Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (16):40-51
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Analysis of the binding and interaction patterns of 100 flavonoids with the Pneumococcal virulent protein pneumolysin: An *in silico* virtual screening approach

Udhaya Lavinya B., Manisha P., Sangeetha N., Premkumar N., Asha Devi S., Gunaseelan D. and Sabina E. P.*

[1]*School of Biosciences and Technology, VIT University, Vellore - 632014, Tamilnadu, India*
[2]*Department of Computer Science, College of Computer Science & Information Systems, JAZAN University, JAZAN-82822-6694, Kingdom of Saudi Arabia.*

ABSTRACT

Pneumococcal infection is one of the major causes of morbidity and mortality among children below 2 years of age in under-developed countries. Current study involves the screening and identification of potent inhibitors of the pneumococcal virulence factor pneumolysin. About 100 flavonoids were chosen from scientific literature and docked with pnuemolysin (PDB Id.: 4QQA) using Patch Dockprogram for molecular docking. The results obtained were analysed and the docked structures visualized using LigPlus software. It was found that flavonoids amurensin, diosmin, robinin, rutin, sophoroflavonoloside, spiraeoside and icariin had hydrogen bond interactions with the receptor protein pneumolysin (4QQA). Among others, robinin had the highest score (7710) revealing that it had the best geometrical fit to the receptor molecule forming 12 hydrogen bonds ranging from 0.8-3.3 Å.

Keywords: Pneumococci, pneumolysin, flavonoids, antimicrobial, virtual screening

---

INTRODUCTION

Streptococcus pneumoniae is a gram positive pathogenic bacterium causing opportunistic infections that may be life-threating[1]. Pneumococcus is the causative agent of pneumonia and is the most common agent causing meningitis. Apart from these two major types of infections, the organism is also known to cause sinusitis (acute), rhinitis, bronchitis, otitis media, bacteraemia, septicaemia, peritonitis, pericarditis and endocarditis[2].Severe infections with pneumococcus may lead to brain damage and death[3]. The bacteria colonize the nasopharynx and upper respiratory tract of the host. Several virulence factors present on the surface of pneumococci contribute to the pathogenic processes involved in disease establishment[4].These factors in addition to rendering protection to the bacteria, also interact with host tissues[5]. Some of the important virulence factor of pneumococci include pneumolysin, autolysin, neuraminidases A and B, choline binding protein A and pneumococcal surface protein A[6]. These virulence factors are potential vaccine targets.

The virulence factor pneumolysin (PLY)is an intracellular protein which exerts its effect when released to surroundings and PLY is toxic to a range of cells and induction of cytokine production[7].

Flavonoids are a group of polyphenolic compounds which are widely present throughout the plant kingdom [8]. They are more commonly found in fruits, vegetables, nuts, seeds, stems, flower, tea, wine, propolis and honey [9]. Bioflavonoids are found in bark, sapwood, heartwood and parts of plants [10–12].

Flavonoids are classified as flavonols, flavonones, flavan 3-ols and isoflavones according to the position present on the parent molecule[13]. They are known to possess various pharmacological effects such as anti-oxidative,anti-inflammatory,anti-coagulation,anti-allergic,cytotoxicity[14–17].They are known to reduce the risk of heart disease or cancer[18]. Several enzymes such as prostaglandin synthase, hypoxygenase, cyclooxygenase and detoxifying enzyme glutathione s-transferase are inhibited by flavonoids [19]. Consumption of flavonoids has effect in cardiovascular disease and cancer [20]. They include anti-oxidant actions, central nervous system effects and have protective effect against cognitive decline, cancer and metabolic disease [21,22].

Quercetin, the most common flavonoid seen in human diet has a role in exerting potent free radical scavenging and cause vasodilation (low blood pressure) in isolated vascular preparations, to detect (anti-diabetics) effects in experimental diabetes [23].In anti-microbial activity of flavonoids, the sophoroflavone G and (-)-epicatechingallate inhibit cytoplasmic membrane function and licochalcones A and C inhibit energy metabolism [24]. Flavonoids inhibit or kill many bacterial strains also restrict the viral enzymes such as protease, reverse transcriptase and they destroy some pathogenic pathogen [25].

MATERIALS AND METHODS

Preparation of flavonoid compounds for docking:
About 100 flavonoids with significant antimicrobial and antioxidant activities were selected from scientific literature. The chemical and structural information of the chosen flavonoids were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The structure of each compound in canonical SMILES format was taken from PubChem and the same was used to generate the 2D structure of the respective flavonoid on Corina molecular network (https://www.mn-am.com/online_demos/corina_demo). The obtained structures were used as ligands in the docking experiments.

Preparation of pneumolysin protein for docking:
The 3D structure of pneumolysin (PDB Id.: 4QQA) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein databank(PDB) (http://www.rcsb.org/pdb/explore.do?structureId=4QQA).

