Trends in Bioinformatics

ISSN 1994-7941

science alert

ANSInet
an open access publisher
http://ansinet.com

Trends in Bioinformatics 6 (3): 91-100, 2013
ISSN 1994-7941 / DOI: 10.3923/tb.2013.91.100

In silico Molecular Interaction Studies of Gamma-hemolysin of *Staphylococcus aureus* with Flavonoid Compounds

Ramadevi Mohan and Subhashree Venugopal
Division of Biomolecules and Genetics, School of Biosciences and Technology, VIT University, Vellore, 632014, Tamilnadu, India

Corresponding Author: Subhashree Venugopal, Division of Biomolecules and Genetics, School of Biosciences and Technology, VIT University, Vellore, 632014, Tamilnadu, India Tel: +919486947377

ABSTRACT

The aim of the study was to predict and analyze the molecular interactions between the drug target named γ-hemolysin of *Staphylococcus aureus* and various flavonoid compounds. Binding affinity for the complexes were calculated based on the free binding energies and the number of interactions. The receptor is a bicomponent, β-barrel pore forming toxin associated with several clinical diseases. The cytolytic and hemolytic activity of the toxin and its involvement in the pathogenesis by damaging the host membranes proved γ-hemolysin to be a valid drug target. The 3D structure of γ-hemolysin downloaded from PDB database (PDB ID: 2QK7) was docked with flavonoid compounds having antimicrobial property. The SMILES of the twenty compounds were obtained from NCBI Pubchem Compound database and downloaded PDB files from Corina tool. Drug-like property and drug-likeness of the compounds were checked by Lipinski's Rule. Molecular docking was done using Autodock 4.0 resulting in binding energies and the docked complexes were analyzed by PyMol which revealed the binding pattern of amino acids of the receptor with the ligands. Docking analysis revealed that the residue SER90 play an important role in binding with the ligands. The binding energy values of the docked complexes ranged between -6.49 to -5.02 kcal moL^{-1}. A more negative binding energy was found with Ponciretin compound with -6.49 kcal moL^{-1}. The lower energy indicated that the compound was in stable conformation and had higher affinity towards the receptor. Thus, Ponciretin compound would be a possible drug compound for inhibiting the cytotoxic and hemolytic activity of the toxin.

Key words: γ-hemolysin, *Staphylococcus aureus*, flavonoids, Lipinski's rule, docking-autodock 4.0, pymol

INTRODUCTION

Staphylococcus aureus which is a pathogenic bacterium is also found as commensal in about 30% of the healthy individuals. The bacterium is able to cause a number of diseases ranging from superficial skin infections to deep infections such as furuncles, impetigo, boils, endocarditis, pneumonia and osteomyelitis. These are related with a large number of virulent components allowing colonization and persistence, dissemination within the host and evasion of host immune system. There is a correlation between the disease caused by the virulence factors and the site of infection (Vandenesch *et al*., 2012). The organism produces a number of exotoxins which are responsible for damaging the host cell membrane. The toxins which are Pore-Forming Toxins (PFT) and 'hole punchers' delivers the toxic materials through pores to particular sites of the cell

membrane and induces cell leakage. The proteins included in this category are α-hemolysin, β-hemolysin, γ-hemolysin, Panton-Valentine leukocidin, Phenol soluble modulin peptides all of which are leukotoxic and hemolytic in nature (Julianelle, 1922; Panton and Valentine, 1932).

Gamma-hemolysin toxins produce pores by the secretion of water-soluble monomeric proteins, binding of the monomers to the cell membrane and oligomerization and transmembrane β-barrel pore formation (Prevost *et al.*, 2005; Tilley and Saibil, 2006). It is a bi-component toxin consisting of two non-associated secretory proteins namely class S (HlgA/HlgC, 36 KDa) and class F (HlgB, 32 KDa) proteins. Due to the synergistic activity of the two components, it possesses cytotoxic and hemolytic activity. It lyses host cells especially monocytes, macrophages and polymorphonuclear cells of human and rabbits as well as erythrocytes of human, rabbit, sheep and horse (Cooney *et al.*, 1993; Supersac *et al.*, 1998; Nilsson *et al.*, 1999; Bohach *et al.*, 1997; Prevost *et al.*, 1995, 2005). In rabbits, the toxin was found to be responsible for cell chemotaxis, vasodilatation and tissue necrosis (Prevost *et al.*, 1998).

