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Original Research Paper

IN SILICO BINDING STUDIES OF OXALIS CORNICULATA COMPOUNDS WITH *RALSTONIA SOLANACEARUM* PROTEINS AND HISTONE DEACETYLASE 8 PROTEIN

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ABSTRACT

Molecular docking with the molecules of Oxalis corniculata was done against *Ralstonia solanacearum* and Histone Deacetylase 8 (HDAC8) protein using LeadIT software. Total of 28 ligands were selected and screened against 20 proteins of *Ralstonia solanacearum* and 3 proteins of HDAC8, the best interaction with energy score-25 KJ/mol or more showed by 15 ligands with Ralstonia solanacearum and HDAC8 respectively.

Keywords: Interaction, Proteins, Ligands, Software, Molecular docking.

INTRODUCTION

Oxalis corniculata is an endangered and medicinally important plant found in tropical and subtropical regions of the world (*Badwaik et al.*, *2011*). *Oxalis corniculata* is the creeping wood sorrel, also called procumbent yellow-sorrel or sleeping beauty, resembles the common yellow wood sorrel. It has a narrow, creeping stem that readily roots at the nodes which belong to the family Oxalidaceae. The trifoliate leaves are subdivided into three rounded leaflets and resemble a cloverin shape. The fruit is a narrow, cylindrical capsule, 1 to 2 cm long and noteworthy for its explosive discharge of the contained, 1 mm long seeds. *Oxalis Corniculata* plant (Kingdom : Plantae; Division : Magnoliophyta; Class : Magnoliopsida; Order : Oxalidale; Family :Oxalidaceae; Genus : *Oxalis*; Species :*corniculata)* is antibacterial, antifungal, antimicrobial, anticancer, antidiabetic, anti-inflammatory, astringent, depurative, diuretic etc It is used in the treatment of several diseases like influenza, fever, urinary tract infections, enteritis, diarrhea, traumatic injuries, sprains and poisonous snake bites. An infusion can be used as a wash to rid children of hookworms. The plant is a good source of vitamin C. It is also rich in niacin and beta carotene. The leaf juice is applied to insect bites, burns and skin eruptions. *Ralstonia solanacearum* is an aerobic non sporing, Gram-negative plant pathogenic bacterium. *R. solanacearum* is soil-borne and motile with a polar flagella tuft. It colonizes the xylem, causing bacterial wilt in a very wide range of potential host plants. *Ralstonia solanacearum*, previously known as *Pseudomonas solanacearum*, was originally described by Smith (1896) as the causative agent of bacterial wilt of solanaceous plants (Owoseni *et al*.). It is internationally recognized as one of the leading models in the analysis of plant pathogenicity (Owoseni *et at*.). This soil bacterium is the causal agent of a severe and devastating disease of major economic importance on solanaceous crops. The bacteria can survive for a long time in water (up to 40 years at 20–25 °C in pure water) and the bacterial population is reduced in extreme conditions

(temperature, pH, salts, e.g.). Infected land sometimes cannot be used again for susceptible crops for several years. *R. solanacearum* can also survive in cool weather and enter a state of being viable but not culturable. In most cases, this stage is not an agricultural threat because the bacteria usually become avirulent after recovering. Histone deacetylation alters chromosome structure and it also affects transcription factors access to DNA. Molecular Docking is an effective and competent tool for *in silico* screening. Its plays an important role in drug designing. It is used to predict accurately the structure of a ligand-protein complex, the position and orientation of the ligand when it bound to a receptor and also to calculate binding energy of any ligand-protein interaction. Such docking technique basically used in pharmaceutical research now a days, primarily for virtual screening of large database of available protein in order to select likely drug candidates. Thus, docking is a computational procedure which is fast and reliable where searching for an appropriate ligand is done that fits in the protein's binding site. The goal of ligand-protein docking is to predict the predominant binding mode of a ligand with a protein of known three-dimensional structure. The process which determine whether the given conformation or orientation fit the active site is called posing and the pose score is the measure of fit of the ligand to the active site. Docking is the formation of protein ligand complexes. Docking can be of three types:

