IJPSR (2023), Volume 14, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 December 2022; received in revised form, 26 March 2023; accepted 30 May 2023; published 01 August 2023

IN-SILICO SCREENING OF POTENTIAL PHYTOCOMPOUNDS AGAINST *STAPHYLOCOCCUS AUREUS* AND AN *IN-VITRO* ANTIBACTERIAL EVALUATION

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Keywords:

Staphylococcus aureus, Antibiotic resistance, Multidrug Resistance, ClfA-Fibrinogen, Phytocompounds, ADME, Molecular docking, Antibacterial

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ABSTRACT: *Staphylococcus aureus*is a Gram-positive facultative pathogenic bacterium responsible for a wide range of infections ranging from skin to life-threatening infections. Antibiotic resistance of *Staphylococcus aureus* is an emerging global concern. Thus, developing viable antibiotics are in high demand. This study identified novel lead compounds from traditionally used medicinal plants *via in-silico* molecular docking and *in-vitro* antibacterial analysis. Thus, we have derived literature-evident phytocompounds from numerous traditional medicinal plants such as *Boerhavia diffusa, Clerodendrum infortunatum, Sida rhombifolia, Tephrosia purpurea, Scoparia dulcis, Breynia retusa, Euphorbia heterophylla, Hemigraphis alternata, Hedyotis corymbose, Imperata cylindrica* and their structures were retrieved from PubChem. Lipinski's rule in ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiles were used to screen derived phytocompounds, followed by *in-silico* docking to the target protein. Clumping factor A (ClfA)-fibrinogen, a key virulence factor in *S. aureus*, was taken as a target protein. ClfA is a cell-wall-anchored protein that causes bacterial adherence to the blood plasma protein fibrinogen, which causes a variety of infections. Thus, an appealing strategy is to discover a novel lead compound with antiadhesive properties to prevent cell adherence. After performing molecular docking, Eupalitin 3-o-galactoside, a natural compound derived from *Boerhavia diffusa*, exhibited strong binding affinity with the least glide score of -8.56 kcal/mol. Antibacterial investigations were carried out using different solvent extractions of plants with phytocompounds that exhibited a significant glide score. Leaf extract of *Boerhavia diffusa, Clerodendrum infortunatum* and *Sida rhombifolia* shows the strongest activity against *Staphylococcus aureus*.

INTRODUCTION: *Staphylococcus aureus* is a Gram-positive spherical bacterium belonging to the Staphylococcaceae family. It is one of the most harmful bacteria, causing diseases ranging from minor skin infections like folliculitis and impetigo to life-threatening infections including bloodstream infection, endocarditis, and pneumonia [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(8).4128-41

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(8).4128-41

S. aureus can create a broad spectrum of virulence factors connected to the cell wall and play a major role in invading microbes into the host tissue. It can also release exotoxins, which promote staphylococcal infections. The adherence of bacteria to host extracellular matrix proteins such as fibrinogen, fibronectin, and collagen triggers the molecular pathogenesis of infections [2].

Antibiotics have been used to treat bacterial illnesses since the early 20th century. Antibiotic exploitation is assumed to be responsible for antibiotic-resistant strains' widespread growth. Most bacteria are now resistant to multiple treatments, making this scenario difficult to treat.

Multidrug-resistant bacteria cause a massive range of bacterial diseases, with *Staphylococcus aureus* being one of the most threatening MDR strains. The emergence of resistant strains poses a persistent hazard to human health, which is now a serious challenge [3]. To combat multidrug resistance strain, a variety of techniques have been implemented. Currently, antibiotics such as ceftaroloine, ceftobiprole, co-trimoxazole, cephalosporin, dalfopristin, tedizolid and linezolid are available but they are not employed in clinical procedures because of their high cost and safety concerns [4]; therefore, there is a compelling need for the development of novel drugs. The conventional drug development method is complex and time-consuming so computer-assisted docking can be utilized to find new lead molecules. Molecular docking is a time-saving method for docking a large number of molecules to a target protein [5].

In the current scenario, virtual screening is employed to develop an efficient therapeutic compound in which structure-based virtual screening will be carried out where screening is based on receptor structure [6]. In this study plant, phytocompounds were used as ligands since plants produce a vast range of secondary metabolites with diverse pharmaceutical properties like antimicrobial, anti-fungal, anti-inflammatory, and antitumor activity and are mostly preferred due to their fewer no side effects [7]. Developing drugs from herbal plant samples would be cost-effective and biocompatible [8].

And as target protein ClfA- fibrinogen was utilized. Clumping factor A (ClfA) is a cell adhesion protein anchored to the surface of *Staphylococcus aureus* that allows bacteria to adhere to fibrinogen in host tissue, thus it is known to be a fibrinogen binding protein. Fibrinogen is a glycoprotein found in the blood that consists of a polypeptide chain (2α, 2β and 2γ chains) [9]. A- region of ClfA binds to fibrinogen by interacting C-terminal of two γ-chains of fibrinogen. ClfA protects *S. aureus* against macrophage phagocytosis, as a result, bacteria become more virulent [10].

Therefore, we have considered ClfA- fibrinogen as a potential target protein and phytocompounds from medicinal plants such as *Boerhavia diffusa, Clerodendrum infortunatum* and *Sida rhombifolia, Scoparia dulcis, Breynia retusa, Euphorbia heterophylla, Hemigraphis alternata, Imperata cylindrica, Hedyotis corymbosa* and *Tephrosia purpurea* were used as ligand. *Boerhavia diffusa*is a well-known Ayurvedic plant and the aerial part of *B. diffusa* are reported to have notable antioxidant and antibacterial activity [11]. The entire plant, including the roots, leaves, and stem of *Clerodendrum infortunatum,* exhibits a variety of biological functions. According to studies, leaf extracts of *C. infortunatum* poses significant antibacterial and antifungal activities than root and stem [12]. Similarly, the aerial part of *Sida rhombifolia* is rich source of phytoconstituents and is known to have antibacterial properties [13]. Therefore, the current study focuses on *in-silico* analyses to determine the phytocompound with the best binding efficiency against the target protein and *in-vitro* antibacterial screening was used to determine the plant extract with optimum antibacterial activity against *Staphylococcus aureus.*

METHODS:

***In-silico* Studies:** Molecular docking is an efficient and expanding method for developing prospective lead drugs. Molecular docking involves various computational procedures, including preparing receptors and ligands, docking and post-docking analyses, *etc.* [14]. The computational software maestro Schrodinger version 9.0.211 was utilized for ADME profiling, LigPrep, Protein preparation, Glide grid generation, and G scoring function.

Structure Retrieval: This study aimed to evaluate the antibacterial activity of phytocompounds from different natural plants. After conducting a literature survey, GC-MS identified 125 phytocompounds from 10 different plants considered ligands. The PubChem database (http://pubchem.ncbi.nlm.nih.gov) was used to derive the chemical structures of phytocompounds [15]. The three-dimensional structure of the protein to be targeted, ClfA- Fibrinogen was retrieved from the PDB- Protein Data Bank database (http://www.rcsb.org/pdb). The target protein's active site region was determined using the LigSite online tool (http://projrct.biotec.tudresden.de/pocket/), which predicts the amino acids with binding pockets [16].

