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IDENTIFICATION OF ANTIMASTITIS COMPONENTS IN ALLIUM CEPA AS AN INHIBITOR OF MASTITIS CAUSING STAPHALOCOCUS AUREUS MONOFUNCTIONAL GLYCOSYL TRANSFERASE - AN INSILICO APPROACH

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ABSTRACT

Bovine mastitis is an infection of the mammary gland of cattle leading to a huge reduction in milk production.This causes severe economic loss in diary industry across the world. Antibiotics routinely used for the treatment has shown minimum efficacy. Any microbe can opportunistically invade the tissue and cause mastitis. Among these Staphalococus aureus infections are most common and is highly resistant to antibiotics currently used for treatment. Crude extract of locally available medicinal plant *Allium cepa* is used traditionally against mastitis and is found to be highly effective. Objective of the present study is to identify the phytochemicals responsible for the curative action for further development to drug. Insilico docking analysis of the phytochemicals present in *Allium cepa* against monofunctional glycosyltransferase of mastitis causing Staphalococusaures bacteria as target proteins is done using Schrodinger Suit v 9.2. Compounds from *Aliiuomcepa* were selected from literature for docking studies. The high resolution crystal structure of the target receptor protein, monofunctional glycosyl transferase of Staphalococus aureus were retrieved from PDB (PDB ID:3VMS) and the phytochemicals and the most commonly used antibiotic against Bovine mastitis were selected from PUB CHEM NCBI. 20 compounds from *Allium cepa* were chosen based on ADMET properties and docked for drug efficacy. The compounds selected from *Allium cepa* showed good docking scores and better interaction with the target proteins used for the evaluation than the most commonly used commercially available drug. Results of this study is important for the development of novel drug for the treatment of mastitis.

KEYWORDS: Staphalococusaureus Monofunctional Glycosyltransferase, Bovinemastitis, Docking, Qik Prop

Wounds or scars happened to the udder of cattle results in Bovine mastitis when subjected to the attack of infectious agents as bacteria, Bovine Herpes virus, non-bacterial pathogens like mycoplasmas, fungi, yeast and chalmydia [Reena Patel et al,2015], [Wellenberg, G.J. et al, 2002] . Among all these infectious pathogens, staphalococusaureus bacterial infections are most common. The quantity of casein, lactoferrin and potassium in milk reduces due to Bovine mastitis. Deterioration of casein in milk decreases the calcium level. Processing and storage also reduces the amount of protein [Harmon, R.J., 1994.].Milk from affected animals exhibit very high somatic cell count which reduces the quality of milk [Kandasamy, S. et al.2011] Hence, Bovine mastitis causes huge economic losses in diary industry. [http://en.wikipedia.org/wiki/peptidoglycan]Current methods used in the treatment of Bovine mastitis are based on antibiotic therapy. Pencillins and semisynthetic pencillin derivatives are the commonly used antibiotics. Thesetargets the pencillin binding proteins of Staphalococusaureus. DD-transpeptidase and Glycosyl transferaseare penciling binding proteins in the bacterial cell wall of Staphalococusaureus. DD- transpeptidaseare associated with the peptidoglycan synthesis. Inhibition of transpeptidase interrupt peptidoglycan synthesis which leads to bacterial cell lysis. [Andrea FeBler ,et al,2010]. A commonly used antibiotic targets these transpeptidase. But mutations in genes coding for transpeptidases leads to decreased interaction with antibiotics resulting in the development of antibiotic resistance in Staphalococusaureus [Apurba Sarker Apu, et al, 2012]. This is the main reason for the reduced antibiotic efficacy. There lies the importance of drug molecules which can inhibit glycosyltransferase associated with the bacterial cell wall of Staphalococusaureus. So Monofunctional glycosyltransferase [Reena Patel et al,2015] is an alternate potent target of Staphalococusaureus, the inhibition of which also leads to bacterial cell lysis.

The immunemodulator compounds derived from medicinal plants has effective application in controlling mastitis[https://phytochem.nal.usda.gov/]. *Allium cepa* is one of the medicinal plants in the whole or its peculiar parts have enormous medicinal value. [http://www.rcsb.org/pdb]

Crude extract of the plant as a whole is used from traditional knowledge as a medicine for controlling mastitis. It is observed that phytochemicals in *Allium cepa* shows excellent inhibitory activity towards

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monofunctional glycosyl transferase compared to the most common commercially available drug.