1. Rigid docking :Rigid docking is where a suitable position for the ligand in receptor is obtained while maintaining its rigidity
2. Flexible docking :Flexible docking is where the receptor remain fixed and a favoured geometry for receptor-ligand interaction is obtained by changing internal torsions of ligand into the active site
3. Full flexible docking: Full flexible docking is where the ligand is flexed via its torsion angles as well as the side chain of active site residues are flexed (Mukesh *et al.)*

One of the very popular software used is LeadIt. LeadIt is the state of the art tool for docking and scoring. LeadIt modules help to investigate possible binding conformations of the receptor ligand complex using state of the art docking software -"FlexX". The receptor structure is uploaded as a PDB file and is prepared for docking in the protein definition wizard. The docking algorithm in FlexX uses flexible ligand and rigid protein (Mukesh *et at.*). Ligand flexibility limited to torsion angles and ring conformations.

MATERIALS AND METHODS

Retrieval of the Three-Dimensional Structure of Target Proteins

The structures of the target receptor binding sites of *Ralstonia solanacearum* and HDAC8 (HIstone deacetylase8) from RCSB protein Data Bank were obtained. The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. Tolal 20 proteins of *Ralstonia solanacearum* were selected viz. 3ZI8, 4I68, 4KF9, 4FDB, 3UMB, 3TMB, 3TOT, 3TOU, 3NPN, 3NPQ, 3LOP, 3GG9, 3GHY, 3EN2, 2QGU, 2CHH, 2BT9, 2BS5, 2BS6, 1UQX and 3 proteins of HDAC8 viz. 2V5W, 2V5X and 3QRD.

Selection of the Ligands

The ligands of *Oxalis corniculata* were selected from various journals and NCBI. Total of 28 different ligands were selected and they are luteolin, quercetin, apigenin, vitexin, isovitexin, orientin, isoorientin, phytosterol, acacetin, 4 hydroxybenzoic acid, flavones, malic acid, tartaric acid, oxalic acid, isoproterenol, ascorbic acid, glycopyrunoside, tannin, linolenic acid harmine, stearic acid, palmitic acid, glycoside, beta sitosterol, betulin, ethyl gallate, oleic acid and 1-octacosanol. All these ligands were selected from Zinc Database. The Zinc database is accurate collection of commercially available chemical compounds prepared especially for virtual screening. In Zinc database the ligand name was uploaded and then downloaded in mol2 format and the URL was saved in note pad and

noted down. In the same way all the 28 ligands in mol2 format was downloaded. After downloading, all the ligands were merged.

Docking Analysis

Docking was performed to interpret the best binding pose of the test ligand in the active site of the receptor and also its binding affinity and conformation in the binding sites. The software used for docking was LeadIt. LeadIt modules help to investigate possible binding conformations of the receptor ligand complex using state of the art docking software – "FlexX". FlexX was the software to predict the protein ligand interaction. At first In LeadIt the docking option has to be selected. Protein of my interest was then uploaded. To select the receptor side there were two methods, the reference ligands and the selection of sphere to get the best score. A reference ligand may be defined as by clicking on the option and in sphere a spherical cut off of the protein was carried out using a reference ligand. In the next step, protonation state of the amino acids were selected. Which was known as chemical ambiguities. It means the crystallographic unresolved region. These are highlighted in orange. Ambiguities affects the rotamers, alternate locations, protonation. H-torsion etc. These were adjusted manually by adjusting the torsion angle. After removing the chemical ambiguities the 'finish' option was clicked. After that the ligands that were already merged were uploaded and the entries were confirmed by clicking 'ok'. After uploading both protein and ligands, the docking was performed by clicking on the option 'Apply & Dock'. After completion of the docking the scores were saved and the docked poses was viewed. The interaction and the position of the ligand in the active site of the protein were viewed in 2-D using dock widget PoseView. Each pose is drawn with hydrogen and metal interaction. Hydrophobic interaction were given in green colour and hydrophilic bonds were given in red colour.

