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COMPUTATIONAL INVESTIGATION OF THE ANTIFUNGAL POTENTIAL OF PHYTOCHEMICALS IN *Senna Alata* AGAINST SIX 1 AND SIX 6 TARGETS IN *Fusarium Oxysporum F. Sp. Lycopersici*

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ABSTRACT

Background: *Fusarium oxysporum f.sp.lycopersici* is a pathogen, causing Fusarium wilt; a major fungi infection of tomatoes in Nigeria. This soil-borne pathogen has proved difficult to control due to its ability to produce chlamydospores in the soil and strong resistance to the environmental factors and chemical fungicides. Several studies have confirmed the antifungal properties of *Senna. alata* extracts and the phytochemicals in the plant's extracts against clinical pathogens and skin infections, but research data on its activities against plant pathogens are rare.
Objectives: The aim of this study was to examine the potential antifungal property of *S. alata* phytochemicals against *F. oxysporum sp.lycopersici* through their binding interactions with SIX1 and SIX6 effector proteins, implicated as virulent agents in *F. oxysporum sp.lycopersici* .
Methods: The study was carried out using computer-aided drug discovery/ design (CADD).
Results: XP docking analysis predicted six flavonoid derivatives as potential fungicides with probable better inhibitory interaction with the protein targets than the five standard antifungal agents used as controls. These hit compounds are kaempferol 3-gentiobioside (CID:9960512), kaempferol 3-o-beta-d-glucofuranoside (CID:72551445), astragalin (CID:5282102), thermopsoside (11294177), rhamnetin (CID: 5281691) and quercetin (CID: 5280343) with docking scores -9.257 & -8.772, -8.373 & -6.565, -7.919 & -6.398, -7.162 & -6.693, -5.318 and -5.403 Kcal/mol respectively. Their respective binding free energy (ΔG) are; -37.77 & -42.68, -50.61 & -27.75, -37.34 & -35.59, -37.57 & -34.5, -33.58 and -39.61 Kcal/mol. The binding scores and ΔG values for the reference antifungal compounds; prothioconazole, prochloraz, bromuconazole, thiophanate-methyl and azoxystrobin, ranged from -4.069 to -1.309 Kcal/mol and -42.84 to -9.57 Kcal/mol respectively.
Conclusion: The higher binding affinity of the six compounds than the reference chemical fungicides in this study suggests their better fungicidal potential, they are therefore recommended for further studies.

Keywords: *Fusarium oxysporum f.sp.lycopersici, Senna Alata,* fungicides, virulent, proteins

INTRODUCTION

Worldwide, plant pathogenic fungi severely damage agricultural products. According to Ortoneda *et al.* (2004) and Di *et al.* (2017), *Fusarium oxysporum* is a soil-borne fungus that infects over 120 different plant species of economic importance, including tomato, potato, banana, cotton, and date palm, at pre- and post-harvest stages. This results in a significant annual loss of commercially important crops for which there is currently no known solution (McGovern, 2015). There are numerous distinct species of this fungi

that are capable of infecting a single or small number of host species; Fusarium wilt disease in tomatoes are caused by *Fusarium oxysporum f.sp. lycopersici.* The use of chemical fungicides and resistant plant cultivars, where available has been the main method of treating this harmful disease in Nigeria (Michielse *et al.,* 2009). However, biopesticide researches aim at producing effective and more environmentally friendly pesticides, as better alternatives to petroleum -based agro-chemicals are ongoing. Several phytochemicals have demonstrated various biochemical activities in several documented researches to suggest their potentials as biopesticides in pest and disease control (Lahlali *et al.*, 2022).

In the quest to investigate the means by which pathogenic fungi break through the defense mechanisms of the host plant, research -based evidences have severally showed that pathogenicity of fungi is controlled by multiple proteins (Jain *et al.,* 2004; Yong *et al*., 2014; Wei *et al.,* 2023). Thus, to investigate their mode of action and subsequently create potent biofungicide products, it is essential to identify these virulence-related proteins, comprehend their pathogenic mechanisms, and understand how they interact with active compounds of plant materials with identified fungicide properties (Yong *et al.,* 2014). The understanding of pathogenic pathways at the molecular level has significantly improved with the use of genomic and computer-aided drug discovery/design (CADD) technology. In contrast to the conventional drug discovery method, the development of new medications in the pharmaceutical business has become less tedious and more intriguing in recent years due to developments in computational and informational technology (Shanmugam and Jeon, 2017). Applying these state-of-the-art technology methods to pesticide research will be necessary for the development of cheap, effective, and environmentally friendly biopesticides for the management and control of plant diseases in sustainable agriculture (Kandakatla and Ramakrishnan, 2014; Pathak *et al*., 2016; Soundararajan *et al.,* 2011; Zhou *et al.,* 2015; Shanmugam and Jeon, 2017).