METHODOLOGY

Bioinformatics Analysis

Preparation of Protein

Crystal structure of the target protein, Staphalococusaureus Monofunctional glycosyltransferase was obtained from RCSB Protein Data Bank (PDB ID: 3VMS) [Schrodingersuit 2009 Protein Preparation Wizard; Epik version 2.0; Impact version] .The Protein Preparation Wizard increased efficiency in structure preparation. Automatically imported PDB files from the RCSB PDB website to the Schrodinger working interface.[Friesner, R.A.,et al,2004], [Friesner, R.A. et al,2006].Missing hydrogen atoms were added automatically. Then rectified metal ionization states to ensure proper formal charge and force field treatment. Co-crystallized water molecules were removed. By means of a systematic, cluster-based approach,optimized the protein's hydrogen bond network which decreased the preparation times and then performed a restrained minimization that allows hydrogen atoms to be freely minimized. Here the crystal structure of target protein was complexed with a ligand. So sitemap creation was excempted.

Ligand Preparation

Twenty phytochemicals present in *Allium cepa* were selected to find out the inhibitory activity towards target protein. Structure of the phytochemicals and routinely used antibiotic pirlimycin hydrochloride were downloaded from pub chemin the (.sdf) format. These ligands were subjected to ligand preparation using the ligand preparation wizard (ligprep) of Schrodinger software in the Maestro interface. Ionization states were generated for the structures.One low energy conformation was generated. The Ligprep ligands were used for the Docking analysis.

Docking Studies

The compounds were screened by Schrodinger docking software to study the inhibitors of target protein. Grid generation was done using the centroid of workspace ligand R0 48-8071.The rigid receptor docking using the Glide program was carried out against the target protein with the set of ligands. The mode of docking was selected as XP (Extra precision) for a high docking accuracy. The glide docking was carried out for the minimised protein [Friesner, R.A, et al, 2004], [Friesner, R.A, et al, 2006]

Ligand Interaction Study

The ligand molecules fit in to the active binding sites of the protein molecules by means of some interaction. The interactions include hydrogen bonding, pi-pi stalking interactions and pi-pi cationinteractions. A detailed information of various interactions of the ligands with aminoacid residues pointing the type of bonds, bond length and various angles can be studiesd using this option.

ADME Properties Prediction

The bioactive compounds from *Allium cepa*were checked for their ADME properties using Qik prop module. [Schrodinger, LLC, New York, NY, 2010]. QikProp helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug likeness. Predicted significant ADME properties are Molecular weight (MW), hydrogen bond donor, hydrogen bond acceptor and $Q_P \log P_{(O/W)}$

RESULTS AND DISCUSSION

Table 1: ADMET Property Satisfied Components in Allium cepa

Protein (PDB ID: 3VMS)	Ligands	Stars	Donor H_B	Acceptor H_B	$Q_p\log P_{o/w}$	Rule of five	Rule of Three
	Myricetin	0	5	6.000	0.293	0	0
	Quercetin	0	4	5.250	0.360	0	0
	Kaempferol	0	3	4.500	1.059	1	0
	3,4-dihydroxybenzoic acid	0	3	3.500	0.042	0	0
	Emodin	1	1	4.250	1.256	0	0
	Diphenylthiosulfinate	1	0	4.000	2.657	1	1
	Quercetin-4'-glucoside	4	7	13.750	-1.627	0	1
	Diosgenin	3	1	3.200	6.124	1	0

Table 2: Phytochemicals in Allium cepa having good docking results with target protein (PDB ID:3VMS)

Protein (PDB ID: 3VMS)	Ligand	Docking Score(Kcal/mol)	Interacting aminoacid residues	Hydrogen bond length (Angstrum)
	Myricetin	-7.012	140LYS,137GLN,141ASN,222LYS,181TYR ,140LYS	1.95255,2.16378,2.16567, 1.89909,2.11591,2.19278
	Quercetin	-5.935	137GLN, 141ASN, 222LYS, 181TYR	1.75271, 2.1716, 1.95177, 2.13182
	Emodin	-4.575	145ASP, 223VAL, 225ALA	1.76847, 1.67299, 1.79181
	3,4-dihydroxybenzoic acid	-4.442	147ASP, 140LYS , 156GLU	1.66149, 1.88018, 1.75058
	Pirlimycinhydrochloride	-2.758	141ASN, 224ASN, 223VAL, 145ASP	1.88965, 1.55245, 1.44589, 1.48796

