 respectively. Grid point spacing was set at 0.987 A^0 in this case. The grid dimension for mecA in grid settings was 126x76x126 in x,y, and z dimensions with x,y, and z center dimensions as -5.314x-13.374x-47.834 respectively. Grid point spacing was set as 1 A^0 in this case. The docking simulation was performed, and the results were visualized and analyzed using Pymol and biovia discovery studio.

Drug likeness and ADMET profiling

ADME/toxic profiling is an important process in selecting a good drug candidate (Zhong, 2017). The drug-likeness and toxicity of compounds were evaluated using two webservers; swissadme and way2drug. The Smiles were obtained from the pubchem database.

Chemoinformatics

2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine, gitogenin, tigogenin, and hecogenin were retrieved from PubChem compound database.

Results and Discussion

Table 1: Compilation of physicochemical properties and drug-likeness assessments for the four selected ligand molecules (pharmacokinetics)

Ligands	M.W	NHA	NAHA	NRB	NHbA	NHbD	MR	TPSA	ILOGP	PAINS	BRENK	BS	SA
2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine	355.35	27	25	2	4	1	102.59	85.57	2.64	0	0	0.55	2.66
Gitogenin	432.64	31	0	0	4	2	123.23	58.92	4.24	0	0	0.55	7

Tigogenin	416.64	30	0	0	3	1	122.07	38.69	4.42	0	0	0.55	6.88
Hecogenin	430.62	31	0	0	4	1	122.07	55.76	4.06	0	0	0.55	6.7

MW: Molecular weight, **NHA**: Number of heavy atoms, **NAHA:** Number of aromatic heavy atoms, **NRB:** Number of Rotatable bonds N**HbA:** Number of H-Bond acceptors, **NHbD:** Number of H-bond Donors, **MR:** Molar refractivity, **TPSA:** Topological Polar Surface Area, **iLOGP:** Octanol/water partition coefficient, **PAINS:** Pan-Assay INterference compoundS**, Brenk:** Reference to Dr. Ruth Brenk, **BS:** Bioavailability score**, SA:** Synthetic accessibility.

Table 2: Compilation of biological activities of the four selected ligands

Ligands	Biological activity
2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine	Bacterial efflux-pump inhibitor, cell wall biosynthesis inhibitor, isopenicillin -N-epimerase inhibitor, anti-epileptic, nootropic, GABA-receptor agonist, HMGCS2-expression enhancer, anxiolytic, anti-convulsant, neuro-degenerative diseases treatment, chloride peroxidase inhibitor, a protein kinase inhibitor, Alzheimer's disease treatment, Endopeptidase inhibitor, dehydro -L-gluconate decarboxylase inhibitor, histamine release inhibitor, formaldehyde trans-ketolase inhibitor, trimethylamine-oxide aldolase inhibitor and anti-diabetic symptomatic.
Gitogenin	Antibiotic, UGT1A-substrate, UGT1A4-substrate, Glyceryl-ether mono oxygenase inhibitor, Dolichyl-diphospho-oligosaccharide-protein glycol-transferase inhibitor, UDP-glucuronosyl-transferase substrate, beta-adrenergic receptor kinase inhibitor, G-protein-coupled receptor kinase inhibitor, bilirubin oxidase inhibitor, anti-fungal, anti-pruritic, CYP3A2-substrate, immune suppressant, H^+- transporting two sector ATPase

	inhibitor, anti-protozoal (Leishmania), anti-eczematic, CYP3A-substrate, Vascular dementia treatment and CYP3A4-substrate.
Tigogenin	Antibacterial, prostate cancer treatment, plasmanyl ethanolamine desaturase inhibitor, UGT2B17 substrate, Anti- seborrheic, maleate isomerase inhibitor, hhydroxylamine reductase (NADH) inhibitor, Signal peptidase-I-inhibitor, Membrane permeability inhibitor, Analeptic phosphatidyl-inositol diacyl glycerol-lyase inhibitor, Membrane integrity antagonist, Membrane permeability enhancer, polarization stimulant, Linoleate diol synthase inhibitor, Alkyl glycerol phosphor ethanolamine-phospho-diesterase inhibitor, Anti-protozoal Lipoprotein disorders treatment, Protein -Npi- phosphor histidine-sugar, phosphor transferase inhibitor, Phospho ino-sitide 5-phosphatase inhibitor, CYP2J2 substrate, Anti-metastatic and CYP2A4 substrate.
Hecogenin	Antibacterial, Menopausal disorders treatment, UGT2B substrate, Cholesterol oxidase inhibitor, CYP2A11 substrate, 1,2- alpha -L- fucosidase inhibitor, Oxido reductase inhibitor, Antinociceptive, Protein-disulfide reductase (glutathione) inhibitor, CYP2J2 substrate, Prostate cancer treatment, P- glycoprotein substrate, Trans -1,2- dihydrobenzene -1,2- diol dehydrogenase inhibitor, Transcription factor NF kappa B stimulant, Transcription factor stimulant, Protein -Npi- phosphor histidine-sugar phosphotransferase inhibitor, Retinol dehydrogenase inhibitor, Ovulation inhibitor, Beta glucuronidase inhibitor.

