

**THEORETICAL APPROACH ON TARGETING PLANT FUNGAL PATHOGENIC PROTEINS AGAINST NATURALLY ISOLATED COMPOUNDS FROM *CHITINIPHILUS SHINANONENSIS***KRITHIKA S<sup>1</sup>, CHELLARAM C<sup>2,3\*</sup>

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**ABSTRACT**

**Objective:** The objective of this study was to find the potency and bioefficacy of Asiatic acid and triterpene against four different plant fungal pathogens using a structure-based drug designing approach.

**Methods:** The pathogenic fungus which causes a dreadful effect on plants is reviewed from literature study, and its three-dimensional structures are retrieved from the protein data bank database. On the other hand, ligands are prepared. Finally, prepared fungal drug targets are docked with naturally isolated compounds using AutoDock tools.

**Results:** Both compounds Asiatic acid and triterpene structures are complementary to bind at the active site of four different drug targets. Comparatively, it is more favorable for Avr2 effector protein from *Fusarium oxysporum* with Ki value of 126.60  $\mu$ M, 1.76  $\mu$ M, and dock score value of -5.32 kcal/mol and -7.85 kcal/mol for Asiatic acid and triterpene, respectively. Thus, interaction analysis was carried out only for these protein-ligand complexes.

**Conclusion:** The computational biology study states that these two compounds can be the lead candidate for treating disease caused by plant fungal pathogen *F. oxysporum*. However, further study has to be done *in vitro* and *in vivo* to prove its same efficacy.

**Keywords:** Fungal pathogen, Bioefficacy, Structure-based drug designing, AutoDock tools, Protein-ligand, Computational biology, Docking.

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**INTRODUCTION**

The agriculture is the major backbone for all the countries from the ancient period to the contemporary century also; the country's global economy is majorly deepened on farming. Hence, people are now turned up for organic farming to preserve the genetics of crops and human health. However, the enormous losses in the yield and quality of fruits, filed crops, and plant edible substance are majorly caused by fungal plant pathogens [1]. To bolster the argument American State of Ohio done an investigation on plant pathogenic fungi for several years and revealed that 1000 plant diseases are caused by fungi, which brings the most rigorous loss for farmers [2]. They are some of the fungal species which predominately affect the plants are *Magnaporthe oryzae*, *Botrytis cinerea*, *Fusarium oxysporum*, *Blumeria graminis*, *Colletotrichum* spp., *Ustilago maydis*, and *Puccinia* spp. Mostly pathogenic fungi belong to the class of basidiomycetes and ascomycetes [3]. Besides, these fungi have different lifestyles and relation with host plants; some are biotrophic, necrotrophic, hemibiotrophic, and obligately biotrophic. Biotrophic plant fungal pathogen will have long-term relation on feeding without destroying the plant. Whereas, necrotrophic pathogens actively kill host tissue as they colonize and thrive on the contents of dead or dying cells [2-4].

In this current study, we are targeting four fungal pathogenic proteins that cause major economies deterioration. *Colletotrichum lindemuthianum* is a common and important genus of a fungal plant pathogen. It is the most susceptible species throughout the world; at least one or two species will affect every crop and causes major losses to the economy; commonly it will affect fruits such as bananas, and vegetable crops such as sorghum and cassava and ornamentals [5]. *C. lindemuthianum* is one of the most important seed-borne diseases of common bean (*Phaseolus vulgaris* L.) commonly it was known as

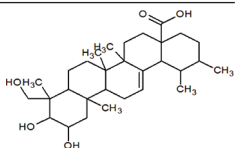
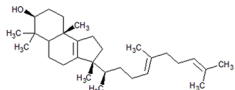
bean anthracnose [6] in the world found extensively in tropical and subtropical regions. *C. lindemuthianum*, the fungal pathogen secretes an enzyme endo-chitin de-N-acetylase to modify exposed hyphal chitin during penetration and infection of plants [7-9]. Thus, chitin deacetylase was considered as an attractive target for diseases caused by this organism.