RESULTS AND DISCUSSION

After analysis of molecular docking for 28 ligands that is luteolin, quercetin, apigenin, vitexin, isovitexin, orientin, isoorientin, phytosterol, acacetin, 4 hydroxybenzoic acid, flavones, malic acid, tartaric acid, oxalic acid, isoproterenol, ascorbic acid, glycopyrunoside, tannin, linolenic acid harmine, stearic acid, palmitic acid, glycoside, beta sitosterol, betulin, ethyl gallate, oleic acid and 1-octacosanol against 20 proteins of *Ralstonia solanacearum* (3ZI8, 4I68, 4KF9, 4FDB, 3UMB, 3TMB, 3TOT, 3TOU, 3NPN, 3NPQ, 3LOP, 3GG9, 3GHY, 3EN2, 2QGU, 2CHH, 2BT9, 2BS5, 2BS6, 1UQX) and HDAC8 (2V5V, 2V5X and 3QRD) . Only 15 ligands out of 28 showed energy score -25KJ/mol or more against *R.solanacearum* protein and the classification of the lagands are given in the Table 1. Most of the ligands whose energy score is -25 KJ/mol or more belongs to flavones. The energy scores are given in Table 2-15. Among all these ligands the best binding showed by luteolin and isoorientin with protein 4KF9 (Table3). In HDAC8 that is (2V5V, 2V5X and 3QRD) only 10 ligands had shown energy score above -25KJ/mol (Table13-15). In case of HDAC8 the best score was shown by vitexin with 2V5W protein. Few amino acid showed good interaction with *Ralstonia solanacearum* protein and the energy score was above -30KJ/mol and the ligands were luteolin, quercetin, isoorientin, vitexin, malic acid. It helps us to indentify the active binding site of the of protein enzyme. The PoseView displays a 2D view of the docked poses. Each PoseView image had hydrogen bond, hydrophobic bond and hydrophilic bond and each pose had hydrogen and metal interactions and its corresponding residues. Hydrophobic interactions were displayed as green contact curves with only the names of the interacting residues attached to the lines. Dashed line represented the hydrogen bond. PoseView image of few best ligands whose energy scores were above -30KJ/mol are given in the Figure 1-7.

CONCLUSION

The ligands of *Oxalis corniculata* which showed best binding with *Ralstonia solanacearum* and HDAC8 proteins were luteolin, quercetin, acacetin, apigenin, isoorientin, vitexin, malic acid

and because of these following compounds *O.corniculata* can be used as antibacterial agent against *Rasltonia solanacearum* ant anticancer agent against HDAC8.

ACKNOWLEGMENT

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Table 1: Classification of ligands

Ligand	Category
Luteolin	Flavone, type of flavonoid
Quercitin	Flavonol, is a flavonoid
4- Hydroxybenzoic acid	PolyPhenolic derivative of benzoic
Aigenin	Flavone
Orientin	Flavone
Acacetin	Flavone
Isoorietin	Flavone
Tartaric Aicd	Carboxylic acid
Ascorbic Acid	Dihydrofurans
Oxalic Acid	Carboxylic acid
Malic Acid	Carboxylic acid
Isovitexin	Flavone
Glucopyranose	Glycoside
Tannin	Polyphenolic
Vitexin	Flavone

Table 2: Energy score for 3GHY

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Luteolin	-	-33.191	-	-6.5375	5.8549	5.6	12
Quercetin	-	-35.6979	-	-6.957	5.8418	7	13
4-hydroxybenzoic	-	-23.409	-	-6.6458	2.3284	1.4	11
Apigenin	-	-29.3808	-	-6.3386	5.3327	4.2	11
Orientin	-26.184	-27.3642	-10.3	-11.2	3.331	14	13

Table 3: Energy score for 4KF9

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Luteolin	-35.3128	-30.8741	-10.72	-9.3961	4.6772	5.6	12
Quercetin	-33.8144	-32.2444	-10.5466	-8.4804	5.057	7	18
Acacetin	-30.1316	-21.8405	-12.9767	-9.5104	4.5961	4.2	9
Apigenin	-30.0394	-25.0363	-10.7698	-8.4262	4.5929	4.2	9
Isoorientin	-35.8203	-37.1391	-16.1988	-	12.6413	14	17