Table 3: Compilation of toxicity or side effects of the four selected ligands

Ligands	Toxicity or Side effects
2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo[4',5'] pyridazine	Neutrophilic dermatosis (Sweet's syndrome), Nail discoloration, Gastro-intestinal hemorrhage, Multiple organ failure, Twitching, Hyperuricemia, Hematemesis, Pure red cell aplasia, Splenomegaly ffibrillation, Atrial aacneiform eruption, Thrombocytopoiesis inhibitor, Adrenal cortex hypoplasia Fibrosis, interstitial ulcer, gastric anemia, sideroblastic Cleft, Palaten ulcer, peptic

	Palpitation, Acidosis, lactic hypnotic, Visual acuity impairment, Occult bleeding, Pseudo porphyria, Bullous pemphigoid, Weight gain, Sialorrhea, Zinc deficiency, Retroperitoneal fibrosis.
Gitogenin	Hematotoxin, Toxic cataract, Reproductive dysfunction, Dermatitis, Pain, Embryotoxic, Necrosis, Teratogen, Respiratory failure, Hyper cholesterolemic, Cytotoxic, Toxic gastrointestinal inflammation, Behavioral disturbance, Glaucoma, Sensitization, Tachycardiac, Ocular toxicity, Endocrine disruptor, Hepatotoxic, Excitability Asthma, Conjunctivitis, Optic neuritis, Euphoria, Neurotoxic, Sensory disturbance, Headache, Consciousness alteration, Cleft palate, Nausea, Toxic, respiration, Sneezing, Optic neuropathy, Dizziness, Paralysis, Toxic, vascular, Emetic, Hypertensive, Cholestasis, Irritation, Xerostomia, Chorea, Thrombophlebitis, Bradycardic, Edema, Telangiectasia, Abortion inducer, Keratopathy, Sleep disturbance, Hypercalcemic, Bronchoconstrictor, Pulmonary edema, Dependence, hypoglycemics ccardio depressant, Dyskinesia, Nephrotoxic, Delirium, Dyspnea, Toxic respiratory center, Muscle weakness, Carcinogenic,
Tigogenin	Hematotoxin, Toxic cataract, Hyper cholesterolemic, Reproductive dysfunction, Embryotoxic, Cytotoxic, Teratogen, Dermatitis, Pain, Irritation, Glaucoma, Necrosis, Behavioral disturbance, Toxic gastro intestinal sensitization, Ocular toxicity, Asthma, Respiratory failure, Inflammation, Hepatotoxic, Optic neuritis, Conjunctivitis, Edema, Neurotoxic, Excitability, Cholestasis, Tachycardiac, Cleft palate, Optic neuropathy, Consciousness alteration, Endocrine disruptor, Abortion inducer, Paralysis, Headache, Nausea, Sensory disturbance, Chorea, Pulmonary edema, Euphoria, Emetic, Hypertensive, Dizziness, Sneezing, Toxic vascular Hypercalcemic, Telangiectasia, Xerostomia, Sleep disturbance, Thrombo-phlebitis, Toxic respiration, Muscle weakness, Delirium, Nephrotoxic, Bradycardic, Broncho constrictor, Toxic

	respiratory center, Cardio depressant, Keratopathy, Hypoglycemiaa
Hecogenin	Hematotoxin, Cataract, Irritation, Cytotoxic, Embryotoxic, Teratogen, Reproductive dysfunction, Hyper cholesterolemic, Sensitization, Respiratory failure, Dermatitis, Excitability, Necrosis, Behavioral disturbance, Toxic gastro intestinal pain, Ocular toxicity, Glaucoma, Hepatotoxic, Cholestasis, Cleft palate, Endocrine disruptor, Neurotoxic, Abortion inducer, Hypertensive, Optic neuritis, Telangiectasia, Tachycardiac, Emetic inflammation, Hyper calcemic, Sensory disturbance, Headache, Optic neuropathy, Edema, Nausea, Asthma, Dizziness, Consciousness alteration, Sleep disturbance, Conjunctivitis, Cardio depressant, Toxic vascular pulmonary edema, Toxic respiratory center, Sneezing, Paralysis, Drowsiness, Chorea, Xerostomia, Carcinogenic

Table 4: Molecular Docking score of the four selected ligands with BlaZ gene

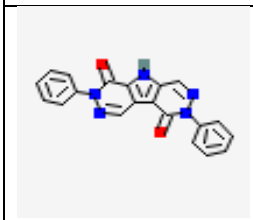
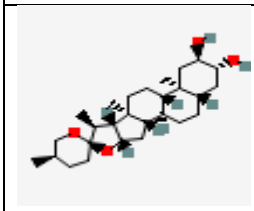
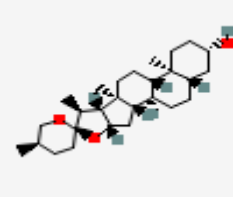
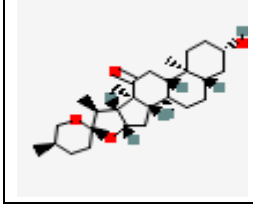
Structure	Compound	Docking Score (kcal/mol)	RMSD
	2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine	-7.9	0.00
	Gitogenin	-8.9	0.00
	Tigogenin	-8.9	0.00
	Hecogenin	-9.0	0.00