Second, *F. oxysporum* is a ubiquitous soil-borne pathogen; the symptom of this fungal disease includes stunting, defoliation, vascular browning, progressive wilting, leaf epinasty, and plant death [10,11]. This genus is turning worldwide attention because most of this species will affect more than 100 plants provoking rigorous losses in crops such as cotton, banana, tomato, and melon [11]. *F. oxysporum* secretes important virulence factor Avr2 in xylem and manipulates their hosts in the process of either suppress or counteract host defenses [12]. Tomato is a common host for *F. oxysporum*, which invades the plant through roots followed by epidermal and endodermal tissues to eventually colonize the xylem vessels and destroys the crop [13,14].

Finally, the other two fungal plant pathogens are *M. oryzae* and *U. maydis*. Rice blast disease is commonly caused by *M. oryzae*. It is the most devastating rice pathogen that will lead to major crop loss worldwide, and it is the topmost fungal pathogen based on economic calamity. *M. oryzae* hypersensitive protein 2 (MoHrip2) is an elicitor protein that induces resistance in rice leaves, so before farming seedling is treated with MoHrip2 will result in significant loss to the rice plants [15-18].

*U. maydis* is a major causative organism for maize smut, which produces cytokines, a hormone responsible for plant development overexpression of the hormones forms a tumor in plant tissue. Serine/threonine protein kinase are the enzyme involved in complex

**Table 1: Isolated natural compounds and its basic chemical properties**

Structure	Number of atoms	Molecular composition	Molecular weight
	83	C30 H48 O5	488.714
Asiatic acid			
	81	C30 H50 O	426.73
Triterpene			

**Table 2: Energy minimization of plant fungal pathogen proteins**

Drug targets	Before energy minimization KJ/mol	After energy minimization KJ/mol
Chitin deacetylase	-6682.23	-11,763.5
Avr2 effector protein	-3567.31	-5534.31
Elicitor <i>Magnaporthe oryzae</i> hypersensitive protein 2	-3072.02	-6371.3
Glycogen synthase kinase 3	-10,624.3	-16,501.1

**Table 3: Free energy of binding of naturally isolated compounds**

Plant fungal pathogen targets	Free energy of binding (kcal/mol)	
	Asiatic acid	Triterpene
Chitin deacetylase	1.13E+03	-0.12
Avr2 effector protein	-5.32	-7.85
Elicitor <i>Magnaporthe oryzae</i> hypersensitive protein 2	-1.91	-1
Glycogen synthase kinase 3	-0.67	66.96

cellular process in all living organisms including signaling pathway, differentiation and proliferation. Glycogen synthase kinase 3 (GSK3) belong to family of serine/threonine protein kinase present in plant fungal pathogen organism *Ustilago maydis* plays major role in diseases molecular pathogenesis in maize was chosen as attractive drug target enzyme [19-21]. Overall, study was mainly concerned towards targeting various plant fungal pathogen treatment using natural isolate compounds from microbe *Chitinophilus shinanonensis* instead of synthetic chemicals.

**METHODS**

**Drug target protein retrieval from protein data bank (PDB)**

The fungal pathogen organisms which play a crucial role in devastating plants were explored through a literature study followed by identifying drug target proteins of the specific fungal pathogen in structural databases. The three-dimensional (3D) structure of the drug target proteins was retrieved from PDB (<http://www.rcsb.org>); this database contains more than 30,000 proteins structure in nuclear magnetic resonance and X-ray. In this study, the 3D structure of chitin deacetylase from the fungal pathogen *C. lindemuthianum* (2IW0), Avr2 effector protein from *F. oxysporum* (5OD4), elicitor MoHrip2 from *M. oryzae* (5FID), and GSK3 from *U. maydis* (4E7W) were retrieved from protein databank was probed for *insilico* binding interaction.

**Preparation of receptor for docking**

Structures of each receptor are prepared manually using Discovery Studio Visualizer 4.5 by removing alternative conformers of amino acids, bound HETATMS or ligands, unbound water molecules, and identical chains subsequently energy minimization was done with steepest descent algorithm 100 cycles in Swiss DeepView project to remove the bad steric clashes.