Senna alata is a member of the Leguminosae family of herbal remedies. It is prevalent in tropical and humid environments. Often called *Cassia alata*, candle brush or candlestick. It is known as "asuwon oyinbo" in Yoruba. A wide range of metabolite substances, including flavones, flavonols, flavonoids glycosides, alatinon, alanonal, and β-sitosterol-β-D-glucoside, have been identified in *S. alata*; primarily located in the leaves, stems, and flowers (Fatmawati *et al.,* 2020; Adelowo and Oladeji, 2020; Oladeji *et al.,* 2020). The antifungal action of *S. alata* extracts against skin infections and clinical pathogens has been shown by several investigations (Jenson *et al.,* 2020; Sule *et al*., 2010). However, there is insufficient information on the extracts' ability to combat plant pathogens. This study aims to explore the potential of *S. alata* phytochemicals as fungicides against *F. oxysporum f.sp. lycopersici,* through their binding interactions with SIX1 and SIX6 effector proteins, using CADD.

2.0 Materials and Methods

2.1 Databases, Tools, and Softwares

The databases, tools, and softwares used include research collaboratory structural bioinformatics protein data bank (RCSB PDB), PubChem database, Schrodinger PyMOL version 2.5.1, Osiris Data Warrior, Schrodinger Maestro Glide LigPrep module, Biovia Discovery Studio suite version 21.1.0.20298 2020, Maestro Glide receptor grid generator module, Maestro Glide Docking Standard Precision Module, and Maestro Molecular Mechanics/Generalized Born Surface Area (MMGBSA) free energy calculator module (Adigun *et al*., 2023).

2.2. Protein preparation

The PDB coordinate files (PDB ID: 6S4T) of 7T69 and 8EBB, the respective crystal structures of the effector proteins SIX 1 and SIX 6 were obtained from the protein data bank (http://www.rcsb.org) and prepared using the protein preparation wizard of Schrodinger maestro v12.5.139. The two proteins which lacked co-crystallized components were produced at a pH of 7.02 in the OPLS2005 force field. The hydrogen bond order was reinstated, all steric conflicts were eliminated, and prime was used to replace the absent side chains and loops. Bond orders were assigned and water molecules having a molecular weight larger than 5.00 and less than three hydrogen bonds to non-waters were removed from the ionized (Het) groups. The polarity charge limit of 0.25 was where this

was energetically minimized (Adigun *et al.,* 2023).

2.3. Ligand preparation

The two-dimensional structures of the thirty two (32) phytochemicals detected from various solvents extracts of *S.alata* as reported by Adelowo and Oladeji, (2017), Fatmawati *et al.,* (2020) and Oladeji *et al.,* (2020) and five commercial antifungal agents (prothioconazole, prochloraz, bromuconazole, thiophanate-methyl and azoxystrobin) were obtained from the PubChem database. The ligands were prepared using the Schrodinger maestro ligand preparation wizard, which involved desalting the ligands, generating tautomers at the target pH of 7.02, and using the MacroModel wizard to minimize energy under the OPLS2005 force field (Adigun *et al*., 2023).

2.4 Receptor Grid Generation

In this investigation, the grid coordinates defining the ligand screening boundaries within the target were constructed using the Maestro receptor grid generating module. To reduce the potential for non-polar regions of the receptor, input partial charges of target atoms and van der Waals radii of receptor atoms with partial atomic charge (absolute value) less than the predetermined cut-off were used. Moreover, during docking, the volumes that shouldn't have contained any ligand atoms were eliminated, and the centroid of particular residues served as the sphere's center. For 7T69, the receptor grid box was created in each direction with X = 28.57 Å, Y = 16.55 Å, Z = 39.57 Å, and for 8ebb, X = 1.88 Å, Y = 10.28 Å, Z = -27.38 Å (Adigun *et al.* 2023).