Table 5: Molecular docking score of the four selected ligands with mecA gene

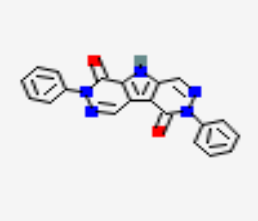
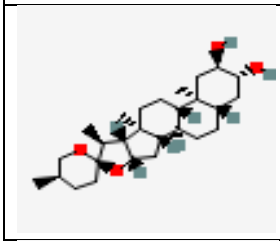
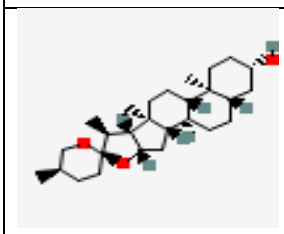
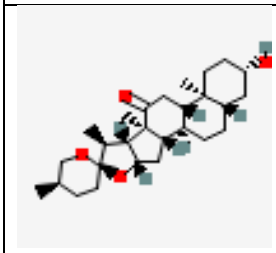
Structure	Compound	Docking score (kcal/mol)	RMSD
	2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine	-9.4	0.00
	Gitogenin	-6.4	0.00
	Tigogenin	-6.5	0.00
	Hecogenin	-6.8	0.00

Table 6: Ligands-BlaZ gene interaction and docking tools

Ligands	Docking	Visualisation	A.A with H-bond	A.A with other bonds
2,7-Diphenyl-1, dioxopyridazino [4,5:2',3'] pyrrolo [4',5'-d] pyridazine	Auto dock vina	Discovery studio and Pymol	SER-216, SER-235, ARG244	TRY-105, TYR-129, ASN-132, GLN-170, LYS-215, ASP-218, THR-219, ILE-239, ASP-276, GLN-273
Gitogenin	Auto dock vina	Discovery studio and Pymol	SER-216, LYS-234, SER-235	ALA-104, TYR105, SER-130, ASN-132,

				ILE-167, GLN-170, GLY-236, GLN-237, ILE-239, ARG-244
Tigogenin	Auto dock vina	Discovery studio and Pymol	SER-70	TYR-105, SER-130, ASN-132, GLN-170, TYR-171, SER-235, GLY-236, GLN-237, ILE-239
Hecogenin	Auto dock vina	Discovery studio and Pymol	ASN-132, GLN-170	SER-70, TYR-105, SER130, ILE-167, GLN-170, SER-235, ALN-244, ILE-239, ARG-244

A.A(Amino Acid), H-Bond (Hydrogen Bond)

Table 7: Ligands-mecA gene interaction and docking tools

Ligands	Docking	Visualisation	A.A with H-bond	A.A with other bonds
2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine	Auto dock vina	Discovery studio and Pymol	ILE-309, ALA-310	ARG-110, TRP-205, THR-210, PHE-211, THR-234, THR-235, ASN-236, HIS-311
Gitogenin	Auto dock vina	Discovery studio and Pymol	HIS-583	TYR-446, GLU-447, VAL-448, THR-582, SER-643
Tigogenin	Auto dock vina	Discovery studio and Pymol	ARG-469	SER-424, LYS-426, ASP-428, TRP-432, GLN-433, LYS-434,

				ASN-442
Hecogenin	Auto dock vina	Discovery studio and Pymol	ARG-469	SER-424, TYR-425, LYS-426, TRP-432, GLN-433, LYS-434

A.A(Amino Acid), H-Bond (Hydrogen Bond)

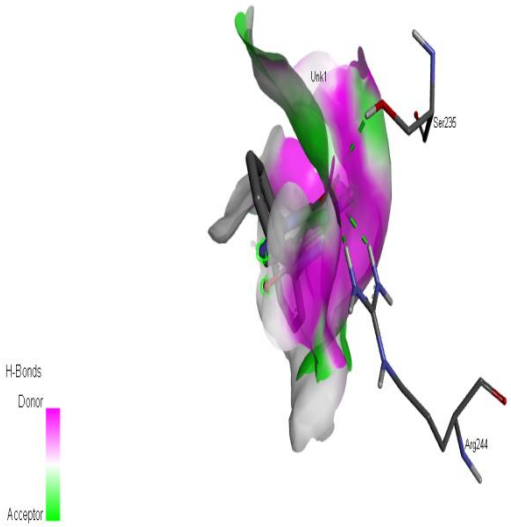


Fig 1

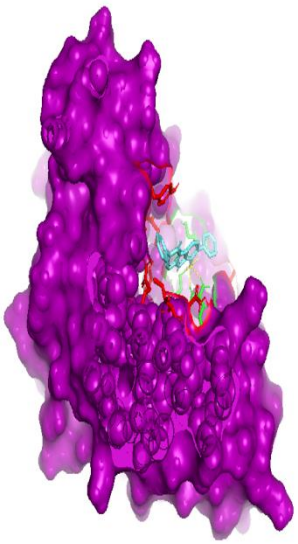


Fig 2

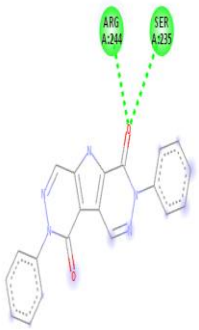


Fig 3

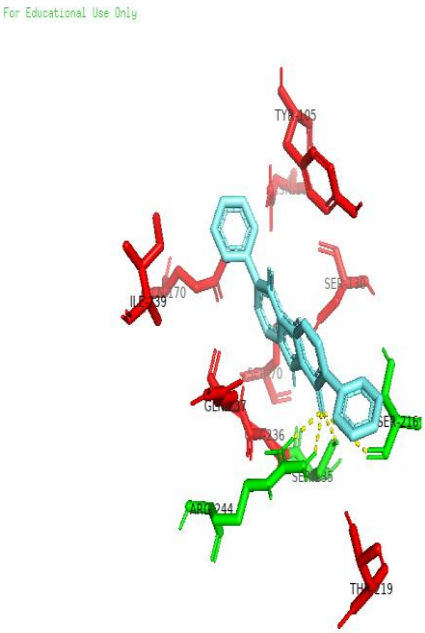


Fig 4

Fig. 1: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with BlaZ 3D structure viewed with discovery studio