The increase in antibiotic resistance and difficulty in treating microbial infections is becoming major problem that leads to find alternatives for the existing drugs. Plants constitute one of the major sources of drugs both in modern as well as traditional medicine throughout the world. Flavonoids, also called as bioflavonoids, (Citrin or Vitamin P) are a group of naturally occurring polyphenolic compounds having a wide range of biological activities occuring as secondary metabolites in plants. It is found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey (Havsteen, 1983; Middleton and Chithan, 1993; Grange and Davey, 1990). The different classes of flavonoids include flavones, flavonols, chalones, xanthones, isoflavones and biflavones. They show anti-inflammatory and anti-microbial properties (Havsteen, 1983) by the inhibition of iNOS in culture medium of LPS-stimulated (e.g., Kaempferol, Naringenin, Catechin, Quercetin, Apigenin, Naringenin).

The objective of the study was to investigate and analyze the interactions between the *S. aureus* toxin (γ-hemolysin) with certain flavonoid compounds which were sources of having anti-microbial and anti-inflammatory like properties using *in silico* approach-molecular docking.

MATERIALS AND METHODS

Target protein: The three-dimensional structure of the protein γ-hemolysin that was stored in the form of X-Ray diffraction data with resolution 2.40 Å was downloaded from Protein DataBank (PDB) database (http://www.rcsb.org) with PDB ID 2QK7. The hetero atoms from the protein were removed and the protein structure was refined by Koba server (http://csb.stanford.edu/kobamin).

Analysis of interactions between two chains: Protein-protein interactions between HlgA and HlgB in the bicomponent toxin were analyzed for the residues involved in interaction that makes the protein active toxin by PDB sum Generate (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html).

Ligands selection: Twenty flavonoid compounds were chosen as ligands for the study. The compounds included in this study were acacetin, apigenin, baicalien, baicalin, catechin, dihydroquercetin, galangin, kaempherol, leucocyanidin, luteolin, morin, noringenin, noringin, pinocembrin, ponciretin, quercetin, quercetagetin, quercitrin, robinetin and rutin. The SMILES (Simplified Molecular-Input Line-Entry System) of the compounds were obtained from the NCBI PUBCHEM Compound database (http://www.ncbi.nlm.nih.gov/pccompound) and submitted in CORINA (http://www.molecular-networks.com/node/84), an online tool to obtain the PDB files of the ligands.

Active sites prediction: The ligand binding sites in the protein receptor were found by Q-Site Finder (http://www.bioinformatics.leeds.ac.uk/qsitefinder). The ligand binding site prediction was carried out by binding hydrophobic (CH_3) probes to the protein and finding clusters of probes with the most favorable binding energy. It uses the interaction energy between the protein and a simple Vander Waal's probe to identify energetically favorable ligand binding sites.

Drug evaluation and drug likeness: The ligands were evaluated and filtered for compounds with drug-like properties using Molinspiration tool (http://www.molinspiration.com/). It determines the drug like activity of the compounds based on Lipinski's Rule of 5 (Lipinski *et al.*, 2001) by evaluating the ligands for drug likeness and determining if a chemical compound with a certain pharmacological or biological activity has property of an active drug. The rule helps in determining the compounds of soluble, permeable and bioavailable. The Lipinski's rule states that those ligands whose molecular weight was less than 500 g moL^{-1}, H bond donors less than 5, H bond acceptors less than 10 and log p-value less than 5 was considered to have drug like property.

Molecular docking: AutoDock 4.0 (Morris *et al.*, 1998) was used for docking which employed the preparation of receptor by converting PDB into PDBQT files by adding hydrogens, setting map types and assigning Kollman charges. Ligands were assigned with Gasteiger charges. The grid was centered in the active site region of the receptor which involved all functional amino acid residues. The most efficient and reliable method of AutoDock known as Lamarckian Genetic algorithm (LGA) was used to run the docking simulations. The grid points for Autogrid calculations were set to be 60×60×60 Å with the active site residues at the centre of the grid box. The default parameters for docking were 10 runs, 2,500,000 energy evaluations 27,000 generations, 150 as population size and 0.02 as the rate of gene mutation and 0.8 as the rate of crossover. The obtained conformations of ligands with top rank poses having more negative energy were used for analysis by Autodock Tool.

Visualizing and analysis of docked complexes: The molecular interactions between the protein and the ligands in docked complexes were viewed and analyzed using PyMol software.