ADME Profiling of Phytocompounds: To assess the drug-likeness of particular ligands, the ADME properties, which include Absorption, Distribution, Metabolism, Excretion, and Toxicity of phytocompound were analyzed. The Schrodinger program's QikProp module version 4.4 was used to predict the ADME properties including, the number of rotatable bonds, molecular weight, number of donor hydrogen bonds, number of acceptor hydrogen bonds and octanal water partition coefficient logP, *etc* as per Lipinski rule of five [17] and the Pass online way2drug online tool (http://way2drug.com/PassOnline/predict.php)was used to further analyze the biological properties of the ligand [18].

Ligand and Receptor Preparation: Before molecular docking, ligands were prepared using the LigPrep module, optimized by bond ordering and angles. In contrast, GLIDE's Protein preparation wizard was used to prepare proteins. In which water molecules were removed from the structure for preparation, hydrogen bonds were optimized and energy was minimized, further structure-based virtual screening was performed [19].

Molecular Docking: Identification of new therapeutic compounds is a critical step in the *in-silico* investigation and is accomplished through molecular docking, where structure-based virtual screening was performed for each screened phytocompound against ClfA-Fibrinogen. The glide module of the Schrodinger program was used to simulate receptor-ligand interaction and binding affinities.

Effective ligands against the target protein will be identified due to molecular docking based on the least glide score value and by the formation of hydrogen bonds and hydrophobic interactions [20]. The PyMol visualization tool was further used to see the hydrogen bond interaction between the ligand and the target protein, where the interaction between the amino acid residues and the hydrogen bonds with bond length can be evaluated [21].

***In-vitro* Studies:**

Sample Collection and Authentication: The three different plant species *Boerhavia diffusa, Clerodendrum infortunatum* and *Sida rhombifolia* were chosen for *in-vitro* antibacterial screening among the remaining ten plants as a result of *in-silico* studies. Those plants were harvested within the Kanyakumari district of Tamil Nadu and the Botanical Survey of India (BSI) in Coimbatore, Tamil Nadu, identified those plants as *Boerhavia diffusa* (BSI/SRC/5/23/2022/Tech/520), *Clerodendrum infortunatum* (BSI/SRC/5/23/2022/Tech/518) and *Sida rhombifolia* (BSI/SRC/5/23/2022/Tech/519).

Sample Preparation: Leaves, stem, root and flowers of *Boerhavia diffusa*, *Clerodendrum infortunatum* and *Sida rhombifolia* were washed, dried and crushed into fine powder. 50 grams of powdered samples were subjected to Soxhlet extraction using hexane, ethyl acetate and methanol at 40°C for roughly 6-8 hours [22]. After extraction excess solvents were evaporated using rotary evaporator under lower pressure. Final extracts were collected and kept for future use in an airtight container.

Anti-Bacterial Screening: The Agar well diffusion method was used to determine the antibacterial activity of various extracts [23]. *Staphylococcus aureus* culture was purchased from MTCC (MTCC-96). 100 µl of bacterial culture were swabbed into MHA plates, followed by 6 mm wells that were created using a sterile cork borer.

50 µl of crude extract from each sample and 50 µl of neomycin sulphate as a positive control were introduced to the wells. Organic solvents hexane, ethyl acetate, and methanol were used as the negative control. Plates were further kept for overnight incubation at 37 °C.

After the incubation period, the sensitivity of the test plates was assessed using a zone of inhibition, with the diameter of the zone surrounding the well determined in millimeters.

RESULT:

Structure Retrieval and Active Site Prediction: 3D Structure of the target protein ClfA-Fibrinogen was retrieved from Protein Data Bank with a PDB ID of 2VR3 (Fig. 1), and its active site pockets ASN 525, ILE 384, GLU 526, ALA 528 was discovered *via* the LigSite online tool.

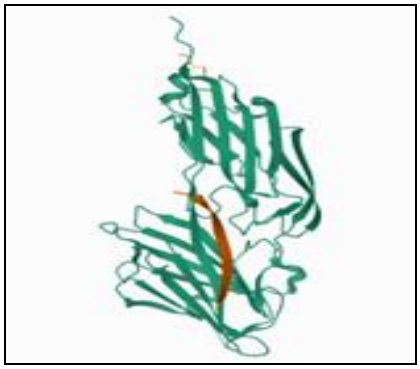


FIG. 1: 3D STRUCTURE OF TARGET PROTEIN 2VR3

ADME Screening Using QIKPROP Module: The bioavailability of selected phytocompounds was predicted using ADME profiling, which is a crucial step in the discovery of potential lead compounds. Out of 125 phytocompounds, only 24 compounds satisfy the Lipinski rule of five and are considered to be drug-likeness. Parameters like lipophilicity, permeability in octanol/ water partition coefficient and brain/ blood barrier along with these, properties like Number of rotatable bonds, Number of metabolic reactions, Molecular weight, Hydrogen bond donor, Hydrogen bond acceptor and Skin permeability were evaluated. The compounds that satisfied the Lipinski rule of five were tabulated in **Table 1** and the pharmacological properties of ADME-cleared compounds were validated using PASSonline Way2Drugand are reported in **Table 2.**

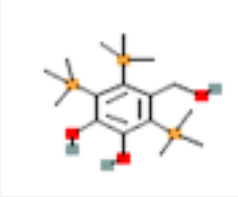
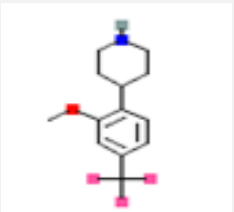
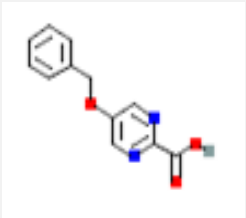
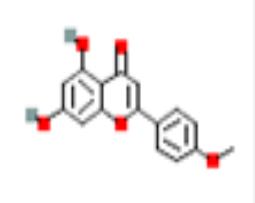
TABLE 1: ANALYSIS OF ADME PROPERTIES FOR THE PLANT COMPOUNDS USING QIKPROP

