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Virtual screening of rice phytochemical scaffolds targeting peptide deformylase of *Xanthomonas Oryzae* pv. *Oryzae* for the control of bacterial leaf blight

Kailash Pati Bhandari[1#], Samayaditya Singh[2#], Prajna Priyadarshini Das[1], Komal Bhati[1], Komararagiri Rajesh Babu[1], Pramod R Somvanshi[2]* & Irfan Ahmad Ghazi[1]*

[1]Department of Plant Sciences; & [2]Department of Systems and Computational Biology, University of Hyderabad, Hyderabad-500 046, Telangana, India

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Bacterial leaf blight (BLB) disease of rice, incited by bacterium *Xanthomonas Oryzae* pv. *Oryzae* (*Xoo*) causes huge loss of rice production. For reviving rice production, various bio-hazardous and eco-unfriendly chemical fertilizers, synthetic pesticides and antibiotics have been employed. Thus, the discovery of safe natural pesticides is an effective approach for managing rice BLB disease. In this study, 235 rice phytochemicals were screened to identify most effective bio-pesticide like molecules against Peptide Deformylase of *Xoo* (XoPDF), which actively participates in the translation process. Through molecular docking and simulation studies, nine potential lead compounds were screened with reference to Actinonin (a known inhibitor). Structural analysis *via* molecular dynamics suggests better structural stability and binding affinity of XoPDF with the screened leads in relative to Actinonin. Notably, nine leads exhibit structural backbone similarity with the subclass of anthocyanins (natural antibacterial polyphenols) and flavones *viz*., pelargonin, isorhamnetin, tricin, peonidin, syringetin, cyanidin, chrysoeriol and isoscoparin. In essence, these lead compounds can serve as potential hits for drug design against BLB disease by targeting XoPDF, thereby limiting *Xoo* survival and alleviating rice BLB. Since, peptide deformylase (PDF) is found in many other plant pathogens, it can be used as a broad - spectrum disease resistance drug.

Keywords: Actinonin, Bio-pesticides, Broad spectrum disease resistance, Molecular docking & simulation

Rice (*Oryza sativa* L.) is popularly known as a model for cereal biology. Rice is a global staple food because it is a source for more than 75% of calories in some economically developing nations[1]. Rice contributes 21% and 15% of human per capita energy and protein worldwide, respectively , thus playing a major role in food and nutritional security. Asia has been playing a major role in ensuring global food security because it alone contributes 90.6% of world's rice production[2]. The Sustainable Development Goals (SDGs), the United Nation's principal plan to eradicate poverty, hunger, and other related issues, are not going to be accomplished by 2030. Rather, it is projected that approximately 660 million people could still be in hunger in the near future or by 2030. Further need for feed nine billion people by the year 2050 in spite of environmental changes brought on by climate change has made it as a necessity to increase yield[3]. In addition to that, pathogens *viz*., bacteria, fungi, nematodes, insect pests and viruses are major threats to rice production. During the rice growth and development stage it affects 30% global yield loss per hotspot[4].

Our targeted pathogen, *Xanthomonas Oryzae* pv. *Oryzae* that causes BLB disease, annually affecting 30 – 50 % yield loss[5-7]. *Xoo* is a gram negative proteobacterium[8], considered as a destructive threat in all rice growing ecologies. In tropical countries and subtropical regions BLB disease is even more destructive. The BLB disease incidence is rising day by day in Kaller belt, a well-known area known for quality rice production such as Basmati rice. But the recent report indicated that all the Basmati varieties are becoming susceptible to the pathogen. In almost all the states of India, 60 – 80 % yield loss has been observed because of severe infestation[32].

The introgression of single R-gene is not enough to defeat the bacterial blight pathogen because of wide genetic diversity among the *Xoo* isolates present in different geographical region of India[9]. Therefore, this current study suggests that the higher variability in *Xoo* population and a frequent emergence of new virulent races cause a high selection pressure,

#Equally contributed
*Correspondence:
E-mail: irfan@uohyd.ac.in (IAG); pramodrs@uohyd.ac.in (PRS)
Suppl. data available on respective page of NOPR

resulting in resistant varieties often losing resistance within a few years of cultivation and becoming susceptible. Till date a total of 48 R-genes (from *Xa*1 to *Xa*48) have been reported from different wild and cultivated rice across the globe[10-12], out of which some R-genes have been cloned and few of them have been physically mapped. But, integration of broad spectrum and durable resistance genes has become a key issue among rice breeders[13]. The three main problems with breeders to make rice resistant against pathogens have been nicely discussed by Ning with his group[14]. Above all the facts suggested as well as demanding the effective control of *Xanthomonas* is extremely important for sustainable rice production.

Further several experimental studies evaluated the antimicrobial potential of chemicals and antibiotics against *Xanthomonas* – such as inorganic bactericide, antibiotic, triazoles, metallic compound biocides (organosulfurs, nitrogen compounds etc.) and Tetramycin, Brestanol, Agromycin 500 and Agrimycin 100 + Fytolan[15,16]. Recently, *in-vitro* study was performed to check the effectiveness of six antibiotics (Ampicillin, Benzylpenecilin, Kanalmycin, Streptomycin, Chloramphenicol and Sinobionic) with four different concentrations against five virulent *Xanthomonas* isolates by Khan and group[17]. They have found the antimicrobial capability of six antibiotics varied among the *Xoo* isolates. Thus, there is a need for exploration of potential natural lead compounds which can attenuate the pathogenicity of *Xoo*. To find out eco-friendly, potential and effective inhibitors of *Xoo,* we have conducted virtual screening of 235 rice phytochemicals to target sole PDF enzyme, which is popularly known for its involvement in protein synthesis pathway. Normally protein synthesis is initiated by incorporation of N-formyl methionine (N-fMet), which is directly bound to the metal ions in the active site for hydrolysis. It functions when it is frequently removed from the nascent protein chain via two consecutive cleavage reactions by peptide deformylase and methionine aminopeptidase[18]. PDF is a remarkable enzyme in bacteria that cuts off the formyl group[19], which is very important for bacterial growth[20]. Because this enzyme is crucial for bacteria and is absent in eukaryotes; therefore, it has become an attractive and potential target for bactericidal studies. The inhibition of bacterial PDF can disrupt the protein synthesis, maturation and eventually inhibit vital cellular processes, leading to bacterial cell death[21,22]. Therefore, PDF can be utilized as a molecular target for the development of effective bactericides against BLB. The present investigation is concentrated on finding novel natural bactericides through molecular docking and simulation studies. Since PDF is found in all other plant pathogens therefore, the targeting of this peptide may result as a broad-spectrum resistance. Through this approach, the attenuation of bacterial pathogenicity and to overcome BLB disease through such identified natural bactericides will enable better crop protection and assists in meeting Rice's growing demand.