RESULTS AND DISCUSSION

Binding site analysis: The structure of γ-hemolysin (Fig. 1) which was a bi-component toxin with S and F subunits was downloaded from PDB database. The protein toxin possessed greater number of β-strands when compared to helices. There were no turns. It was submitted to QSITE finder. The following aminoacids were found at binding site. GLY52, PHE53, ILE100, ASP101, SER102, ALA103, ASP104, VAL105, SER136, TYR137, ASN138, GLN139, LYS140, ASN141, TYR142, VAL143, THR144, VAL158, LEU200, GLY204, PHE205, ASN206, PRO207, SER208, GLN89, SER90, ASN91, CYS156, THR157, ASN158, TYR159, LYS160, ASN161.

Analyzing interactions between the two chains of γ-hemolysin: The analysis of the results produced by PDBsum Generate (Fig. 2) revealed 1 disulphide bond at Cys28-Cys156 positions of chain A and B, respectively. There were 13 hydrogen bond interactions between the two components. The residual pairs involved were GLN18-ASN297, THR17-ASN297, THR17-THR295, LYS273-ASN298, GLY204-TYR159, ASN206-TYR159, ASN206-ASN158, ARG16-GLU296, SER136-SER90, SER36-ASN91, LYS23-ARG155 and ASP19-GLN246.

Filtering of drugs for docking based on Lipinski's rule: The flavonoid compounds that were screened and filtered for drug-likeness based on Lipinski's rule (Lipinski *et al.*, 2001) revealed

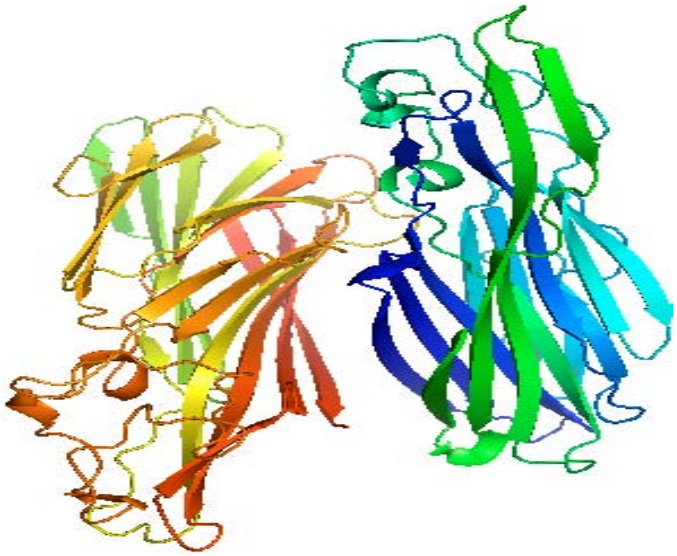

Fig. 1: Structure of gamma hemolysin (PDB ID: 2QK7)

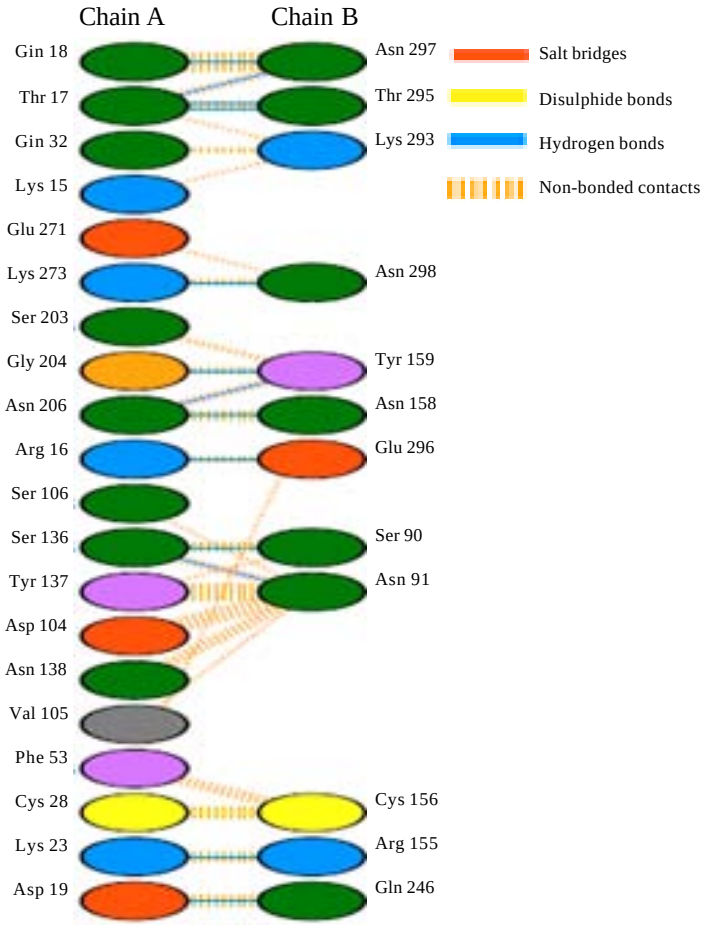

Fig. 2: Interactions between the two chains (HlgA and HlgB) of γ-hemolysin

14 compounds to possess drug-like property (Fig. 3). The molecular properties shown in Table 1 such as Molecular Weight, HB-A, HB-D and logP of each of the 14 compounds satisfied the Lipinski's rule which signified its role as drugs.

