Post harvest management of fruits and vegetable spoilage by Aspergillus niger employing some selected natural compounds: An Insilico study

[1]KVN Rajeshwari, [2]Shravan Kumar Gunda, [1]K. Susmitha, [3]G. Shyam Prasad*,[1]S. Suma

[1]Department of Biochemistry, Chaitanya (Deemed to be) University, Kishanpura, Hanamkonda, Warangal-506001, Telangana State, India.

[2]Bioinformatics Division, PGRRCDE, Osmania University, Hyderabad – 500007, Telangana State, India.

[3]Department of Microbiology, Chaitanya (Deemed to be) University, Kishanpura, Hanamkonda, Warangal-506001, Telangana State, India.

Email: shyamprasad1919@yahoo.com

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Abstract

The enzyme Polygalacturonase is produced by fungal pathogens for their success and survival during host infection. Among fungal pathogens, Aspergillus niger is the key fungus which infects post harvested fruits and vegetables leading to their decay. In the present investigation, polygalacturonase inhibition potentials of some selected natural compounds were studied insilico. The putative protein three dimensional structure of polygalacturonase was built by Homology modelling using MODELLER software. An advanced molecular docking software Auto Dock was used for docking study. Fifteen natural compounds were tested targeting the Polygalacturonase protein of Aspergillus niger. The putative polygalacturonase protein for Aspergillus niger was built by homology modeling using MODELLER software. Among fifteen natural compounds studied, Withanolide L, Withanolide G, Withanolide M, Withanolide O have shown strong interaction with Polygalacturonase compared to other compounds in the study.

Hence, from the above results, it can be concluded that polygalacturonase enzyme which is involved in fruit ripening and spoilage of fruits can be inhibited by spraying above natural compounds. However, further invitro and invivo investigations are needed to confirm these observations.

Keywords: Aspergillus niger, Polygalacturonase, Homology modelling, Docking.

1. INTRODUCTION

Fruits and vegetables are good sources of vitamins, minerals, phytochemicals, dietary fibre and antioxidants (Slavin & Lloyd, 2012)[1]. The change in fruits and vegetables losing their quality leading to unfit for human consumption is called spoilage. About 20 to 40% of the fruit and vegetables undergo spoilage after harvest due to lack of post harvest management skills and technologies (Poonam et al., 2022) [2]. Fungal spoilage of fruits may also occur during growing, harvesting, handling, transport and marketing conditions and even after purchase by the consumer (Al-Hind, 2013) [3]. Fresh fruits and vegetables contain natural microflora whose growth and spoilage is favoured by many extrinsic factors viz. presence of air, high humidity and high temperature during storage (Erkmen and Bozoglu, 2012) [4].The spoilage of fruits and vegetables also has broad socio-economic implications as it directly correlates with food shortages, food waste and hunger in some parts of the world, water stress, needless biodiversity loss and increased greenhouse gas emission (Alegbeleye et al., 2022) [5]. Different microorganisms viz. Bacteria, fungi are involved in spoilage of fruits and vegetables. Among fungal species Aspergillus niger a widely distributed saprophytic fungus in soil, air and cereals causing postharvest diseases in fruits (Liu et al.,2012) [6]. The fungus Aspergillus niger was reported to highest occurance incidence in fruits like pineapple, watermelon, oranges, pawpaw and tomatoes with a frequency of 38% (Oza et al.,2020) [7] leading to their spoilage. This fungal species are also reported to produce several toxic compounds like malformins, naphthopyrones and it was also reported to produce ochratoxins (OTA), a mycotoxin which is a very important toxin worldwide because of the harm it causes to human and animal health (Al-hind,2011) [8].

Numerous cell wall degrading enzymes are produced by fungi to use fruit cell wall as nutrient source which reduce post harvest life leading to spoilage. Among the fruit cell wall degrading enzymes polygalacturonase (PG) is one of the main enzymes in fungal pathogens. These are the pectinolytic which catalyze the hydrolytic cleavage of the pectin chain with the

introduction of water across the oxygen bridge. Pectinases are the first enzymes to be secreted by fungal pathogens when they attack plant cell wall (Collmer and Keen,1986;Idnurm and Howlett,2011) [9-10].These enzymes are essential for fungal pathogens that do not have specialised penetration structures as well as for necrotrophic pathogens during the late stages of the invasion process (DeLorenzo et al.,1997) [11].

Hence, present investigation is directed to design a bioformulation in silico using some selected natural compounds to serve as antifungal agents, a promising sustainable alternate to chemical products targeting polygalacturonase enzyme. Due to lack of experimental structures for Polygalacturonidase, a homology medel of the enzyme was build using modeller software and molecular docking was performing using Autodock software.

2. EXPERIMENTAL

2.1. Materials and methods

Molecular docking studies were performed using an interactive graphics program for protein-ligand interaction (available from http://viba.scripps.edu/). Fifteen natural compounds were used as ligands and homology modelled Polygalacturonase emzyme of Aspergillus niger was used as protein.

