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MOLECULAR DOCKING STUDIES OF OMP6 PROTEIN OF *HAEMOPHILUS INFLUENZAE* WITH PHYTOLIGANDS

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ABSTRACT

Haemophilus influenzae is an important human pathogen affecting infants and children often infecting ear and eye causing otitis media, conjunctivitis, sinus infections, pneumonia, epiglottitis and meningitis. OMP6 (Outer membrane protein 6) protein of the organism is an important antigen involved in adhesion with Toll-like receptor 2 of the host. In this study, we downloaded the 3D structure of OMP6 protein from PDB database with PDB code 2AIZ and the structure was refined by submitting to KOBA^{MIN} server. Forty different phytoligands were identified from Pubchem compound database and the corresponding PDB files were drawn from CORINA by submitting SMILES of the compounds into the tool. Docking analysis was performed for the target protein with those ligands to study the binding interactions between them using Patchdock server. The analysis revealed that the compounds Silibinin and Silimarin with highest patchdock score of 4298 could be a potential drug lead to treat OMP6 virulence.

KEYWORDS: ***Haemophilus influenzae*, OMP6 protein, molecular docking, phytoligands, PyMol.**

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INTRODUCTION

Haemophilus influenza is a gram-negative bacterium which is found as normal nasopharyngeal flora of most humans. It is a pleomorphic bacillus that is associated with localized and invasive infections such as bronchitis, otitis, pneumonia, meningitis, septicemia, and epiglottitis [1, 2, 3, 4, 5]. The organism is found in two species - *H. influenza* type B and NTHi (Non-Typeable *Haemophilus influenzae*) both of which are virulent. The organism contains upto 36 proteins in the outer membrane of which P6 is one of the proteins present in major content [6]. P6 is a 28KDa protein, a peptidoglycan-associated lipoprotein [7]. It is a surface-exposed protein and an important antigen of the organism and thus is a potential vaccine candidate[8]. The OMP6 protein of NTHi has its ability to bind to human Toll-like receptor (TLR2) and promote virulence by inducing NF-kB [9]. Plants are the traditional source of many chemicals such as secondary metabolites that are used as pharmaceuticals [10]. The secondary metabolites compounds are the most beneficial to mankind which are polyphenolic in nature called flavonoids. The flavonoids include flavones, flavonols, flavanones, chalones, xanthones, isoflavones and biflavones. These naturally occurring compounds act as anti-oxidants and free radical scavengers and are also anti-inflammatory, anti-allergic, anti-spasmodic and anti-microbial [11]. Some flavonoids show inhibitory activity against many bacteria and viruses. Xu and Lee tested 38 flavonoids against MRSA, Vancomycin Resistant *Enterococci* (VRE) and also some antibiotic resistant Gram negative bacteria. They found that myricetin was active against VRE, *K. pneumoniae, Burkholderia cepacia* and *Ps. aeruginosa* and also that only aglycones of flavonols and flavones inhibited the growth of MRSA [12]. Ali *et al* reported the inhibitory activity of chrysin against *E. coli* and *Ps. Aeruginosa* [13].

MATERIALS AND METHODS

Protein structure download

The three-dimensional structure of the protein was downloaded from the Protein DataBank database available at http://www.rcsb.org/ using the ID 2AIZ. The protein structure was refined by submitting the PDB file to the KoBA^{MIN} server (http://csb.stanford.edu/kobamin/). The 3D structure of the protein 2AIZ is shown in the Fig. 1.

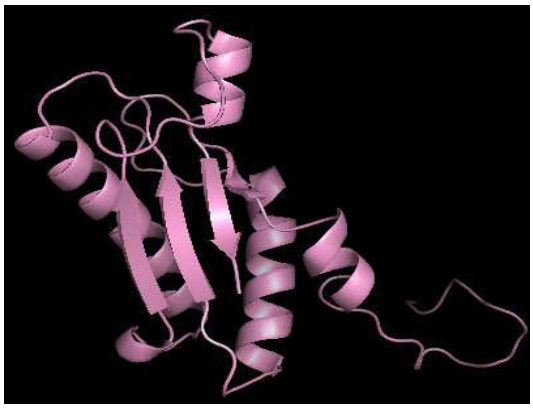


Figure 1
Structure of Outer Membrane Protein 6 of Hemophilus influenzae (PDB ID: 2AIZ)

Preparation of ligands

The study included 40 ligands identified from Pubchem compound database available at (http://www.ncbi.nlm.nih.gov.pccompound). The SMILES of the compounds obtained from the

database were submitted to CORINA, an online server (http://www.molecular-networks.com) that generates 3D structures and downloaded the PDB files. The structure of some of the compounds used in the study is shown in the Fig 2.