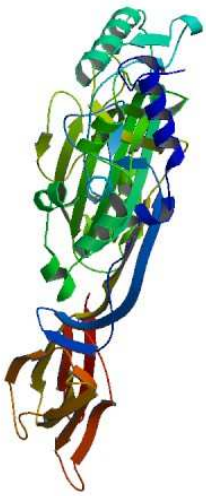

Fig 1.Structure of pneumolysin from Streptococcus pneumonia (PDB Id. 4QQA)

Determination of the active site of 4QQA:
The active site residues of 4QQA were identified by using 3DLiganSite, an online tool for the prediction of ligand binding sites. The pdb file of 4QQA was submitted on 3DLigandSite (http://www.sbg.bio.ic.ac.uk/3dligandsite/). The results were obtained through the link provided through the user's e-mail.

Docking and Visualization of flavonoids with 4QQA:
Docking of each flavonoid with 4QQA was carried out on PatchDock molecular server (http://bioinfo3d.cs.tau.ac.il/PatchDock/). The interacting amino acid residues of each docked complex were analysed using LigPlus software. Docking scores, area and atomic contact energy (ACE) were noted for each experiment.

RESULTS AND DISCUSSION

Predicted binding site of 4QQA had the following residues: TYR371, ALA373, GLN374, ILE425, ARG426, GLU427, VAL439, ILE455 (Figure 2). Table 1 shows the score, atomic contact energy (ACE), area and the number of hydrogen bonds for each flavonoid.

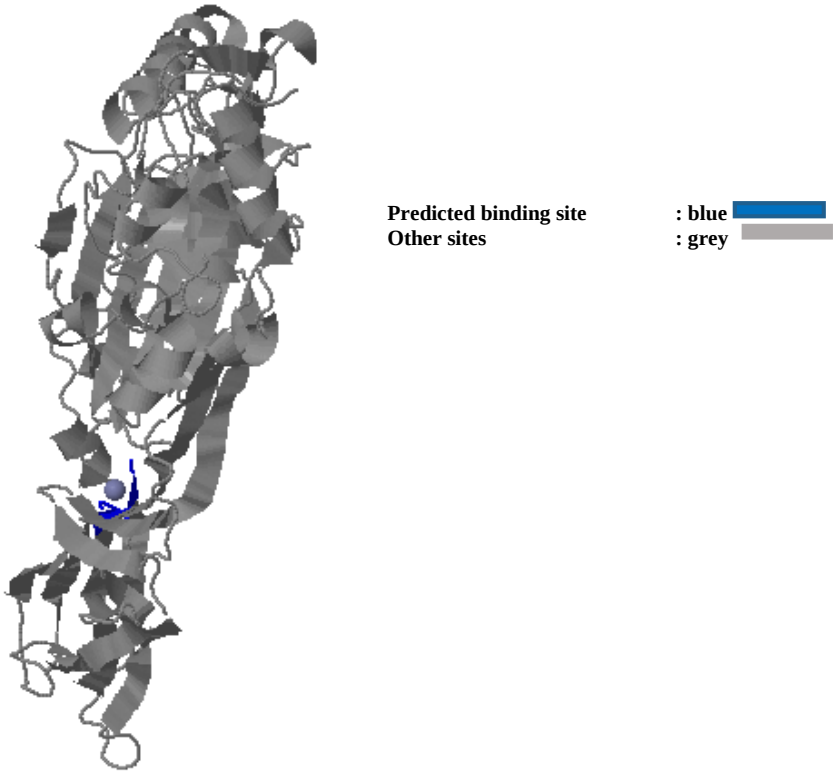

Fig 2.Predicted binding site of 4QQA: TYR 371, ALA 373, GLN 374, ILE 425, ARG 426, GLU 427, VAL 439, ILE 455

Table 1: Docking results of 100 flavonoids with pneumolysin (4QQA)

Flavonoids name	Score	Area	Ace	Bonds
7,8-dihydroxyflavonehydrate	4384	504.9	-216.58	2
7-hydroxyflavone	3886	456	-170.75	3
Afzelin	5110	576.2	-175.94	7
Allicin	3032	360.3	-183.61	2
Amuresin	5806	734.2	-211.06	11
Apigenin	4188	485.1	-186.59	6
Apigertin	5338	658.6	-221.57	6
Astragalin	5044	589.8	-91.32	6
Azalein	5184	608.8	-157.38	6
Bacopaside 1	6690	769.6	-40.89	7
Bacopaside 2	7016	933.7	-26.62	7
Bacopaside X	6694	971.2	-264.05	5
Baicalein	4218	492.6	-194.59	3
Broussonal A	5538	681	-270.66	3
Broussonal E	5752	720.5	-237.65	5
Canthaxanthin	6542	809.2	-186.57	0
Chrysin	4094	474	-195.25	2
Cianidanol	4324	498.4	-189.3	2
Curcumin	4802	548	-66.17	1
Cyanidin chloride	4756	550.6	-210.49	4
Delphindin	4368	492.2	-242.84	2
Diadzein	4056	476.4	-206.81	0
Dihydrokampferol	4354	487.5	-205.68	3
Diosmetin	4778	522.1	-198.81	2
Diosmin	6172	742.5	-102.66	10
Dl-catechin	4324	498.4	-189.3	2
Dorsilurin h	6224	815	-175.18	2
Dorsilurin k	6350	746.5	-195.75	2