Material and Methods

Preparation of phytochemical library and ligands

In this study, we have selected 235 phytochemicals from different part of rice such as leaves, stems, root exudate, bran, husk, endosperm, embryo, grains, seedlings, rice flour, kernels, coleoptiles, stems, seeds, hulls and even some others were identified in whole rice plants through searching from last 5 decades of scientific literature. The nature of these phytochemicals and their diverse range of biological activities are tabulated in supplementary file (see Suppl. Table S1). The respective SMILES of compounds were downloaded from PubChem repository (https://pubchem.ncbi.nlm.nih.gov)[23], and afterwards imported on the OpenBabel[24] for the generation of refined 3D structure. Actinonin was selected as a reference inhibitor and added in the phytochemical library for further comparative analysis. Actinonin is a well-studied antibiotic against the bacterial peptide deformylase at the biochemical and structural level reported in many crystal structure studies on the XoPDF.

Preparation of target protein

The 3D-atomic structure of XoPDF, (PDB ID: 5CVQ) reported by Nago and group[25], was extracted from the PDB database (https://www.rcsb.org/). The water and other molecules such as ions and ligands were removed from the XoPDF structure and a clean monomeric structure was retrieved for the docking studies. The conformational data of Actinonin along with protein interaction information from 5CVQ were recorded and further used as a reference for lead screening study. Subsequently, the docking-based screening of compounds was carried out by the virtual screening protocol of AutoDock Vina[26], wherein XoPDF was considered as a receptor.

Virtual screening

The XoPDF is mainly associated with biosynthesis of proteins which is important for their growth, metabolism, and for virulence against rice. Previous studies mentioned PDF as a potential target for designing the antibacterial activity against the rice invading pathogens. Virtual screening is an indispensable technique in the field of lead identification for the drug discovery, against the proteins of plant pathogens. The technique is much more cost effective and time-efficient than high-through-put screening of large libraries of chemical and phytochemical compounds against the known protein target. In structure based virtual screening (SVS), the hits are identified based on the predefined active site of the target protein. In this study, we have used SVS technique to screen the potential phytochemicals for the actinonin binding site of XoPDF. First, the blind docking protocol of AutoDockvina was performed as the primary screening, in which whole protein was considered as a receptor (Center: 8.435, -17.177, 4.615; Size: 40, 40, 40; spacing: 1 Å) against the phytochemical library. Later, only 83 phytochemicals were selected for the active-site docking that have energetically preferred the *Xo*PDF substrate pocket repetitively during the blind docking. The actinonin binding domain (substrate pocket) of the XoPDF was used as the active site for the docking of 83 (see Suppl. Table S2) phytochemical compounds. The actinonin binding pocket of the gram-negative PDF subdivided into S1', S2', and S3' sites, wherein S1' is a hydrophobic side and near to metal binding region. The S2' and S3' mainly remain near to the opening of the pocket to facilitate the entry and hinging of ligands within the pocket. These details will further be used for screening of potential leads from the phytochemical library. The respective coordinates for the active-site boundary were defined by the grid size (23,18,28) and center (2.689, -13.43, -2.705), with a spacing of 1Å. The same boundary parameters were used for calculating the binding score of our interest of target receptor/protein and reported actinonin conformation. The enumerated binding scores of the phytochemicals were further compared against the actinonin binding score to screen out of those compounds which had higher binding affinity than the actinonin. Further, filtration was based on the similarity with actinonin interacting residues and their conformational resemblance with the crystal defined pose of actinonin. The hydrophobic and polar interactions were analyzed by PyMOL and LigPlot+ [27].

Molecular dynamics simulations

The complex stability and other dynamic environment related analysis was done by the molecular dynamic simulations (MDSs) through GROMACS v5.4.1 software[28]. Each of the simulation systems of apo and complex (protein-ligand) was solvated by the water molecules. The GROMOS force field was used to parametrize the topological parameters of XoPDF, and the water parameterization was done according to the SPC/E water model. The topological parameters for each ligand were generated by the Automated-Topology-Builder (ATB) v3.0 server[29,30] based on GROMOS forcefield. The Particle Mesh Ewald (PME) method was used to compute the electrostatic interactions with a cut-off of 1.2 nm. Further the partial charge on each system *viz*Apo (-7), XoPDF-Actinonin (-7), XoPDF-lead60 (-7), XoPDF-lead144 (-6), XoPDF-lead145 (-7), XoPDF-lead191 (-7), XoPDF-lead222 (-7), XoPDF-lead225 (-6), XoPDF-lead226 (-7), XoPDF-lead230 (-7), XoPDF-lead232 (-7) was calculated by the GROMACS, which are afterwards neutralized by the equal number of Na^+/Cl^- ions. The steepest descent algorithm (with maximum number of steps = 50000 ps) was used for energy minimization of these systems. Before production simulation, each system was brought to a desired temperature (300K) and pressure (1 bar) by performing the NVT and NPT ensemble equilibrations for 1000 ps. After equilibration, the production simulation of 100 ns was run for all systems, and simulation data was recorded for every 10 ps in a main trajectory file. Further to infer the structural changes and stability of protein-ligand based on the simulation data, the various MD parameters were enumerated by using the GROMACS algorithms like Root-Mean-Square-Deviation (RMSD), Radius of gyration (Rg), Root-Mean-Square-Fluctuation (RMSF), Hydrogen bonds and Binding energy to infer the structural changes for comparative analysis. These parameters provide insight on the average amount of local & global changes, the stability of protein-ligand interaction, and change in fluctuation of active-site residues in the protein structure during the simulation. The simulation trajectories were further implemented for the Molecular Mechanics Poisson Boltzmann Surface Area (MMPBSA) analysis by following the protocol defined by Rashmi Kumari (https://rashmikumari.github.io/g_mmpbsa/single_protein_ligand_binding_energy.html) on the GROMACS[31]. The MMPBSA

analysis aids in determining the binding energy between the protein and ligand based on the MDS data. This is helpful in comparative analysis between the phytochemical compounds and actinonin to find the higher affinity leads or backbone structures. For this study we have selected the stable 50-100 ns data of the MDS to calculate the Binding energy (BE) for each complex. Then the average contribution of the XoPDF residues in the BE was analyzed by using a Python script of Rashmi Kumari i.e., MmPbSa Decomp.py (https://github.com/RashmiKumari/g_mmpbsa/blob/master/tools/MmPbSaDecomp.py).