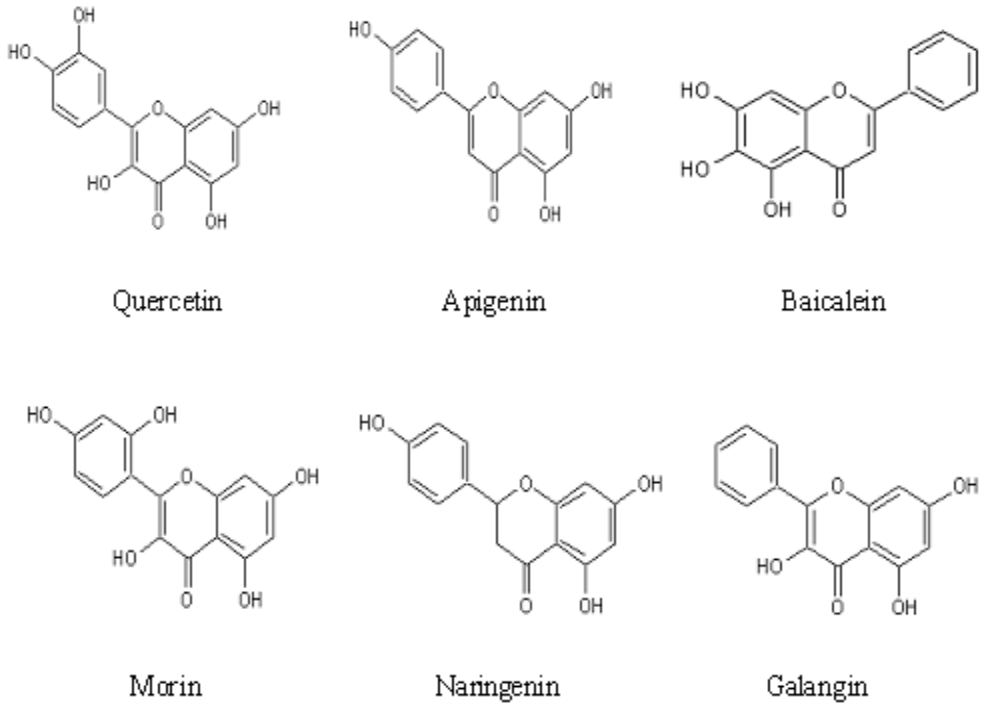


Figure 2
2D structures of some of the phytoligands used in the study

ADME/toxicity prediction

Absorption, Distribution, Metabolism and Excretion (ADME) determines drug likeness of the ligand molecules based on Lipinski's Rule of 5. The rule states that a ligand could be a valid drug candidate if it possesses molecular weight less than 500, hydrogen bond acceptors and donors less than or equal to 10 and 5 respectively and logP less than or equal to 5 [14].

Molecular docking studies

The docking analysis was performed using Patchdock server. The server performs both protein-protein and protein- ligand docking. The PatchDock algorithm is based on shape complementarity principle [15]. The receptor and ligand surfaces are divided into concave, convex and flat surfaces and the complimentary patches are matched resulting in the possible complexes that are evaluated by a scoring function. The scoring function takes into account both geometric fit and atomic desolvation energies [16].

Analysis and visualization of interactions in docked complexes

The binding interactions in the docked complexes obtained by Patchdock server were analyzed by PyMol software (http://www.pymol.org/).

RESULTS AND DISCUSSION

The target 2AIZ which is an Outer membrane Protein (OMP6) of *H. influenzae* contained 5 helices and 6 strands made up of 51 and 21 residues respectively. The protein was docked with various drug compounds having antimicrobial activity. According to Lipinski's rule, any compound violating more than one parameter will not be considered as a drug lead and thus the compounds Quercimeritrin,