Epicatechingallate (ECG)	5268	622.6	-220.12	4
Epigallocatechingallate (EGCG)	5480	667.9	-203.09	2
Eriodictyol	4152	484	-213.25	0
Ermanin	4536	514.8	-173.57	2
Fistein	4278	489.4	-181.2	3
Flavoxate	5530	668	-103.78	1
Galangin	4084	482.7	-228.64	3
Gallocatechin	4494	516	-206.39	3
Gallocatechin 3 gallate	5480	667.9	-203.09	2
Genistein	4494	516	-206.39	3
Genistin	5550	694.7	-233.21	7
Genkwanin	4482	530.5	-173.99	2
Gingerol	4844	583.5	-173.68	2
Glabrene	4806	582	-195.66	2
Glycitein	4504	517.4	-180.85	5
Gossypetin	4188	485.6	-224.32	7
Hagnin a	4572	557.7	-245.9	0
Hagnin d	4088	485.1	-233.27	4
Hagnin e	3958	468.9	-235.49	1
Hesperetin	4556	516.1	-203.29	1
Homoeriodictyol	4358	499	-226.57	2
Hyperoside	4908	614	-240.75	6
Icariin	6322	768.2	-128	8
Isoluteolin	4188	471.3	-185.39	1
Isoquercetin	4908	614	-240.75	6
Isorhamnetin	4450	535.1	-213.48	4
Kaempferide 3 glucoside	5104	586.6	-91.54	3
Kaempferol	4334	492.7	-222.87	5
Laxifloranone	5772	828	-220.44	2
Licochalcone a	4918	627.5	-175.96	1
Liquiritin	5832	674.6	-182.95	1
Lonchocarpan	4496	542.3	-119.53	1
Lutein	6682	995.9	-346.87	0
Luteolin	4292	486.7	-207.11	4
Luteolin-7-o-glucoside	5282	651	-188.38	5
Malvidin	4242	490.9	-142.41	1
Morin	4402	517.6	-217.64	6
Myricetin	4482	515.5	-210.4	4
Myricitrin	5038	609.5	-186.21	6
Narcissoside	5238	745	-152.71	5
Naringenin	4070	461.9	-186.95	2
Natsudaidain	5224	656.6	-232.41	4
Nicotiflorin	5528	708.5	-73.01	6
Pachypodol	4746	589.6	-256.69	4
Pelargonidin	4242	477.5	-244.59	3
Peonidin	4424	518.4	-213.09	3
Petunidin chloride	4708	577.5	-225.06	2
Pinocembrin	4204	504.6	-223.5	3
Ponciretin	4462	514.4	-208.48	1
Proanthocyanidens	5774	793.6	-188.28	4
Pruning	5874	682	-170.33	2
Quercetiin	4240	494.3	-196.08	4
Retusin	5308	612.4	-214.99	1
Rhamnazin	4968	572.4	-216.57	6
Rhamnetin	4560	541.1	-225.71	4
Robinetin	4334	498.9	-251.43	4
Robinin	7710	909.1	-184.69	12
Santin	4736	580.9	-195.17	4
Scutellarein	4192	486.8	-229.26	3
Senegalensin	5262	676.7	-230.02	3
Silibinin	5884	699.1	-163.89	6
Sophoraflavone b	5282	654.9	-186.14	7
Sophoroflavonoloside	6036	719.8	-208.76	8
Spiraeoside	5252	639.6	-186.46	9
Tangeretin	4900	646.2	-180.53	2
Taxifolin	4194	503.1	-265.14	6
Theaflavin	5120	620.9	-10.12	7
Theaflavin-3'-o-gallate	6514	800.4	-138.51	5
Trifolin	5044	589.8	-91.32	6
Troxerutin	6358	768.4	-18.99	5
Wogonin	3904	502.3	-247.39	3

The results obtained on carrying out docking experiments show that the flavonoids robinin, amurensin, diosmin, rutin, spiraeoside, sophoroflavonolosideandicariin had 12, 11, 10, 9, 9, 8 and 8 hydrogen bond interactions respectively with the receptor protein pneumolysin (4QQA) (Figure 3-9). Among others, robinin had the highest score (7710) revealing that it had the best geometrical fit to the receptor molecule forming 12 hydrogen bonds ranging from 0.8-3.3 Å.

