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Research article

Antibacterial Activity and *In-silico* Analysis of *Rumex nepalensis* Leaf Extract Against *Staphylococcus aureus*

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ABSTRACT

The use of medicinal plants in traditional healthcare practices presents an exciting opportunity for novel antimicrobial agents. This research study investigated the antibacterial properties of *Rumex nepalensis*, a common ethnomedicinal plant, against *Staphylococcus aureus*, which is a clinically significant pathogen that causes a wide variety of human infections. The crude leaf extracts of *R. nepalensis* were tested using the agar well diffusion test. and the results showed significant inhibitory activity against *S. aureus*. Following phytochemical screening, compounds were identified using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Seven of the twelve bioactive compounds that were found - chrysophanol, hastatusides A, L-phenylalanine, schisandrin C, Cis-p-coumaric acid, pinoresinol, and βeta-caryophyllene - met the requirements for drug-likeness and were chosen for further analysis. The interaction of these drugs with *S. aureus* virulence-associated protein targets, including gamma haemolysin, exfoliative toxin, lysostaphin-type metalloendopeptidase, and toxic shock syndrome toxin-1, was evaluated using *in-silico* molecular docking experiments. Chyrsophanol showed strong binding affinities with all four targets, notably with exfoliative toxin, lysostaphin, and toxic shock syndrome toxin. These results suggest that chrysophanol is a potential bioactive component that supports *R. nepalensis* antibacterial action.

Introduction

Plants are a vital source of medicine and have a significant role in elevating global health outcomes.[1] Plants have been utilised for the treatment of various illnesses by the indigenous people. In the recent decade, there has been an expanding enthusiasm for the investigation of medicinal plants and their uses.[2-4] The health-promoting properties of the medicinal plants are usually derived from the interaction of several phytochemicals present in the phytocomplex.[5]

The Polygonaceae family is a taxonomically isolated group with the presence of a stipulated sheath and ochrea.[6] This family is rich in catechins, alkaloids, tannins, saponins, phenols, and anthraquinones.[7] With 200 species distributed across Europe, Asia, Africa and North America, *Rumex* is the second largest genus of Polygonaceae. The *Rumex L.* species, also referred to as '*Dock*', are well-known for their use in traditional healing practices because of their remedial and organic viability.[8] Antioxidant property is found in *Rumex* sp., including *R. acetosa* L., *R. acetosella* L., *R. crispus* L., *R. hydrolapathum* Huds, and *R. obtusifolius* L.[9] The root and leaf extracts of *R. dentatus* are used for curing constipation,[10] root and leaf of *R. hastatus* have been used in the treatment of jaundice,[11] and the roots of *R. nepalensis* are used for the treatment of pain, inflammation, bleeding, tinea, tumours, and constipation in Chinese folk medicine.[12] In the Khasi indigenous community of Meghalaya, it is used as a vegetable.[13] Additionally, the rootstock of *R. nepalensis* is used as an antibacterial agent[14] and in the form of a decoction. However, information on the use of its leaf extract remains limited.[15] Specifically, information on the antibacterial activity present in the leaf of the plant has not been well documented.

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Staphylococcus aureus represents an infectious bacterium pathogenic to humans and is liable for the larger part of the community-acquired and hospital-acquired staphylococcal infections. It is known to cause skin diseases, soft tissue abscesses, sepsis, endocarditis, pneumonia, and toxic shock syndrome.[16] Owing to the emergence of antibiotic resistance in *S. aureus*, treatment of the same has become a challenge.[17] Infections caused by antibiotic-resistant strains of S. *aureus* have reached epidemic proportions globally,[18] therefore, there is an urgent need for the discovery of new drugs against it.

Drug targets such as the exfoliative toxins (ETs) of *S. aureus* are responsible for the skin lesions. These ETs, which are epidermolytic toxins, are serine proteases hydrolysed by *S. aureus* that further hydrolyse desmosome proteins in the skin[19]. Lysostaphin-type metalloendopeptidase (LytM) exhibits peptidase activity and has effects on cell division by influencing cleavage and remodelling of peptidoglycan.[20] Another drug target, gamma haemolysin a two-component toxin for the disruption and lysis of erythrocytes and leukocytes.[21] Toxic shock syndrome toxin (TSST), an exotoxin, is another potential drug target in *S. aureus* [22].