Results

Docking and interaction analysis of actinonin

The phytochemical library was subjected to virtual screening according to the defined methodology of SVS. The binding score for a crystal structure defined actinonin conformation was found -8.0 Kcal/mol that was considered as threshold binding energy and further used for the screening of phytochemicals that have more negative binding energy than -8.0 Kcal/mol. The interaction analysis of actinonin in 5CVQ infers the involvement of (GLU97, LEU100, PHE134, ARG137, VAL138) and (VAL45, GLY46, GLN51, GLY98, LEU100) in hydrophobic and hydrogen bond interactions,, respectively (see Fig. 1). Crystal structure-based studies stated the importance of S1' site for accommodating the hydrophobic chain of actinonin and MAS (Met-Ala-Ser) substrate, wherein the site was lined by the hydrophobic part of V45, E97, F134, R137, V138, H141 residues[25]. In previous inhibitor studies S1' site is mentioned as an important selection property for the leads, and the amide group of S2' site residues (G44, V45, E97, G98) was involved in providing the hydrogen donor and acceptor for the ligands.

Selection of compounds from docking analysis study

To gain significance on the AutoDock binding score of phytochemicals, we have performed three consecutive dockings, and considered the average of their respective docking scores. This average-scoring was further used for the rank-based screening, to filter out the top 20 compounds. Afterwards, the top 20 compounds were again screened to 9 compounds based on the similarity with the actinonin interacting residue and its conformation in the active site of XoPDF (refer to Table 1 & Fig. 2). These nine phytochemicals mainly have the backbone of anthocyanin or flavones, which are the flavonoid groups that have potential antimicrobial compounds. Afterward, the stability of these protein-compound complexes within a solvent system was verified by using the molecular dynamic simulation along with their binding energy enumerated by MMPBSA.

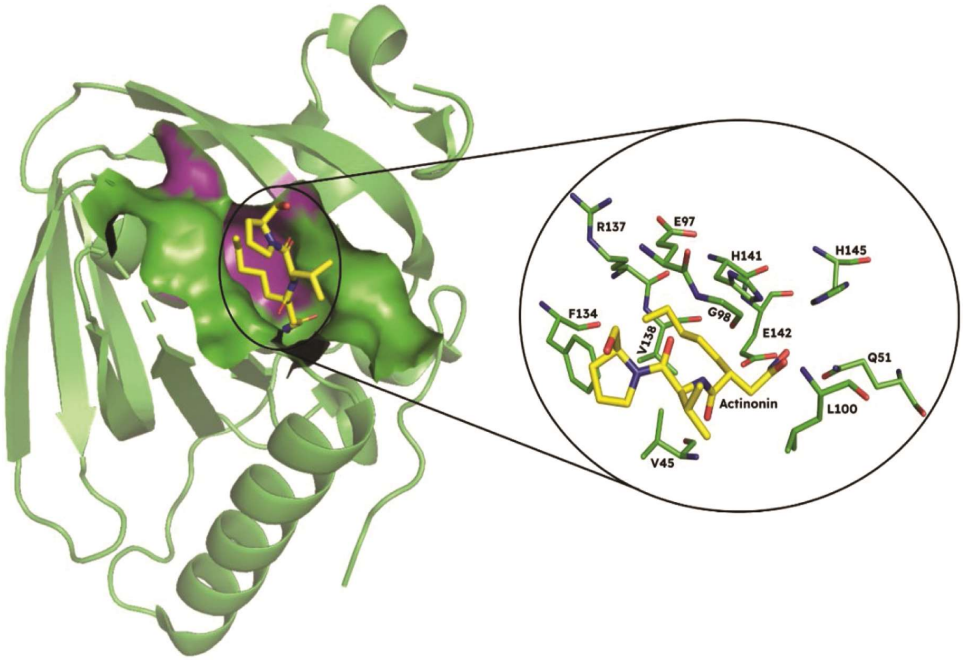
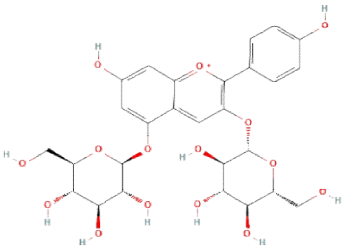
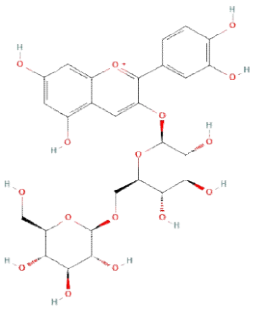
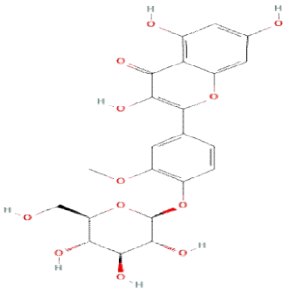
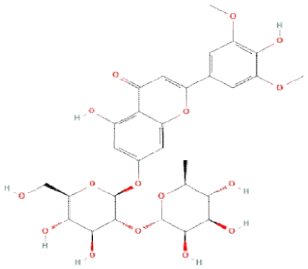
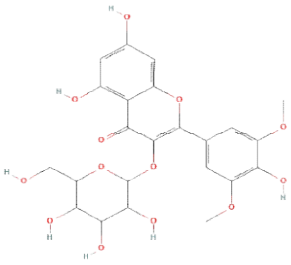