Fig 2: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with BlaZ 3D ligand-protein complex viewed with pymol

Fig 3: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with BlaZ 2D structure viewed with discovery studio

Fig 4: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with BlaZ ligand-amino acid interaction 3D structure viewed with pymol

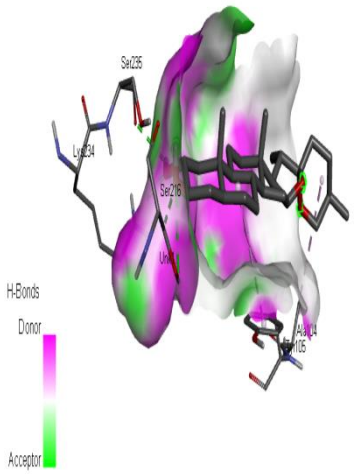


Fig 5

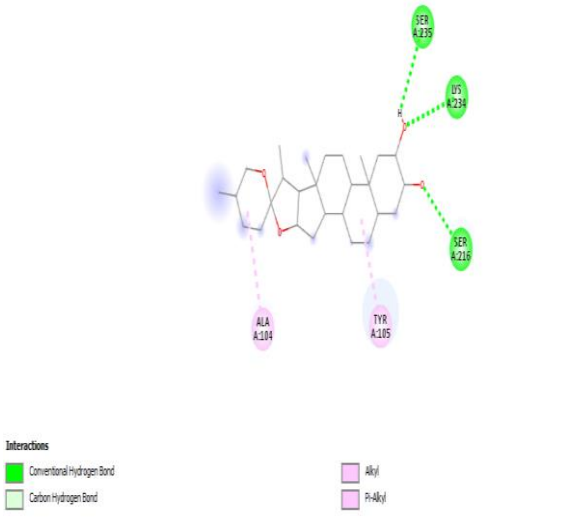


Fig 6

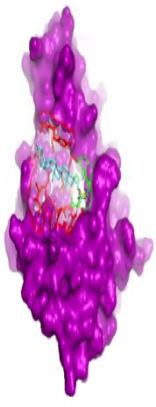


Fig 7

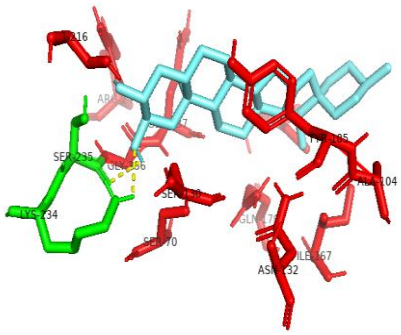


Fig 8

Fig. 5: Gitogenin with Blaz ligand-amino acid interaction 3D structure viewed with discovery studio

Fig. 6: Gitogenin with Blaz ligand-amino acid interaction 2D structure viewed with discovery studio

Fig. 7: Gitogenin with Blaz ligand-protein complex interaction 3D structure viewed with pymol

Fig. 8: Gitogenin with Blaz ligand-amino acid interaction 3D structure viewed with pymol

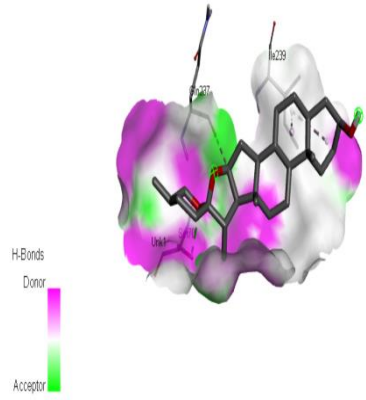


Fig 9

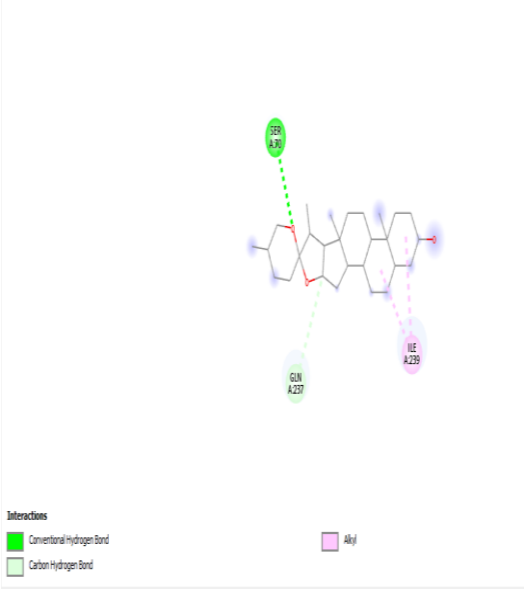


Fig 10

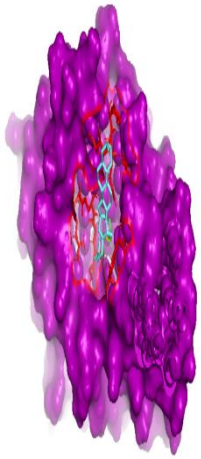


Fig 11

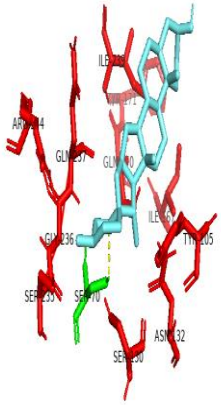


Fig 12

Fig. 9: Tigogenin with Blaz ligand-amino acid interaction 3D structure viewed with discovery studio
Fig. 10: Tigogenin with Blaz ligand-amino acid interaction 2D structure viewed with discovery studio
Fig. 11: Tigogenin with BlaZ ligand-protein complex 3D structure viewed with pymol
Fig. 12: Tigogenin with BlaZ ligand- amino acid interaction 3D structure viewed with pymol