2.2. Preparation of Ligands:

Fifteen natural compounds were selected viz.Withanolide L, Withanolide G, Withanolide M, Withanolide O, Withanolide P, Withanolide D,16760705, Withanolide R, Withanolide A,(+)- Withanolide A,3-HDH- Withanolide F,3-beta-Methoxy-2,3-dihydrowithaferin A, Withanolide, Withanolide N. The selected compounds used as ligands were drawn using Chem Draw and were converted to 3D PDB format from mol format by Accelrys Discovery Studio Visualizer (DS Visualizer).Later Gasteiger charges and hydrogen atoms were added using Auto dock software.

3. PREPARATION OF TARGET PROTEIN:

The crystal structure of Polygalacturonase was constructed using homology modelling with MEDELLER software version 10.3 using following steps.

3.1. Homology modeling of Exopolygalacturonase:

Template selection and structure prediction

The protein sequence of Exopolygalacturonase from Aspergillus niger (Uniprot accession number: A2QW66) was retrieved from the UniProtKB database (http://www.uniprot.org/)[12]. To select the template BLAST[13] (Basic Local Alignment Search Tool) search was performed resulted with the best match Crystal Structure of Endo-Xylogalacturonan hydrolase from Aspergillus tubingenesis (PDB ID: 4C2L) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [13] with 35.68% identity, having a resolution of 1.75 Å making it an excellent template for further steps. Using ClustalX, a pairwise sequence alignment was performed for Target template protein sequences [14]. To obtain models with an automated homology modelling approach, MODELLER 10.3 was used which performs modelling by satisfaction of spatial restraints [15]. To build 3D structure of target protein using MODELLER 10.3 software these aligned sequences were used. After manually modifying the alignment input file in MODELLER 10.3 to match the query and template sequences, twenty models were generated. The best model for the target putative protein was selected on the basis of MODELLER objective function value out of these 20 models. PROCHECK was used for the validation of the model[16]. The RMSD (root mean square deviation) was calculated by superimposing (4C2L) over the generated model to assess the accuracy and reliability of the generated model using SPDBV[17] by selecting the main chain atom (i.e. the backbone atoms of alpha carbon). These models were then checked in detail for the protein structure stereochemistry by using PROCHECK[16], which generates Ramachandran plot and comprehensive residue by residue listing, which facilitates the in depth assessment of Psi/Phi angles and the backbone conformation of the models.

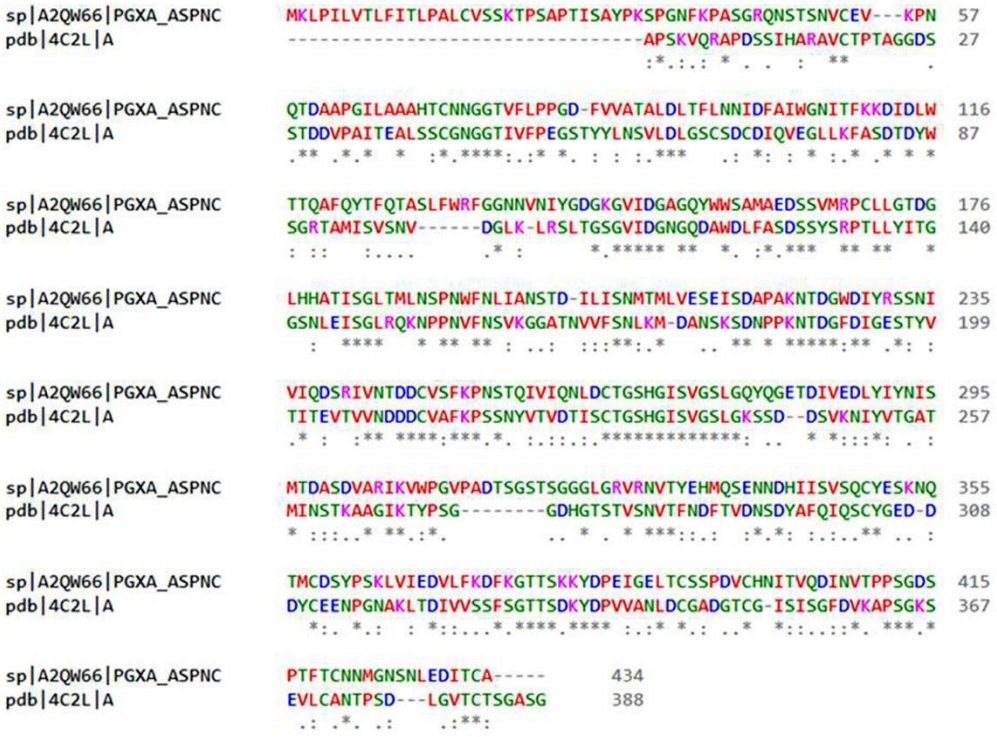
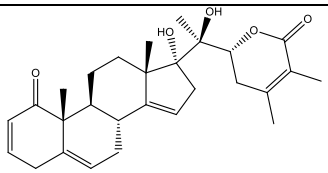
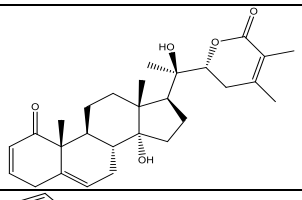
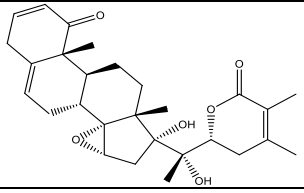