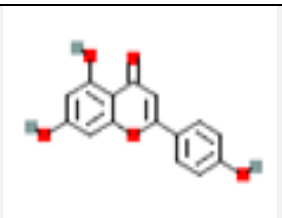
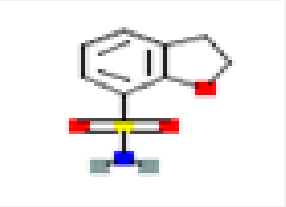
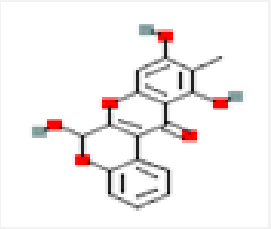
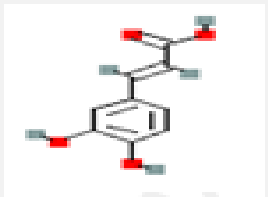
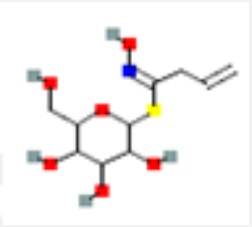
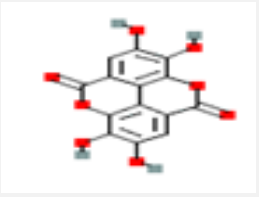
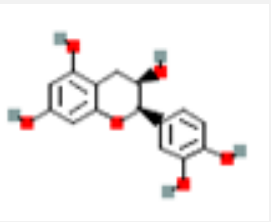
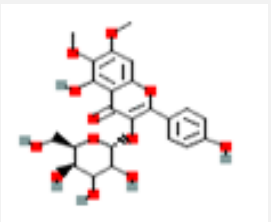
Molecule Name	No. of rotatable bonds	Molecular weight	Dipole moment	SASA	Donor Hydrogen bonds	Acceptor Hydrogen bonds	QPlogP for Octanol/gas
Normal Range	**0-15**	**130.0-725.0**	**1.0-12.5**	**300.0-1000.0**	**0.0-6.0**	**2.0-20.0**	**8.0-35.0**
3,4-Dihydroxy-benzyl alcohol	7	356.683	2.689	618.238	3	3.2	17.553
4-(2-methoxy phenyl) piperidine	1	191.272	2.518	440.293	1	2.25	9.851
5-Benzyloxypyrimidine-2-carboxylic acid	4	230.223	9.473	475.807	1	4.75	13.762
Acacetin	3	284.268	6.881	517.54	1	3.75	13.963
Apigenin	3	270.241	6.07	537.139	2	3.75	13.963
Benzofuran 2,3, dihydro	2	199.224	7.011	385.618	2	5.25	12.53
Boeravinone B	3	312.278	4.885	516.43	2	5.45	16.195
Caffeic acid	5	180.16	7.175	392.531	3	3.5	12.706
Desulphosingrin	11	279.307	6.977	491.931	5	11.2	20.811
Ellagic acid	4	302.197	4.916	446.65	4	8	18.761
Epicatechin	5	290.272	2.921	509.455	5	5.45	19.681
Eupalitin 3-O-galactoside	11	492.435	8.321	695.424	5	13.75	29.318
Ferulic acid	5	194.187	6.295	420.153	2	3.5	11.367
Furon-2-ylmethanol	3	216.279	2.385	455.662	1	2.2	10.219
Gallic acid monohydrate	4	170.121	5.716	342.782	4	4.25	13.283
Kaempferol	4	286.24	5.622	501.402	3	4.5	16.695
Malic acid	7	350.633	2.463	633.865	0	4.85	14.129
Purpurin	3	256.214	3.14	445.607	1	4.25	12.061
Quercetin	5	302.24	3.533	512.235	4	5.25	18.32
Semiglabrin	1	392.407	8.159	572.912	0	6	17.46
Succinic acid	5	262.452	3.975	587.602	0	4	11.448
Ursolic acid	2	456.707	6.246	694.702	2	3.7	21.246
Vasicinol	2	204.228	5.644	422.555	2	3.95	12.316
Vasicinone	1	202.212	1.481	412.623	1	5.7	11.374

Molecule Name	QPlog P Water /Gas	QPlogP Octanol /Water	QPlog BB for brain /Blood	No. of Metabolic reactions	QPlogKp for skin permeability	Human Oral absorption	Rule of Five	Rule of Three
Normal Range	**4.0-45.0**	**-2.0-6.5**	**-3.0-1.2**	**1.0-8.0**	**-8.0 to -1.0**	**1,2 (or)3 L, M, H**	**Max 4**	**Max 3**

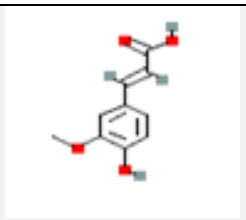
3,4-Dihydroxy-benzyl alcohol	17.553	7.834	-0.519	3	-2.169	3	0	0
4-(2-methoxy phenyl) piperidine	9.851	5.453	0.656	3	-3.498	3	0	0
5-Benzyloxypyrimidine-2-carboxylic acid	13.762	9.154	-1.033	4	-2.84	3	0	0
Acacetin	13.963	8.351	-0.976	3	-3.002	3	0	0
Apigenin	13.963	8.351	-0.976	3	-3.002	3	0	0
Benzofuran 2,3, dihydro	12.53	10.124	-0.679	1	-3.441	3	0	0
Boeravinone B	16.195	11.412	-1.073	3	-3.386	3	0	0
Caffeic acid	12.706	9.871	-1.569	2	-4.524	2	0	1
Desulphosingrin	20.811	18.74	-2.122	6	-4.733	2	0	0
Ellagic acid	18.761	16.688	-2.333	4	-6.753	2	0	1
Epicatechin	19.681	15.562	-1.845	7	-4.686	2	0	1
Eupalitin 3-O-galactoside	29.318	22.69	-2.488	8	-4.47	1	2	1
Ferulic acid	11.367	1.378	-1.189	2	-3.697	2	0	0
Furon-2-ylmethanol	10.219	3.179	0.087	5	-1.206	5	0	0
Gallic acid monohydrate	13.283	-0.567	-1.669	3	-5.486	3	0	1
Kaempferol	16.695	1.06	-1.803	4	-4.533	4	0	0
Malic acid	14.129	4.506	-0.182	2	-1.51	2	0	0
Purpurin	12.061	1.025	-1.39	3	-4.32	3	0	0
Quercetin	18.32	0.387	-2.309	5	-5.422	5	0	1
Semiglabrin	17.46	3.368	-0.144	1	-1.889	1	0	0
Succinic acid	11.448	3.446	-0.332	2	-2.15	2	0	0
Ursolic acid	21.246	6.142	-0.455	3	-3.152	3	1	1
Vasicinol	12.316	1.187	-0.559	3	-3.045	3	0	0
Vasicinone	11.374	0.646	-0.413	1	-2.833	1	0	0