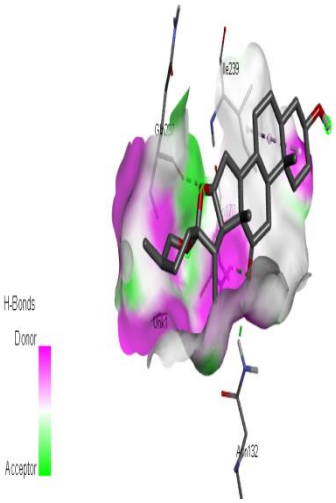


Fig 13

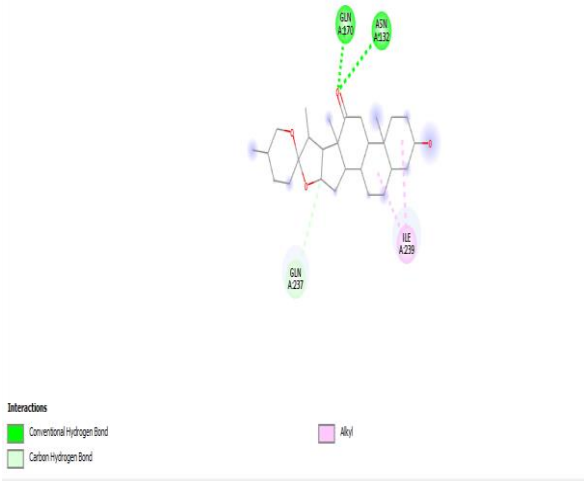


Fig 14

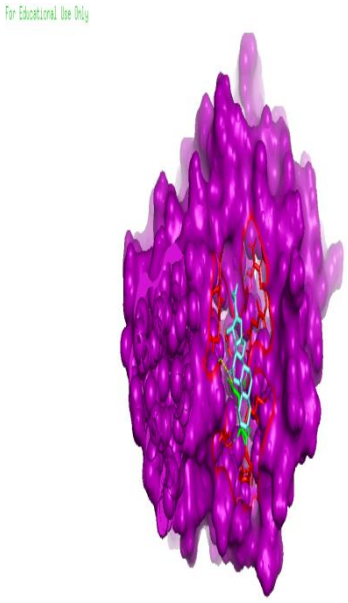


Fig 15

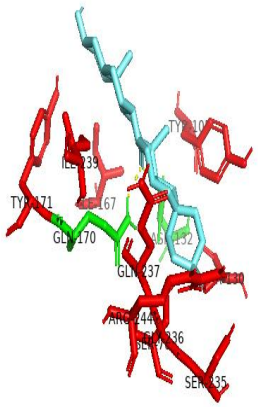


Fig 16

Fig. 13: Hecogenin with BlaZ ligand-amino acid interaction 3D structure viewed with discovery studio

Fig. 14: Hecogenin with BlaZ ligand-amino acid interaction 2D structure viewed with discovery studio

Fig. 15: Hecogenin ligand-protein complex 3D structure viewed with pymol

Fig. 16: Hecogenin with BlaZ ligand-amino acid interaction 3D structure viewed with pymol