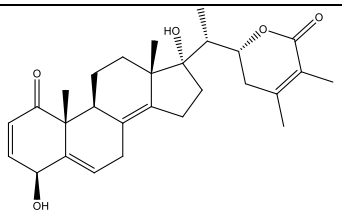
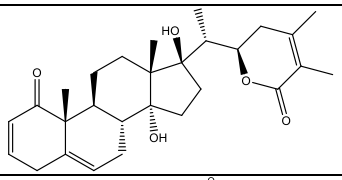
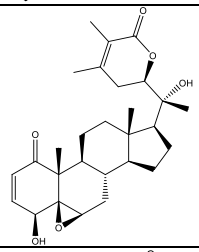
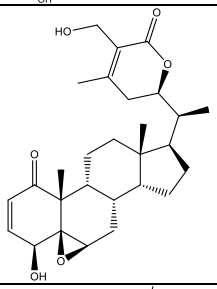
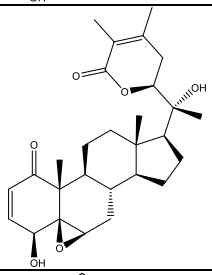
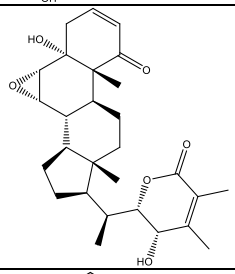
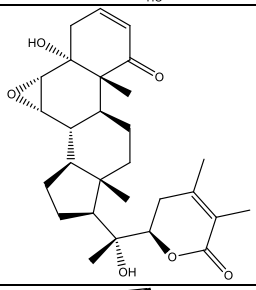
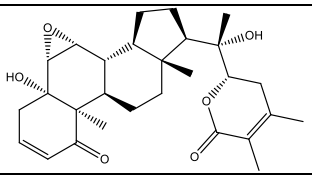
Fig.1: Sequence alignment of Target protein (A2QW66) and template protein (4C2L)

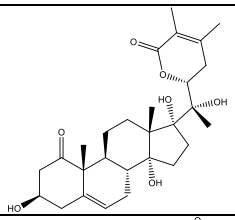
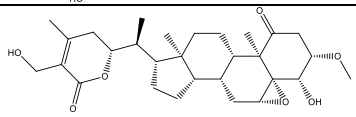
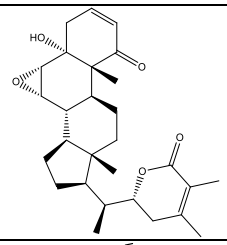
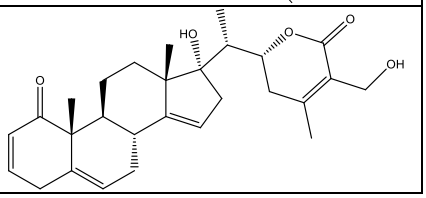
4. Docking studies: Interaction of 15 natural compounds with Polygalacturonase protein by molecular docking

After preparing the ligands as well as the receptor polygalacturonase, both were converted into the pdbqt format using the automated docking tool Autodock which was later used for docking. A grid box was prepared to convert the pocket with the main residues of the protein binding site by maintaining the grid size of X=40,Y= 40 and Z=40. An advanced molecular docking program Autodock 4.2 was used for docking against the receptors and also to estimate the binding affinities (Kcal mol-1).The ligands were evaluated in silico against homology modelled Polygalacturonase in triplicates and the average of the best conformation was chosen with the lowest docking energy, based on a complete docking search (tenruns). The interaction of Polygalacturonase with the ligands, hydrogen bonds, bond lengths and Root Mean Square Difference (RMSD) was analyzed using PyMol software (http://pymol.sourceforge.net/).