TABLE 2: 2D STRUCTURE OF ADME CLEARED PHYTOCOMPOUNDS WITH PHARMACOLOGICAL PREDICTION

S. no.	Phytocompound with PubChem ID	2D structure	Pass prediction
1	3'4-dihydroxy-benzyl alcohol (101663520)		,237 0,090 Antibacterial 0,237 0,090 Anticarcinogenic 0,173 0,027 D-glutamate oxidase inhibitor 0,161 0,015 Anthranilate synthase inhibitor 0,160 0,015 Amino-acid racemase inhibitor 0,186 0,041 Glutathione S-transferase substrate 0,183 0,039 Quinoline-4-carboxylate 2-oxidoreductase inhibitor
2	4-(2-methoxy phenyl) piperidine		0,329 0,082 Calcium regulator 0,392 0,146 General pump inhibitor ,248 0,005 Antibacterial, ophthalmic 0,260 0,017 Menstruation disorders treatment 0,288 0,047 Imidazoline I1 receptor agonist 0,284 0,042 Age-related macular degeneration treatment 0,360 0,119 Antianginal 0,265 0,025 Sickle-cell anemia treatment
3	5- Benzyloxypyrimidine (561874)		0,133 0,023 inhibitor 0,142 0,032 2-Dehydropantolactone reductase (A-specific) inhibitor 0,153 0,044 Lysine 2-monooxygenase inhibitor 0,157 0,047 Antibacterial, ophthalmic 0,161 0,052 2-Dehydropantoate aldolase inhibitor 0,182 0,073 Gout treatment 0,276 0,167 CDK9/cyclin T1 inhibitor 0,126 0,017 3-Dehydroquinate dehydratase inhibitor
4	Acacetin (5280442)		0,339 0,003 Antithyroid 0,365 0,030 Bone diseases treatment 0,343 0,009 Cell wall biosynthesis inhibitor 0,410 0,078 Spasmolytic, urinary 0,393 0,064 Lactase inhibitor 0,369 0,041 Vanilloid 1 agonist ,367 0,039 Antibacterial 0,384 0,056 EIF4E expression inhibitor

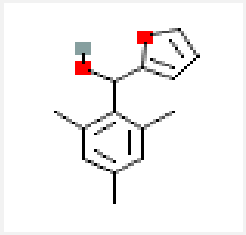
5	Apigenin (5280443)		0,425	0,065	Fibrolase inhibitor
			0,370	0,011	Glutathione S-transferase substrate
			0,366	0,007	Alkaline phosphatase inhibitor
			0,394	0,035	Glucan 1,4-alpha-maltotetraohydrolase inhibitor
			0,391	0,032	Antibacterial
			0,360	0,003	Aldose reductase inhibitor
			0,396	0,039	Isopenicillin-N epimerase inhibitor
			0,367	0,011	CYP2B2 substrate
6	Benzofuran 2,3 dihydro (20209882)		0,231	0,187	Opioid kappa 3 receptor antagonist
			0,085	0,041	Cushing's syndrome treatment
			,133	0,090	Antibacterial, ophthalmic
			0,137	0,094	Thioredoxin reductase inhibitor
			0,134	0,090	Oligopeptidase B inhibitor
			0,082	0,039	15-Lipoxygenase inhibitor
			0,179	0,136	Carbon-monoxide dehydrogenase inhibitor
			0,111	0,069	Pectin lyase inhibitor
7	Boeravinone B (14018348)		0,423	0,049	CYP2B5 substrate
			0,447	0,074	Antiinflammatory
			0,372	0,002	Nicotinic acid receptor 2 agonist
			0,373	0,005	Skin whitener
			0,397	0,031	Antibacterial
			0,376	0,011	Aryl hydrocarbon receptor agonist
			0,399	0,036	Nitrite reductase [NAD(P)H] inhibitor
			0,435	0,073	JAK2 expression inhibitor
8	Caffeic acid (689043)		0,520	0,055	Sorbitol-6-phosphate 2-dehydrogenase inhibitor
			0,323	0,006	HMG CoA synthase inhibitor
			,358	0,041	Antibacterial
			0,324	0,008	Peptidylglycine monooxygenase inhibitor
			0,320	0,003	Urocanate hydratase inhibitor
			0,327	0,011	Acetolactate decarboxylase inhibitor
9	Desulphosinigrin (9601716)		0,535	0,016	Dolichyl-diphosphooligosaccharide-protein glycotransferase inhibitor
			0,540	0,022	Aspartyltransferase inhibitor
			0,524	0,014	Antibacterial
			0,519	0,019	Transcription factor stimulant
			0,519	0,019	Transcription factor NF kappa B stimulant
			0,526	0,027	Glucan 1,4-alpha-maltotriohydrolase inhibitor
			0,507	0,008	Endo-1,3(4)-beta-glucanase inhibitor
			0,507	0,013	CDP-diacylglycerol-serine O-phosphatidyltransferase inhibitor
10	Ellagic acid (5281855)		0,351	0,005	Oligo-1,6-glucosidase inhibitor
			0,360	0,014	Glucan 1,6-alpha-glucosidase inhibitor
			0,365	0,020	Imulinase inhibitor
			0,370	0,024	Acetylornithine deacetylase inhibitor
			0,391	0,046	Leukopoiesis inhibitor
			0,367	0,022	Sclerosant
			,380	0,035	Antibacterial
			0,364	0,020	Polygalacturonase inhibitor
			0,419	0,074	Spasmolytic, urinary
11	Epicatechin (72276)		0,281	0,010	Antiviral (HIV)
			0,317	0,046	Endopeptidase La inhibitor
			0,336	0,067	Immunostimulant
			0,288	0,019	Keratolytic
			0,271	0,002	Squalene epoxidase inhibitor
			0,387	0,120	NADPH-cytochrome-c2 reductase inhibitor
			0,320	0,053	Antibacterial
			0,284	0,016	Antiprotozoal (Plasmodium)
			0,359	0,092	CYP2C8 substrate
12	Eupalitin 3-O- galactoside (44259727)		0,590	0,010	GABA aminotransferase inhibitor
			0,602	0,024	Eucosterol-epoxide lyase inhibitor
			0,584	0,010	Antibacterial
			0,578	0,005	Chemoprotective
			0,571	0,004	DNA ligase (ATP) inhibitor
			0,583	0,017	Radioprotector
			0,564	0,002	Severe acute respiratory syndrome treatment

13 Ferulic acid (445858)



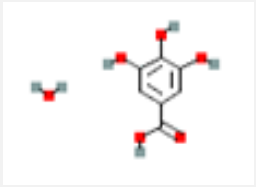
0,360	0,074	CYP2A4 substrate
0,296	0,011	Thioredoxin reductase inhibitor
0,335	0,050	Venom exonuclease inhibitor
0,331	0,046	Arylesterase inhibitor
0,323	0,038	Oxidizing agent
0,329	0,044	Transcription factor inhibitor
0,333	0,048	Antibacterial
0,391	0,107	Limulus clotting factor B inhibitor
0,334	0,050	CYP2C10 substrate

14 Furon- 2yl methanol (49962474)



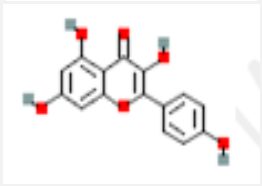
0,374	0,070	Gluconate 5-dehydrogenase inhibitor
0,372	0,071	Adenomatous polyposis treatment
0,334	0,033	ICAM1 expression inhibitor
0,352	0,054	Nitrite reductase (NO-forming) inhibitor
0,342	0,046	Cyclomaltodextrinase inhibitor
0,364	0,069	Peptidoglycan glycosyltransferase inhibitor
,340	0,046	Antibacterial
0,331	0,038	Di-trans,poly-cis-decaprenylcistransferase inhibitor
0,364	0,071	NAD(P)+-arginine ADP-ribosyltransferase inhibitor