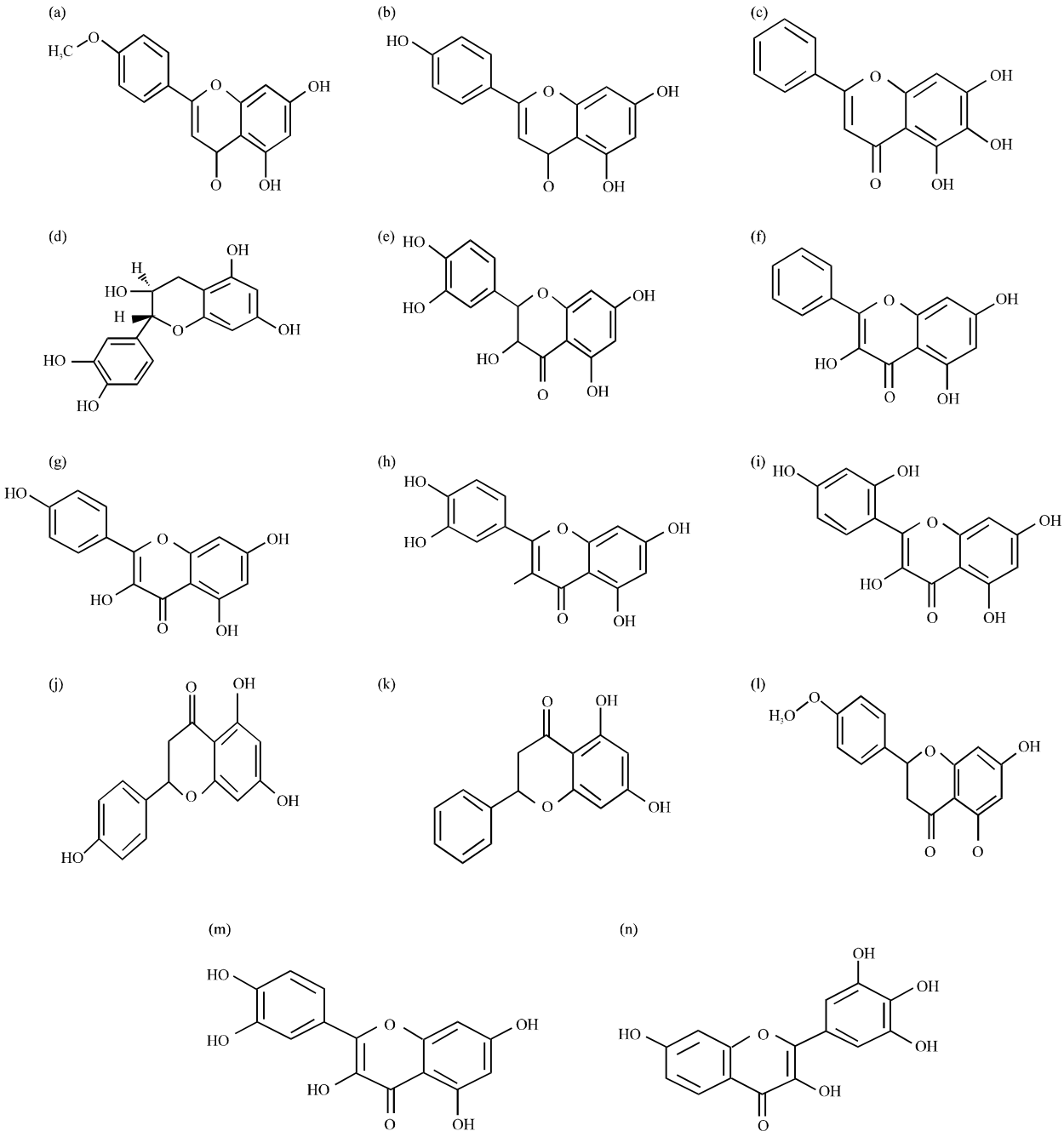

Fig. 3(a-n): 2D structures of flavonoid compounds used for docking (a) Acacetin, (b) Apigenin, (c) Baicalein, (d) Catechin, (e) Dihydroquercetin, (f) Galangin, (g) Kaempherol, (h) Luteolin, (i) Morin, (j) Narigenin, (k) Pinocembrin, (l) Poniciretin, (m) Quercetin and (n) Robinetin