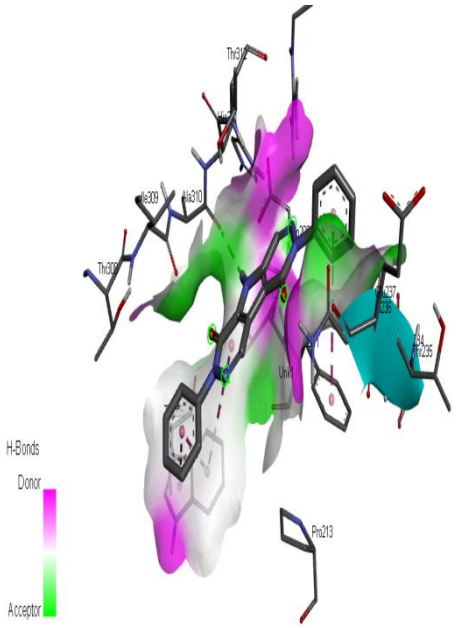


Fig 17

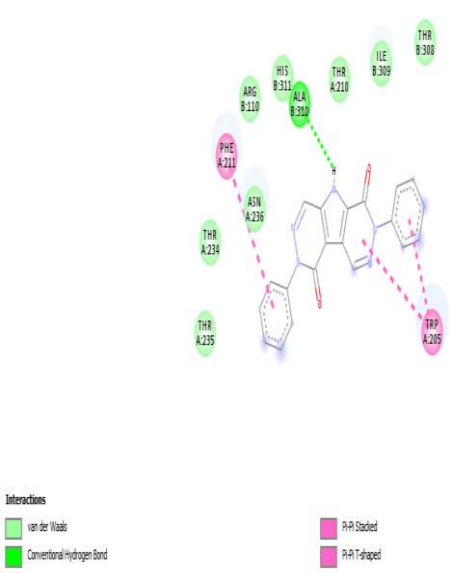


Fig 18

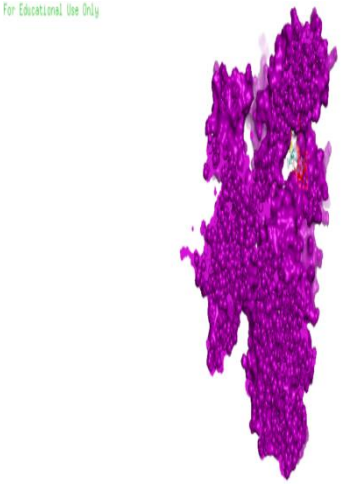


Fig 19

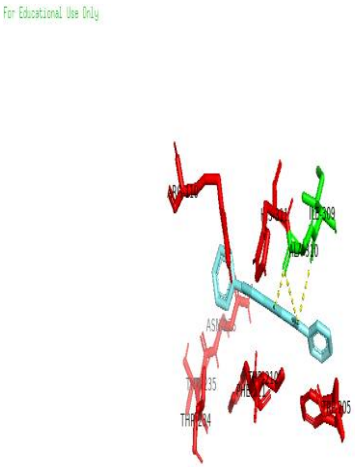


Fig 20

Fig. 17: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with mecA ligand-amino acid interaction 3D structure viewed with discovery studio

Fig. 18: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with mecA ligand-amino acid interaction 2D structure viewed with discovery studio

Fig. 19: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with mecA ligand-protein complex 3D structure viewed with pymol

Fig. 20: 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine with mecA ligand-amino acid interaction 3D structure viewed with pymol

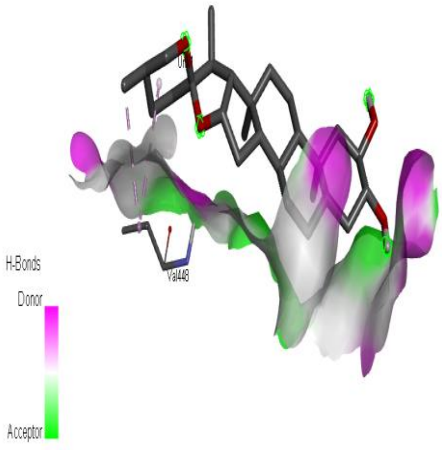


Fig 21

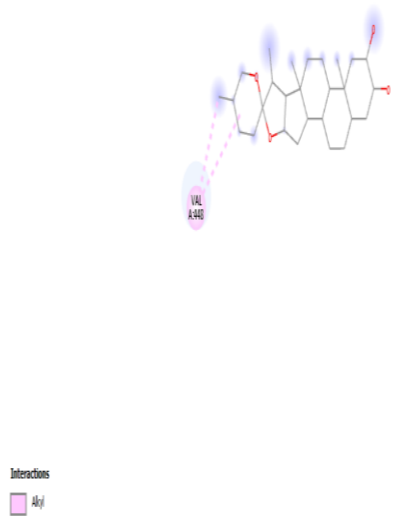


Fig 22

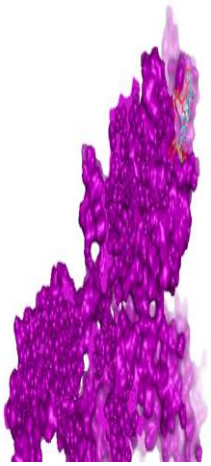


Fig 23

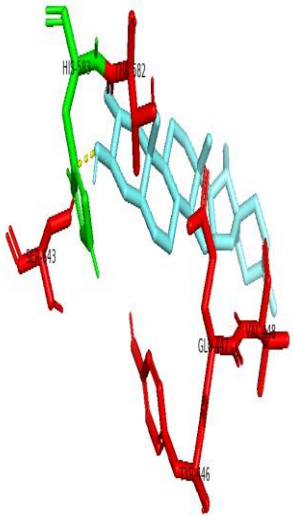


Fig 24

Fig. 21: Gitogenin with mecA ligand-amino acid interaction 3D structures viewed with discovery studio
Fig. 22: Gitogenin with mecA ligand-amino acid interaction 2D structures viewed with discovery studio
Fig. 23: Gitogenin with mecA ligand-protein complex 3D structure viewed pymol
Fig. 24: Gitogenin with mecA ligand-amino acid interaction 3D structure viewed with pymol