15 Gallic acid monohydrate (24721416)



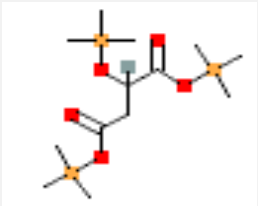
0,255	0,004	2-Methyleneglutarate mutase inhibitor
0,265	0,015	Transcription factor NF kappa B inhibitor
,255	0,005	Antibacterial, ophthalmic
0,254	0,005	Aminolevulinate transaminase inhibitor
0,254	0,005	2-Nitrophenol 2-monooxygenase inhibitor
0,254	0,005	Glutamate (mGluR6) antagonist
0,293	0,045	Thiamine-triphosphatase inhibitor
0,253	0,006	Aerobactin synthase inhibitor

16 Kaempferol (5280863)



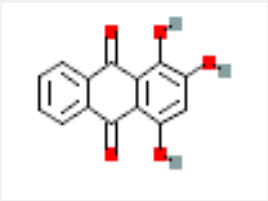
0,375	0,005	Lipoxygenase inhibitor
0,440	0,072	Fructose 5-dehydrogenase inhibitor
0,374	0,007	Histidine decarboxylase inhibitor
0,377	0,012	Phenylpyruvate decarboxylase inhibitor
0,373	0,007	Laxative
0,369	0,005	Catechol 1,2-dioxygenase inhibitor
,395	0,031	Antibacterial
0,402	0,038	Antiprotozoal (Trypanosoma)
0,439	0,074	GST A substrate

17 Malic acid (522155)



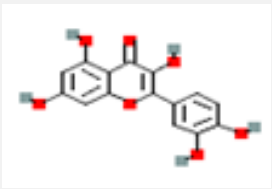
0,095	0,040	Glycine N-methyltransferase inhibitor
0,061	0,007	Membrane-oligosaccharide glycerophosphotransferase inhibitor
0,063	0,009	Sucrose phosphorylase inhibitor
0,137	0,082	Antibacterial, ophthalmic
0,151	0,097	Nitric-oxide synthase stimulant
0,241	0,187	CYP3A5 substrate
0,082	0,028	Methylthioadenosine nucleosidase inhibitor
0,195	0,142	Antiparkinsonian, rigidity relieving

18 Purpurin (6683)



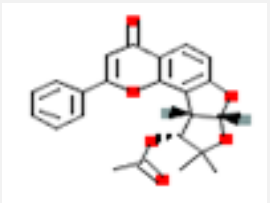
0,411	0,027	Opine dehydrogenase inhibitor
0,431	0,048	Lactase inhibitor
0,412	0,028	Coccolysin inhibitor
0,399	0,015	Antiviral (Hepatitis B)
0,411	0,028	Antibacterial
0,406	0,024	2,3,4,5-Tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase inhibitor
0,401	0,019	Urease inhibitor
0,416	0,034	Sulfite dehydrogenase inhibitor

19 Quercetin (5280343)

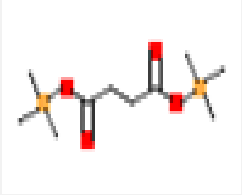
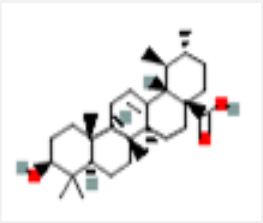
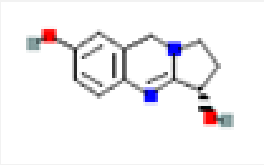
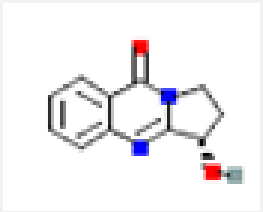


0,410	0,055	CYP2D6 substrate
0,379	0,024	Myosin ATPase inhibitor
0,382	0,028	Opioid kappa 3 receptor antagonist
0,358	0,003	MAO A inhibitor
0,387	0,033	Antibacterial
0,370	0,017	CYP1A2 inducer
0,412	0,060	Aspartate-phenylpyruvate transaminase inhibitor
0,408	0,057	Lactase inhibitor
0,475	0,124	Chymosin inhibitor

20 Semiglabrin (156341)



0,328	0,025	Free radical scavenger
0,302	0,006	Protein kinase stimulant
0,333	0,037	RNA synthesis inhibitor
0,329	0,035	DNA ligase (ATP) inhibitor
0,339	0,046	Antibacterial
0,312	0,019	Paraoxonase substrate
0,295	0,004	Demethylsterigmatocystin 6-O-methyltransferase inhibitor
0,378	0,089	Vasoprotector

21	Succinic acid (520988)		0,167 0,053 Candidapepsin inhibitor 0,127 0,014 Lactaldehyde dehydrogenase inhibitor 0,124 0,011 Alpha,alpha-phosphotrehalase inhibitor 0,119 0,006 5-Aminovalerate transaminase inhibitor 0,158 0,046 Antibacterial, ophthalmic 0,135 0,022 Globoside alpha-N-acetylgalactosaminyltransferase inhibitor 0,223 0,111 Cytostatic 0,133 0,021 Succinate-semialdehyde dehydrogenase inhibitor
22	Ursolic acid (64945)		0,285 0,093 H+-transporting two-sector ATPase inhibitor 0,258 0,066 Antiprotozoal 0,248 0,059 Oxytocic 0,216 0,028 Endoglycosylceramidase inhibitor 0,268 0,081 Hydroxylamine reductase (NADH) inhibitor 0,256 0,069 Thyroxine 5-deiodinase inhibitor 0,262 0,076 Antibacterial 0,307 0,123 Beta-adrenergic receptor kinase inhibitor 0,307 0,123 G-protein-coupled receptor kinase inhibitor
23	Vasicinol (442934)		0,248 0,059 Oxytocic 0,216 0,028 Endoglycosylceramidase inhibitor 0,268 0,081 Hydroxylamine reductase (NADH) inhibitor 0,256 0,069 Thyroxine 5-deiodinase inhibitor 0,262 0,076 Antibacterial 0,307 0,123 Beta-adrenergic receptor kinase inhibitor 0,307 0,123 G-protein-coupled receptor kinase inhibitor 0,228 0,045 Protein-synthesizing GTPase inhibitor
24	Vasicinone (442935)		0,154 0,108 Cyclopentanone monooxygenase inhibitor 0,183 0,137 Photosensitizer 0,121 0,075 Quercetin 2,3-dioxygenase inhibitor 0,116 0,070 Mevalonate kinase inhibitor 0,134 0,088 Antibacterial, ophthalmic 0,115 0,069 NAD(P)+ transhydrogenase (AB-specific) inhibitor 0,088 0,043 5 Hydroxytryptamine 3 agonist 0,112 0,068 Formaldehyde dehydrogenase (glutathione) inhibitor

Molecular Docking Studies: Molecular docking investigation predicts the interaction of bioactive compounds against the target protein in *Staphylococcus aureus*. Molecular docking was carried out using the Glide module of maestro Schrodingerr software.