Table 4: Energy score for 2BS5

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Luteolin	-26.742	-26.0225	-8.891	-5.5711	2.7428	5.6	16
Quercetin	-26.1941	-26.3701	-6.9319	-7.7952	2.5031	7	19
Isoorientin	-26.6636	-32.1113	-9.8695	-6.8361	2.733	14	17
Tartaric acid	-25.1314	-31.8423	-2.3584	-3.786	0.4553	7	16

Table 5: Energy score for 2BS6

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Orientin	-26.4625	-34.4747	-4.5544	-9.0533	2.22	14	8

Table 6: Energy score for 3 GG9

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Tartaric Acid	-28.2113	-36.8971	-1.1003	-2.6587	0.0448	7	8
Ascorbic Acid	-25.9335	-37.0079	-1.8943	-4.6017	2.3704	9.8	10
Orientin	-25.3285	-34.5941	-4.2195	-8.1219	2.2071	14	11
Isoorientin	-26.1208	-32.0384	-13.7	-13.9	14.1	14	13
Malic Acid	-25.3967	-33.2656	-1.6626	3.3649	-1.8958	5.6	5
Oxalic Acid	-25.5059	-25.7107	-1.9308	-4.1854	0.921	0	10

Table 7: Energy score for 3TOU

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Orientin	-27.1447	-33.5382	-12.093	-9.7075	8.794	14	13
4-Hydroxybenzoic Acid	-26.0691	-26.9628	-3.7281	-3.9799	1.5317	14	13
Isoorientin	-25.0989	-35.3145	-8.8138	7.385	7.0144	0	21
Oxalic Acid	-25.4359	-25.4773	-2.2106	-4.1559	1.0079	0	6
Luteolin	-26.3513	-29.3362	-7.0564	-5.5986	4.6398	5.6	20
Quercetin	-25.1805	-29.3275	-7.0188	-6.2151	4.9808	7	26
Isovitexin	-25.2196	-23.1621	-10.9346	-8.8752	3.9523	5.6	6
Malic Acid	-27.4926	-34.2661	-3.599	-4.184	3.5565	5.6	6

Table 8: Energy score for 3LOP

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
4-Hydroxybenzoic Acid	-25.684	-25.422	-5.5891	-5.5532	4.0803	1.4	6
Malic Acid	-26.3098	-29.9234	-2.9874	-5.8512	1.4521	5.6	6
Tartaric Acid	-26.7947	-31.9593	-2.8355	-6.9381	2.5382	7	7

Table 9: Energy score for 3TOT

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Luteolin	-25.3697	-28.8794	-6.9132	-6.6454	6.0683	5.6	17
Acacetin	-26.7635	-26.7635	-8.5335	-6.3681	6.7772	4.2	18
Orientin	-29.3622	-33.1547	-14.9832	-8.523	-7.8988	14	7
Vitexin	-30.4469	-33.1547	-14.9313	-8.2605	7.9039	12.6	17
Glycopyanoside	-30.5585	-33.0106	-12.0756	-6.43	4.3577	11.2	27
Oxalic Acid	-26.2418	-27.1576	-1.6449	-3.93	1.0907	0	4
Malic Acid	-27.1062	-32.865	-3.2819	-4.6371	2.6478	5.6	6

Table 10: Energy score for 4S16

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Quercetin	-25.7173	-29.3166	-4.8356	7.0764	3.1113	7	14
Luteolin	-27.8414	-26.8281	-6.6603	-6.5505	1.9975	5.6	15
Oxalic Acid	-26.0522	-26.2268	-1.5352	-3.7092	0.0189	0	5
Isovitexin	-27.4107	-26.8936	-9.2311	-7.4522	2.3662	8.4	12
Malic Acid	-26.9454	-29.5239	-3.73	5.0452	0.3537	5.6	6