Table.1showing IUPAC names and structure of natural compounds

S.No.	Name of the Compound	IUPAC Name	Molecular Structure
1.	Withanolide L	(2R)-2-[(1S)-1-hydroxy-1-[(8R,9S,10R,13S,17R)-17-hydroxy-10,13-dimethyl-1-oxo-7,8,9,11,12,16-hexahydro-4H-cyclopenta[a]phenanthren-17-yl]ethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	
2.	Withanolide G	(2R)-2-[(1R)-1-hydroxy-1-[(8R,9S,10R,13R,14R,17S)-14-hydroxy-10,13-dimethyl-1-oxo-7,8,9,11,12,15,16,17-octahydro-4H-cyclopenta[a]phenanthren-17-yl]ethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	
3.	Withanolide M	(1R,2R,4S,6R,7R,10S,11R)-6-[(1S)-1-[(2R)-4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl]-1-hydroxyethyl]-6-hydroxy-7,11-dimethyl-3-oxapentacyclo[8.8.0.02,4.02,7.011,16]octadeca-13,16-dien-12-one	

4.	Withanolide O	(2R)-2-[(1R)-1-[(4S,9S,10R,13S,17S)-4,17-dihydroxy-10,13-dimethyl-1-oxo-7,9,11,12,15,16-hexahydro-4H-cyclopenta[a]phenanthren-17-yl]ethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	
5.	Withanolide P	(2R)-2-[(1R)-1-[(8R,9S,10R,13S,14R,17R)-14,17-dihydroxy-10,13-dimethyl-1-oxo-4,7,8,9,11,12,15,16-octahydrocyclopenta[a]phenanthren-17-yl]ethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	
6.	Withanolide D	(1S,2R,6S,7R,9R,11S,12S,15S,16S)-15-[(1R)-1-[(2R)-4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl]-1-hydroxyethyl]-6-hydroxy-2,16-dimethyl-8-oxapentacyclo[9.7.0.02,7.07,9.012,16]octadec-4-en-3-one	
7.	16760705	(2*R*,6*S*,7*R*,9*R*,11*S*,15*R*,16*S*)-6-hydroxy-15-[(1*S*)-1-[(2*R*)-5-(hydroxymethyl)-4-methyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-2,16-dimethyl-8-oxapentacyclo[9.7.0.0^{2,7}.0^{7,9}.0^{12,16}]octadec-4-en-3-one	
8.	Withanolide	(1S,2R,6S,7R,9R,11S,12S,15S,16S)-15-[(1R)-1-[(2S)-4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl]-1-hydroxyethyl]-6-hydroxy-2,16-dimethyl-8-oxapentacyclo[9.7.0.02,7.07,9.012,16]octadec-4-en-3-one	
9.	Withanolide R	(1S,2S,4S,5R,10R,11S,14R,15R,18S)-5-hydroxy-15-[(1S)-1-[(2S,3S)-3-hydroxy-4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-10,14-dimethyl-3-oxapentacyclo[9.7.0.02,4.05,10.014,18]octadec-7-en-9-one	
10.	Withanolide A	(1S,2S,4S,5R,10R,11S,14S,15S,18S)-15-[(1R)-1-[(2R)-4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl]-1-hydroxyethyl]-5-hydroxy-10,14-dimethyl-3-oxapentacyclo[9.7.0.02,4.05,10.014,18]octadec-7-en-9-one	
11.	(+)-Withanolide A	15-[1-(4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl)-1-hydroxyethyl]-5-hydroxy-10,14-dimethyl-3-oxapentacyclo[9.7.0.02,4.05,10.014,18]octadec-	

		7-en-9-one	
12.	3-HDH-Withanolide F	(2R)-2-[(1S)-1-hydroxy-1-[(3R,8R,9S,10R,13S,14R,17R)-3,14,17-trihydroxy-10,13-dimethyl-1-oxo-2,3,4,7,8,9,11,12,15,16-decahydrocyclopenta[a]phenanthren-17-yl]ethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	
13.	3-beta-Methoxy-2,3-dihydrowithaferin A	(1S,2R,5S,6S,7R,9R,11S,12S,15R,16S)-6-hydroxy-15-[(1S)-1-[(2R)-5-(hydroxymethyl)-4-methyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-5-methoxy-2,16-dimethyl-8-oxapentacyclo[9.7.0.02,7.07,9.012,16]octadecan-3-one	
14.	Withanolide B	(1S,2S,4S,5R,10R,11S,14R,15R,18S)-15-[(1S)-1-[(2R)-4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-5-hydroxy-10,14-dimethyl-3-oxapentacyclo[9.7.0.02,4.05,10.014,18]octadec-7-en-9-one	
15.	Withanolide N	(2R)-2-[(1R)-1-[(8R,9S,10R,13S,17S)-17-hydroxy-10,13-dimethyl-1-oxo-7,8,9,11,12,16-hexahydro-4H-cyclopenta[a]phenanthren-17-yl]ethyl]-5-(hydroxymethyl)-4-methyl-2,3-dihydropyran-6-one	

5. RESULTS AND DISCUSSION:

In the present in silico study, molecular docking of 15 natural products was performed with homology modelled Polygalacturonase enzyme of Aspergillus niger using Autodock software.