Kaempferide-3-glucoside, Kaempferide-3-glucuronide were not used for docking analysis. The remaining 37 compounds were docked and analysed for exploring the binding mechanism between the receptor and the drug compounds. The binding affinity between the target protein and the ligands was evaluated by its score and number of hydrogen bond interactions formed between them. The results of docking were shown in the Table 2. The analysis of docked complexes by using PyMol revealed the number of hydrogen bonds and residues involved in it. The highest docking score was found with Silibinin and Silimarin compounds which were 4298 forming interactions with GLY12 and THR16. The Quercimeritrin compound obtained 4284 score which was nearly equal to the Silimarin and Silibinin compounds. It made complex with the target protein at sites GLU67, ASN69 and ARG129. Eight hydrogen bond interactions were found with Robinetin compound and the aminoacids involved in forming stable complex were TYR20, ARG28, TYR29, THR31, GLU44, TYR45 and GLN47. Kaemferide-3-glucoside possessed 7 hydrogen bond interactions with aminoacids at positions ASN30, THR31, TYR45, GLN47, and ARG129 and scored 3802. 72.5 % of compounds contained hydrogen bond interactions less than or equal to 3 and 27.5 % of compounds greater than 3 hydrogen bonds in complexes. The least score was found in naringenin-2AIZ complex having a value of 2882. The number of interactions found in compounds was given in the Fig. 3.

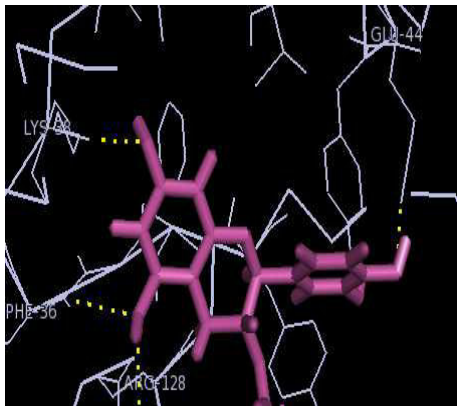


Figure 3
Hydrogen bond interactions involving with aminoacids PHE36, LYS38, GLU44 and ARG128 in 2AIZ-ECG complex.

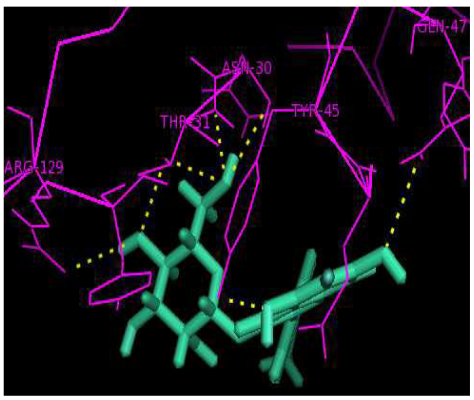


Figure 4
2AIZ-kaemferide3glucoside complex is formed with 7 hydrogen bonds at aminoacids positions ASN30, THR31, TYR45, GLN47 and ARG129.

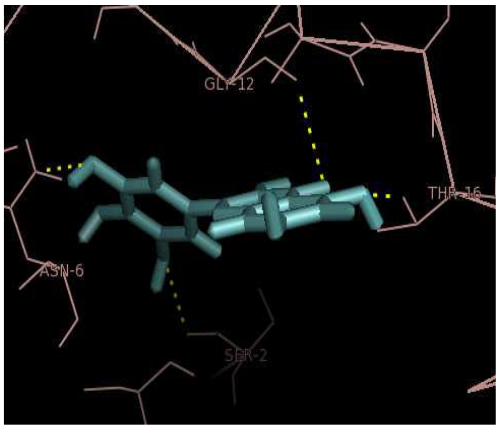


Figure 5
Complex formations by 2AIZ and Myricetin compound with SER2, ASN6, GLN12 and THR16 residues creating 4 hydrogen bond interactions.

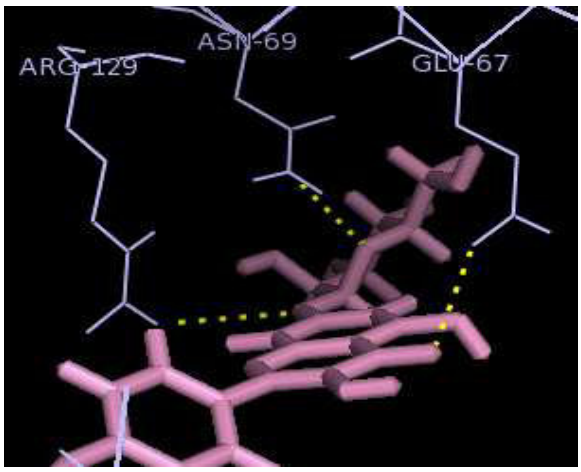


Figure 6
Interaction of Quercimeritrin compound with 2AIZ protein and the hydrogen bond formation with the corresponding aminoacids.