Table 11: Energy score for 3NPQ

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Luteolin	-27.1355	-32.1008	-4.0264	-7.27	5.2618	5.6	11
Tannin	-26.7533	-42.6715	-1.6352	-8.9775	2.931	18.2	13
Quercetin	-25.6146	-31.6533	-4.1961	-6.9304	4.7659	7	13
Orientin	-27.9175	-38.2779	-5.2775	-11.2798	7.5178	14	18
Isoorientin	-26.5347	-34.3931	-7.237	-8.7466	4.442	14	12

Table12: Energy score for 3UMB

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Malic Acid	-32.6745	-37.7291	-4.2738	-5.6518	3.9801	5.6	7
Orientin	-28.5136	-35.6965	-9.3854	-10.1717	7.34	14	15
Tartaric Acid	-29.5572	-35.1815	-2.6352	-6.5571	2.4165	7	8
Glycopyanoside	-28.337	-35.99	-8.4128	-7.583	7.0435	11.2	16
Vitexin	-26.2237	-31.302	-9.3432	-9.7367	6.1582	12.6	14

Table13: Energy score for 2V5W

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Luteolin	-29.9341	-32.5899	-8.1581	-6.5611	6.375	5.6	12
Tannin	-32.8799	-40.9541	-8.987	-13.989	-7.4503	18.2	17
Quercetin	-27.2461	-27.6193	-7.1467	-8.0893	3.2061	7	10
Orientin	-29.1637	-34.7324	-9.125	-10.1589	5.4525	14	16
Vitexin	-33.1826	-36.2502	-10.2763	-10.9644	6.3082	12.6	16

Table 14: Energy score for 2V5X

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Orientin	-28.3841	-38.2893	-6.2846	-8.3962	5.1861	14	16
Vitexin	-27.294	-34.4729	-7.0717	-7.4922	3.7427	12.6	18
Isoorientin	-29.2193	-34.4444	-6.7069	-9.5768	2.1087	14	12
Isovitexin	-27.4628	-22.849	-12.9368	-12.3696	6.8926	8.4	9
Malic Acid	-31.4084	-36.007	-2.3398	-5.4681	1.4064	5.6	8
Tartaric Acid	-27.3869	-32.8595	-2.9803	-6.4679	2.5202	7.006	8
Quercetin	-26.324	-26.2573	-6.1491	-8.2405	1.9229	7	11

Table15: Energy score for 3QRD

LIGAND	SCORE	MATCH	LIPO	AMBIG	CLASH	ROT	#MATCH
Isoorientin	-29.5945	-35.7223	-8.0396	-11	5.8828	14	15
Tartaric Acid	-25.0568	-32.7018	-3.66	-4.3377	3.2427	7	5
Isovitexin	-25.9064	-27.9615	-7.8822	-11.2	7.3476	8.4	