2.5 Schrodinger Maestro Glide Standard and Extra Precision Dockings

Before the ligand was docked into the receptor grid using the Maestro Glide standard precision module, the various resultant generated conformations of each compound were retrieved and rigorously sampled using flexible sampling of nitrogen inversions and ring conformations (with bias sampling of torsions for all predefined functional groups). Prior to using the Maestro Glide standard precision module to dock the ligand into the built receptor grid, the maximal shared substructure of the generated conformers as well as conjugated Pi group planarity were identified.

The docking process was restricted to the grid's reference point with a tolerance position of 0.10, intramolecular hydrogen bonds were rewarded in the scoring function, strain correction terms were employed, and Epik state penalties were applied to the docking score value. A threshold of 0.50 Kcal/mol was set for rejecting the reduced pose. Extra Precision Docking was also carried out for more accurate and flexible docking (Adigun *et al.,* 2023).

2.6 Molecular Mechanics-Generalized Born Surface Area Calculations

The VSGB solvation model, the input ligand partial charges, and prime were utilized to compute the molecular mechanics generalized Born surface area free energy change (MMGBSA dG) of the docked ligands. In addition to employing the Glide extra precision (XP) capability to facilitate more accurate and flexible docking on the designated receptor grid, the flexible residues were confined and all processed ligands were used in the energy computations (Adigun *et al.,* 2023).

3.0. Results and Discussion

3.1 Molecular docking

The six hit compounds out of the thirty-*two* screened that had better binding (docking) scores with the protein target*s* 7t69 and 8ebb; than the five standard antifungal agents were kaempferol 3-gentiobioside, kaempferol 3-o-beta-d-glucofuranoside, astragalin, thermopsoside, rhamnetin and quercetin with docking scores of -9.257 & -8.772, -8.373 & -6.565, -7.919 & -6.398, -7.162 & -6.693, -5.318 and -5.403 Kcal/mol respectively. This is an indication of better inhibitory interaction potential. The binding scores for the reference antifungal compounds; prothioconazole, prochloraz, bromuconazole, thiophanate-methyl and azoxystrobin, ranged from -4.069 to -1.309 Kcal/mol.

3.2 Molecular Interactions

The interactions of the amino acid residues with the hit compounds in the ligand binding domain of the SIX1 (Avr3) and SIX6 are presented in Figures. 1A-F. and 2A-F. In the ligand binding domain of 7t69, kaempferol 3-gentiobioside with the highest docking score of -8.22 kcal/mol had hydrogen bond interactions with GLU 213, SER 282, CYS 188, GLN 166, TYR 280 and MET 111 of 7t69. Strong pi-cation interactions were observed with ARG 142. Its

Table 1: Retrieved *S. alata* Phyto-compounds with their CID Nummers

S/N	Name	CID
1	Adenine	190
2	Coumarin	323
3	4-Aminoantipyrine	2151
4	Emodin	3220
5	P-Benzoquinone	4650
6	Anthraquinone	6780
7	Anthraquinone	6780
8	Isoquinoline	8405
9	Anthracene	8418
10	Rhein	10168
11	Chrysophanol	10208
12	Anthranol	10731
13	6-Hydroxy-4,4,7a-Trimethyl-5,6,7,7a-Tetrahydrobenzofuran-2(4H)-One	14334
14	Butylhydroxytoluene	31404
15	Campesterol	173183
16	Pyrazolinone	351317
17	Cannabinolic Acid	3081990
18	Apigenin	5280443
19	Luteolin	5280445
20	Chryseriol	5280666
21	Robigenin	5280863
22	Diosmetin	5281612
23	Rhamnetin	5281691
24	Astragalin	5282102
25	6-Hydroxyrubiadin	5319801
26	Kaempferol 3-Gentiobioside	9960512
27	Thermopsoside	11294177
28	4,5,7-Trihydroxyflavanone	17873337
29	2,5,7,4'-Tetrahydroxyisoflavone	54728923
30	Kaempferol 3-O-Beta-D-Glucofuranoside	72551445
31	Laurifolin	102301875
32	Quercetin	5280343

Table 2: Retrieved reference compounds with their CID Nummers

S/N	Name	CID
32	Prothioconazole	6451142
33	Prochloraz	73665
34	Bromuconazole	3444
35	Thiophanate-Methyl	3032791
36	Azoxystrobin	3034285