5.1. Homology modelling and model evaluation:

In the present study, the template protein (PDB ID: 4C2L) was selected based on high degree of homology with target protein A2QW66 and good atomic resolution of its crystal structure. The target sequence of Exo-Polygalacturonase having 434 amino acid residues was retrieved from the Uniprot protein sequence database. PDB id: 42CL was identified and selected as template using BLAST having 35.68 % identity Fig.1. The structure was modelled using MODELLER 10.3. The generated structure was validated using PROCHECK. The predicted model shows 83.8% of amino acid residues in core region, 14.4% of amino acid residues in additionally allowed region, 0% of amino acid residues in generously favored region and disallowed region Fig. 2. Both the target and templates were superimposed to get RMSD value Fig. 3.

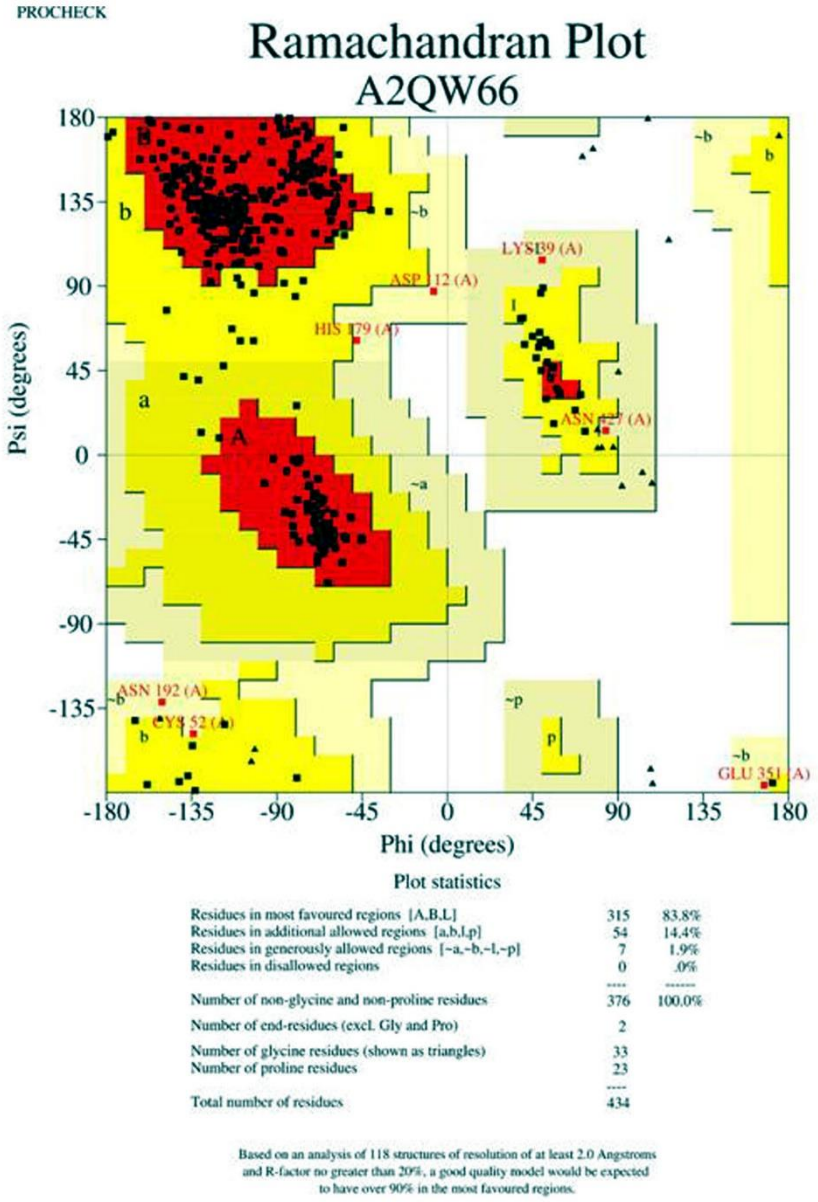


Fig 2: Ramachandran plot analysis of the backbone dihedral angles PSI (Ψ) and PHI (ϕ) of the target model (A2QW66)