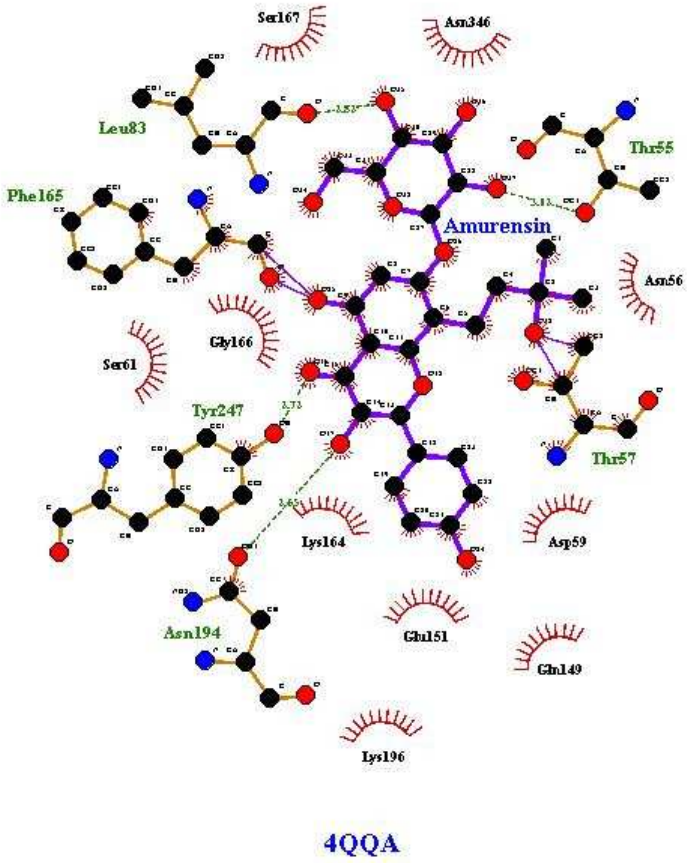

Fig 3. 4QQA-amurensin docked complex

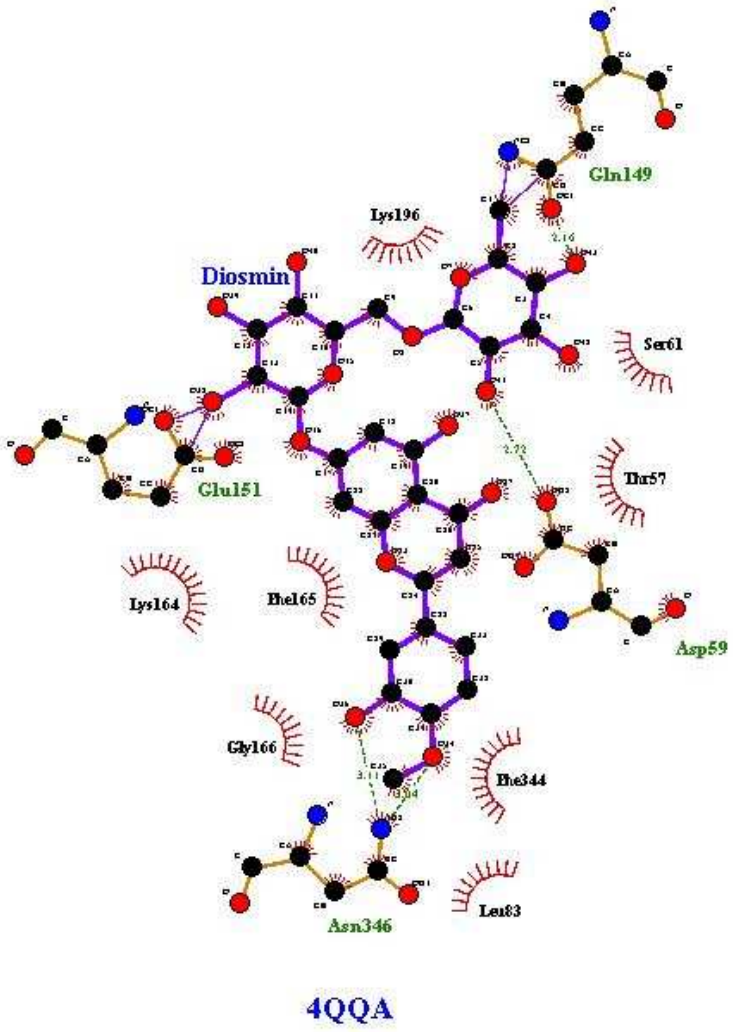

Fig 4. 4QQA-diosmin docked complex

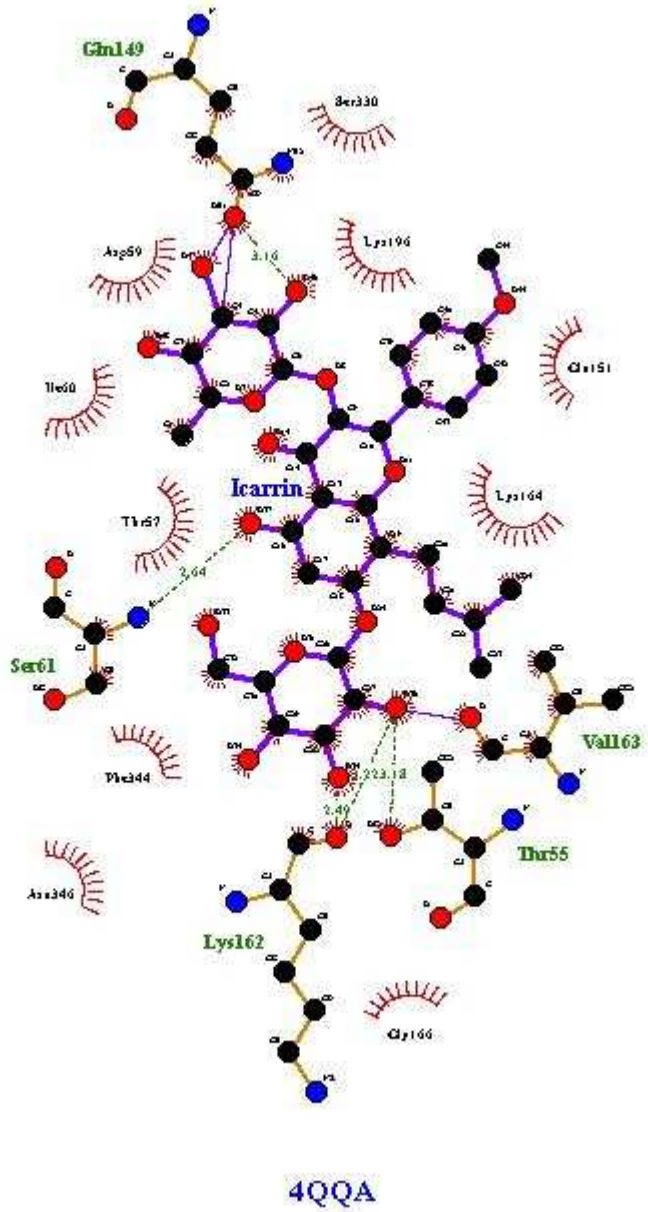

Fig 5. 4QQA-icariin docked complex

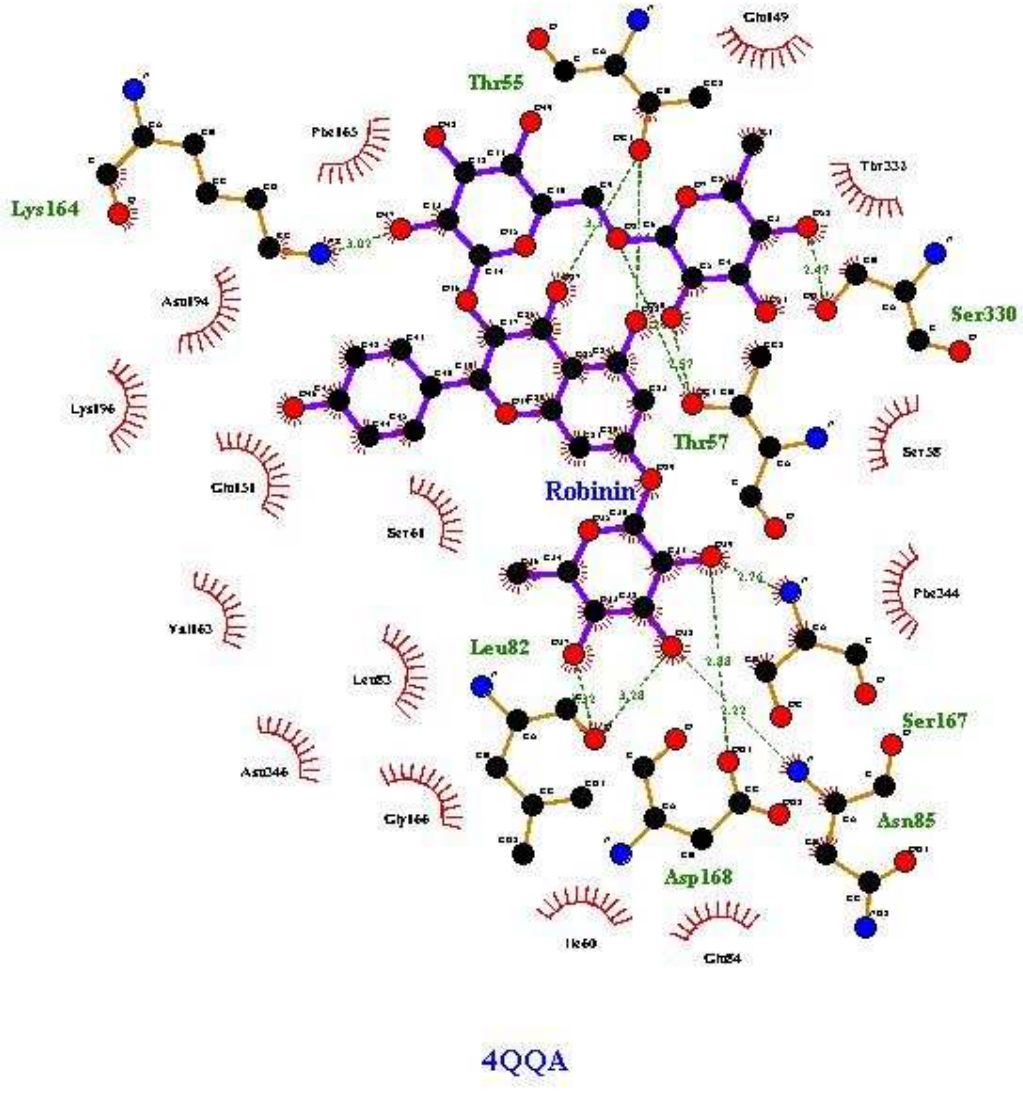

Fig 6. 4QQA-robinin docked complex

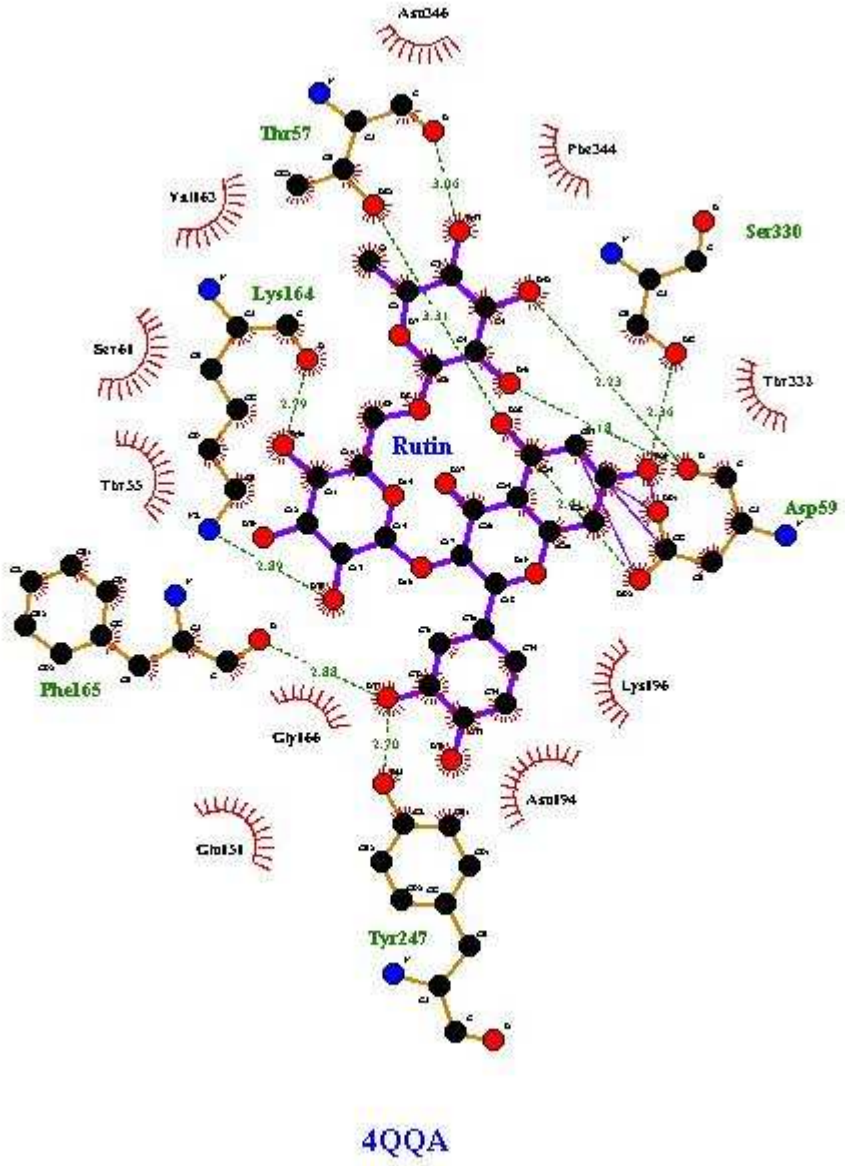

Fig 7. 4QQA-rutin docked complex

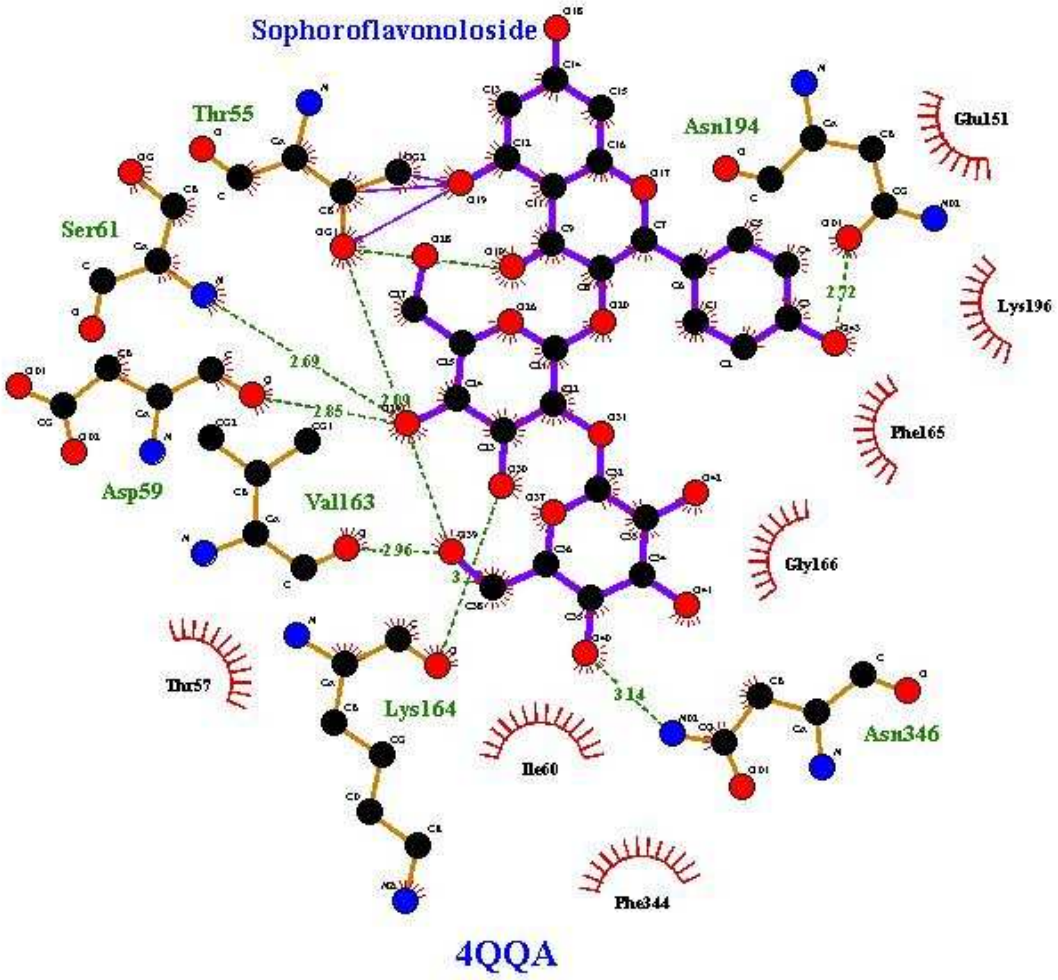

Fig 8. 4QQA-sophoroflavonoloside docked complex

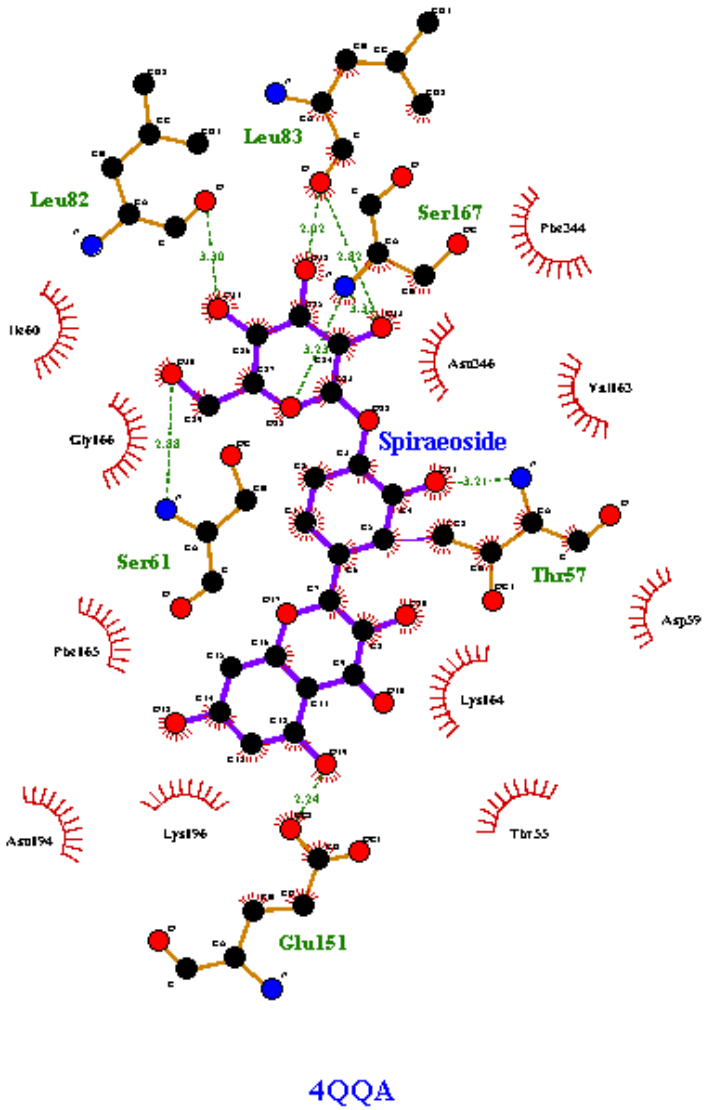