The pharmacological significance of the present study focused on the identification of *Rumex nepalensis* leaf extract as a promising source of natural antibacterial agents against *S. aureus*. Research findings on this plant have exhibited pharmacological properties in root extracts, whereas the present study is among the first to establish the efficacy of leaf-derived bioactive compounds. Phytochemical screening followed by LC-HRMS and *in-silico* docking revealed several compounds with drug-like properties, notably chrysophanol, which demonstrated strong binding affinities to multiple virulence factors of *S. aureus*, including exfoliative toxin, lysostaphin-type metalloendopeptidase, gamma haemolysin, and toxic shock syndrome toxin-1. These findings suggest a multi-target antibacterial mechanism that may reduce the likelihood of resistance development. The *in-silico* results, supported by well diffusion assays, highlight the therapeutic potential of chrysophanol and related compounds as lead candidates for novel antimicrobial drug development. Thus, this study advances the pharmacognostic profile of *R. nepalensis* as a novel candidate for the development of avenues for plant-derived antimicrobial agents effective against antibiotic-resistant strains of *S. aureus*

Materials and Methods

Plant sample preparation

The plant sample was obtained from the campus of St. Edmund's College, Meghalaya, India, and was identified by its morphological characteristics, including basal leaves, broad leaf blade, ovate-cauline leaves, short petiolate, ovate-lanceolate and ocrea fugacious. The leaf extract was prepared as per the protocol provided by Odey *et al*.[23] The leaves were washed, shade dried, and ground into powder. Solvent extract was prepared by dissolving the powder in methanol and incubating for 72 hours at room temperature. The crude extract was filtered through Whatman filter paper No. 2 and briefly evaporated at 60°C.

Antibacterial action

The methanolic concentrate was studied for its antibacterial action against *S. aureus* (MTCC 9886) obtained from IMTECH, Chandigarh, Punjab. Following the agar well diffusion method,[24] leaf extract treatment was administered at two different concentrations: 50 and 80 mg/mL and incubated at 37°C for 24 hours.

Phytochemical screening

The presence of anthraquinones, phytosterols, alkaloids, tannins, cardiac glycosides, triterpenoids, saponins, terpenoids, flavonoids and phenols[25–31] was tested following the standard protocols for each phytochemical.

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS)

LC-HRMS (Xevo G2-XS QT, Waters, USA) analysis was performed at CSIR-NEIST, Jorhat, India. The data generated in the form of peaks from the LC-HRMS experiment was further analysed using the software Mestrenova[32], and after manual calculation of the identified peaks, the mass of the corresponding compounds was determined.

IN-SILICO Evaluation

Retrieval of the ligands and screening

The structure and other data about ligands (LC-HRMS detected compounds) were downloaded from the PubChem Database (NCBI) and then evaluated for drug likeness following Lipinski's rule of five[33].

Retrieval of the drug target, the human homology search against human proteome

Four drug targets, including exfoliative toxin[34], lysostaphin-type metalloendo-peptidase[35], gamma-haemolysin[36], and toxic shock syndrome toxin-1[37] in *S. aureus,* were revealed through a literature search. The structures of the drug targets were retrieved from the protein data bank (PDB).[38] Furthermore, these drug targets were evaluated for similarity throughout the human proteome using BLASTp.[39]

Exfoliative toxin (PDB ID-1EXF) was discovered to be in monomeric form, having chain A, which was used for the interaction studies. Lysostaphin-type metallopeptidase (PDB ID-2B44) existed in dimeric form and was deduced to monomeric form before studying the interaction. Gamma haemolysin (PDB ID-3B07) has two unique protein components in octameric forms. Both components were studied, and the gene located in component B was deduced into monomeric forms retaining only chain E, which was

used for interaction studies. Toxic shock syndrome toxin-1 **(**PDB ID-4OHJ) has a dimeric form and was deduced to a single chain-A.

Drug target validation

Validation of the drug target proteins was carried out via Ramachandran plot using RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Molecular Docking

To study the interaction between the ligand and the drug target, as well as to identify the energetically most favourable binding state, molecular docking was carried out employing Autodock 4.2 v1.5.6.[40] A docking experiment was performed using the Lamarckian Genetic Algorithm. Fifty independent docking runs were performed for each of the compounds. The docking parameters and protocols were validated by re-docking the co-crystal ligand and considering a root mean square deviation (RMSD) value between the native and docked poses. Prior to docking, the ligand file format obtained from PubChem (.sdf) was converted to .mol2 using the application Openbabel[41] followed by final visualisation in Chimaera[42].

Results

The leaf extract was obtained from the dried, powdered leaf, which showed maximum solubility in organic solvents and partial solubility in water. The antibacterial effect of the extract on the growth of *S. aureus* was demonstrated by the well diffusion assay, which showed a significant zone of inhibition. The zone of inhibition was measured to be 16 and 12 mm for 80 and 50 mg/mL concentrations, respectively (Fig. 1).