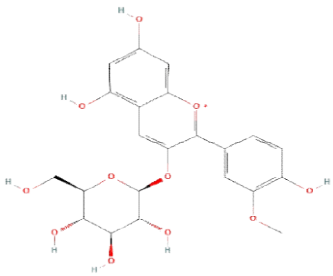
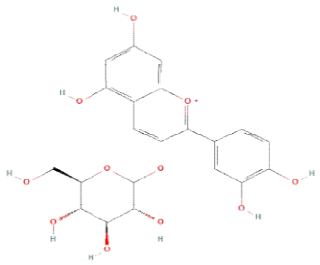
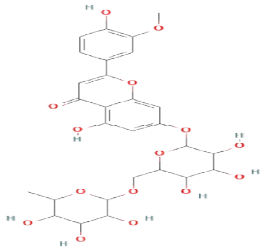
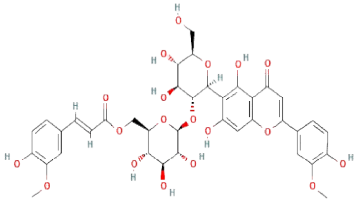
Fig. 1 — The crystal structure of XoPDF with Actinonin (PDB ID: 5CVQ). The green stick structures are those PDF residues that make hydrophobic and Hydrogen-bond interactions with the Actinonin (Yellow; stick structure). In surface structure, the purple region represents the S1' site of binding pocket, which is decorated by the [V45, E97, F134, R137, V138, and H141]

Table 1 — The molecular docking and interaction data of virtually screened top 9 compounds along with their CID and 2D structural information.

PubChem CIDs	Compound name and CID	Docking Score (kcal/mol)	Interacting residues	Compound 2D structure
Lead60	Pelargonin (441772)	-8.7	V45, G46, W96, E97, G98, C99, L100, I102, P103, G104, R106, F134, N137, V138, H141	
Lead144	Cyanidin 3-gentiobioside (11968393)	-8.37	H43, V45, G46, F63, W96, E97, G98, C99, L100, R106, F134, V138, H141, E142	
Lead145	Isorhamnetin 4'-O-beta-D-glucopyranoside (11968470)	-8.3	A42, V45, G46, W96, E97, G98, C99, L100, P103, G104, R106, F134, N137, V138, H141	
Lead191	tricin 7-O-neohesperidoside (102158614)	-8.4	I5, A42, V45, G46, G98, L100, P103, G104, R106, H141, E142, D164	
Lead222	Syringetin-3-o-glucoside (14524434)	-8.6	A42, H43, G44, V45, G46, W96, E97, G98, C99, L100, I102, P103, G104, R106, H141, D164	

(*Contd.*)

Table 1 — The molecular docking and interaction data of virtually screened top 9 compounds along with their CID and 2D structural information.

PubChem CIDs	Compound name and CID	Docking Score (kcal/mol)	Interacting residues	Compound 2D structure
Lead226	Peonidin-3-glucoside (443654)	-8.6	H43, V45, W96, E97, G98, C99, L100, I102, R106, R137, V138, F134, V138, H141, E142	
Lead225	Cyanidin 3-O-beta-D-glucoside (12303203)	-8.5	H43, V45, G46, W96, E97, G98, C99, I102, G104, R106, H141, E142, D164	
Lead230	Chrysoeriol 7-rutinoside (14374725)	-8.5	V45, G46, E93, N94, G95, W96, E97, G98, C99, R106, F134, R137, H141, E142, D164	
Lead232	Isoscoparin 2"-[6-(E)-feruloylglucoside] (21722000)	-8.97	H43, G44, V45, G46, Q51, W96, E97, C99, L100, G104, R106, F134, H141, E142, D164	

Molecular dynamic simulation analysis of screened compounds

The molecular dynamic simulations were implemented on the 9 compounds to screen out further those scaffolds that showed similar or better structural properties than the reference compound (actinonin) with the XoPDF in the solvent system. All compounds remained intact within the pocket throughout the 100 ns simulation. The root mean squared deviation (RMSD) is generally used to observe the conformational changes in the protein structure in the dynamic environment, relative to the starting conformation, to infer the stability of protein structure after interacting with the ligands. The average RMSD of each MD system is tabulated in (Table 2), and the degree of deviation incurred in the XoPDF backbone during each simulation is visualized by the graph (see Fig. 3). RMSD analysis depicted that most of the leads (except XoPDF-lead222) induced less conformational changes in the XoPDF structure in comparison to actinonin. Further, to inspect the perturbations in protein compactness after ligand interaction we have calculated the average radius of gyration (Rg) for protein's backbone, which help in inferring the effects of ligand on folding or unfolding of the protein backbone, and also illustrate the stability of structure compactness during simulation. The estimated average Rg for each system referred in (Table 2) and their respective simulation trajectories are visualized in (Fig. 4). Rg data of