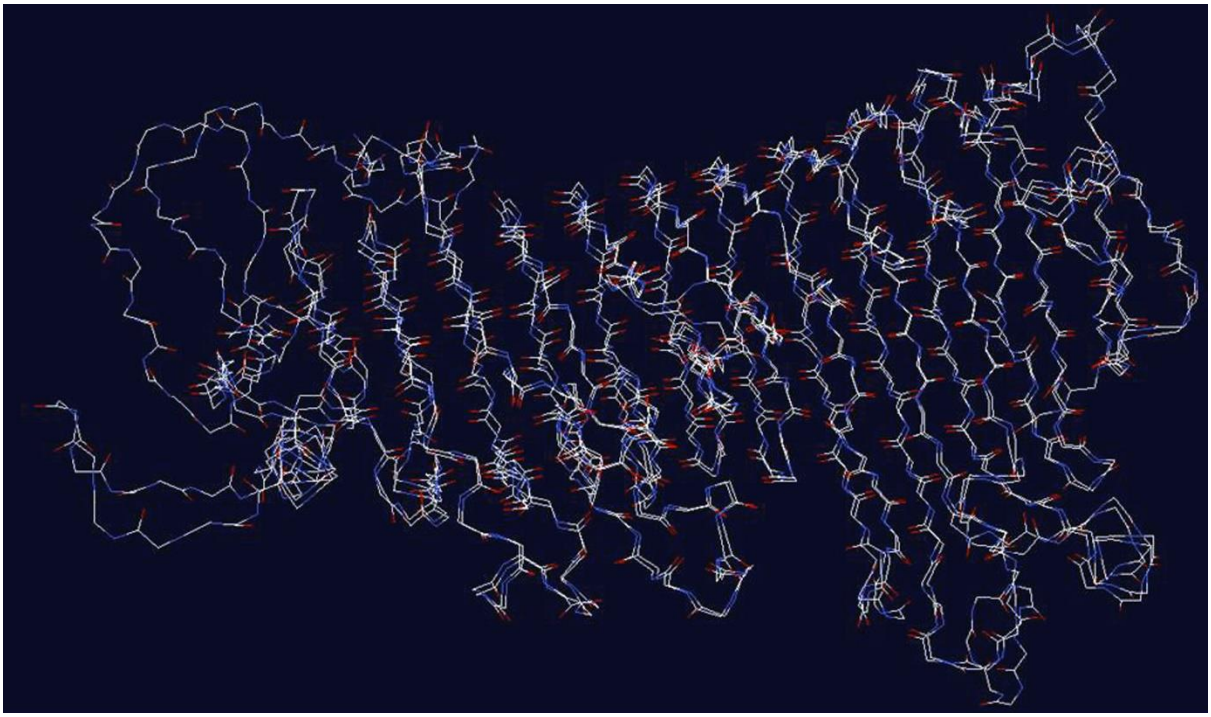


Fig 3: Superimposed model of target and template proteins

5.2. Docking Results

Molecular docking of the 15 test compounds into the active site of Polygalacturonase protein was found to be successful based on the formation of complexes of Polygalacturonase with ligands. The hydrogen bond interactions, bond length, binding energies, RMSD, active site residues and orientation of the docking compound within the active site were visualized. All the test compounds screened showed best fit RMSD values of 0.0000, indicating statistically significant interaction. The negative and low value of ΔG indicates a strong and favorable bonding between Polygalacturonase and the ligands in their most favorable conformations.

Withanolides are secondary metabolites found primarily in genera of the nightshades, a family of flowering plants. They are a group of at least 300 naturally occurring C28 steroids built on an ergostane skeleton functionalized at carbons 1, 22 and 26 commonly known as the withanolide skeleton. Among 300 naturally occurring withanolides 15 natural compounds were selected for the study (table.1). Among the selected natural compounds as ligands against homology modelled Polygalacturonase enzyme withanolide L was found to show strong interaction followed by withanolide G, withanolide M and withanolide O with binding energies of -9.8,-9.2,-9.2 and 9.1 respectively. Withanolide L was found to show strong interaction with aminoacid Ala-25 followed by Withanolide G with interacting to Ser-34,Lys-39, Withanolide M with binding score of -9.2 and interacting to Lys-25. Withanolide O showed interaction with score of -9.1 interacting with Lys-39, Pro-440.Rest of the compounds under study showed moderate degree of interaction with Polygalacturonase enzyme.

The details of the binding energies, the number of hydrogen bonds formed and the catalytic site residues involved in the protein –ligand complex of Polygalacturonase with different ligands is depicted in table 2 and fig.4.

Table 2: Showing binding energy and predicted contacting residues of homology modelled Polygalacturonase with selected natural compounds.

Name of the Compound	Binding Affinity	Interacting amino acids
Withanolide L	-9.80	Ala25
Withanolide G	-9.20	Ser34, Lys39
Withanolide M	-9.20	Lys25
Withanolide O	-9.10	Lys39, Pro40
Withanolide P	-9.00	Lys39, Pro40
Withanolide D	-8.90	Ala25, Ser34
16760705	-8.90	Ala25

Withanolide	-8.90	Pro40
Withanolide R	-8.80	Ser42
Withanolide A	-8.70	Ser42
(+)-Withanolide A	-8.70	Ala25
3-HDH-Withanolide F	-8.60	Ser34
3-beta-Methoxy-2,3-dihydrowithaferin A	-8.60	Ala25, Ser34
Withanolide B	-8.60	Ser42
Withanolide N	-8.50	Pro82

6. ADME PROPERTIES

This is used to describe the absorption, distribution, metabolism and elimination of drugs. ADME properties are useful to predict the pharmacological properties of drugs [18]. All the compounds follow Lipinski rule of five. ADME properties of selected compounds are shown in table 3.

Table 3. Results of the phytochemical molecules drug-likeness properties.