Phytochemical screening revealed the presence of anthraquinones, terpenoids, alkaloids, saponins, reducing sugars, tannins, cardiac glycosides, triterpenes, flavonoids and steroids. LC-HRMS analysis resulted in several mass peaks (Fig. 2), which were resolved in Mestrenova, which detected smaller peaks that were unidentifiable in LC-HRMS (Fig. 3). Data generated from LC-HRMS led to the identification of 12 compounds that were found present in the leaf extract (Table 1). These 12 compounds were then subjected to *in-silico* evaluation for drug likeness using Lipinski's Rule of Five. Of the 12, 7 compounds passed the evaluation and were predicted as potential bioactive compounds bearing antibacterial properties (Table 2). These 7 compounds were then used as ligands for molecular docking.

Literature search revealed the presence of several drug target proteins in *S. aureus;* however, only 4 widely reported proteins were considered (Table 3). Validation of the selected proteins using the Ramachandran Plot revealed Exfoliative toxin A (1EXF) to be 96.7% within the favourable regions. Lysostaphin-type metalloendopeptidase (2B44),

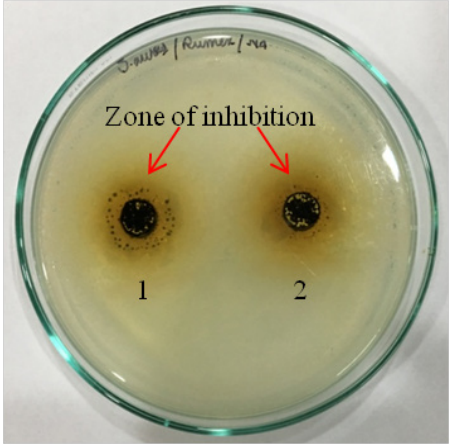


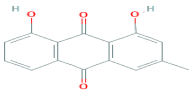
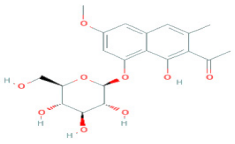
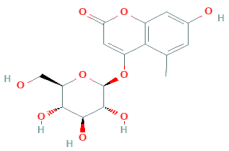
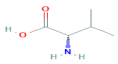
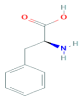
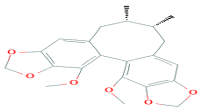
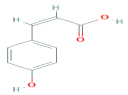
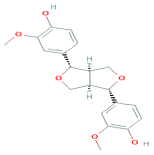
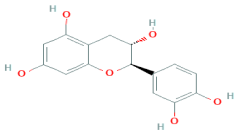
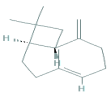
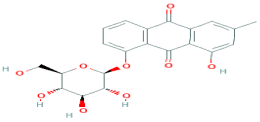
Fig. 1: *Well Diffusion Assay: Leaf Extract of R. nepalensis against S. Aureus*
1. *Treatment with 80 mg/mL concentration*
2. *Treatment with 50 mg/mL concentration*

gamma-haemolysin (3B07) and toxic shock syndrome toxin-1 (4OHJ) to be 96.6, 97.0 and 98.9% within the favorable regions, respectively. The compounds evaluated by Lipinski's test were docked against the drug targets.

The results obtained from BLASTp revealed that there is negligible similarity between the target protein sequence of *S. aureus* and the human proteome. Except for exfoliative toxin A (P09331), which showed a single hit with a query cover of 57% and identification of 23.98%, lysostaphin-like metalloprotein (O33599), gamma haemolysin (P0A071) and toxic shock syndrome toxin (P06886) showed no significant hit. The protein drug targets were subjected to molecular docking analyses with the compounds identified as potential drug candidates. The output of the docking analyses in terms of their binding energies, inhibition constant (Ki) and molecular interactions are summarised in Table 4.

Chrysophanol was found to bind with all four drug targets, Hastatusides A with two drug targets, whereas Pinoresinol and Schisandrin C interacted with one drug target each. Each of these interactions was supported with lower inhibition constants and binding energy values (Table 5). The best docking result of chrysophanol was seen with the Toxic Shock Syndrome Toxin target protein. The binding with the receptor molecule was mediated by a single hydrogen bond with the amino acid residue, GLU213, having a bond length of 1.924 Å (Fig. 4a). Chrysophanol showed interaction with the enterotoxin lysostaphin-like metalloprotein facilitated by a single hydrogen bond via the residue ASN303 and with a bond length of 2.113 Å (Fig. 4b). Chrysophanol and exfoliative toxin also showed significant interaction, which resulted from three hydrogen bonds via the residues GLN154, HIS156 and HIS145 with a bond length of 2.130 Å, 2.264 and 1.905