Table 1: Molecular properties of ligands

Compounds	Molecular weight (g moL^{-1})	logP	H bond donor	H bond acceptor
Acacetin	284.26348	2.1	2	5
Apigenin	270.23690	1.7	3	5
Baicalein	270.23690	1.1	3	5
Catechin	290.26806	0.4	5	6
Dihydroquercetin	304.25158	1.5	5	7
Galangin	270.23690	2.3	3	5
Kaempherol	286.23630	1.9	4	6
Luteolin	286.23630	1.4	4	6
Morin	302.23570	1.5	5	7
Naringenin	272.25278	2.4	3	5
Pinocembrin	256.25338	2.7	2	4
Ponciretin	285.27142	2.8	1	5
Quercetin	302.23570	1.5	5	7
Robinetin	302.23570	1.6	5	7

Molecular interactions of the toxin with the flavonoid compounds: The gamma toxin was docked with 14 flavonoid compounds. The analysis of the docked complexes formed by the target protein and flavonoid compounds revealed favorable docking poses of the ligands with the receptor protein as shown in Fig. 4. Throughout the study, the target protein was kept rigid and the ligands were made to rotate freely inside the grid to find the best conformers. The energetically favorable pose with lowest binding energy conformation in the first cluster was considered as the most favorable docking pose that could bind well with the receptor. PyMOL was used to visualize, identify and analyze the interactions that contributed to ligand binding to the protein receptor.

The binding free energies of the docked complexes and the residues involved in the interaction between the toxin and the drug compounds resulted in negative binding energy value (Table 2-3). The binding affinity of the complexes was evaluated by its binding energy values which inferred that the complexes were in favorable poses and stable conformations. Binding poses with the lowest docked energy belonging to the top-ranked cluster was selected as the final model for post-docking analysis with AutoDock Tools and PyMOL (Suvannang *et al.*, 2011). Results obtained from AutoDock provided pertinent information on the binding orientation of ligand-receptor interactions.

The compound Acacetin interacted with the protein receptor by forming 6 hydrogen bond interactions at amino acid positions GLY52, THR134, PRO207, SER87, 88 and 90. The binding energy value for making the complex stable was -6.06 kcal moL^{-1}. Apigenin showed 5 hydrogen bond interactions with 2QK7 as of Acacetin except PRO and predicted binding energy was -5.76 kcal moL^{-1}. The target protein and Baicalein made complex by interacting with aminoacids such as GLY52, ASN206, PRO207, SER208 and SER87 by giving binding energy -6.0 kcal moL^{-1}. Catechin which interacted with protein receptor at GLY52, THR134, ASN206, GLN89 and SER90 to form interactions showed binding energy value of -5.49 kcal moL^{-1}. An efficient docking was seen with Dihydroquercetin-2QK7 complex with binding energy value of -6.11 kcal moL^{-1}. It formed eight hydrogen bond interactions with residues GLN51, SER136, PRO207, SER208, 87, 89 and 90 and LYS160.

The toxin and Galangin compound were in contact with LYS49, THR134, SER136, 208 and 90 residues by giving binding energy value of -5.66 kcal moL^{-1}. Kaempherol showed 5 hydrogen bond interactions with the protein receptor whose residues involved in bonding were THR134,