Table 3: Docking Scores of Hit and Reference compounds

Hit Compounds	**Docking score (Kcal/ mol)**	**Hit Compounds**	**Docking score (Kcal/mol)**
	7t69		**8ebb**
Kaempferol 3-Gentiobioside	-9.257	Kaempferol 3-Gentiobioside	-8.772
Kaempferol 3-O-Beta-D-Glucofuranoside	-8.373	Thermopsoside	-6.693
Astragalin	-7.919	Kaempferol 3-O-Beta-D-Glucofuranoside	-6.565
Thermopsoside	-7.162	Astragalin	-6.398
Rhamnetin	-5.318	Quercetin	-5.403
Reference Compounds	Docking score (Kcal/ mol)	Reference Com-pounds	Docking score (Kcal/mol)
	7t69	8ebb	
Prothioconazole	-4.069	Prothioconazole	-4.069
Prochloraz	-3.176	Prochloraz	-1.946
Bromuconazole	-2.599	Bromuconazole	-2.505
Thiophanate-Methyl	-2.089	Thiophanate-Methyl	-3.391
Azoxystrobin	-1.309	Azoxystrobin	-1.347

interactions profile showed a positive correlation to its relative high binding score. Kaempferol 3-O-beta-D-glucofuranoside (-8.373) bonded to GLU 213, GLN 166, THR 281, PRO 186, and ARG 142 via hydrogen interactions. Protein- astragaline hydrogen bond interactions occur at GLU 213, GLN 166, ARG 142, PRO 186. Next to astragaline is thermopsoside -7.162 kcal/mol), with hydrogen bond interaction at GLN 166, ASP 117, SER 282, and a strong one at GLU 213. For rhamnetin (-5.318), hydrogen bond interactions occur at ARG 142, GLN 166, THR 281, and SER 282. Strong hydrogen interaction with GLU 213 was a common trend with the flavonoids except rhamnetin, indicating that GLU 213 of the SIX1 (Avr3) is a very active site for hydrogen interaction with ligands. Compounds with the strongest receptor-ligand interaction should normally be the most effective, however, other pharmacokinetic factors like polarity, chemical and metabolic stability and hypophilic/hypohobic balance play crucial roles that determine the ability of the compounds to reach their target and have an acceptable interaction time.

The 8ebb protein has three chains, A, B and C, of which A and B are homologous with removed pro-domain. Chain A was removed since it was shorter chain B. Chain C which was actually a pro-domain was also removed in the protein preparation because it was in isolation from B after removing A. The predicted probable inhibitors of the protein are similar to those predicted for the SIX1 with an exception of rhamnetin for SIX 1 and quercetin for six 6. Kaempferol 3-gentiobioside interacted via hydrogen bond with the binding domain of 8ebb at THR 189, SER 190, CYS 76**,** GLU 168, more strongly at GLN 110 and pi-pi stacking at TYR 77. An observed stronger interaction of

8ebb with thermopsoside than kaempferol 3-O-Beta-D-Glucofuranoside is a notable distinction from their interaction with 7t69 where the opposite was observed, however, 8ebb bonded with hydrogen bonds in a similar pattern with both ligands. Thermopsoside bonded more strongly at SER 190 than Kaempferol 3-O-Beta-D-Glucofuranoside, both at THR 189, GLU 168 and ARG 73. The major distinction is the interaction of the protein with thermopsoside at TYR 191 and with Kaempferol 3-O-Beta-D-Glucofuranoside at ARG 83. A stronger hydrogen bond interactions of astragalin than Kaempferol 3-O-Beta -D-Glucofuranoside at SER 190 is the only observed difference between the two. For quercetin, hydrogen bond interaction was observed with CYS 76, GLN 110, GLU 168, TYR 191, and unlike other hit compounds, formed bonds with PRO 166 and ASN 165. Prothioconazole, a known fungicide with the best binding score among the reference compounds has the highest docking score (-4.069) compared with the hit compounds. Like other triazole compounds with antifungal properties, it formed a hydrogen bond with SIX 1 protein at MET 111, and with SIX 6 at CYS 76, SER 190 and GLU 168 via its heterocyclic ring. By implication, the six predicted hit compounds in this study can inhibit the virulent proteins better than prothioconazole and possibly exert antifungal activity by binding to the virulence protein to inhibit their actions against the defense mechanisms of the host plants as predicted by other studies (Michielse, *et al.*, 2009).