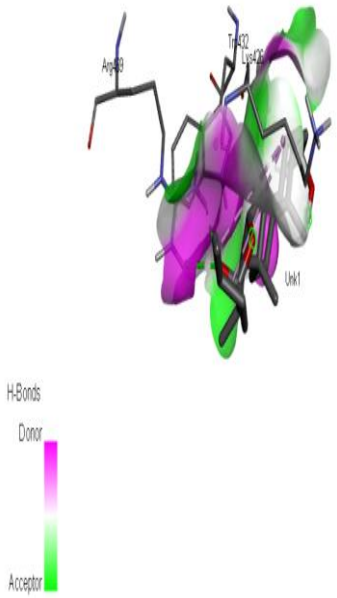

H-Bonds
Donor
Acceptor

Fig 25

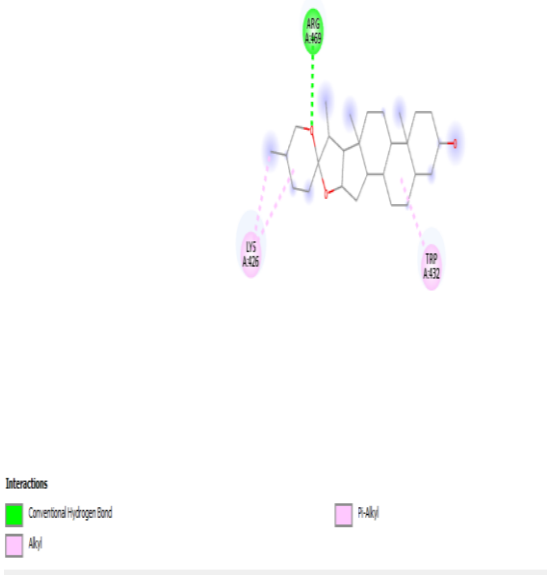

Interactions
Conventional Hydrogen Bond
Pi-Alkyl
Alkyl

Fig 26

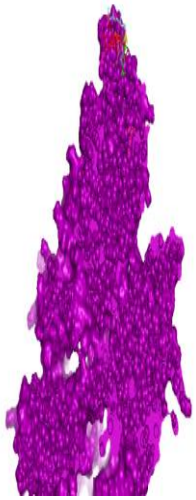


Fig 27

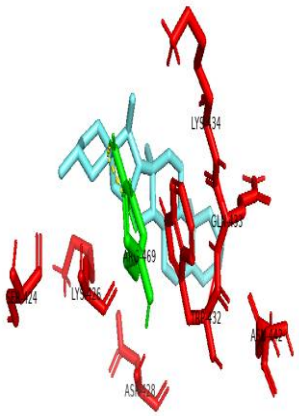


Fig 28

Fig. 25: Tigogenin with mecA ligand-amino acid interaction 3D structures viewed with discovery studio

Fig. 26: Tigogenin with mecA ligand-amino acid interaction 3D structures viewed with discovery studio

Fig. 27: Tigogenin with mecA ligand-protein complex 3D structure viewed with pymol

Fig. 28: Tigogenin with mecA ligand-amino acid interaction 3D structure viewed with pymol

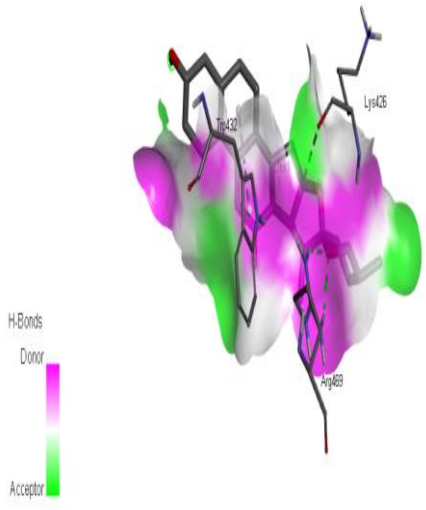

H-Bonds
Donor
Acceptor
Lys426
Arg469

Fig 29

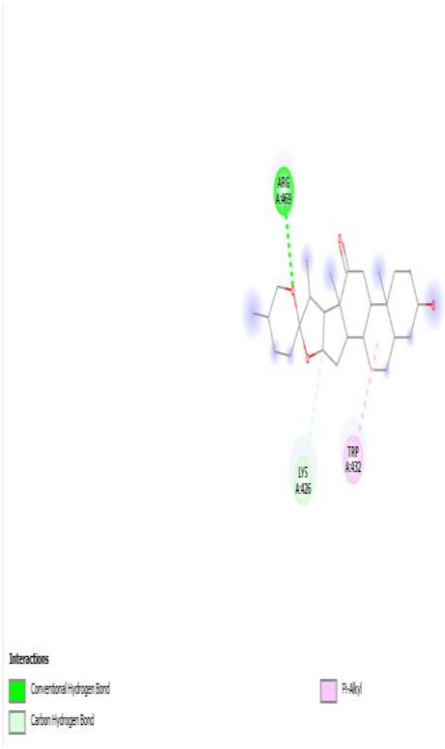

ARG A:469
TRP A:432
LYS A:426
Interactions
Conventional Hydrogen Bond
Pi-Alkyl
Carbon Hydrogen Bond

Fig 30

Fig 31

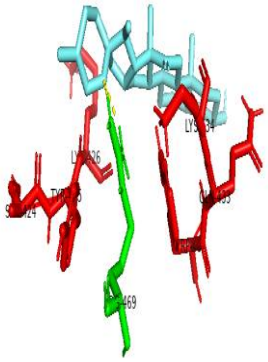


Fig 32

Fig. 29: Hecogenin with mecA ligand-amino acid interaction 3D structure viewed with discovery studio
Fig. 30: Hecogenin with mecA ligand-amino acid interaction 2D structure viewed with discovery studio
Fig. 31: Hecogenin with mecA ligand-protein complex 3D structure viewed with pymol
Fig. 32: Hecogenin with mecA ligand-amino acid interaction 3D structure viewed with pymol

Description of figures

The ligands are colored in aquamarine colour. The red-colored structures represent the non-hydrogen bonds. The yellow-colored structures represent the hydrogen bonds. The purple-colored structure represents the whole protein structure with binding pockets showing the red, yellow and

Discussion

Staphylococcus aureus (S. aureus) is among the Gram-positive bacteria known as one of the prevalent human pathogens and one of the important bacteria that causes bovine mastitis (Hnini *et al.,* 2024). The methicillin resistant staphylococcus aureus (MRSA) is a serious threat to dairy industries causing loss by formation of antibiotic resistance mechanism against certain antibiotics among which are ceftriaxone, taxobactum, oxacillin, amoxycillin, methicillin, penicillin, ampicillin and sulbactum which inhibits the production of beta lactamase enzyme in BlaZ gene associated with mecA gene (Siddiqui and Koirala, 2024). It prevents the formation of penicillin binding proteins (PBP2a) in the bacterial cell wall (Idrees *et al.,* 2023). Staphylococcus aureus is among the class-D beta lactamase enzyme which uses serine residue to hydrolyse antibiotics (Leonard *et al.,* 2013).