Table 1
Molecular property of phytoligands used in this study for docking analysis

S. No.	Compounds	ID	MW	HBD	HBA	xLogP3
1	Apigenin	5280443	270.236	3	5	1.7
2	Apigetrin	5280704	432.377	6	10	-0.1
3	Baicalein	5281605	270.236	3	5	1.7
4	Catechin	9064	290.268	5	6	0.4
5	Chrysin	5281607	254.237	2	4	2.1
6	Cyanidin	68247	322.697	5	6	-0.7
7	Delphinidin	68245	338.697	6	7	-1
8	Diadzein	5281708	254.237	2	4	2.5
9	Diosmetin	5281612	300.262	3	6	1.7
10	ECG	107905	442.372	7	10	1.5
11	EGCG	65064	458.371	8	11	1.2

12	Eridicityol	11095	288.252	4	6	2
13	Fisetin	5281614	286.236	4	6	2
14	Galangin	5281616	270.236	3	5	2.3
15	Genestein	5280961	270.236	3	5	2.7
16	Genistin	5281377	434.377	6	10	0.9
17	Genkwanin	5281617	284.263	2	5	2.1
18	Hesperetin	72281	302.278	3	6	2.4
19	Isoluteolin	5281801	286.236	4	6	2.3
20	Kaemferide-3-glucoside	44259083	462.403	6	11	1
21	kaemferide-3-glucuronide	44259089	476.387	6	12	1.3
22	Licochalcone A	5318998	338.396	2	4	4.9
23	Liqvirtin	503737	418.393	5	9	0.4
24	Luteolin-7-O-glucoside	5291488	448.376	7	11	0.5
25	Morin	5281670	302.235	5	7	1.5
26	Myricetin	5281672	318.235	6	8	1.2
27	Naringenin	932	272.252	3	5	2.4
28	Pinocembrin	68071	256.253	2	4	2.7
29	Ponciretin	25201019	285.27	1	5	2.8
30	Prunin	282013	434.393	6	10	0.6
31	Quercetin	5280343	302.235	5	7	1.5
32	Quercimeritrin	5282160	464.376	8	12	0.4
33	Rhamnetin	5281691	316.262	4	7	1.9
34	Robinetin	5281692	302.235	5	7	1.6
35	Senegalensin	124035	408.486	3	5	6.2
36	Silibinin	31553	482.436	5	10	2.4
37	Silimarin	1548994	482.436	5	10	2.4
38	Sophoraflavone B	44257572	416.378	5	9	1.5
39	Tangeritin	68077	372.368	0	7	3
40	Taxifolin	439533	304.251	5	7	1.5

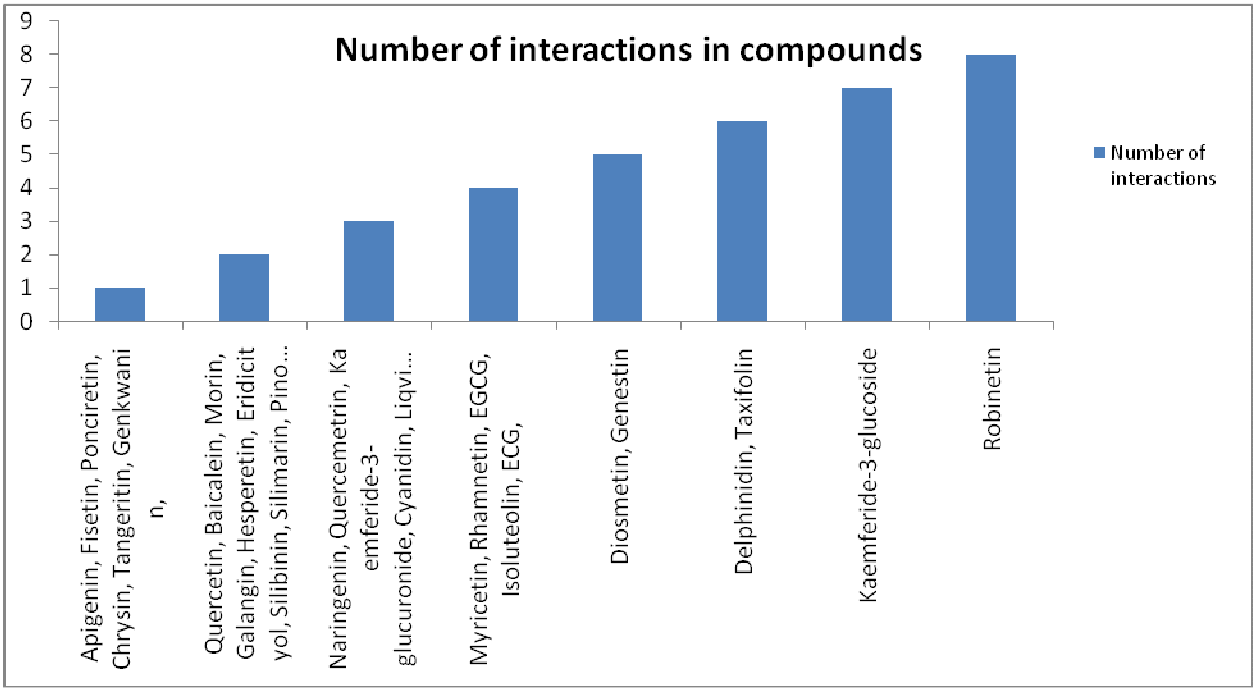
Table 2
Patchdock score results of 2AIZ with various phytoligands