**Ligand preparation for docking**

The ligand or compounds in the study was isolated from the source of microorganism *C. shinanonensis* the two natural compounds (Table 1), and its basic chemical properties are calculated as follows. The ligands were initially drawn using ChemSketch tool, followed by cleaning structure, and the chemical language format is converted into PDB format for the docking.

**Molecular docking**

Molecular docking is an effective tool in theoretical structural biology and computer-aided drug design [22]. The objective of protein-ligand or protein-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known 3D structure. The docking methods search high dimensional spaces effectively and use a scoring function that correctly ranks candidate docking of different drug target proteins of various plant fungal pathogens are consider as receptors, and naturally isolated compounds are ligands using AutoDock 4.2. A concept of lock key was employed in structure-based drug designing; docking was carried out with genetic algorithm with a population size of 150 and 2,500,000 maximum number of energy evaluations, 27,000 maximum number of generations with 10 runs before docking, AutoDock wants AutoGrid needs to get processed for atomic positions. Hence, the AutoGrid calculations are performed placing the box (XYZ) in 3D directions, the dimension of the grid will differ from one protein to other drug targets. Chitin deacetylase (2IW0) box is placed in 13.364 X, 12.584 Y, and 11.392 Z with 0.405 Å and Avr2 effector protein (5OD4) dimensions are 6.609 X, 11.938 Y, and 66.96 Z with 0.425 Å. Similarly, elicitor MoHrip2 (5FID) dimensions of box 6.688 X, 11.841 Y, and 67.029 Z with 0.375 Å and GSK3 (4E7W) dimensions include 16.58 X, -62.67 Y, and 20.35 Z with 0.375 Å. The best run in the AutoDock with negative binding energy is considered the best results [23]. The interaction results are visualized using discovery studio 2017 visualizer.

**RESULTS AND DISCUSSION**

**Receptor protein loop building**

The drug target proteins taken in this study belong to various molecular functions and biological activity. GSK3 and chitin deacetylase fall into protein kinase activity and catalytic activity, respectively. While elicitor MoHrip2 is toxin protein, whereas the function of Avr2 effector protein is considered as putative. Protein structures have a lack of amino acid residues in loop regions that are identified through SEQRES data of PDB, and it was automatically processed using protein preparation tools. It was observed that there is no changes loop region for other three-drug target proteins except 5OD4 structure loop regions amino acid is inserted form position of ARG90 to GLY93, built loops are minimized for structure stability. Finally, drug target proteins without any loop and side-chain flaws are taken for further proceedings [24] (Fig. 1).

**Protein structure stability analysis**

The energy of protein is important in theoretical biology any proteins with positive energy or least negative energy regarded as non-stable, so the stability of proteins was achieved through energy minimization technique. Table 2 shows the energy minimization of drug target proteins before and after 100 cycles of standard deviation minimization. The steepest descent method is typically used at initial stages for slow convergence of the protein. As a default, the structures retrieved from PDB database will have the least energy further subjected to minimum steps of minimization will increase its stability and local minima energy value.

Table 4: Receptor-ligand interaction of Asiatic acid – Avr2 effector complex

Active site amino acid and compound (atom-atom bonding)	Distance in Å	Category	Type of bond
A:THR54:HG1-:Asiatic acid:O1	1.71288	Hydrogen Bond	CHB*
Asiatic acid:H78-A:SER55:O	2.81559	Hydrogen Bond	CHB*
Asiatic acid:C30-A:GLU74:OE1	3.26396	Hydrogen Bond	CAHB <sup>#</sup>
A:PRO71-:Asiatic acid	5.4319	Hydrophobic	Alkyl
A:PRO72-:Asiatic acid	5.39586	Hydrophobic	Alkyl
A:ILE85-:Asiatic acid	5.36522	Hydrophobic	Alkyl
A:ILE85-:Asiatic acid	5.15608	Hydrophobic	Alkyl
Asiatic acid:C26-A:ARG84	4.85148	Hydrophobic	Alkyl
Asiatic acid:C26-A:ILE85	5.20135	Hydrophobic	Alkyl
Asiatic acid:C35-A:ARG65	4.35983	Hydrophobic	Alkyl