3.3 Binding free energy

The binding free energy (ΔG) is a measure of spontaneity and thus feasibility of a reaction. Kaempferol 3-O-Beta-D-Glucofuranoside exhibited the lowest binding energy to the SIX1(Avr3) and with ΔG value of -50 kcal/mol, followed by Kaempferol 3-Gentiobioside with -37.77 kcal/mol, at the same level of pose. This implies that the reactions of the binding pockets of the protein with Kaempferol 3-O-Beta-D-Glucofuranoside (-8.402) are predictably more spontaneous than with Kaempferol 3-Gentiobioside (-9.286). The ΔG values of -37.34, -37.57, and -33.58 Kcal/mol were obtained for astragalin, thermopsoside, and rhamnetin respectively. Relative to the binding affinity of prothioconazole with ΔG value of -16.55, all the hit compounds in this study demonstrated better binding pose with outstanding docking scores and binding energies. For 8ebb protein, the ΔG value for the complexes in increasing order is kaempferol 3-gentiobioside < thermopsoside < kaempferol 3-o-beta-d-glucofuranoside < astragalin < quercetin. The ΔG values for the reference antifungal compounds ranged from -42.84 to -9.57 Kcal/mol. It's interesting to note that hit chemicals in this study are all derivatives of flavonoids. The polyphenolic chemicals known as flavonoids are further classified in to anthocyanines, isoflavones, chalcones, flavones, flavonols, flavonones, and flavononols. In addition to having distinct biochemical activities, these subgroups are also unique in the plants in which they are distributed (Havsteen, 2002, Alzand, and Mohamed, 2012).
Scientific literature has demonstrated the antimicrobial properties of flavonoids derived from a variety of medicinal plants, as well as the synergy between these properties and the antimicrobial activities of multiple groups of flavonoids (Cushnie and Lamb, 2005). Additionally, their potential as food product biopreservatives has been documented (Tzanova *et al.,* 2020).

Conclusion

The antimicrobial properties of the six flavonoids; kaempferol 3-gentiobioside, kaempferol 3-o-beta-d-glucofuranoside, astragalin, thermopsoside, rhamnetin and quercetin, predicted as hit compounds with potential antifungi property against *F. oxysporum f. sp. lycopersici* had been established by literatures. The specific binding interactions between the implicated proteins in the pathogenesis of fusarium wilt disease and *S,alata* phytocompounds revealed in this study will provide some insights in the quest for the development of more effective biofungicides for the control of the plant disease. This research on the interactions between phytochemicals in *S.alata* extracts and *F. oxysporum f. sp. lycopersici*, will aid in the creation of innovative management and engineering techniques to tackle the fungi.