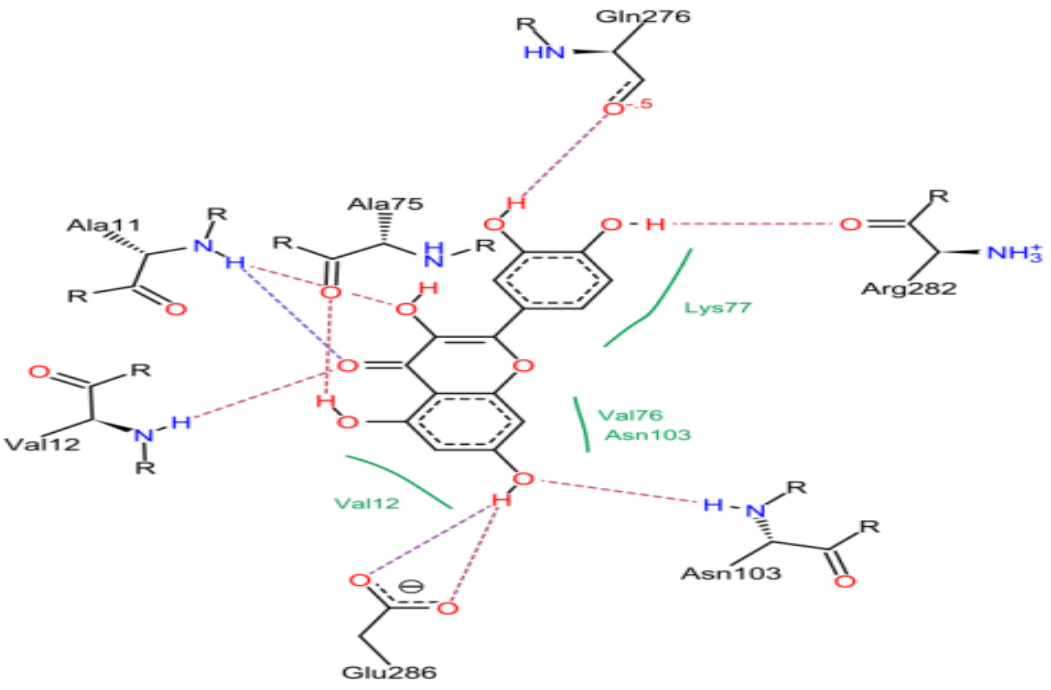

Figure 1: Pose View image of luteolin with *R. solanacearum* protein 3GHY

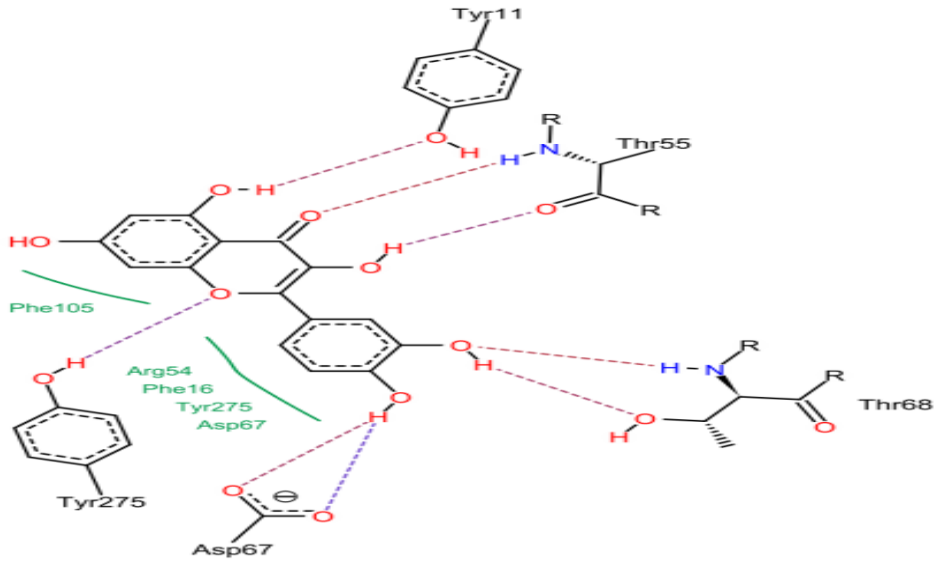

Figure 2: Pose View image of quercetin with *R. solanacearum* 4KF9

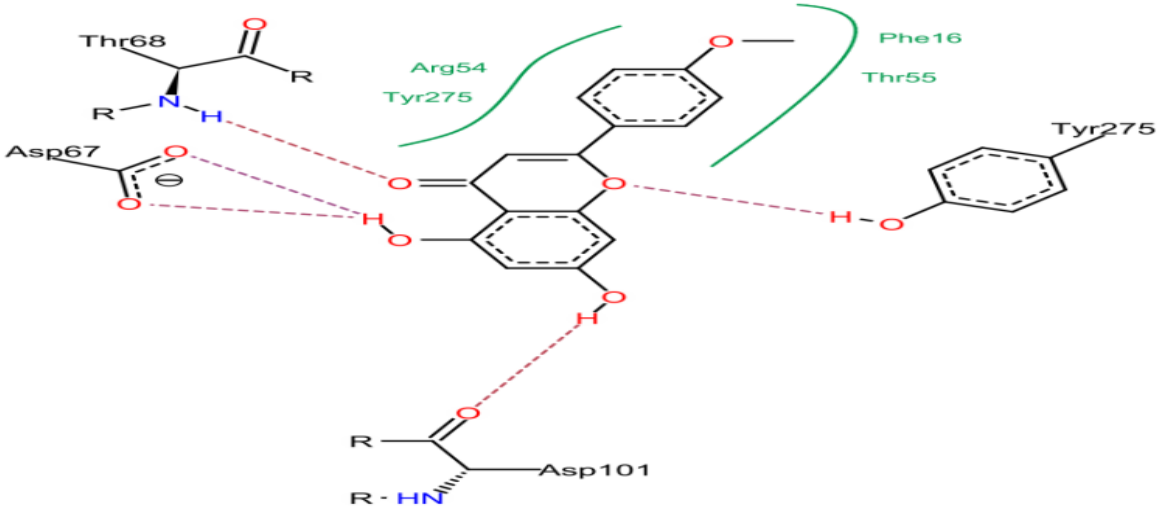