\*CHB: Conventional hydrogen bond, <sup>#</sup>CAHB: Carbon hydrogen bond

Table 5: Receptor-ligand interaction of triterpene – Avr2 effector complex

Active site amino acid and compound (atom-atom bonding)	Distance in Å	Category	Type of bond
A:HIS83:HE2-:Triterpene:O29	1.82383	Hydrogen bond	CHB*
Triterpene:C27-A:PHE56	3.96694	Hydrophobic	Pi-Sigma
A:PRO72-:Triterpene	4.59219	Hydrophobic	Alkyl
A:ARG84-:Triterpene	5.28147	Hydrophobic	Alkyl
A:ILE85-:Triterpene	4.65986	Hydrophobic	Alkyl
:Triterpene:C12-A:ILE85	3.84799	Hydrophobic	Alkyl
:Triterpene:C30-A:PRO71	4.59704	Hydrophobic	Alkyl
:Triterpene:C30-A:ARG84	4.06396	Hydrophobic	Alkyl
:Triterpene:C31-A:ILE85	4.66029	Hydrophobic	Alkyl
:Triterpene:C20-A:PRO71	4.5908	Hydrophobic	Alkyl
:Triterpene:C20-A:PRO72	4.13047	Hydrophobic	Alkyl
:Triterpene:C27-A:ILE85	3.92151	Hydrophobic	Alkyl
A:PHE56-:Triterpene:C12	5.04537	Hydrophobic	Pi-Alkyl
A:PHE56-:Triterpene:C28	5.02283	Hydrophobic	Pi-Alkyl
A:HIS83-:Triterpene:C31	3.67345	Hydrophobic	Pi-Alkyl
A:TYR86-:Triterpene:C30	5.43767	Hydrophobic	Pi-Alkyl