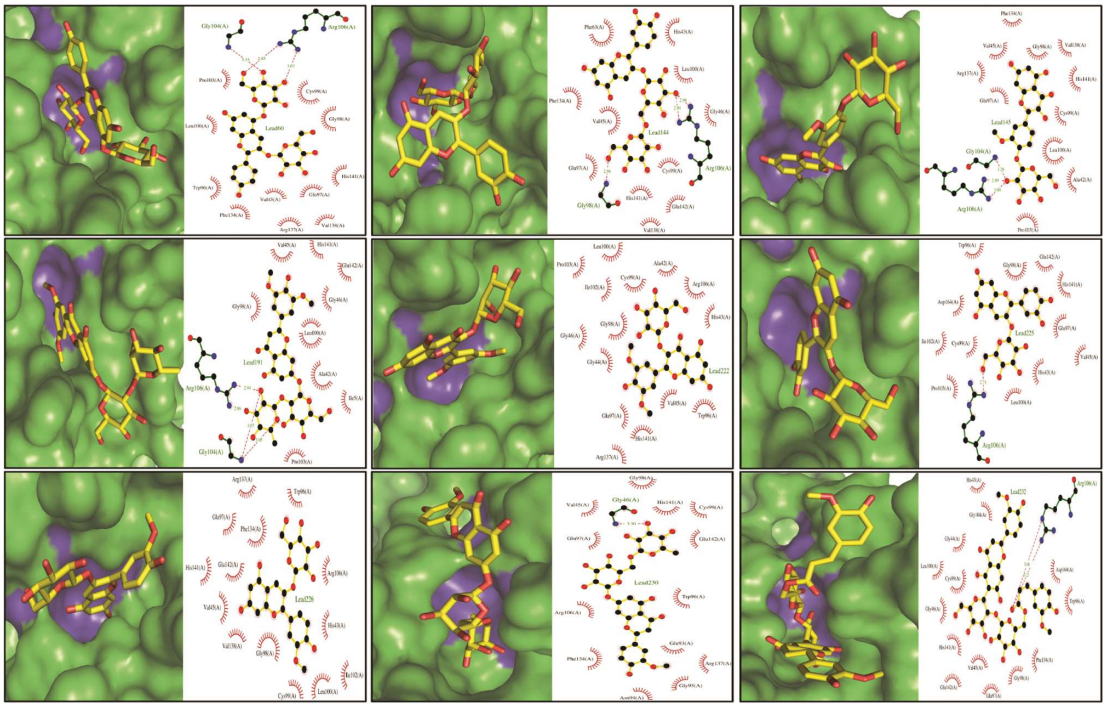


Fig. 2 — The conformation of top 9 compounds in their respective XoPDF-complex is represented in the surface format (Purple color = S1' site; Green color = XoPDF; Yellow color = Ligand). The hydrophobic (red arc) and hydrogen-bond interactions (red dashes) analyzed by the LigPlot+ for each XoPDF-Ligand complex, wherein the green and yellow stick structure represents the protein residues and ligands

Table 2 — The MDS parameters calculated by GROMACS functions for each molecular system.

Molecular Dynamic Systems	MD parameters for the Backbone of *Xo*PDF		
	RMSD (nm)	Rg (nm)	RMSF (nm)
APO	0.249 ± 0.033	1.489 ± 0.012	0.102
*Xo*PDF-Actinonin	0.282 ± 0.056	1.486 ± 0.012	0.11
*Xo*PDF-lead60	0.226 ± 0.040	1.49 ± 0.011	0.118
*Xo*PDF-lead144	0.230 ± 0.023	1.48 ± 0.007	0.084
*Xo*PDF-lead145	0.257± 0.031	1.47 ± 0.009	0.086
*Xo*PDF-lead191	0.198 ± 0.030	1.496 ± 0.01	0.120
*Xo*PDF-lead222	0.298 ± 0.048	1.499 ± 0.008	0.103
*Xo*PDF-lead225	0.238 ± 0.024	1.471 ± 0.010	0.094
*Xo*PDF-lead226	0.227 ± 0.033	1.517 ± 0.009	0.108
*Xo*PDF-lead230	0.223 ± 0.033	1.496 ± 0.009	0.106
*Xo*PDF-lead232	0.235 ± 0.038	1.496 ± 0.010	0.104

each phytochemical-XoPDF complexes (except XoPDF-lead226) showed almost similar amount of compactness as shown in the backbone of XoPDF-actinonin, and infers no significant changes in the native compactness of the XoPDF after interacting with the phytochemical compounds. An average fluctuation in the backbone of residues during the simulation was measured as root-mean-square-fluctuation (RMSF) with respect to the reference frame, which informs about the induced changes in the residue conformation after ligand interaction with the protein. The average RMSF values of each system along with their visualization mentioned in (Table 2 and Fig 5),, respectively . In comparison to XoPDF-actinonin complex, all the XoPDF-phytochemical complexes showed no significant changes in the average RMSF. Whereas, the average RMSF of active site residues of the complexes XoPDF-lead144, XoPDF-lead145, XoPDF-lead225, XoPDF-lead232 showed a lower level of fluctuations in comparison to the XoPDF-actinonin (see Fig. 6).

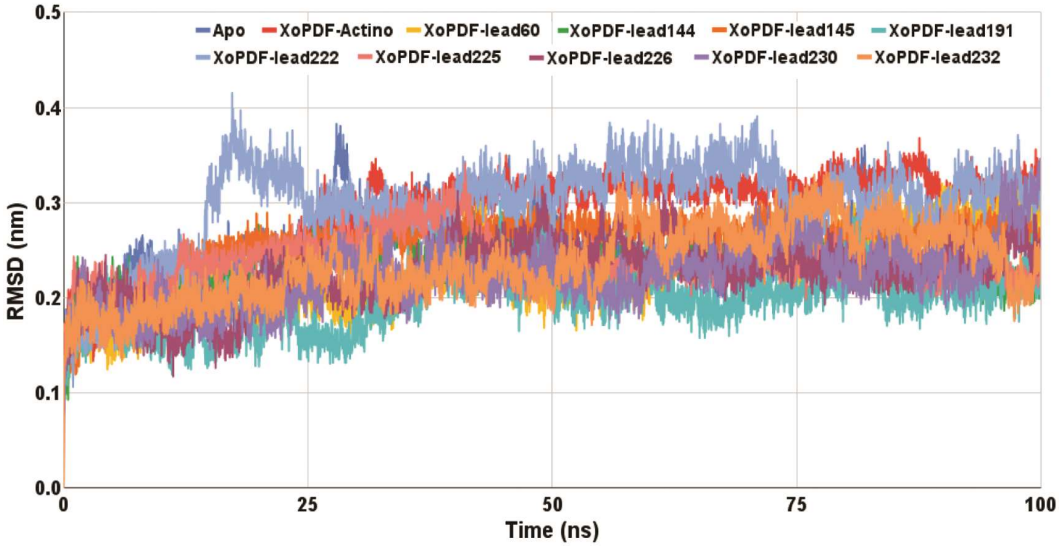


Fig. 3 — The plot represents the backbone RMSD (nm) of each XoPDF system based on their individual simulation data of 100 ns. In which blue, red, dark yellow, green, dark-orange, moderate-cyan, bright-blue, light-red, dark-magenta, light-purple, and orange color depicts the backbone RMSD of Apo, XoPDF-Actino, XoPDF-lead60, XoPDF-lead144, XoPDF-lead145, XoPDF-lead191, XoPDF-lead222, XoPDF-lead225, XoPDF-lead226, XoPDF-lead230, and XoPDF-lead232, respectively . (Actino stand for Actinonin)