Table 1: *Compounds Identified based on LC-HRMS Mass Peak*

Sl. No	*Name of Compound*	*M+ions*	*Peak*	*MW*	*Structure*	*PubChem ID*
1	Chrysophanol	M(254)+Na((23)	277	254		10208
2	Torachysone-8-O-Beta –D-Glucoside	M(408)+Na(23)	431	408.40		11972479
3	Hastatuside A	M(354)+Na(23)	378.21	354		102480464
4	Valine	M(165)+Na(23)	141.96	117		6287
5	L-phenylalanine	M(384)+Na(23)	188.07	165		6140
6	Schisandrin C	M(164)+Na(23)	407.29	384		443027
7	cis-p-Coumaric acid	M(204)+Na(23)	187	164		1549106
8	Pinoresinol	M(290)+Na(23)	381.08	358		73399
9	Iodotridecane	M(311)+Na(23)	334	311		545617
10	Catechin	M(358)+Na(23)	313	290		9064
11	Beta Caryophyllene	M(416)+Na(23)	227	204		5281515
12	Pulmatin	M(117)+Na(23)+H(1)	439	416		442731

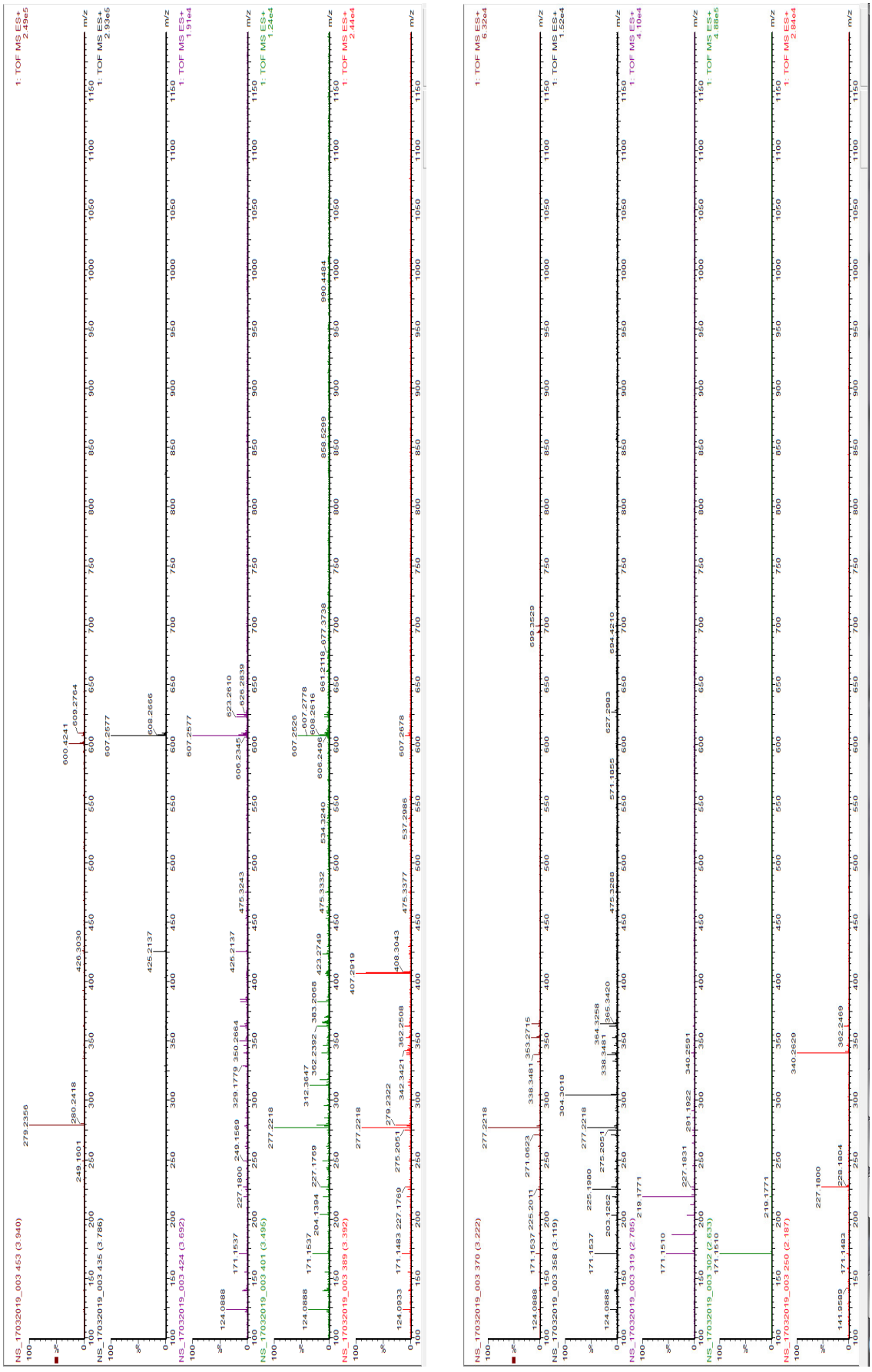