Drug likeliness properties / Phytochemicals	MW g/mol	Consensus Log Po/w	No. of H-bond Acceptors	No. of H-bond Donors	Molar Refractivity	Lipinski	Veber	Bioavailability Score	Synthetic accessibility (SA)	TPSA (Å2)	No of rotatable bonds	solubility (mg/ml)
Withanolide L	452.58	3.68	5	2	127.61	Yes	Yes	0.55	6.19	83.83	2	2.03e-02
Withanolide G	454.60	3.96	5	2	128.08	Yes	Yes	0.55	6.28	83.83	2	8.81e-03
Withanolide M	468.58	3.23	6	2	127.09	Yes	Yes	0.55	6.78	96.36	2	3.36e-02
Withanolide O	452.58	3.70	5	2	127.57	Yes	Yes	0.55	6.15	83.83	2	3.01e-02
Withanolide P	454.60	4.22	5	2	128.08	Yes	Yes	0.55	6.28	83.83	2	6.89e.03
Withanolide D	470.60	3.39	6	2	127.53	Yes	Yes	0.55	6.85	96.36	2	1.21e-02
16760705	470.60	3.45	6	2	127.49	Yes	Yes	0.55	6.83	96.36	2	5.01e-03
Withanolide	470.60	3.39	6	2	127.53	Yes	Yes	0.55	6.85	96.36	2	1.21e-02
Withanolide R	470.60	3.41	6	2	127.49	Yes	Yes	0.55	6.51	96.36	2	6.46e-03
Withanolide A	470.60	3.33	6	2	127.53	Yes	Yes	0.55	6.39	96.36	2	9.99e-03
(+)-Withanolide A	470.60	3.33	6	2	127.53	Yes	Yes	0.55	6.39	96.36	2	9.99e-03
3-HDH-Withanolide F	488.61	2.57	7	2	130.92	Yes	Yes	0.55	6.50	124.29	2	1.50e-01

3-beta-Methoxy-2,3-dihydrowithaferin A	502.64	3.30	7	2	133.86	Yes	Yes	0.55	7.02	105.59	2	7.58-03
Withanolide B	454.60	4.22	5	1	126.33	Yes	Yes	0.55	6.34	76.13	2	1.57e-03
Withanolide N	452.58	3.79	5	2	127.57	Yes	Yes	0.55	6.16	83.83	3	1.10e-03