Figure 3: Pose View image of acacetin with *R. solanacearum* protein 4KF9

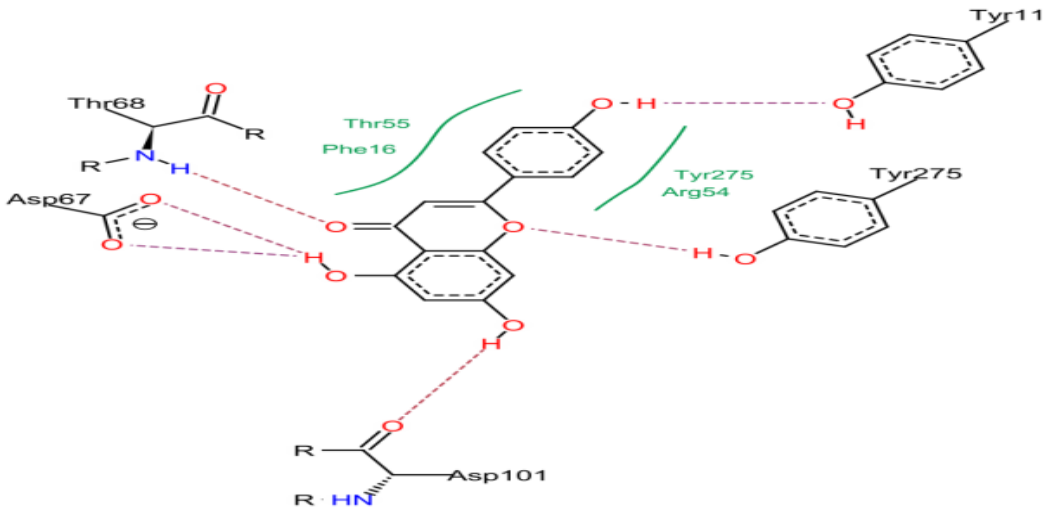

Figure 4: Pose View image of apigenin with *R. solanacearum* proein 4KF9

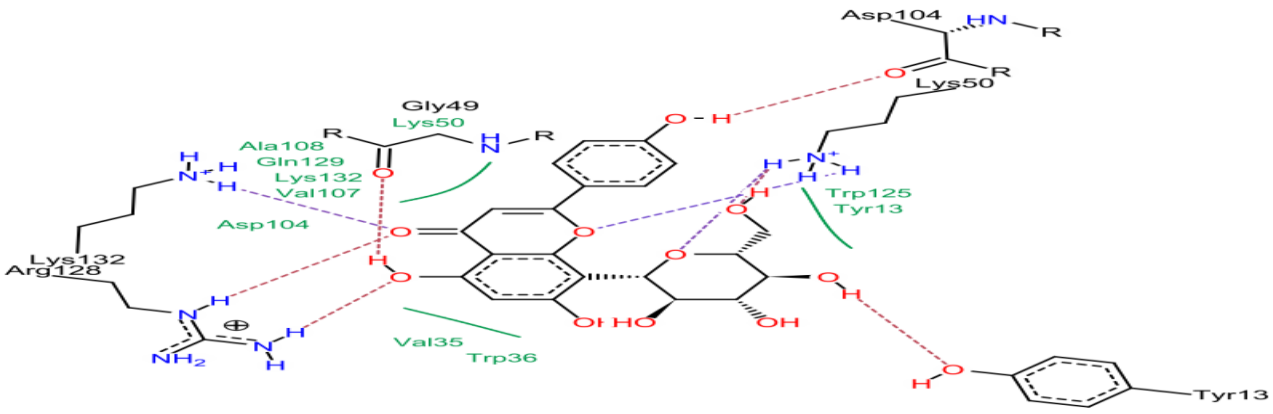

Figure 5: PoseView image of isoorientin with *R. solanacearum* proein 4KF9