Fig. 2: *Raw mass file obtained from LC-HRMS*

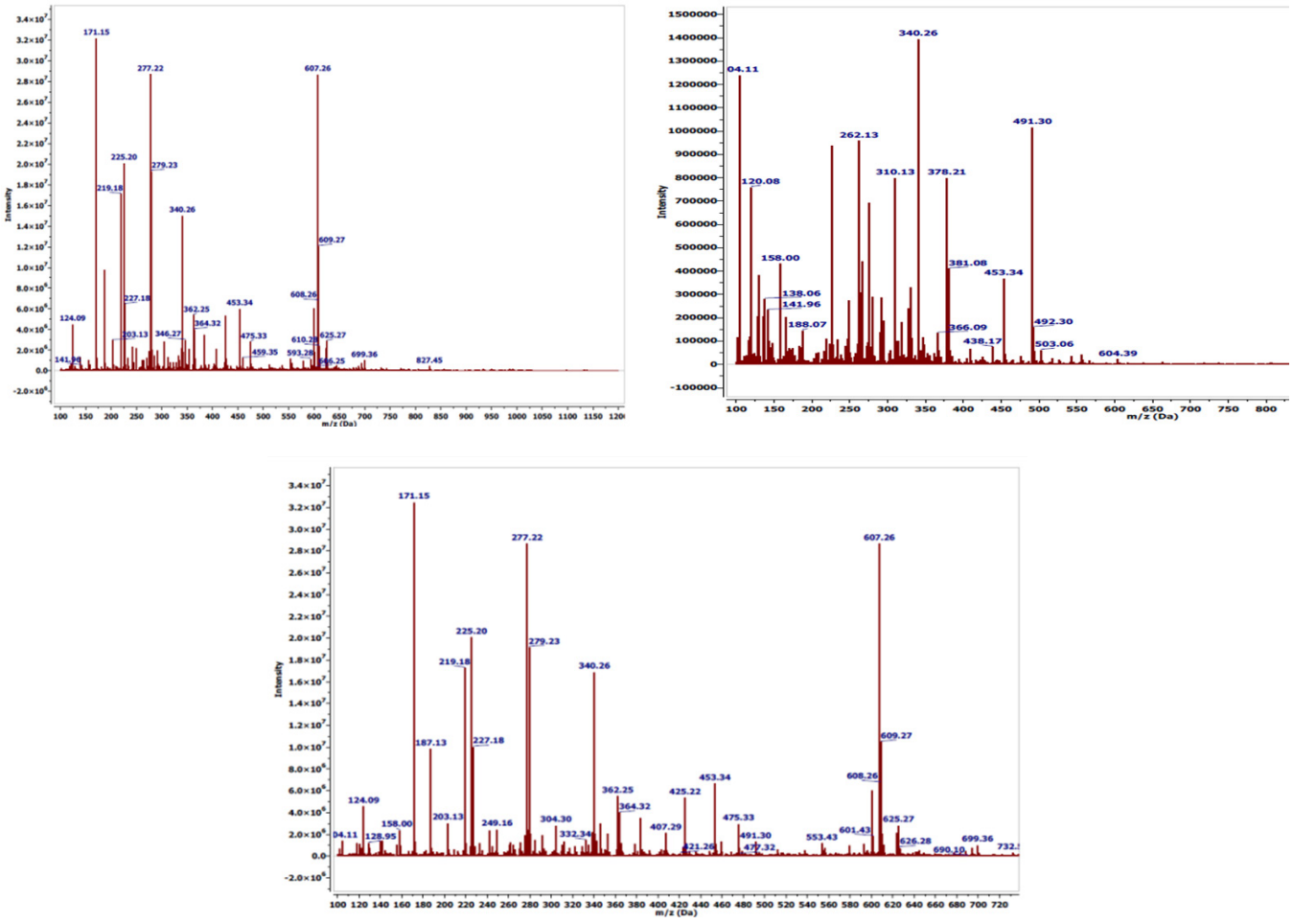


Fig. 3: Mass files obtained from Mestrenova

Table 2: Physicochemical properties of the compounds identified from *LC-HRMS.*

Sl. No.	*Name of Compound*	*Mass*	*H-bond donor*	*H-bond acceptor*	*LogP*	*Molar Refractivity*
1	Chrysophanol*	254	2	4	2.181	67.815
2	Torachrysone 8-O-glucoside	408	5	9	0.243	101.301
3	Hastatusides A*	354	5	9	-1.222	81.307
4	Valine	117	3	3	0.054	30.449
5	L- phenylalanine*	165	3	3	0.641	45.757
6	Schisandrin C*	384	0	6	4.198	102.96
7	Cis-p-coumaric acid*	164	2	3	1.49	44.776
8	Pinoresinol*	358	2	6	3.19	93.68
9	Iodotridecane	311	0	0	5.732	75.34
10	Catechin	290	5	6	1.546	72.622
11	Beta- Caryophyllene*	204	0	0	4.72	66.742
12	Pulmatin	416	5	9	-0.345	100.00