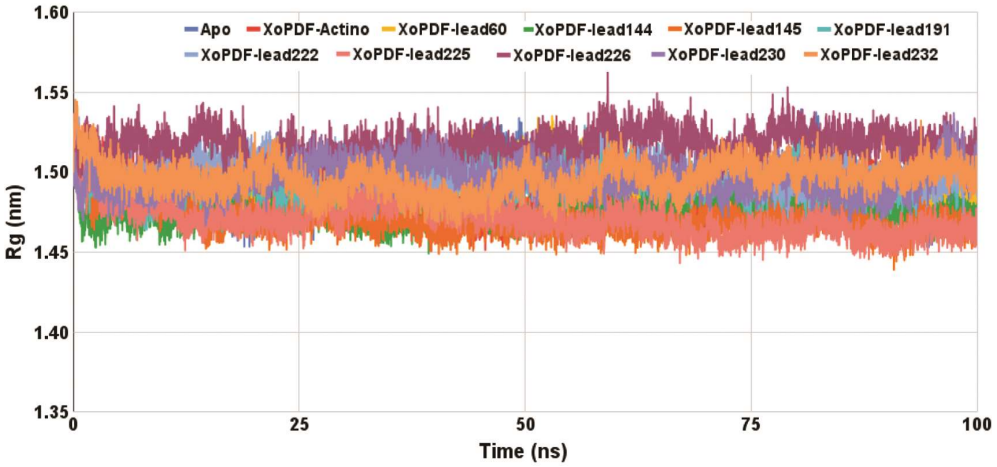


Fig. 4 — The plot represents the backbone Rg (nm) of each XoPDF system based on their individual simulation data of 100 ns. In which blue, red, dark yellow, green, dark-orange, moderate-cyan, bright-blue, light-red, dark-magenta, light-purple, and orange color depicts the backbone Rg of Apo, XoPDF-Actino, XoPDF-lead60, XoPDF-lead144, XoPDF-lead145, XoPDF-lead191, XoPDF-lead222, XoPDF-lead225, XoPDF-lead226, XoPDF-lead230, and XoPDF-lead232, respectively . (Actino stand for Actinonin)

Calculation of binding energy via MMPBSA

The BE of the stable trajectory (50-100 ns) for each complex was calculated as per the predefined commands of Rashmi K *et al,* for MMPBSA. This will help in determining the strength of ligand-active site interaction based on the MDS data, which provides a more accurate binding affinity understanding than a rigid docking protocol. Also gives insight about the contribution of different binding forces *viz* Van der Waals, Electrostatic, Polar solvation, Solvent-Accessible-Area (SASA) in the binding energy of a complex, and their impact on the interaction of a ligand with the active site residues (refer to Table 3). The analysis infers a BE of -57.65 ± 10.21 kJ/mol between the actinonin and XoPDF, wherein the electrostatic and Van der Waals forces played the major contribution for stable protein-ligand interaction. As depicted by the MMPBSA analysis, all leads (except lead-222) have higher binding affinity for the active site than actinonin, moreover, the lead-144, lead-225, lead-226, lead-230, and lead-232 depicted better affinity in comparison to the other

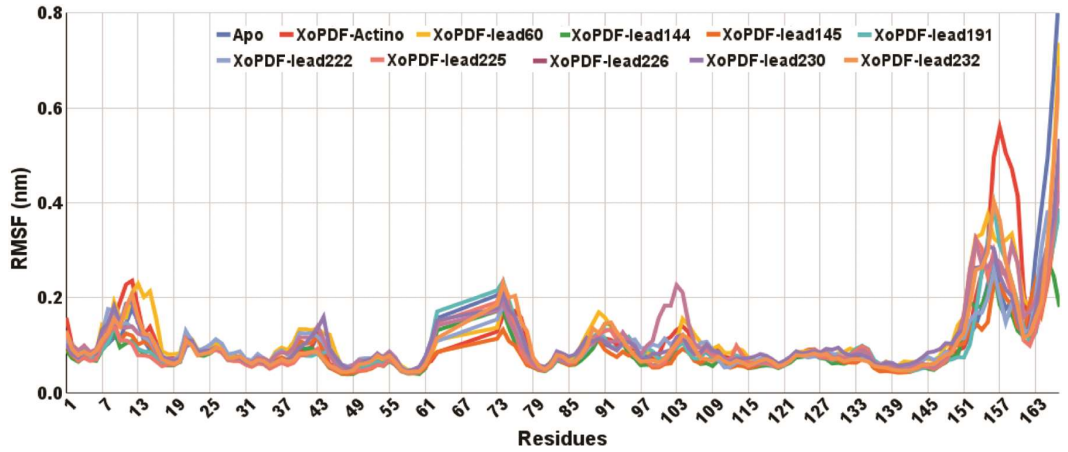


Fig. 5 — The plot represents the backbone RMSF (nm) of each XoPDF system based on their individual simulation data of 100 ns. In which blue, red, dark yellow, green, dark-orange, moderate-cyan, bright-blue, light-red, dark-magenta, light-purple, and orange color depicts the backbone RMSF of Apo, XoPDF-Actino, XoPDF-lead60, XoPDF-lead144, XoPDF-lead145, XoPDF-lead191, XoPDF-lead222, XoPDF-lead225, XoPDF-lead226, XoPDF-lead230, and XoPDF-lead232, respectively (Actino stand for Actinonin)