3D Docking Interaction of Ligands with Staphalococus Aureus Monofunctional Glycosyltransferase (Figure 1 to figure 5)

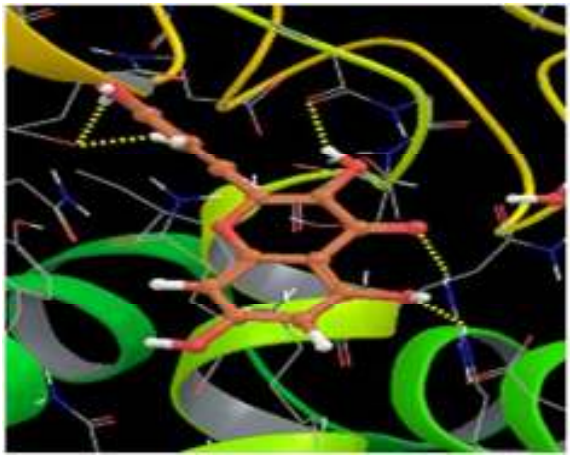

Figure 1: 3VMS and Myricetin

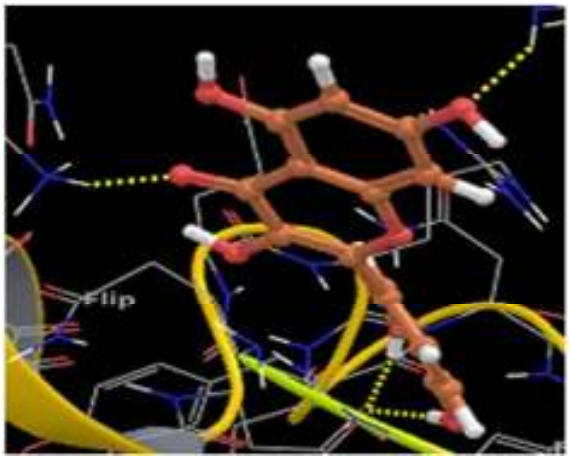

Figure 2: 3VMS and Quercetin

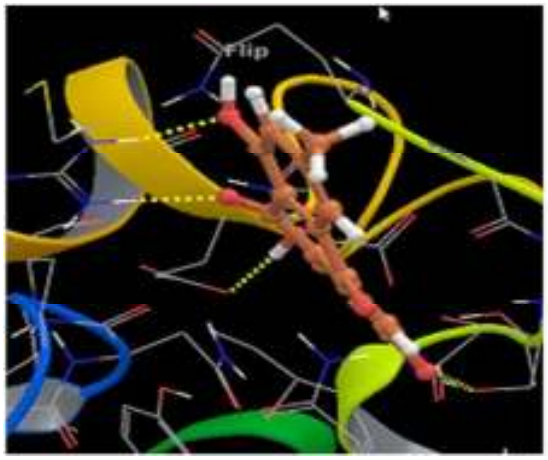

Figure 3: 3VMS and Emodin

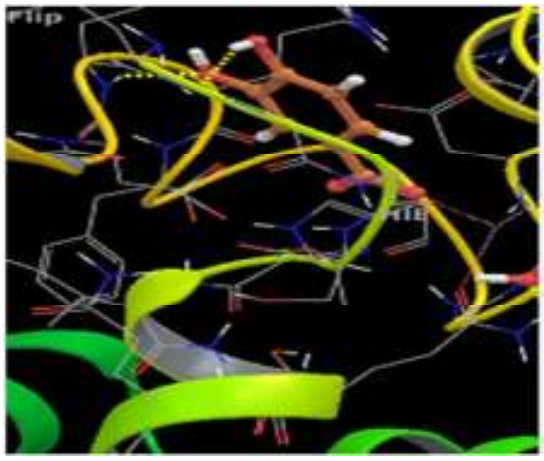

Figure 4: 3VMS and 3, 4-dihydroxybenzoic acid

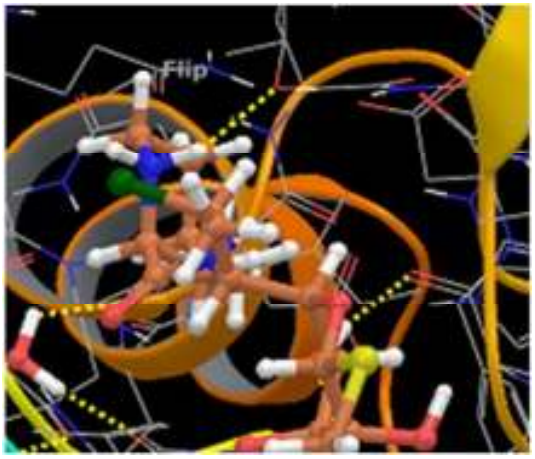

Figure 5: 3VMS and Pirlimycin hydrochloride

2D Interaction of Ligands with Target Protein (Figure 6 to figure 10)

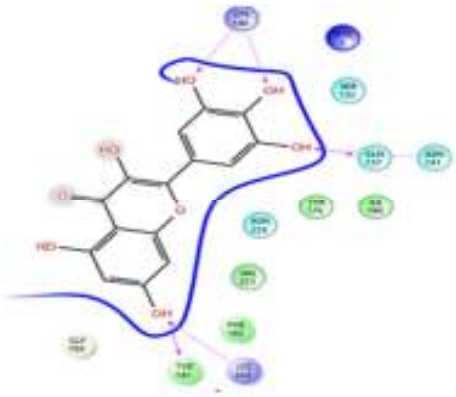

Figure 6: 3VMS and Myricetin

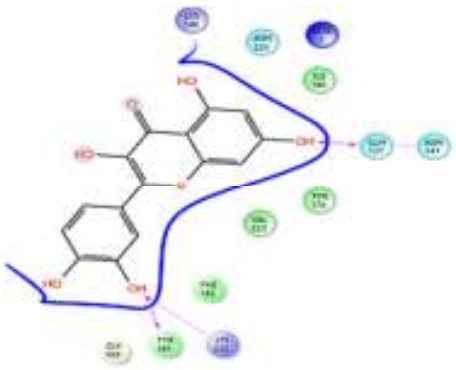

Figure 7: 3VMSand Quercetin

Figure 8: 3VMS and Emodin

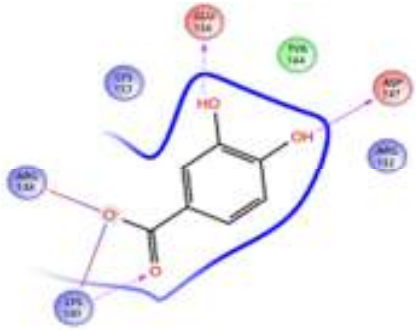

Figure 9: 3VMS and 3,4-dihydroxybenzoic acid

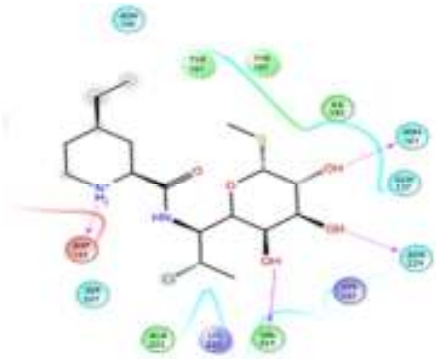

Figure 10: 3VMS and Pirlimycin hydrochloride

Table -1 illustrates the ADMET profile of the phytochemicals in *Allium cepa.* Among the 20 compounds selected from literature, eight of them satisfied the ADMET conditions predicted by Qik prop option. Least star value indicates more drug likeness of the compound. The accepted range of star is 0-5. Donor H_B indicates the number of hydrogen bonds donated by the ligand and the permissible limit is 0-6. Acceptor H_B refers to the number of hydrogen bonds accepted by the ligand and the range of value is 2-20. $Q_Plogp_{o/w}$ indicates predicted octanol/water partition coefficient. The accepted

range is -2 - 6.5. Rule of five indicates number of violations of Lipinski's rule of five. Compounds obeying this is said to be more drug like. The maximum accepted value is 4. Rule of three measures the number of violations in the Jorgenson's rule of three. Maximum permitted value is 3.