**Compounds selected based on Lipinski's ROF test for docking studies.*

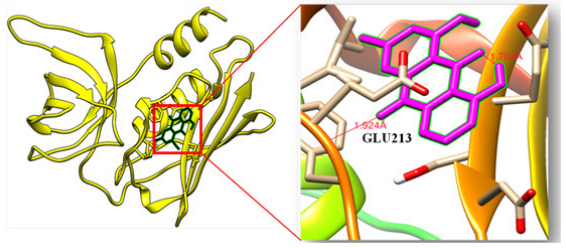
Table 3: Potential protein drug targets reported in *S. aureus* along with their PDB ID and other relevant information.

Sl No.	*Protein name*	*Gene*	*PDB ID*	*Resolution*	*Reference*
1	Exfoliative toxin	eta	1EXF	2.1 Å	Vath et al. 1997.
2	Lysostaphin-type Metalloendopeptidase	lytM	2B44	1.83 Å	Firczuk et al. 2005.
3	Gamma-hemolysin	hlgB	3B07	2.495 Å	Yamashita et al. 2011.
4	Toxic shock syndrome toxin-1	tst	4OHJ	1.28 Å	Sospedra et al. 2012.

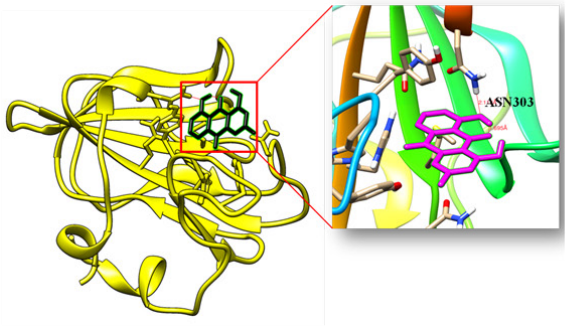
Table 4: Docking score for each of the four drug targets with the ligand

Sl no.	*Protein name*	*Drug target (PDB ID)*	*Ligand*	*Binding Energy (kcal/mol)*	*Inhibition constant, Ki (µM/mol)*
1	Exfoliative toxin	1EXF	Chrysophanol*	-6.14	31.55
			Schisandrin C	-6.13	32.16
			Pinoresinol	-5.94	43.99
			Beta Caryophyllene	-5.57	82.96
	Cis-p-Coumarin			-5.06	197.07
			Phenyalanine	-4.9	257.99
			Hastatusides A	-3.88	1130
2	Lysostaphin like metalloprotein, LytM	2B44	Beta Caryophyllene	-7.78	1.97
			Pinoresinol*	-7.54	2.96
			Schisandrin C*	-6.8	10.45
			Chrysophanol*	-6.63	13.86
			Hastatusides A*	-6.16	30.63
			Coumarin	-5.74	61.76
			Phenylalanine	-5.72	63.79
3	Toxic shock syndrome toxin	4OHJ	Chrysophanol*	-6.81	10.23
			Hastatusides A*	-6.41	20.1
			Pinoresinol	-6.92	8.53
			Schisandrin C	-6.75	11.3
			Phenylalanine	-5.97	41.92
			Coumarin	-5.86	50.75
			Beta Caryophyllene	-7.35	4.07
4	Gamma hemolysin	3B07	Hastatusides A*	-6.79	10.52
			Beta Caryophyllene	-6.81	10.14
			Chrysophanol	-6.39	20.55
			Pinoresinol	-6.25	26.09
			Schisandrin C	-6.71	29.78
			Phenylalanine	-3.94	1300
			Coumarin	-3.9	1400

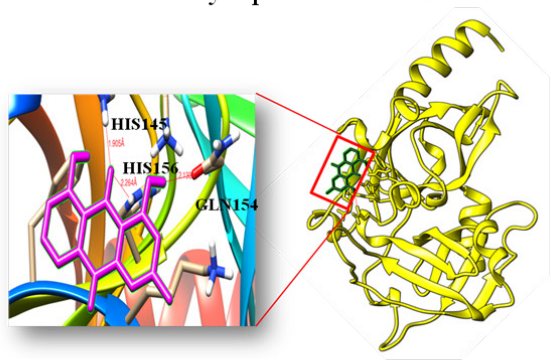
*Significant interaction between the ligand and the receptor molecule.