The docking result interprets the active site and binding efficiency of phytocompounds against the target protein 2VR3. Phytoconstituents from *Boerhavia diffusa, Clerodendrum infortunatum* and *Sida rhombifolia* were found to be potential lead compounds against 2VR3.

Each phytocompounds were allowed to dock with the target protein and the binding efficiency that is the formation of hydrogen bonds was visualized using the PyMol visualization tool. The bioactive compound Eupalitin 3-O- galactoside showed efficient binding interaction against the target protein with theleast Glide score is -8.56 Kcal/mol and the residues interacted were GLN 253 (O-H), GLN 253 (H-O), GLN 253 (H-O), HIS 252 (H-O), GLU 526 (H-O), GLU 526 (O-H), ASN 525 (O-H), ARG 506 (O-H) and ILE 384 (O-H) and with a bond length of 2.6Å, 2.0Å, 2.5Å, 2.1Å, 1.8Å, 1.8Å, 1.9Å and 2.3Å, respectively. Additionally, the compound quercetin and vasicinone from the plants *Clerodendrum infortunatum* and *Sida rhombifolia* also hada better binding ability with a G. score of -8.35 and -5.29 Kcal/mol respectively.

Apart from these phytocompounds from other plants such as *Tephrosia purpurea, Scoparia dulcis, Breynia retusa* and *Euphorbia Herterophylla* also had significant binding properties with the target protein. The phytocompound Ursolic acid from the plant *Boerhavia diffusa* has shown poor binding interaction with the target protein with a G score of -1.92 Kcal/mol.

The binding interactions with the glide score value was reported in **Table 3. Fig. 2** represents the binding efficiency of Eupalitin 3-O-galactoside with the target protein 2VR3. Therefore, the molecular docking studies reveal that phytocompounds from *Boerhavia diffusa, Clerodendrum infortunatum* and *Sida rhombifolia* have significant inhibition against 2VR3 and further *in-vitro* antibacterial studies were carried out to investigate its antibacterial activity.

TABLE 3: MOLECULAR DOCKING OF PHYTOCOMPOUNDS AGAINST TARGET PROTEIN CLFA-FIBRINOGEN

S. no.	Name of the Ligand (Pubchem ID	Residues Interaction	Bond Length (Å)	No. of Hydrogen Bonds	G-Score (Kcal/mol)
		Boerhavia diffusa			
1	Eupalitin 3-O- galactoside (44259727)	GLN 253 (O-H)	2.6	8	-8.56
		GLN 253 (H-O)	2.0		
		HIS 252 (H-O)	2.5		
		GLU 526 (H-O)	2.1		
		GLU 526 (O-H)	1.8		
		ASN 525 (O-H)	1.8		
		ARG 506 (O-H)	1.9		
		ILE 384 (O-H)	2.3		
2	Kaempferol (5280863)	ASP 385 (H-O)	2.0	6	-8.37
		ILE 384 (O-H)	2.3		
		ILE 384 (H-O)	2.0		
		ASN 525 (O-H)	2.1		
		ASP 340 (H-O)	1.8		
		ILE 339 (O-H)	1.8		
3	Gallic acid monohydrate (24721416)	ILE 384 (H-O)	1.8	4	-7.53
		SER 447 (H-O)	2.1		
		SER 447 (H-O)	2.0		
		HIS 252 (O-H)	2.1		
4	Boeravinone B (14018348)	TRP 523 (O-H)	1.8	2	-7.14
		ASP 524 (H-O)	1.8		
5	3'4-dihydroxy-benzyl alcohol (101663520)	GLU 526 (H-O)	2.0	2	-5.43
		ILE 384 (O-H)	2.2		
6	Ferulic acid (445858)	ALA 528 (H-O)	2.2	4	-5.07
		ILE 389 (O-H)	2.0		
		ILE 389 (O-H)	2.4		
		ASN 525 (H-O)	2.0		
7	Malic acid (522155)	ILE 384 (O-H)	2.1	1	-4.68
8	Succinic acid (520988)	ALA 254 (O-H)	2.2	5	-4.61
		GLU 526 (O-H)	2.5		
		ASN 525 (O-H)	2.2		
		ILE 384 (O-H)	2.2		
		ASN 525 (O-H)	2.1		
9	Ursolic acid (64945)	ARG 395 (O-H)	2.1	1	-1.92
		Clerodendrum infortunatum			
10	Quercetin (5280343)	ASN 525 (O-H)	2.1	5	-8.35
		ILE 384 (O-H)	2.4		
		ILE 384 (H-O)	2.3		
		ILE 339 (H-O)	1.9		
		GLY 287 (H-O)	2.1		
11	Ellagic acid (5281855)	ALA 528 (O-H)	2.8	5	-7.53
		GLU 382 (H-O)	2.5		
		TRP 523 (O-H)	2.2		
		ASP 524 (H-O)	2.0		
		ASN 525 (O-H)	2.7		
12	Desulphosinigrin (9601716)	PRO 251 (H-O)	1.8	5	-7.52
		ILE 339 (H-O)	2.2		
		ASN 284 (O-H)	2.3		
		HIS 252 (O-H)	2.6		
		ILE 384 (H-O)	2.1		
13	Acacetin (5280442)	ASN 525 (O-H)	2.2	4	-6.28
		ASN 525 (O-H)	2.7		
		ASN 524 (H-O)	2.0		
		GLU 526 (H-O)	2.0		
14	Apigenin (5280443)	ALA 528 (H-O)	2.7	5	-5.96
		GLU 526 (H-O)	2.0		

		ASP 524 (H-O)	2.0		
		ASN 525 (O-H)	2.5		
		ASN 525 (O-H)	2.7		
15	Caffeic acid (689043)	ILE 339 (H-O)	1.9	3	-5.77
		GLY 287 (H-O)	2.6		
		ILE 384 (H-O)	1.9		
		Sida rhombifolia			
	Vasicinone (442935)	ALA 254 (O-H)	2.2	3	-5.29
16		PRO 251 (H-O)	1.9		
		ILE 384 (H-O)	3.4		
	Vasicinol (442934)	ALA 258 (H-O)	2.4	2	-5.25
17		ILE 339 (H-O)	1.9		
		Tephrosia purpurea			
18	Purpurin (6683)	ILE 384 (H-O)	2.1	2	-6.92
		ILE 339 (H-O)	2.0		
19	Semiglabrin (156341)	ILE 384 (O-H)	2.4	3	-5.86
		ALA 254 (O-H)	2.3		
		ILE 339 (O-O)	2.8		
		Scoparia dulcis			
20	5- Benzyloxypyrimidine	ILE 384 (N-H)	2.6	4	-4.84
	(561874)	ILE 384 (O-H)	2.2		
		ASN525 (O-H)	2.3		
		ASN 525 (O-H)	2.0		
21	Benzofuran 2,3 dihydro	TYR 338 (O-H)	2.2	4	-4.66
	(20209882	LEU 295 (H-O)	2.4		
		LYS 293 (H-O)	2.6		
		GLY 532 (O-H)	2.3		
		Breynia retusa			
22	Epicatechin (72276)	ILE 339 (H-O)	2.2	5	-5.75
		PRO 251 (H-O)	1.9		
		GLU 526 (H-O)	1.8		
		GLU 526 (O-H)	1.9		
		TRP 523 (O-H)	2.5		
		Euphorbia Herterophylla			
23	Furon- 2yl methanol (49962474)	ASN 525 (O-H)	2.7	4	-5.23
		GLU 526 (O-H)	2.4		
		GLU 526 (H-O)	1.9		
		ASN 525 (O-H)	2.0		