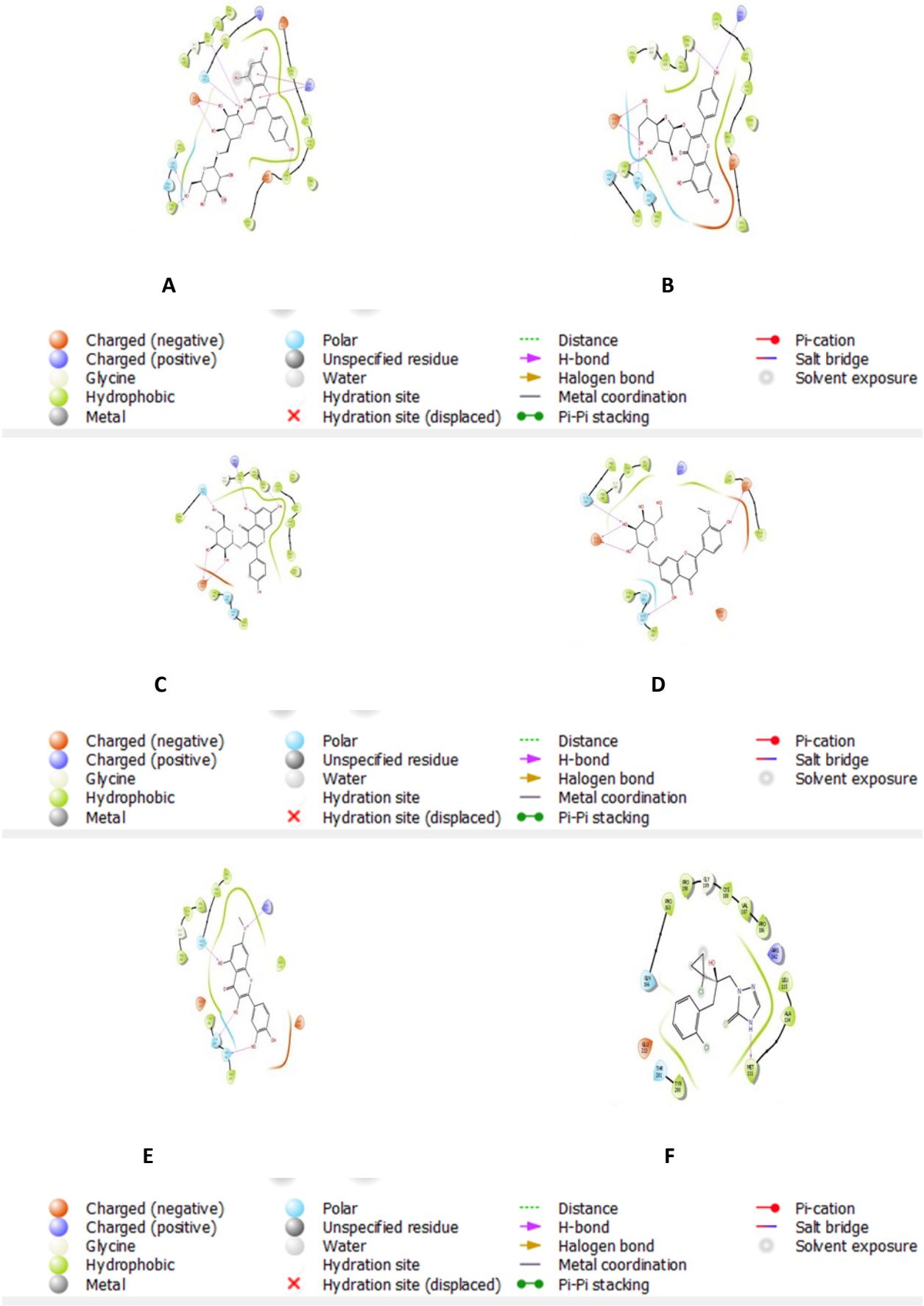

Figure 1: The 2D Amino acid interaction of Ligands with the active site of SIX1(7t69). A = Kaempferol 3-Gentiobioside- 7t69 complex, B = Kaempferol 3-O-Beta-D-Glucofuranoside-7t69_complex, C = Astragaline-7t69 complex, D= Thermopsoside-7t69 complex, E = Rhamnetin-7t69 complex, F= Prothioconazole-7t69 complex