\*CHB: Conventional hydrogen bond

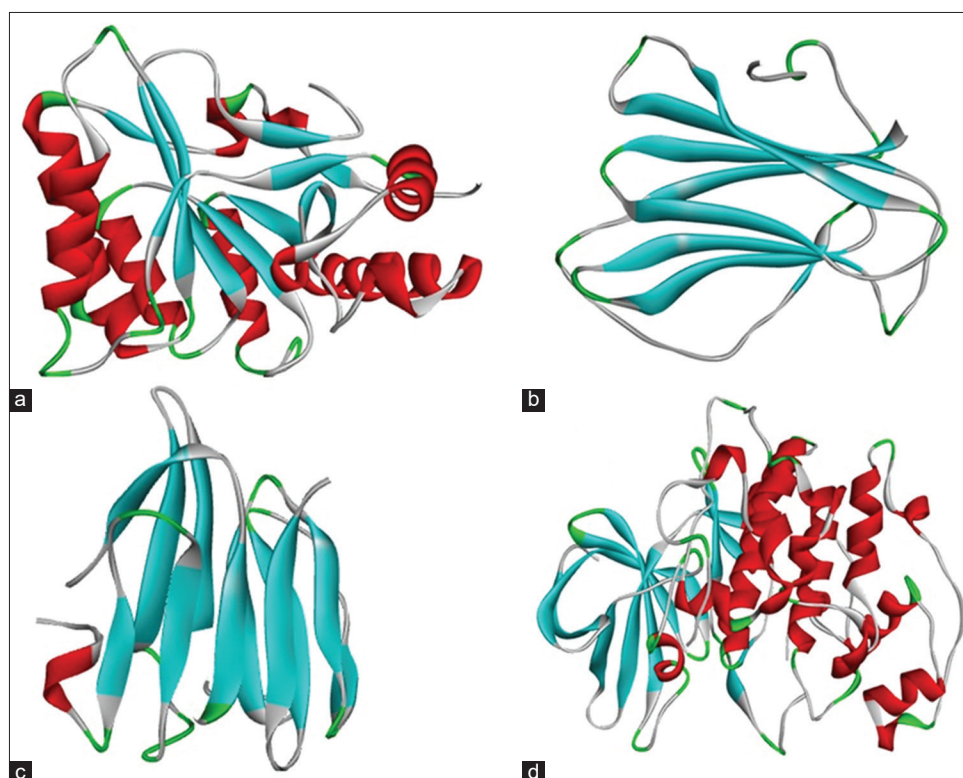


Fig. 1: (a) Chitin deacetylase, (b) Avr2 effector protein, (c) Elicitor *Magnaporthe oryzae* hypersensitive protein 2, (d) glycogen synthase kinase 3, prepared plant fungal pathogen proteins for docking studies