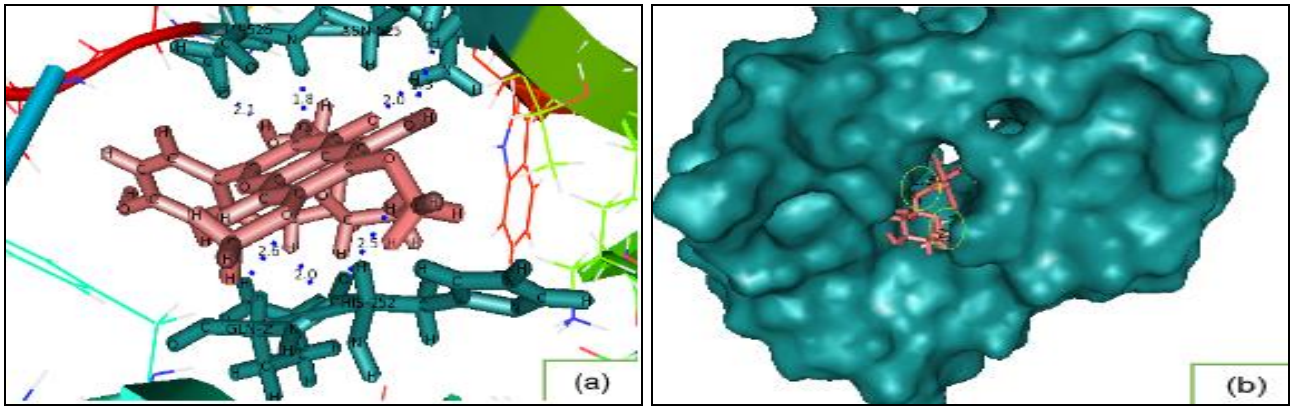


FIG. 2: MOLECULAR INTERACTION OF EUPALITIN 3-O- GALACTOSIDE WITH THE TARGET PROTEIN (A) AND DOCKED COMPLEX (B). Note: The deep teal color represents the target protein and the deep salmon color indicates the Eupalitin 3-O-galactoside. Blue dots represent the hydrogen bond interaction of Eupalitin 3-O-galactoside with the active site region of the target protein.

Antibacterial Screening: As a result of *in-silico* studies, we conclude that phytocompounds from *Boerhavia diffusa* have shown significant binding interaction with the target protein, followed by the plants *Clerodendrum infortunatum* and *Sida rhombifolia*. Thus, the antibacterial activity of these

pants was performed using the agar well diffusion method. **Fig. 3** depicts the antibacterial activity of different plant extracts in which crude ethyl acetate leaf extract of all three plants showed efficient antibacterial activity. The zone of inhibition was used to determine the sensitivity of test plates. The maximum zone of inhibition was 24 mm observed in ethyl acetate leaf extract of *Boerhavia diffusa* and a minimum zone of inhibition was 10 mm observed for hexane root extract as illustrated in **Table 4**. Neomycin sulphate was used as a positive control and exhibited good antibacterial activity. Whereas all the negative controls hexane, ethyl acetate and methanol revealed no activity against *Staphylococcus aureus*.

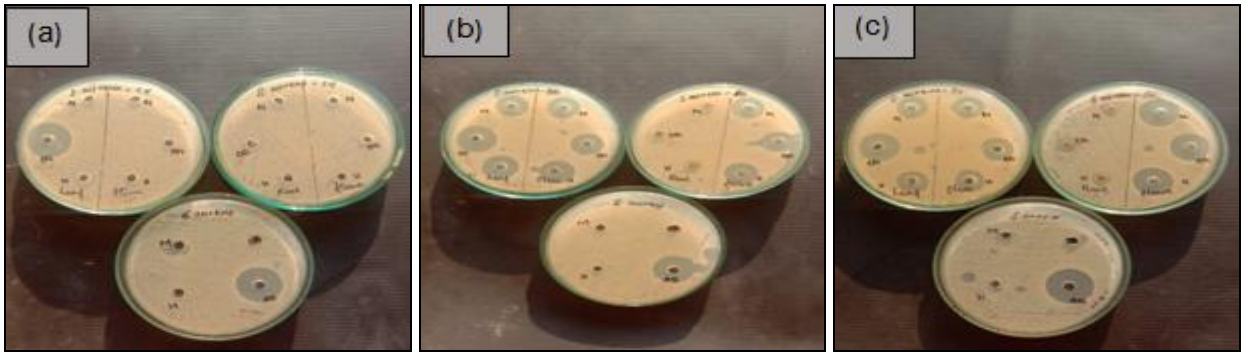


FIG. 3: ANTIBACTERIAL ACTIVITY OF PLANTS EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS.* Note: Agar well diffusion method was used to evaluate the Antibacterial activity of leaf, stem, root, and flower extracts against *Staphylococcus aureus*. (a) represents the activity of the plant *Clerodendrum infortunatum*, followed by *Boerhavia diffusa* (b), and *Sida rhombifolia* (c)

TABLE 4: DIAMETER OF ZONE OF INHIBITION OF DIFFERENT PLANT EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS*

S. no.	Sample (100 µL)		Zone of Inhibition (mm)					
			Leaf	Stem	Root	Flower	Negative Control	Antibiotic (NS)
1	*Boerhavia diffusa*	M	18	18	-	18	-	
		EA	24	18	-	18	-	23
		H	20	20	10	18	-	
2	*Clerodendrum infortunatum*	M	-	-	-	-	-	
		EA	23	-	-	-	-	22
		H	-	-	-	-	-	
3	*Sida rhombifolia*	M	16	17	10	21	-	
		EA	21	17	10	21	-	23
		H	16	17	10	21	-	

Note: (M): Methanol, (EA): Ethyl acetate, (H): Hexane, (NS): Neomycin sulphate. For each extract's average zone of inhibition, the diameter was calculated from the triplicates.