The findings in this research are in line with that reported by (Bouabdallah *et al.,* 2024) where docking scores of 15 compounds against AChe ranged from -11.22 to -24.68 kcal/mol. According to Bouabdallah *et al.,* docking score below -24 kcal/mol reveals that terrestrosin C, protodioscin, rutin, and saponin C were the most stable docked compounds, with rutin as the highest and most stable docked compound having a score of -24.68. It was also reported by (Bouabdallah *et al.,* 2024) where docking scores of four flavonoids (rutin, quercetin, kaemfperol and luteolin) against alzheimer's disease ranged from -6.64 to -7.50, with rutin having the highest docking score of -7.5.

It was reported by (Parimala et al., 2021) that *Tribulus terrestris* leaves extract was proven to have potential antibacterial activity against certain disease pathogens including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhii* and *Klebsialla pneumoniae.* Another finding reported by (Pandey, 2015) in her research of preliminary screening of phytochemicals and anti-bacterial potential activity of *Tribulus terrestris* reveals that the plant has the potential to inhibit bacterial growth.

Earlier studies revealed that there is a variation between the active sites of the different MRSA strain (blaZ and mecA) due to its variant virulent characteristic which make it very difficult to treat (Hnini *et al.,* 2024). Though, this variation still exists but the mechanism of killing is identical, with acylation of the active-site serine in the transpeptidase domain of PBP as the definitive characteristic of the inhibitory activity (Bush and Bradford, 2020). PBPs are serine acyltransferases that facilitate the production of cross-linked peptidoglycan and serve as targets for β-lactam antibiotics due to their transpeptidase-related catalytic activity (Turner et al., 2022). After adhering to the PBP catalytic cleavage, β-lactam antibiotics covalently attach to the active site serine of PBPs, resulting in the creation of a slowly hydrolyzed acyl-enzyme complex that decreases peptidoglycan cross-linking (Ambade et al., 2023).

Among all the four ligands used in this research, only tigogenin binds with serine-70 (SER-70), which is the active-site in the transpeptidase domain of PBP where acylation occurs with docking score of -8.9. Though all other ligands show good binding score, none of three (2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'-d] pyridazine, gitogenin and hecogenin) binds with active-site serine PBPs transpeptidase domain.

3.2.1 Pharmacokinetics of investigated compounds

All the four selected molecules (2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine, gitogenin, tigogenin and hecogenin) exhibit favourable ADME properties, suggesting their potential as drug candidates. A summary of ADME and drug likeliness properties is provided in Table 4.1, chapter four. In essence, all the four met the criteria for molecular weight (MW<500), hydrogen bond donor (HBD≤5), hydrogen bond acceptor (HBA≤10), octanol water partition coefficient (iLOGP) and molar refractivity (MR 40-130). Furthermore, the bioavailabity score was accessed as shown in table 1, chapter four.

Swiss ADME gives extra information and tools relevant to molecular docking analysis. Along this section, Pan-Assay interference compounds (PAINS) are structures commonly found in screening libraries that are known to interfere with many biological assays, often leading to false-positive results. All the four compounds have zero number of PAINS.As for "Brenk," it's likely a reference to the work of Dr Ruth Brenk, a medicinal chemist known for her outstanding contribution to drug design and structure-based drug discovery. It's possible that Swiss ADME includes tools or references related to her work or methodologies in medicinal chemistry. Overall, the Medicinal Chemistry section of Swiss ADME likely provides valuable resources and tools for medicinal chemists to assess and optimize potential drug candidates. Zero brenk alert was also recorded in all the four compounds. The synthetic accessibility of 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'] pyridazine is 2.66, hecogenin has 6.7, tigogenin has 6.8 and gitogenin have the highest 7, gitogenin, tigogenin and hecogenin have similar synthetic accessibility score. All the four compounds have the same bioavailability score of 0.55.

Conclusion

This research employs computational molecular docking and drug likeness assessment to evaluate four selected ligands effectiveness against methicillin resistance staphylococcus aureus genes (blaZ and mecA). The four phytoconstituents exhibits good binding scores ranging from -7.9 to -9.0 for blaz and -6.8 to -9.4 for mecA but only tigogenin binds with serine-70 which is the active site for PBPs transpeptidase domain of blaZ. Though the rest ligands forms hydrogen bonds with amino acids of the blaZ and mecA, the residues show no effect in targeting the active site. According to these findings we can suggest that tigogenin might serves as a potential compound in production of MRSA antibiotic.

Conflict of interest

This research was solely performed to explore the potential of Tribulus terrestris phytochemical as an antibacterial agent against methicillin resistant staphylococcus aureus.

Acknowledgment

This research was achieved through the contribution of the remaining authors and with the guidance of Dr. Ramgopal Dhakar and the entire staff of the department of life science at Mewar University Rajasthan, Chittorgarh, india.

Declarations

Ethical Approval

This study was approved by Life Science Department of Mewar University Chittorgarh Rajasthan, India and is inline with the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA) in which no animals were used physically in the study and does not violet the act of the Indian Parliament under the Prevention of Cruelty to Animals Act 1960, formed in 1964, and revived in 1998, under the committed chairpersonship Maneka Gandhi.

Funding
No funding received for this study

Data availability
Derived data supporting the findings of this study are available from the corresponding author on request.

Consent for publication
All authors on the research paper have approved the manuscript for submission

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