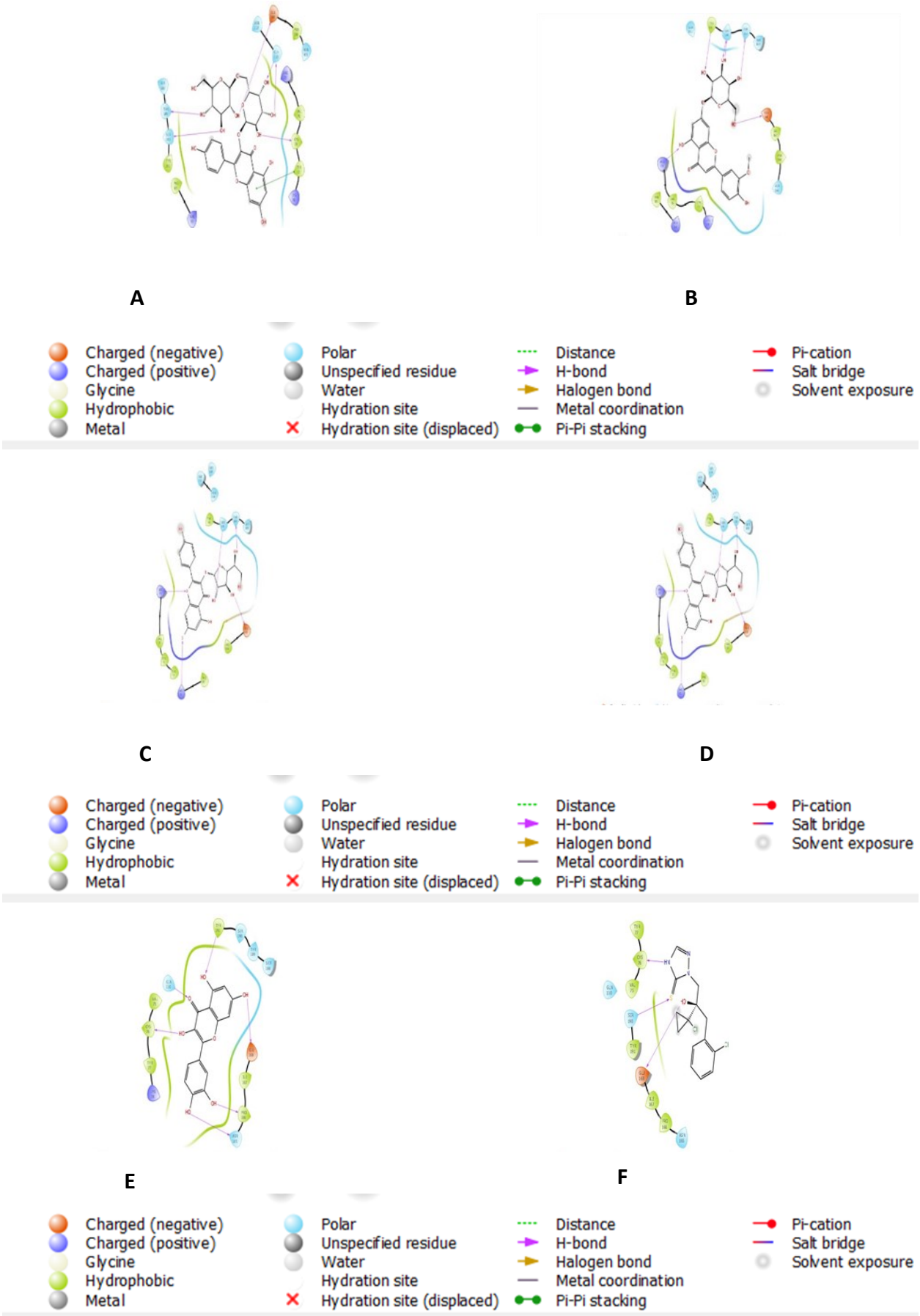

Figure 2: The 2D Amino acid interaction of Ligands with the active site of SIX6. A = Kaempferol 3-Gentiobioside - 8ebb complex, B = Thermopsoside-8ebb complex C = Kaempferol 3-O-Beta-D-Glucofuranoside-8ebb_complex, D = Astragaline-8ebb complex, E = quercetin-8ebb complex, F = Prothioconazole-8ebb complex