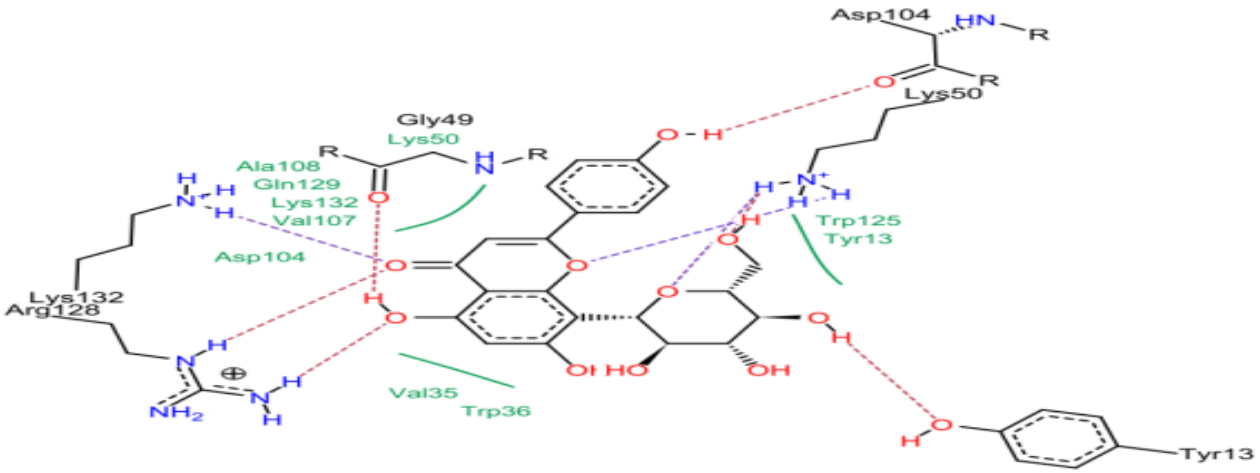

Figure 6: Pose view image of vitexin with *R.solanacearum* protein 3TOT

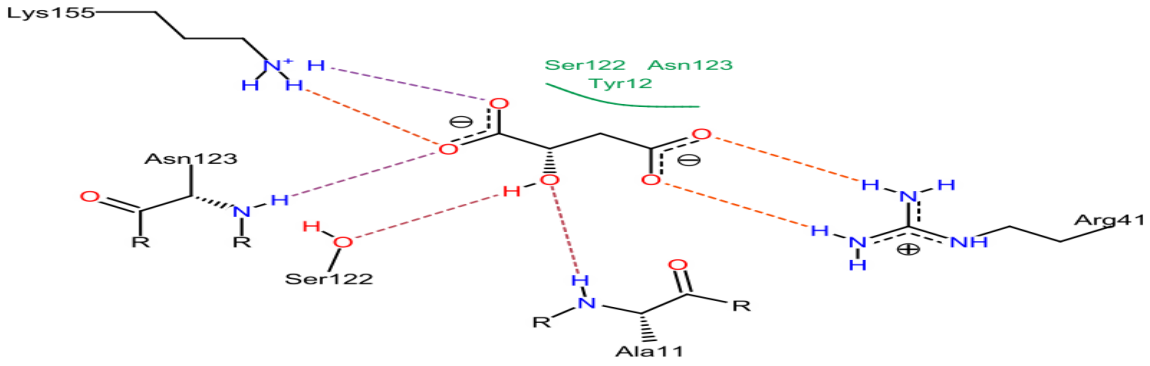

Figure7: Pose View image of malic acid with R. solanacearum protein 3UMB

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