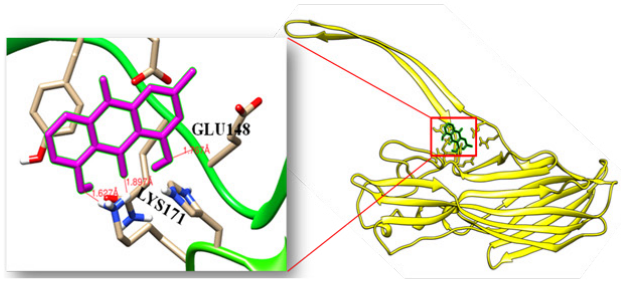
a. Chrysophanol-4OHJ



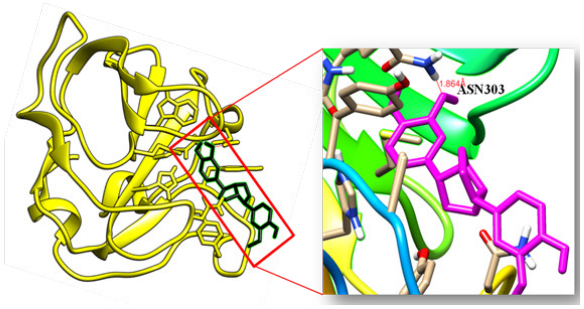
b. Chrysophanol-2B44



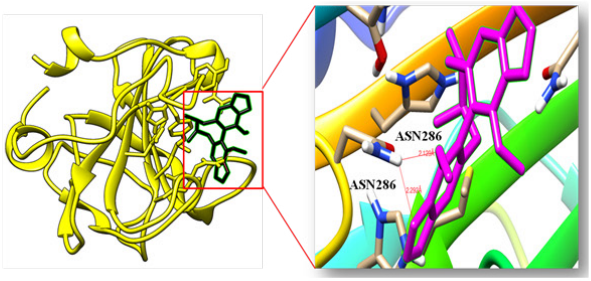
c. Chrysophanol-1EXF



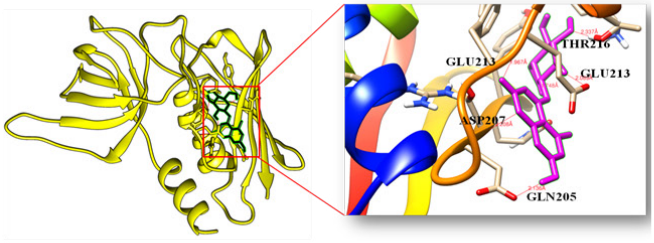
d. Chrysophanol- 3B07



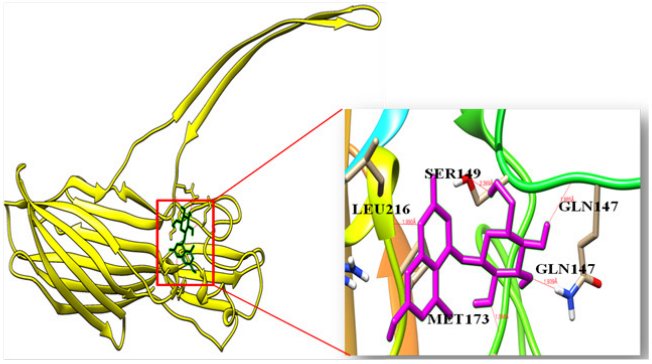
e. Pinoresinol-2B44



f. Schisandrin C-2B44



g. Hastatusides A-4OHJ



h. Hastatusides A-3B07

Fig. 4: Conformations of the ligands with four receptor molecules. The ligands are represented in magenta in the enlarged view. The hydrogen bonds and their respective bond lengths via the amino acid residues of the receptor molecule are also shown.

Table 5: Summary of the Best Docking Scores of the Protein-Ligand Complex.

Sl. no.	*Protein-ligand complex*	*Binding energy (kcal/mol)*	*Inhibition constant (μM/mol)*
1	4OHJ_chrysophanol	-6.81	10.35
2	2B44_chrysophanol	-6.63	13.86
3	1EXF_chrysophanol	-6.14	31.55
4	3B07_chrysophanol	-6.39	20.55
5	2B44_pinoresinol	-7.54	2.96
6	3B07_hastatusidesA	-6.79	10.52
7	4OHJ_hastatusidesA	-6.41	20.1
8	2B44_hastatusidesA	-6.16	30.63

Source: https://link.springer.com/referenceworkentry/10.1007/978-3-030-45597-2_209-2

Fig 5*: R. nepalensis plant*

Å, respectively (Fig. 4c). Moderate binding was evident between chrysophanol and gamma haemolysin through three hydrogen bonds via the residue GLU148 with the bond length of 1.767 Å and LYS171 having a bond length of 1.897 Å and 1.627 Å, respectively (Fig. 4d).