Table 2 refers the docking parameters of the ADMET property satisfied components in *Allium cepa.* From the results obtained, it has been observed that there exist excellent binding interactions between phytochemicals in *Allium cepa* and target protein (PDB ID:3VMS)compared to the commercial drug(Pirlimycin hydrochloride) with good binding scores. The phytochemical Myricetin shows a docking score -7.012kCal/mol and forms six hydrogen bonds with the aminoacid residues in the binding pocket.Five hydroxyl groups in the ligand forms strong side-to-side hydrogen bonds with 140LYS(Bond Length-1.95255 and Donor angle-125.78),137GLN(Bond Length-2.16378 and Donor angle-146.594),141ASN(Bond Length-2.16564 and Donor angle-105.2),222LYS(Bond Length-1.89909 and Donor Angle-129.589) and 140LYS(Bond Length-2.19278 and Donor Angle-122.764). One of the hydroxyl group in Myricetin forms a back bone hydrogen bond with amide nitrogen of 181TYR amino acid residue.The phytochemical Quercetinexhibitfour hydrogen bonding interactions with a docking score -5.935kCal/mol. Three strong side-to-side hydrogen bonds are formed between the hydroxyl group in the ligand and 137GLN(Bond Length-1.75271 and Donor Angle-146.839), 141ASN (Bond Length-2.1716 and Donor Angle-99.7627), 222LYS (Bond Length-1.95177 and Donor Angle-133.647). Also exist a back bone hydrogen bonding between hydroxyl group in Quercetin and 181TYR (Bond Length-2.13182 and Donor Angle-127.743).Emodin forms three hydrogen bonds with a docking score -4.575kCal/mol. One strong side-to-side hydrogen bonding between hydroxyl group in Emodin and 145ASP(Bond Length-1.76847 and Donor Angle-142.399). Two hydroxyl groups in the ligand forms back bone hydrogen bonding with 223VAL (Bond Length-1.67299 and Donor Angle-142.399) and 225ALA (Bond Length-1.79181 and Donor Angle-160.441).3,4-dihydroxybenzoic acid forms three hydrogen bonds with the aminoacid residues in the active site with a docking score -4.442Kcal/mol. Two hydroxyl groups in the ligand forms side-to-side hydrogen bonds with 140LYS (Bond Length-1.88018 and DonorAngle-111.154) and 156GLU (Bond Length-1.75058 and Donor Angle-109.742).There is a strong back bone hydrogen bond between hydroxyl group in the ligand and 147ASP amino acids residue. (Bond Length-1.66149 and Donor Angle-156.273). In addition to that two salt bridges are formed between the ligand and protein(140LYS-distance:4.17227A^{0}and 148ARG-distance:3.6697)

The most commonly used antibiotic Pirlimycin hydrochloride shows least docking score(-2.758Kcal/mol) with four side-to-side hydrogen bonding interactions. Three hydroxyl groups forms hydrogen bonds with 141ASN (Bond Length-1.88965and Donor Angle-146.253), 224ASN (Bond Length-1.55245 and Donor Angle-137.219) and 223 VAL (Bond Length-1.44589 and Donor Angle-141.723). The Nitrogen atom in the ligand forms hydrogen bond with 145ASP (Bond Length-1.48796 and Donor Angle-110.812)

CONCLUSION

The phytochemicals Myricetin, Quercetin. Emodin and 3,4-dihydroxybenzoic acid has potent inhibitory activity towards the staphalococusaureus monofunctional glycosyl transferase compared to the commercial drug Pirlimycin hydrochloride. These phytochemicals are responsible for the antimastitis activity of *Allium Cepa.* The antibiotic resistance developed in Staphalococusaureus transpeptidase became a major problem in the treatment of mastitis. So drug molecules which can inhibit glycosyltransferase took major attention. Present study reveals the inhibitory activity of the components from Allium cepa towards monofunctional glycosyl transferase of Staphalococusaureus. This in Silico analysis will be helpful in designing novel drugs for the treatment of mastitis.

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