S. No.	Compounds	Score	Number of hydrogen bonds	Residues involved in interactions
1	Apigenin	3462	1	SER4
2	Apigetrin	3892	2	GLY74, GLU121
3	Baicalein	3106	2	GLU67, ASN69
4	Catechin	2960	3	GLY74, GLU121, ARG128
5	Chrysin	3006	1	HIS119
6	Cyanidin	3880	3	SER3, ASP7, THR116
7	Delphinidin	3642	6	GLN27, THR31, TYR33, GLU44, TYR45, GLN47
8	Diadzein	2942	3	SER3, ASN5
9	Diosmetin	3392	5	SER3, SER4, ASN6, ASP7, THR16
10	ECG	3786	4	PHE36, LYS38, GLU44, ARG128
11	EGCG	3602	4	GLN27, THR31, TYR45, ARG129

12	Eridicityol	3150	2	ARG73
13	Fisetin	3064	1	ARG129
14	Galangin	3016	2	GLY12, ASN6
15	Genestein	2898	3	PHE34, ARG128
16	Genistin	4152	5	GLU67, ASN69, LYS113, ARG129
17	Genkwanin	3302	1	ARG73
18	Hesperetin	3176	2	GLY74, GLN121
19	Isoluteolin	2846	4	TYR33, TYR45, ARG128
20	Kaemferide-3-glucoside	3802	7	ASN30, THR31, TYR45, GLN47, ARG129
21	kaemferide-3-glucuronide	3800	3	TYR124, HIS119
22	Licochalcone A	4108	3	ASP74, ARG73, GLU121
23	Liqvirtin	3630	3	GLN15, THR16, SER21
24	Luteolin-7-O-glucoside	3844	3	SER4, ASN5, ASP7
25	Morin	3026	2	HIS119, TYR124
26	Myricetin	3098	4	SER2, ASN6, GLN12, THR16
27	Naringenin	2882	3	PHE34, GLU44, ARG128
28	Pinocembrin	3000	2	ARG73, TYR124
29	Ponciretin	3196	1	ARG128
30	Prunin	4022	3	ARG73, HIS119, TYR124
31	Quercetin	2964	2	LYS113, ARG129
32	Quercimeritrin	4284	3	GLU67, ASN69, ARG129
33	Rhamnetin	3168	4	GLY35, THR42, GLU44, TYR45
34	Robinetin	3144	8	TYR20, ARG28, TYR29, THR31, GLU44, TYR45, GLN47
35	Senegalensin	4106	3	ARG73, HIS119, TYR124
36	Silibinin	4298	2	GLY12, THR16
37	Silimarin	4298	2	GLY12, THR16
38	Sophoraflavone B	3946	3	ASP40, GLU44, TYR45
39	Tangeritin	3972	1	GLU67
40	Taxifolin	3188	6	SER3, ASP7, GLY12, THR16

Graph 1

Graphical representation of number of interactions found in docked complexes



CONCLUSION

In conclusion, the present study involving molecular docking analysis with phytoligands resulted in binding interactions between them which are needed for the development of potent inhibitors for the treatment of OMP6 protein virulence. The results clearly indicate that the flavonoids Silibinin and Silimarin have the similar binding sites and interactions with the target OMP6 and they could be potent inhibitors.

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