Pinoresinol showed a significant interaction with the enterotoxin-lysostaphin-like metalloprotein via a single hydrogen bond with the ASN303 residue of the receptor molecule, with a bond length of 1.864 Å (Fig. 4e). The compound Schisandrin C also showed considerable binding with the receptor molecule, enterotoxin-lysostaphin-like metalloprotein. The binding occurred via two hydrogen bonds with the ASN286 residue, with the bond lengths of 2.129 Å and 2.292 Å (Fig. 4f).

Interactions were seen between the compound hastatusides A and the proteins toxic shock syndrome toxin and the gamma haemolysin. The binding with toxic shock syndrome toxin resulted in five hydrogen bonds with the residues GLN205, ASP207, and THR216 having bond lengths of 2.138, 2.208, and 2.337 Å, respectively, whereas the interaction with gamma haemolysin was also mediated via five hydrogen bonds with the residues MET173, SER149, and LEU216 with bond lengths of 1.968, 2.369, and 1.990 Å, respectively (Figs. 4g & h).

Other compounds, including L-phenylalanine, Cis-p-coumaric acid and beta-caryophyllene, did not show any significant interactions and demonstrated higher inhibition constant and binding energy values.

Discussion

Plants have been widely used for the treatment of human diseases owing to their promising therapeutic potential without adverse effects on health[43,44]. *S. aureus* poses a challenge to human health [16]. Methicillin-resistant *S. aureus* (MRSA) infection is on the rise in hospital and community settings[45,46], which highlights the need for the discovery of new drugs. In the present study, the leaf extract of *R. nepalensis* was tested for antibacterial potential, and the bioactive compounds present in the extract were explored. *In-silico* evaluation to understand the interaction between the compounds and the drug targets was also conducted.

Existing literature focuses on the potential of the root extract of *R. nepalensis* against a variety of bacterial pathogens[14,47–49]. Studies have also been carried out on the phytochemical constituents in the root extracts[50,51]. However, the antibacterial potential of the leaf extract and its phytoconstituents was not well established[52].

The well diffusion assay revealed the leaf extract to have antibacterial potential against *S. aureus*. The phytochemical screening revealed the presence of the common secondary metabolites in the leaf extract, like those reported from the roots of *R. nepalensis* and other *Rumex* species[53,54]. LC-HRMS resulted in the identification of 12 compounds, including 3 phenolic compounds, 2 lignans, 2 non-polar amino acids and 1 each of anthraquinone, oxo acid, iodotridecane and sesquiterpene. However, only 7 compounds passed the drug likeness by Lipinski's test and were identified as potential drug candidates. Interaction of the 7 compounds with the drug targets in *S. aureus*, as demonstrated by the molecular docking, revealed 4 compounds to be suitable for potent antibiotics against *S. aureus*. These compounds are chrysophanol, an anthraquinone; Hastatusides A, a phenolic glucoside; and Schisandrin C and Pinoresinol, both lignans. Among these, anthraquinones have been widely studied for antimicrobial potential[55–57]. Aloe-emodin, another anthraquinone from the roots of *R. nepalensis,* has been reported to demonstrate antibacterial

activity.[58] Since aloe-emodin was not identified in the leaf extract, it may be assumed that chrysophanol present in the leaf is responsible for the antibacterial action against *S. aureus*. Moreover, the antibacterial action of chrysophanol has also been asserted previously.[59]

In this study, chrysophanol was seen to bind with all the drug target proteins in *S. aureus*. It interacts well with targets such as toxic shock syndrome toxin, exfoliative toxin and lysostaphin-type metalloendopeptidase, further strengthening that chrysophanol in the leaf extract induces its antibacterial action by inhibiting the drug targets. Its functional hydroxyl groups in the anthraquinone molecule probably make it easier for it to form hydrogen bonds with important amino acid residues. Additionally, chrysophanol has also been suggested to possess anti-diabetic and anti-inflammatory properties.[60,61] Other compounds, including Hastatusides A, Schisandrin C and Pinoresinol identified in the leaf extract, are known for their anti-diabetic, antioxidant and anti-inflammatory properties.[50,62,63]

These results provide a promising foundation for the development of plant-derived medicines and support the traditional use of *R. nepalensis.* Toxicological profiles, *in-vitro* efficacy testing, and formulation strategies for clinical translation should all be included in future research.

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