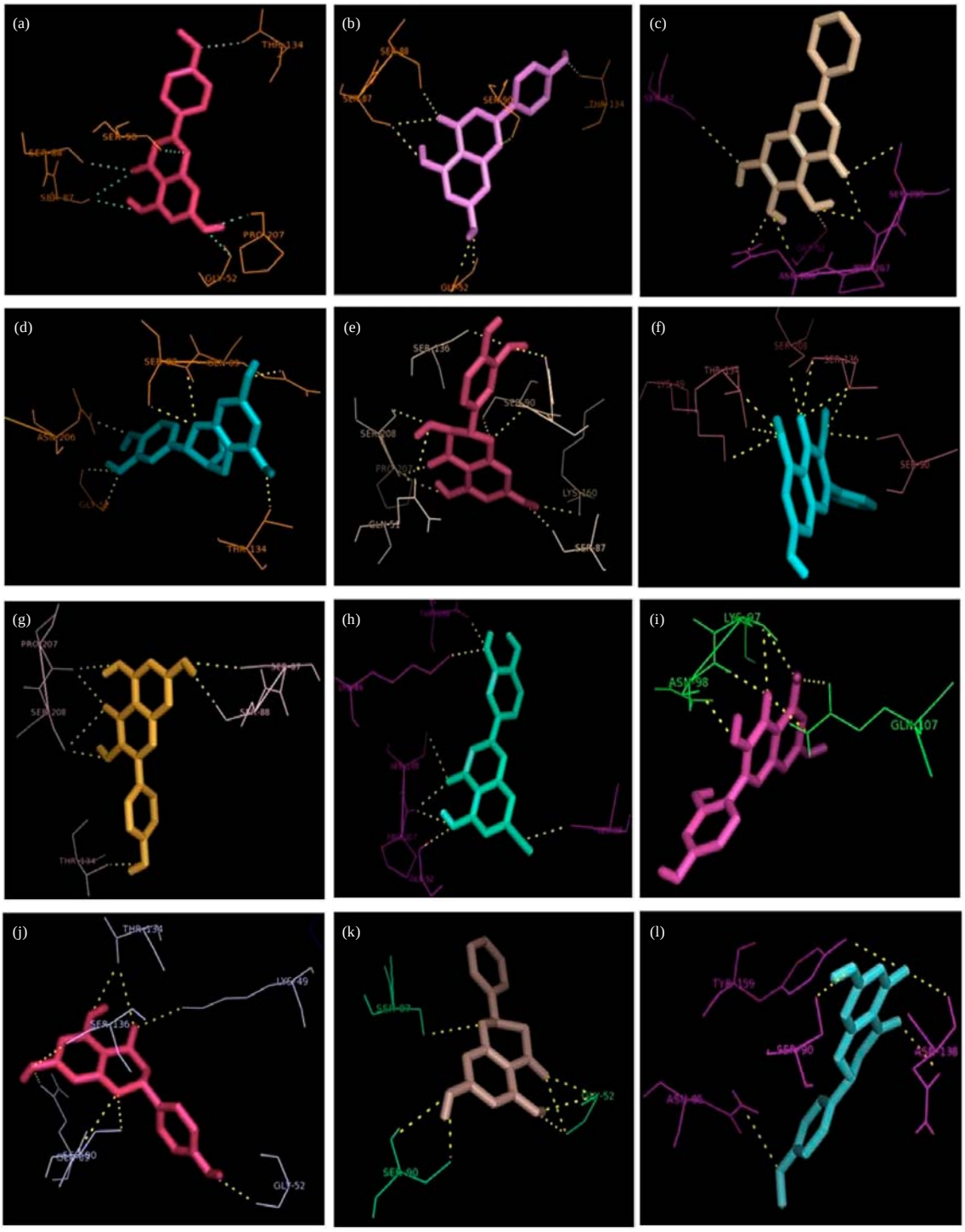

Fig. 4(a-l): Binding modes of docked complexes viewed by PyMol (a) Acacetin, (b) Apigenin, (c) Baicalein, (d) Catechin, (e) Dihydroquercetin, (f) Galangin, (g) Kaempherol, (h) Luteolin, (i) Morin, (j) Naringenin, (k) Pinocembrin and (l) Ponciretin

Table 2: Binding energies and RMSD values of docked complexes

Compounds	Binding energy (kcal moL^{-1})	Ref RMSD
Acacetin	-6.06	44.63
Apigenin	-5.76	44.69
Baicalein	-6.00	49.02
Catechin	-5.49	48.36
Dihydroquercetin	-6.11	48.10
Galangin	-5.66	49.34
Kaempherol	-5.85	44.47
Luteolin	-6.14	44.29
Morin	-5.30	44.83
Naringenin	-6.21	49.37
Pinocembrin	-5.63	49.51
Ponciretin	-6.49	55.31
Quercetin	-5.55	49.37
Robinetin	-5.02	60.02