DISCUSSION: Antibiotic resistance is a massive issue. Overuse or abuse of antibiotics leads bacteria to become more resistant to the inhibitory effects of antibiotics [24]. Every year 700,000 people die due to diseases caused by multidrug-resistant bacteria [25]. *Staphylococcus aureus* is one of the multidrug-resistant bacteria and is resistant to penicillin and methicillin. The lack of treatment options for MRSA infections is also a major global concern [26]. Thus, the current research mainly focuses on developing efficient drug molecules against *Staphylococcus aureus* from traditional medicinal plants. Here we used bioinformatics tools such as molecular docking to find efficient lead molecules and further validation was done using *in-vitro* antibacterial evaluation. *S. aureus* infections are usually caused by the adhesion of multiple surface-anchored virulence proteins, which interact with the host tissue [27]. Studies have revealed that ClfA is important in developing staphylococcal infections [28]. Additionally, ClfA is responsible for infective endocarditis, which is initiated by platelet aggregation in the host [29]. It was reported that the extracellular matrix protein ClfA found in *Staphylococcus aureus* protects the bacterium from phagocytosis, rendering them more virulent [10]. Thus, targeting ClfA could be a promising way to identify the effective lead molecule. Hence, in this work, ClfA-Fibrinogen is considered the target protein. The literature identified that

phytocompounds from different plants were considered ligands since phytochemicals have a wide range of biological activities [30]. These bioactive compounds were evaluated for ADME characteristics, and their drug-likeness was determined using the Lipinski rule of five (RO5) [31]. This study used structure-based virtual screening to estimate phytocompound interaction and binding affinities with the target protein by which efficient drug molecules will be identified based on scoring function and glide energy [32].

As a result of docking studies, phytocompounds from the plants *Boerhavia diffusa*, *Clerodendrum infortunatum,* and *Sida rhombifoli*a have shown excellent glide scores. And all these three plants are known for their biological properties. Docking studies revealed that the compound eupalitin 3-O-galactoside from the plant *Boerhavia diffusa* had remarkable binding interaction with the target protein. A study reported that the *Boerhavia diffusa* contains phytoconstituents with a wide range of therapeutic benefits, including antioxidant and anticancer properties. Methanolic extracts are found to have strong antioxidant activity [33]. According to reports, it was revealed that roots and aerial parts of *B. diffusa* contain methylated eupalitin in both free and glycoside forms [34].

It has anti-inflammatory and immunosuppressive properties as a consequence of the high content of polyphenols in it. Additionally, Eupalitin was found to have improved cancer chemopreventive properties. it induces ROS levels which leads to apoptosis in prostate cancer [35]. *In-silico* studies have revealed that the anti-inflammatory compound eupalitin-3-O-galactoside has a dual effect on cancer, one by inhibiting the target protein aldose reductase enzyme (ALR2) and other by suppressing cancer-mediating pathways [36]. This suggests that eupalitin 3-O- galactoside can be used to develop efficient drug molecule against *S. aureus*. To confirm the *in-silico* studies we have *evaluated invitro* antibacterial activity of *Boerhavia diffusa* along with the other two plants whose bioactive compounds also showed efficient interaction with the target protein. In a study, various solvents were used to conduct an antibacterial study on the roots, stems and leaves of *B. diffusa*. where There was no indication of antibiotic resistance in the aqueous or chloroform extracts. The root extract shows a maximum level of inhibition. At 200 µg of extract concentration, the greatest inhibition of bacterial growth was discovered with a zone of roughly 8 mm[37]. In this study, crude ethyl acetate leaf extract showed significant activity with a maximum zone of inhibition of 24mm, followed by stem and flower extract.

Another study observed that a low polar ethanolic extract of *B. diffusa* had a higher concentration of phytochemicals than an aqueous extract. Since, phytocompounds are more soluble in less polar solvents. In addition, leaf extracts of *B. diffusa* were found to have effective antibacterial efficacy against *S. aureus*[38]. This supports our outcome that the mid-polar solvent, ethyl acetate leaf extract of *B. diffusa* has efficient antibacterial activity. Ethanolic and chloroform extracts of *Clerodendrum infortunatum* were studied to have efficient inhibitory efficacy against *S. aureus* when compared to the common drugs tetracycline and fluconazole [39]. Similarly, in our study, when compared to the standard antibiotic neomycin sulphate, the ethyl acetate leaf extract of *C. infortunatum* demonstrated effective inhibitory activity against *S. aureus*.

A study reported that the ethyl acetate and chloroform extracts had the strongest antibacterial activity against *S. aureus*, whereas the petroleum ether extract had the weakest. Furthermore, their findings indicate active compounds in plant extracts are more likely to be found in mid-polar solvents [40]. Likewise, in our study, when compared to other solvent extracts, the mid-polar ethyl acetate leaf extract demonstrated significant inhibition, thereby validating our result. Studies shows that, aerial part of *Sida rhombifolia* has been effective against a wide range of gram-positive and gram-negative bacteria[41]. Altogether, the *in-silico* molecular docking studies demonstrated the binding interaction of phytocompound against the target protein. From findings, it was identified that the bioactive compound eupalitin 3-O- galactoside can be usedto develop potential inhibitors against *S, aureus* by targeting ClfA- Fibrinogen protein. From *in-vitro* antibacterial studies, it was found that the leaf extracts of all three plants *Boerhavia diffusa*, *Clerodendrum infortunatum* and *Sida rhombifolia* have shown efficient antibacterial activity against the growth of *Staphylococcus aureus*.

CONCLUSION: The present study clearly indicates that phytocompounds from the plants *Boerhavia diffusa*, *Clerodendrum infortunatum*, and *Sida rhombifolia* are found to be potential inhibitors of the ClfA-fibrinogen protein. The phytocompound Eupalitin 3-O- galactoside from *Boerhavia diffusa* effectively binds to target protein 2VR3 with a least G score of -8.56 Kcal/mol. Our *in-vitro* research revealed that the crude ethyl acetate extract of *Boerhavia diffusa* exhibited significant antibacterial activity with a zone of inhibition of 24mm. Future studies will concentrate on the molecular dynamic study to understand the stability and structural behaviour of the identified compound. Further exploration into concentration-based antibacterial analyses and their mode of action on Staphylococcus aureus needs to be done.

Declarations:

Ethics Approval and Consent to Participate: This article does not contain any authors' studies involving animals and human participants.

Consent for Publications: Not applicable

Availability of Data and Materials: The Data used and/or analyzed in the present study will be available from the corresponding author upon reasonable request.

Funding: No funding was received for conducting this study.

Author Contribution: Vidya S. L- Performed the *in-silico* and *in-vitro* studies and drafted the manuscript. R. Sathishkumar- Designed the study, edited the manuscript and guided throughout the study. The final manuscript was read and approved by all authors

ACKNOWLEDGMENT: The authors are grateful to the Kongunadu Arts and Science College administration for supplying all software required to complete this project.

CONFLICTS OF INTEREST: The authors declared that they have no competing interest in the studies.

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How to cite this article:
Vidya SL and Kumar R: *In-silico* screening of potential phytocompounds against *Staphylococcus aureus* and an *in-vitro* antibacterial evaluation. Int J Pharm Sci & Res 2023; 14(8): 4128-41. doi: 10.13040/IJPSR.0975-8232.14(8).4128-41.