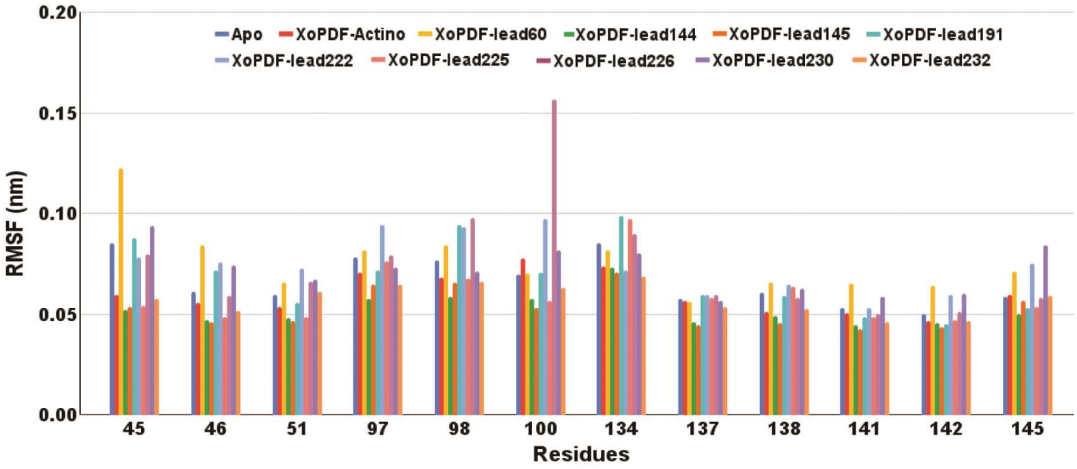


Fig. 6 — The plot represents the backbone RMSF (nm) of binding pocket residues of each XoPDF system based on their individual simulation data of 100 ns. In which blue, red, dark yellow, green, dark-orange, moderate-cyan, bright-blue, light-red, dark-magenta, light-purple, and orange color depicts the binding pocket residues RMSF of Apo, XoPDF-Actino, XoPDF-lead60, XoPDF-lead144, XoPDF-lead145, XoPDF-lead191, XoPDF-lead222, XoPDF-lead225, XoPDF-lead226, XoPDF-lead230, and XoPDF-lead232, respectively (Actino stand for Actinonin)

Table 3—The Binding energy data from MMPBSA analysis for top 9 compounds is tabulated along with the respective Van der Waal, Electrostatic, Polar Solvation, and SASA energies

Compounds	van der Waal energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
Actinonin	-67.86 ± 11.78	-52.01 ± 8.84	69.26 ± 12.65	-7.88 ± 1.48	-57.65 ± 10.21
lead60	-117.81 ± 16.42	-54.33 ± 8.32	90.96 ± 10.91	-11.92 ± 1.65	-91.88 ± 16.36
lead144	-237.26 ± 2.09	-932.56 ± 8.31	369.54 ± 4.90	-26.21 ± 0.20	-826.27 ± 4.70
lead145	-140.61 ± 15.62	-78.14 ± 9.79	124.34 ± 12.36	-14.26 ± 1.59	-109.15 ± 14.37
lead191	-138.81 ± 10.22	-56.68 ± 5.74	105.91 ± 7.54	-15.36 ± 1.16	-104.67 ± 10.06
lead222	-41.14 ± 11.25	-13.56 ± 4.19	30.34 ± 9.43	-4.50 ± 1.17	-29.73 ± 7.79
lead225	-53.94 ± 12.29	-352.26 ± 47.88	88.86 ± 19.50	-6.04 ± 1.47	-325.91 ± 43.82
lead226	-149.37 ± 9.97	-262.60 ± 20.25	237.03 ± 15.16	-17.50 ± 1.17	-192.48 ± 15.13
lead230	-200.35 ± 6.05	-146.40 ± 6.46	207.34 ± 6.84	-22.38 ± 0.59	-161.77 ± 5.98
lead232	-266.02 ± 2.36	-124.38 ± 4.73	224.29 ± 3.59	-28.02 ± 0.28	-193.84 ± 3.38

remaining phytochemicals. The major contributors of their BE are mainly from Electrostatic and Van der Waal forces, which together reduced the effects of polar solvation on the interaction of phytochemicals with XoPDF active site. Whereas, SASA infers the accessibility of ligand with the solvent after interacting with the protein active site, less accessibility with solvent makes SASA energy more negative or vice versa.

Solvent accessibility and hydrogen bond analysis of XoPDF-ligand complexes

All the leads and actinonin showed a low solvent accessibility, which is favorable for their stable interaction with active site residues within a solvent (water) system. The analysis for an average contribution of residues in the binding of ligand and XoPDF depicted the involvement of S1' (hydrophobic region of the binding pocket) residues [V45, G46, F134, V138, E142] repeatedly in each complex (refer to Suppl. Table S3), infer their importance in the maintenance of stable interaction between the ligand and protein within the solvent system during the dynamics. Further the hydrogen bond (HB) analysis revealed that the XoPDF-Actinonin, XoPDF-lead60, XoPDF-lead144, XoPDF-lead145, XoPDF-lead191, XoPDF-lead222, XoPDF-lead225, XoPDF-lead226, XoPDF-lead230, and XoPDF-lead232 have the average number of HBs 5, 5, 6, 3, 3, 3, 4, 3, 4, and 3, respectively , for simulation period of 50 ns to 100 ns (refer to Suppl. Table S4). The data also shows that lead222 relatively has lesser number of hydrogen bonds in the stable trajectory (50 - 100 ns), which states for the lowering of electrostatic free energy along with total contribution in BE. To compensate that a lowering of polar solvation energy found in the lead222 complex, that aids the phytochemical in stabilizing the interaction with XoPDF during the simulation study.

Discussion

The pathogen, *Xoo* is very destructive for rice production[32]. The nature of *Xoo* to become the vascular wilt along with its propagation in the rice promotes the difficulties in finding a cure for rice wilting. The chemical control method primarily involves the use of metallic copper, often in combination with other fungicides or pesticides, to effectively reduce the incidence of BLB in rice. However, the excessive demand and higher cost of these chemicals may reduce their usage by farmers. Additionally, during the rainy season, majority of these chemicals are washed away by water, offers an advantage for bacterial proliferation and supports the wilting processes. Other than this, these synthetic pesticides pose a threat to non-targeted organisms and the environment[33,34]. To tackle these hurdles the field of phytochemical based pesticides paves a promising pathway to overcome these issues. These phytochemicals-based bactericides pose a minimal or no risk to the non-targeted organisms and the particular ecosystem of the crop, and also due to their complex molecular functioning reduces the chances of pest resistance. Based on these assumptions, we have screened out a set of phytochemicals with the backbone of anthocyanin or flavone, and these hits significantly showed the inhibitor type properties and better interactions with the peptide deformylase of *Xanthomonas Oryzae* pv. *Oryzae.* The peptide deformylase plays a vital role in maintaining the activity of different proteins in the *Xanthomonas*, and is crucially required for the survival of other plant pathogens[35,36]. Previous studies depicted that the potential of this protein as a drug target due to its different functioning in the prokaryotic system in comparison to the eukaryotic system. That provides a major rationale for the XoPDF to be exploited as a potential target for the bio-pesticide studies against the rice BLB disease[37,38]. Better resolution (~2.5 Å) crystal structures of XoPDF were reported by Ngo *et al*.[25] in which they reported the solved crystal structures of the XoPDF interacting with Tri-peptide (Met-Ala-Ser), Di-peptide (Met-Ala), and Actinonin (antibiotic). The structural information from these crystal structures elucidated the important physicochemical understanding regarding the preferences of various chemical groups for the S1', S2', and S3' sites of the binding pocket. Also, informs the involvement of those binding pocket residues, which ensure the proper interaction of substrate and antibiotic with the XoPDF. The hydrophobic region of XoPDF binding pocket (S1') showed a promising region for designing the drug molecules having the bulkier rigid groups (like polycyclic group), as this side is mainly preferred for the binding of the N-formylated methionine[25]. Based on these previous studies we have selected the 5CVQ as a representative structure for the virtual screening (VS) study. The conformational as well as interaction details of its ligand *i.e*., Actinonin were used as a reference for filtering the probable leads from the output of