Table 3: Interacting residues involved in forming protein-ligand complexes

Flavonoids	Residues involved in H bond interactions
Acacetin	GLY52, THR134, PRO207, SER87, SER88, SER 90
Apigenin	GLY52, THR134, SER87, SER88, SER90
Baicalein	GLY52, ASN206, PRO207, SER208, SER87
Catechin	GLY52, THR134, ASN206, GLN89, SER90
Dihydroquercetin	GLN51, SER136, PRO207, SER208, SER87, SER89, SER90, LYS160
Galangin	LYS49, THR134, SER136, SER208, SER90
Kaempherol	THR134, PRO207, SER208, SER87, SER 88
Luteolin	LYS49, GLY52, THR134, PRO207, SER208, SER87
Morin	LYS97, ASN98, GLN107
Naringenin	LYS49, GLY52, THR134, SER136, GLN89, SER90
Pinocembrin	GLY52, SER87 and 90
Ponciretin	ASN138, SER90, ASN95, TYR159
Quercetin	LYS81, SER85, ARG150, THR152, SER154, ARG155
Robinetin	SER55 and 56, ARG57, THR58, ARG188, ASP194, GLN202, SER203, THR157

PRO207 and SER208, 87 and 88. Docking analysis for the compound Luteolin with the toxin revealed 6 interactions with LYS49, GLY52, THR134, PRO207, SER208 and 87 and the complex was stabilized by the binding energy value of -6.14 kcal moL^{-1}. Morin compound showed 3 hydrogen bond interactions with LYS97, ASN98 and GLN107 with binding energy value of -5.30 kcal moL^{-1}. The Naringenin compound when complexed with 2QK7 showed binding energy value of -6.21 kcal moL^{-1} with 6 hydrogen bond interactions at LYS49, GLY52, THR134, SER136, GLN89 and SER90. Aminoacid residues such as GLY52, SER87 and 90 were involved in forming interactions in the Pinocembrin-2QK7 complex. The complex used -5.63 kcal moL^{-1} energy for binding. Binding energy value of -6.49 kcal moL^{-1} was required to form complex for 2QK7 and Ponciretin. The amino acids involved in interactions were ASN95, SER90, TYR159 and ASN138. Quercetin compound which was known to be beneficial having anti-inflammatory properties showed binding energy value of -5.55 kcal moL^{-1} which was lesser than the other compounds. It had interactions with the receptor at LYS81, SER85, ARG150, THR152, SER154 and ARG15. Robinetin showed hydrogen bond interactions with SER55 and 56, ARG57, THR58, ARG188,

ASP194, GLN202, SER203 and THR157 aminoacids. The interaction between the target protein and various ligands was different which might be due to differences in the conformational changes of the protein when it was bound to the drugs after docking.

The low energy values of these compounds with the gamma hemolysin toxin predicted by Autodock 4.0, indicated that the ligands were in most favorable regions of the protein and that the protein had more affinity towards the particular compounds. Thus, all the above studied compounds were potential inhibitors of γ-hemolysin toxin. Molecular docking of a target protein involves finding the best orientations or conformations of a protein at possible binding site of the receptor, evaluating its energy score and ranking according to their scores (Brooijmans and Kuntz, 2003; Halperin *et al*., 2002).

Mechanisms by which a protein receptor recognizes its ligand and interacts with those molecular substrates are of much importance in docking. Binding and interaction of protein and ligand enables prediction and ranking of substrates (Sousa *et al*., 2006). The protein-ligand interaction was compared to the lock-and-key mechanism by considering the lock as the protein and the key as the ligand. Hydrogen bonding interactions plays an important role in binding (Kubinyi, 1998). From the docking analysis, it was concluded that the aminoacid residue Ser90 to be important as it was seen in the binding site and interacting with most of the ligands. The potential inhibitor was found as Ponciretin compound as it showed the best binding energy value as -6.49 kcal moL^{-1} when docked with the target receptor.

CONCLUSION

Gamma-hemolysin toxin of *Staphylococcus aureus* plays an important role in damaging cell membrane in host cells by the formation of pores. Flavonoid compounds which are known to be potent anti-microbial and anti-inflammatory drugs were used for docking with the target protein. The analysis of the docked complexes revealed the molecular interactions between them. The binding pattern of the amino acid residues participating in the interaction were identified and validated by the site of interaction and predicted the interaction energies of the docked complexes. Applying Lipinski's rule, fourteen flavonoid compounds were validated as valid drugs and docked with the toxin which showed good negative binding energy values. A more negative binding energy value could form stable complex with the receptor and it was seen in Ponciretin compound which was -6.49 kcal moL^{-1}. The lesser binding energy value inferred that the compound had higher affinity towards the γ-hemolysin toxin and the binding was in stable conformation and thus may inhibit the hemolytic and cytolytic action of the toxin.