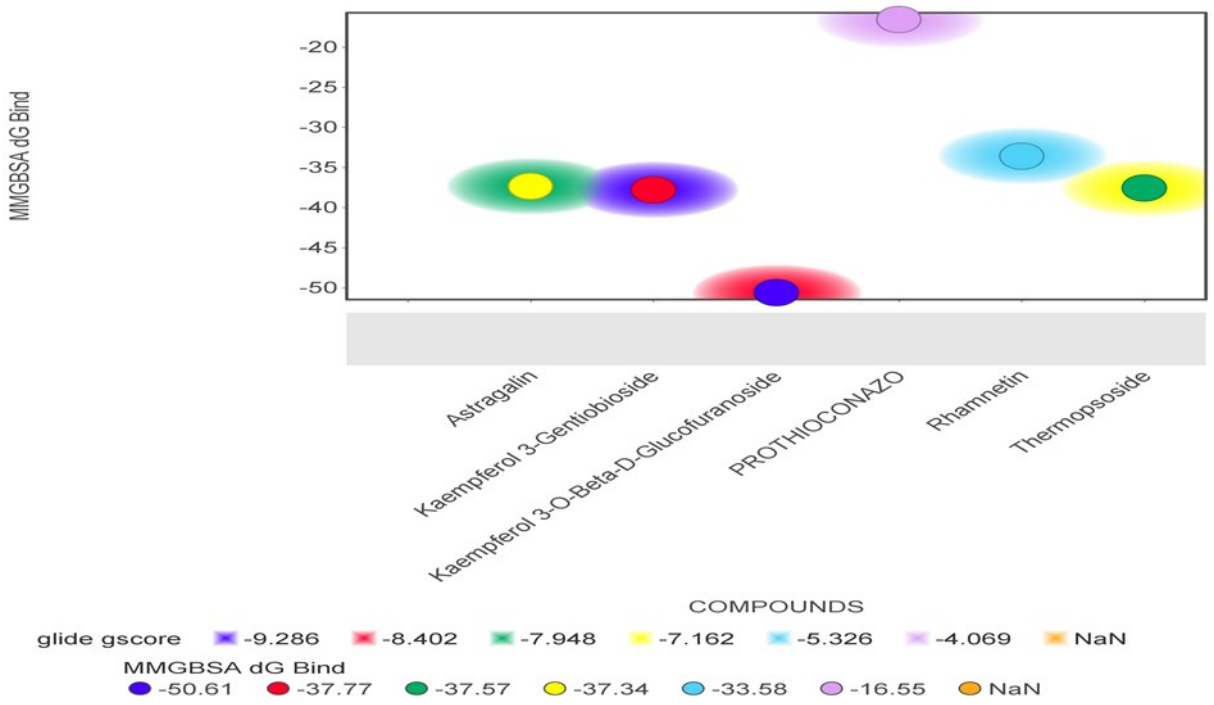

Figure 3: Comparative binding affinities of the hit compounds of *S.alata extracts* on SIX1 (Avr3) protein of *F. oxysporum f. sp.*

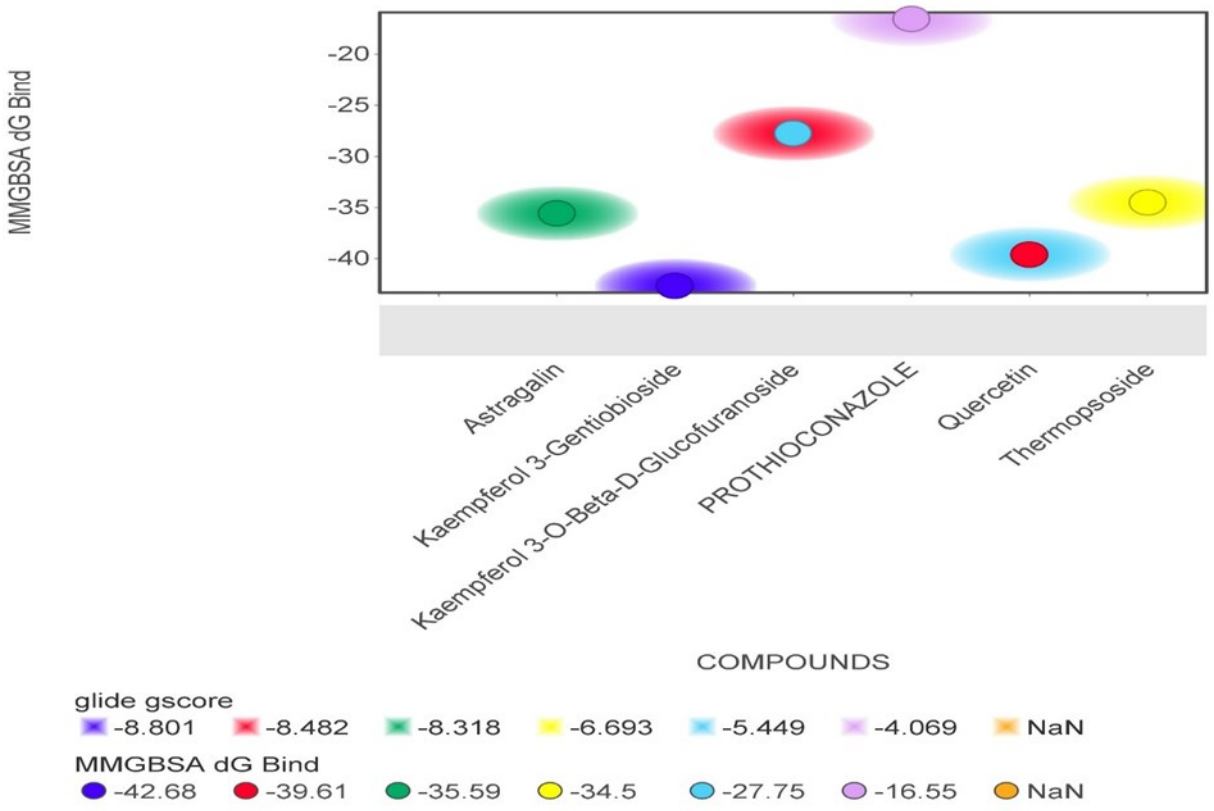

Figure 4: Comparative binding affinities of the hit compounds of *S.alata extracts* on SIX 6 protein of *F. oxysporum f. sp.*

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