Fig 9. 4QQA-spiraeoside docked complex

Flavonoids are potential antimicrobial agents. Recently, there has been increased resistance to antibiotics. The emergence of multi-drug resistant bacterial strains has brought about the need to explore the efficacy of flavonoids in preventing bacterial infections or protecting against the same [26]. The identification of such potential agents involves the screening of their enzyme inhibiting potential or ability to bind to bacterial virulent factors such as pneumolysin. The results of current study reveal that amurensin, diosmin, robinin, rutin, sophoroflavonoloside, spiraeoside, icariin show significant interaction with the receptor protein pneumolysin (4QQA). These flavonoids are known to possess significant beneficial properties including antimicrobial activity [27–29].Studies have been carried out to evaluate the antimicrobial activity of nanoparticles synthesised using flavonoids such as diosmin, naringinin and hesperidin [30]. Amurensin has also shown significant anti-tumor property[31].

CONCLUSION

Identification of potent pneumolysin binding flavonoids would prevent the adhesion of Pneumococci to human airway cells thereby minimizing the chance of Pneumococcal infection. In addition, the effective binding of flavonoids might reduce the extent of cytotoxicity, complement activity and other harmful effects of pneumolysin. Current shows that robinin has highest binding affinity to pneumolysin among the 100 chosen flavonoids. Appropriate *invitro* studies would analyse the antimicrobial effect of robinin against Pneumoccocal strains.