REFERENCES

Bohach, G.A., M.M. Dinges, D.T. Mitchell, D.H. Ohlendorf and P.M. Schlievert, 1997. Exotoxins. In: The Staphylococci in Human Disease. Crossley, K.B. and G.L. Archer (Eds.). Churchill Livingstone, New York, pp: 93-111.

Brooijmans, N. and I.D. Kuntz, 2003. Molecular recognition and docking algorithms. Annu. Rev. Biophys. Biomol. Struct., 32: 335-373.

Cooney, J., Z. Kienle, T.J. Foster and P.W. O'Toole, 1993. The gamma-hemolysin locus of *Staphylococcus aureus* comprises three genes for the F and S components of leukocidin. Infect. Immun., 61: 768-771.

Grange, J.M. and R.W. Davey, 1990. Antibacterial properties of propolis (bee glue). J. R. Soc. Med., 83: 159-160.

Halperin, I., B. Ma, H. Wolfson and R. Nussinov, 2002. Principles of docking: An overview of search algorithms and a guide to scoring functions. Proteins: Struct. Funct. Genet., 47: 409-443.

Havsteen, B., 1983. Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol., 32: 1141-1148.

Julianelle, J.L., 1922. Studies of hemolytic staphylococci: Hemolytic activity-biochemical reactions-serologic reactions. J. Infect. Dis., 31: 256-284.

Kubinyi, H., 1998. Structure-based design of enzyme inhibitors and receptor ligands. Curr. Opin. Drug Discov. Dev., 1: 4-15.

Lipinski, C.A., F. Lombardo, B.W. Dominy and P.J. Feeney, 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev., 46: 3-26.

Middleton, E. and K. Chithan, 1993. The Impact of Plant Flavonoids on Mammalian Biology: Implications for Immunity Infammation and Cancer. In: The Flavonoids: Advances in Research Since 1986, Harborne, J.B. (Ed.). Chapman and Hall, London, UK., pp: 619-652.

Morris, G.M., D.S. Goodsell, R.S. Halliday , R. Huey, W.E. Hart, R.K. Belew and A.J. Olson, 1998. Automated docking using a Lamarkian genetic algorithm and an empirical binding free energy function. J. Comput. Chem., 19: 1639-1662.

Nilsson, I.M., O. Hartford, T. Foster and A. Tarkowski, 1999. Alpha-toxin and gamma-toxin jointly promote *Staphylococcus aureus* virulence in murine septic arthritis. Infect. Immun., 67: 1045-1049.

Panton, P.N. and F.C.O. Valentine, 1932. Staphylococcal toxin. Lancet, 219: 506-508.

Prevost, G., B. Cribier, P. Couppie, P. Petiau and G. Supersac *et al.*, 1995. Panton-Valentine leucocidin and gamma-hemolysin from *Staphylococcus aureus* ATTC 49775 are encoded by distinct biological activities. Infect. Immun., 63: 4121-4129.

Prevost, G., D.A. Colin, L. Staali, L.B. Moussa and A. Gravet *et al.*, 1998. Pore-forming leukotoxins from *Staphylococcus aureus*: Variability of the target cells and 2 pharmacological processes. Pathol. Biol., 46: 435-441.

Prevost, G., L. Mourey, D.A. Colin, H. Monteil, M. Dalla and G. Menestrina, 2005. A-Toxin and β-Barrel Pore-Forming Toxins (Leucocidins, α-γ-and o-Cytolysins) of *Staphylococcus aureus*. In: The Comprehensive Source Book of Bacterial Protein Toxins, Alouf, J.E. and M.R. Popoff (Eds.). Academic Press, London, pp: 588-605.

Sousa, S.F., P.A. Fernandes and M.J. Ramos, 2006. Protein-ligand docking: Current status and future challenges. Proteins, 65: 15-26.

Supersac, G., Y. Piemont, M. Kubina, G. Prevost and T.J. Foster, 1998. Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of *Staphylococcus aureus*. Microb. Pathog., 24: 241-251.

Suvannang, N., C. Nantasenamat, C. Isarankura-Na-Ayudhya and V. Prachayasittikul, 2011. Molecular docking of aromatase inhibitors. Molecules, 16: 3597-3617.

Tilley, S.J. and H.R. Saibil, 2006. The mechanism of pore formation by bacterial toxins. Curr. Opin. Struct. Biol., 16: 230-236.

Vandenesch, F., G. Lina and T. Henry, 2012. *Staphylococcus aureus* hemolysins bi-component leukocidins and cytolytic peptides: A redundant arsenal of membrane-damaging virulence factors? Front Cell. Infect. Microbiol., 2: 1-12.