VS protocol. Phytochemicals are the secondary metabolites of the plant, which are expressed in different parts of the plant as a protective response against any pathogenic invasion. These secondary metabolites showed a greater potential as fungicide and pesticides for different plant pathologies in previous studies[33,34]. Being a natural compound, they pose no harm to nature, also showed no significant aftereffects on the non-targeted organisms, and their inexpensive commercialized production make phytochemicals a better repertoire for finding the drug leads against the plant pathogens[39]. According to this we collected the secondary metabolites expressed in the various parts of rice lines (like root, bran, husk, leaf, grain, seedling etc.). Out of that top nine leads were further screened based on the virtual screening against the XoPDF via AutoDockVina along with the reference-based filtration. These compounds generally have the backbone of anthocyanin and flavone, whose derivatives mainly come under antibiotics or antimicrobials. The leads also showed a better binding affinity (less than –8.0 kcal/mol) in comparison to the actinonin. The LigPlot analysis also showed the similarity between the interacting residues of 10 leads with the reference compound. Also, their polycyclic groups are positioned in the hydrophobic region (S1') of the binding pocket. These results depict the better binding affinity of these leads in comparison to the actinonin for the XoPDF binding pocket.

Molecular dynamics help in determining the cause of structural changes in protein after an interaction with a ligand in a solvent system (like water). Each MD parameter (RMSD, RMSF, Rg) gives an insight into the ligand induced changes in the protein structure and provides reasoning on deviation in the native fold along with the stability in the structure during simulation. The MD analysis of nine leads reveals a similar amount of average RMSD, Rg, and RMSF values in comparison to the MD parameters of the Apo and reference compound (Actinonin). The RMSF of binding pocket residues also showed a better result with the majority of the leads in comparison of apo and reference. The data surmise the significant structural stability of the screened leads with the XoPDF within a water system. Further, the MMPBSA analysis of leads also showed better MD based binding energies with the XoPDF binding pocket. This study also inferred that a major contribution to the binding energy was mainly provided by the S1' site residues [V45, G46, F134, V138, E142]. However, in a previous study, the importance of F134 is reported for maintaining the interaction with hydrophobic moiety of the groups having cyclic rings[25]. These results ensure the potential of the scaffold represented by the top 9 leads, for designing the botanical bactericide against the *Xanthomanas Oryzae* pv. *Oryzae* of the rice BLB disease.

Our study screened those Anthocyanins and Flavones derivatives that are from the subclass of pelargonin, isorhamnetin, tricin, peonidin, syringetin, cyanidin, chrysoeriol, and isoscoparin. These are the potential leads for the designing of natural bactericidal against the XoPDF protein, for improving the BLB condition in rice. These groups containing derivatives of anthocyanin and flavone are well reported as the potential antimicrobial compounds that are found naturally in the plant extracts. Polyphenolics derivatives exhibit the diverse mechanism of antimicrobial activity on gram positive and negative bacteria by perturbing the various molecular machineries of pathogens (like cell-membrane disruption, ceasing enzyme activity, interfering DNA/RNA/Protein synthesis, etc.)[40]. Previous studies state the potential of cyanidin, pelargonidin, isoscoparin, and peonidin subclass of anthocyanin in antimicrobial or antibacterial activity[41]. Tricin, a subclass of flavonoid is well reported for possessing antibacterial, antifungal and insecticidal properties due to a greater number of hydroxyl groups per molecule compound, as well as these flavonoids are highly abundant in the bran of rice[42]. Chrysoeriol is a type of methylated flavonoids that have been reported as a best defensive secondary metabolite of plants for effective antimicrobial or more specifically antibacterial activity during pathogenesis[43]. From previous literature we can infer a significant antimicrobial potential of these groups bearing derivatives found in our studies. Our findings support that the PDF protein could be a potential target of these compounds, which further perturbs the activation machinery of proteins in the pathogens. This may lead to systemic failure in the pathogen molecular network and lower the chances of survival.

Conclusion

Rice is considered as one of the major cereal crops. The control of BLB disease in rice via natural compounds will be eco-friendly, potential and an effective risk-free method for crop disease management in today's agriculture component.

The result of the present study undoubtedly predicted the 9 natural candidate- pelargonin, cyanidin 3-gentiobioside, isorhamnetin 4'-O-beta-D-glucopyranoside, tricin 7-O-neohesperidoside, syringetin-3-o-glucoside, peonidin-3-glucoside, cyanidin 3-O-beta-D-glucoside, chrysoeriol 7-rutinoside and isoscoparin 2"-[6-(E)-feruloylglucoside] as the potential bactericide against *Xanthomonas*. All these compounds showed excellent binding affinities to target protein XoPDF along with good H-bond and bioactivity scores. Therefore, it is predicted that these compounds could cross the cell membranes and can inhibit the target XoPDF enzyme that is involved in their crucial mechanism to help in preventing bacterial growth and functions. Although PDF is found in many other plant pathogens therefore, these lead compounds could be performed as broad-spectrum inhibitors, enabling plants to have broad spectrum disease resistance by inhibiting translation machinery of pathogens. Further in vivo and field studies are required to confirm the potential activity of this *in silico* study before recommending these nine natural candidates as pesticides against *Xanthomonas Oryzae* pv. *Oryzae*.

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Conflict of interest

All authors declare no conflict of interest.

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