Acknowledgement
The authors are thankful to VIT University for providing the opportunity to carry out this research work.

REFERENCES

[1] R.F. Schneider and M.J. Rosen, *Semin Respir Infect*, **1999**, 14, 237–242.
[2] R.A. Siemieniuk, D.B. Gregson and M.J. Gill, *BMC Infectious Diseases*, **2011**, 11, 314.
[3] B.B. Mook-Kanamori, M. Geldhoff, T. van der Poll and D. van de Beek, *Clin. Microbiol. Rev.*, **2011**, 24, 557–591.
[4] A. Kadioglu, J.N. Weiser, J.C. Paton and P.W. Andrew, *Nat Rev Micro*, **2008**, 6, 288–301.
[5] C.J. Orihuela, G. Gao, K.P. Francis, J. Yu and E.I. Tuomanen, *J Infect Dis.*, **2004**, 190, 1661–1669.
[6] M.J. Jedrzejas, *Microbiol. Mol. Biol. Rev.*, **2001**, 65, 187–207.
[7] R.A. HIRST, A. KADIOGLU, C. O'CALLAGHAN and P.W. ANDREW, *Clin Exp Immunol*, **2004**, 138, 195–201.
[8] N.K. Raj, R.M. Sripal, M.R. Chaluvadi and D.R. Krishna, *Indian Journal of Pharmacology*, **2001**, 33, 2.
[9] T.P.T. Cushnie and A.J. Lamb, *Int. J. Antimicrob. Agents*, **2011**, 38, 99–107.
[10] J. McNulty, J.J. Nair, E. Bollareddy, K. Keskar, A. Thorat, D.J. Crankshaw, A.C. Holloway, G. Khan, G.D. Wright and L. Ejim, *Phytochemistry*, **2009**, 70, 2040–2046.
[11] N. Kavian-Jahromi, L. Schagerl, B. Dürschmied, S. Enzinger, C. Schnabl, T. Schnabel and A. Petutschnigg, *European Journal of Wood and Wood Products*, **2015**, 73, 841–844.
[12] T. Tanaka, M. Iinuma, K. Yuki, Y. Fujii and M. Mizuno, *Phytochemistry*, **1992**, 31, 993–998.
[13] R. Tsao, *Nutrients*, **2010**, 2, 1231–1246.
[14] H.A. Guglielmone, A.M. Agnese, S.C. Núñez Montoya and J.L. Cabrera, *Thromb. Res.*, **2002**, 105, 183–188.
[15] K. Sak, *Pharmacogn Rev*, **2014**, 8, 122–146.
[16] M. Serafini, I. Peluso and A. Raguzzini, *Proc Nutr Soc*, **2010**, 69, 273–278.
[17] A. Sato and H. Tamura, *Fitoterapia*, **2015**, 102, 74–83.
[18] Hertog ML, Kromhout D, Aravanis C and et al, *Arch Intern Med*, **1995**, 155, 381–386.
[19] I.E. Orhan and M.T.H. Khan, *Curr Top Med Chem*, **2014**, 14, 1486–1493.
[20] H.D. Sesso, J.M. Gaziano, S. Liu and J.E. Buring, *Am J Clin Nutr*, **2003**, 77, 1400–1408.
[21] S.-L. Hwang, P.-H. Shih and G.-C. Yen, *J. Agric. Food Chem.*, **2012**, 60, 877–885.
[22] A. Vijayalakshmi, P.R. Kumar, S. Sakthi Priyadarsini and C. Meenaxshi, *Journal of Chemistry*, **2013**, 2013, e150675.
[23] A. Machha and M.R. Mustafa, *J. Cardiovasc. Pharmacol.*, **2005**, 46, 36–40.
[24] T.P.T. Cushnie and A.J. Lamb, *Int. J. Antimicrob. Agents*, **2005**, 26, 343–356.
[25] B.H. Havsteen, *Pharmacol. Ther.*, **2002**, 96, 67–202.
[26] G.G.F. Nascimento, J. Locatelli, P.C. Freitas and G.L. Silva, *Brazilian Journal of Microbiology*, **2000**, 31, 247–256.
[27] T. Zhang, X. Wei, Z. Miao, H. Hassan, Y. Song and M. Fan, *Chem Cent J*, **2016**, 10, 47.
[28] T. Coenye, G. Brackman, P. Rigole, E. De Witte, K. Honraet, B. Rossel and H.J. Nelis, *Phytomedicine*, **2012**, 19, 409–412.
[29] H. Arima, H. Ashida and G. Danno, *Biosci. Biotechnol. Biochem.*, **2002**, 66, 1009–1014.
[30] N. Sahu, D. Soni, B. Chandrashekhar, D.B. Satpute, S. Saravanadevi, B.K. Sarangi and R.A. Pandey, *Int Nano Lett*, **2016**, 6, 173–181.
[31] S.-H. Lee, M.-J. Kim, D.-W. Kim, C.-D. Kang and S.-H. Kim, *Cancer Sci*, **2013**, 104, 1632–1639.