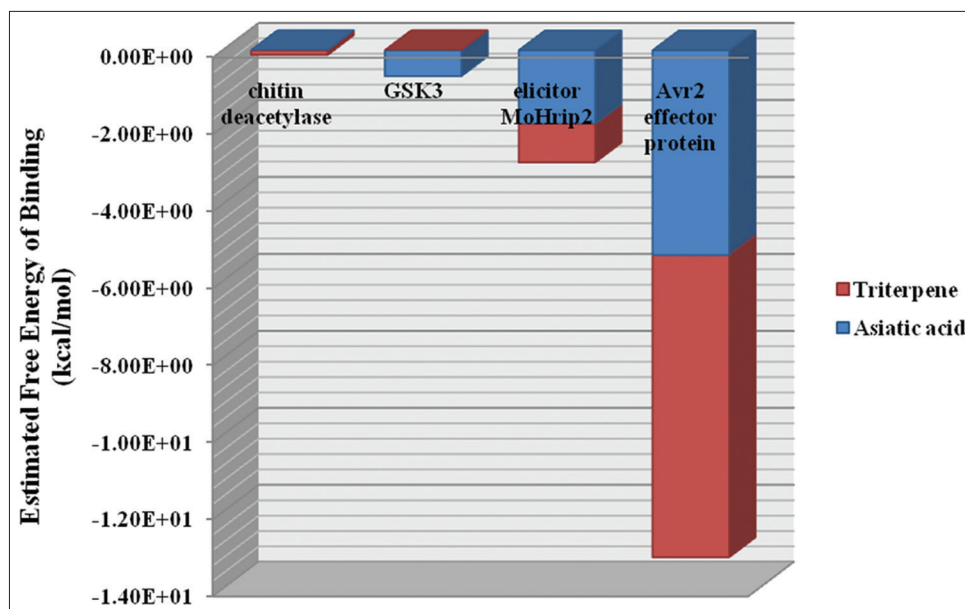


Fig. 2: Comparative graphical representation of binding energy

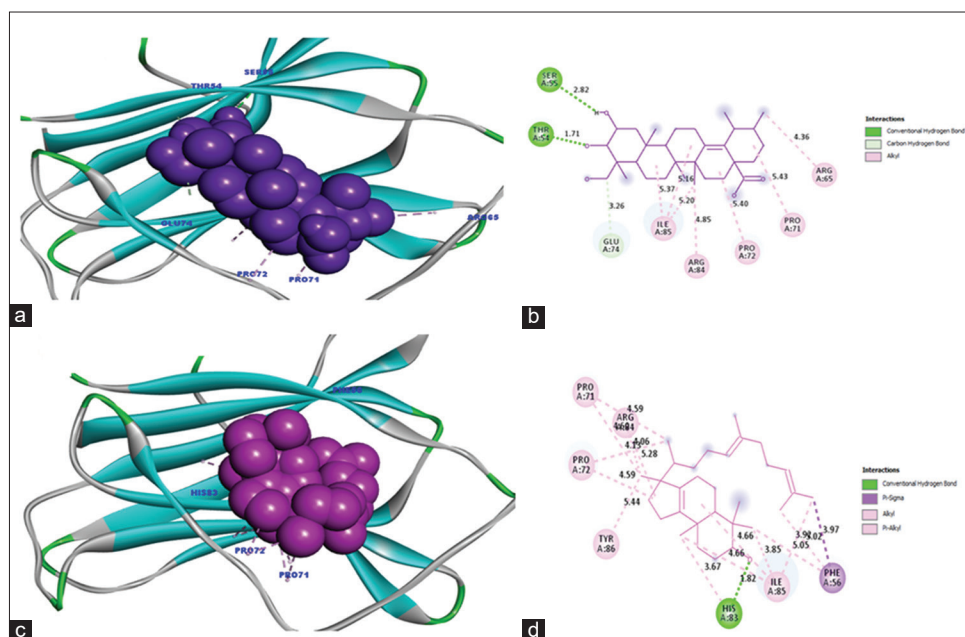


Fig. 3: (a and b) Active site amino acid and Asiatic acid binding interaction, (c and d) active site amino acid and triterpene binding interaction

**Comparative analysis of compounds with different plant fungal pathogens**

Asiatic acid and triterpene compounds docked with four different fungal pathogens show a difference in binding energy value. Overall, only two positive binding energies were observed for Asiatic acid bound to chitin deacetylase (1.13E+03) and GSK3 with triterpene (66.96). However, both compounds show the least stable binding energy and strong bonding interaction with Avr2 effector protein followed by negative binding energy for drug target elicitor MoHrip2 (Table 3).

To depict a better representation of the graph, positive free-energy binding values are not considered during plotting (Fig. 2). Biological significantly these two compounds show good potency on targeting Avr2 effector protein. This protein will be secreted in tomato plants during infection caused by *F. oxysporum*.

This fungus predominately infects a wide variety of plant species by results; it forms wilt or root. Moreover, this fungus colonizes in the region xylem tissue of tomato plant, an important part of the plants where the mechanism of transporting nutrients, water from the roots to the leaves will take place [12]. Thus, treating the Asiatic acid and triterpene will inhibit the xylem colonization and improves biological transport. However, *in vivo* and *in vitro* studies are recommended to study the same mechanism based on theoretical docking.

**Interaction analysis**

Analyzing the protein-ligand complex was done only for compounds with high estimated free binding energy. Among all drug targets, Avr2 effector protein binding site favors more complementary for both compounds Asiatic acid and triterpene. Hence, only this interaction is elaborately discussed. The biological or physiological process is regulated by protein-protein and protein/receptor-ligand interactions, this mechanism was observed in all living cells on,



compound binding to the active site will exchange atoms to make the bond stronger [22,25].

Receptor-ligand complex with more hydrogen and hydrophobic bonds will have good stability as compared electrostatic and Van der Waals bonding. Asiatic acid – Avr2 effector complex shows maximum count of interaction with amino acids such as Thr, Ser, Glu, Pro, Arg, and Ile residues (Table 4) present at the active site of the drug target protein and it forms a carbon-hydrogen bond and conventional hydrogen bond along with alkyl interaction (Fig. 3).

Based on interaction inhibition (Ki) constant of Asiatic acid is estimated as 126.60  $\mu$ M. Similarly, triterpene interacts with amino acid His, Phe, Tyr, Pro, Arg, and Ile forms alkyl, pi-alkyl, pi-sigma, and conventional hydrogen bond with Ki value of 1.76  $\mu$ M (Table 5). Hence, these compounds can be the lead candidate for treating Avr2 effector protein of *F. oxysporum*.

## CONCLUSION

Receptor-ligand docking through computational biology study is a powerful tool to screen compounds based on energy and interaction. This will save time and energy in research also; it assists one to investigate atom-level interaction between two molecules. To improve compounds efficacy, structure-activity relationship studies and fragment-based drug designing are recommended. Hence, among four different fungal pathogens, isolated compounds are more favorable for treating Avr2 effector protein of *F. oxysporum*. Unlike, synthetic compounds which causes resistance and affect the plant genetic diversity the natural isolated compound from *Chitinophilus shinanonensis* treating plant fungal pathogen are free from lethal effects. Furthermore, molecular interaction bolsters the study, but further investigation in *in vivo* and *in vitro* is necessary to prove the same.

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## AUTHORS' CONTRIBUTIONS

Both authors are equally contributed to this work.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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