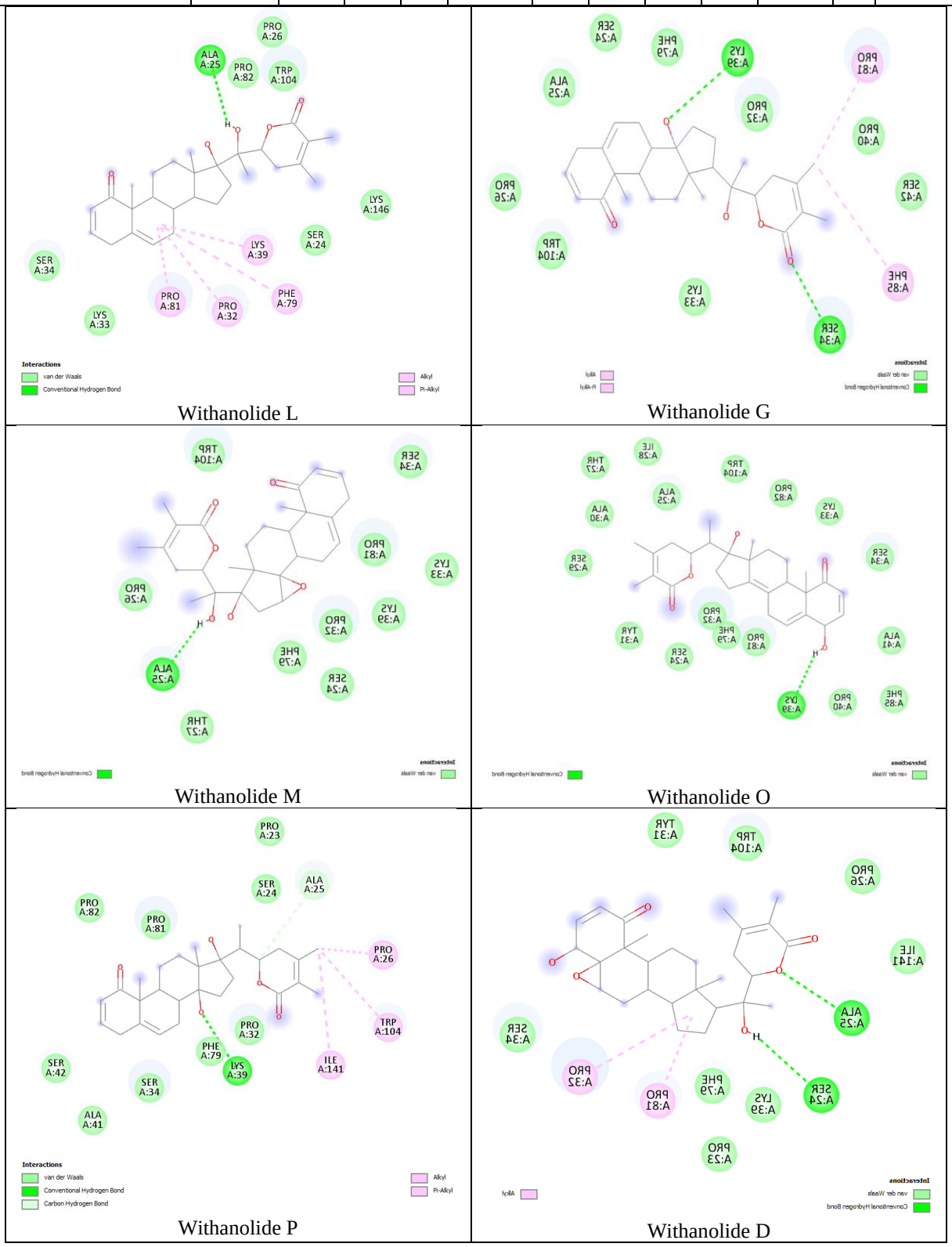


Fig. 4: Docking pose of ligands showing high affinity with the active site of Polygalacturonase enzyme.

7. Conclusion

The cell wall degrading enzyme Polygalacturonase secreted by Aspergillus niger triggers decaying of the fruits and vegetables thereby decreasing their shelf life and also their quality by degradation of pectin. Hence post-harvest control of Aspergillus niger is considered to be one of the major insights to be looked after. Chemical or synthetic fungicides used for controlling these postharvest diseases have been reported negative effects on environment and human health. Withanolide group of natural compounds were found to show strong inhibition potentials of Aspergillus niger polygalacturonase enzyme insilico. Hence, from the above results it is concluded that the withanolide natural compounds would be promising alternate for chemical antifungal drugs. However further invitro and invivo investigation is required.

Declaration: The authors declare that there is conflict of interest

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References

1. Slavin JL, Lloyd B. Health benefits of fruits and vegetables. Advances in Nutrition. 2012, 3:506-516.
2. Poonam, & Jakhar, Raj & Kumawat, Priyanka & Prajapat, Amit & Kumawat, Subhita. Post-Harvest Management of Fruits and Vegetables. 2022, 148-160
3. Al-Hindi RR. Cell Wall Degrading Enzymes of Fruit Spoilage Fungi. Life Science Journal, 2012, 2456-2463.
4. Erkmen and Bozoglu. Spoilage of Vegetables and Fruits. In Food Microbiology: Principles into Practice 2016, ISBN: 9781119237761 pp337-363. John Wiley & Sons, Ltd.
5. Alegbeleye O, Odeyemi O.A, Strateva M, Stratev D. Microbial spoilage of vegetables, fruits and cereals. Applied Food Research. 2022, 2: 100122.
6. Liu CQ, Hu KD, Li TT, Yang Y, Yang F, Li YH, Liu HP, Chen XY, Zhang H. Polygalacturonase gene pgxB in Aspergillus niger is a virulence factor in apple fruit. PLoS One. 2017, 12:e0173277
7. Oza K, Jain BK, Maitreya B. Spoilage of fruits and vegetables due to fungal infection and its post-harvest control: A review. 694 International Journal of Botany Studies. 2020, 5: 694-697.
8. Al-Hindi RR, Ahmed R, Al-Najada and A. S. Mohamed. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. African Journal of Microbiology Research. 2011, 5(4), 443-448.
9. Collmer A, Keen N. The role of pectic enzymes in plant pathogenesis. Ann. Rev. Phytopathol. 1996, 24, 383-409.
10. Idnurm A, Howlett BJ. Pathogenicity genes of phyto-pathogenic fungi. Mol. Plant Pathol. 2001, 2: 241-255.
11. De Lorenzo G, Castoria R, Bellincampi D, Cervone F. Fungal invasion enzymes and their inhibition. In: G.C.Carroll, P. Tudzynski (Eds.), The Mycota V, Part B: Plant Relationship. Springer, Berlin, 1997, 61-83 (1997).
12. The UniProt Consortium, UniProt: a worldwide hub of protein knowledge, Nucleic Acids Research. 2019, 47(D1), D506–D515 (2019).
13. M. Johnson, I. Zaretskaya, Y. Raytselis, Y. Merezhuk, S. McGinnis, T.L. Madden. NCBI BLAST: a better web interface. Nucleic Acids Res. 2008, 36(Web Server issue), W5-9.
14. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H et.al. Clustal W and Clustal X version 2.0. Bioinformatics.2007, 23: 2947-8
15. Webb B, Sali A. Protein Structure Modeling with MODELLER. Methods Mol Biol. 2021, 2199: 239-255
16. Laskowski RA, MacArthur MW, Thornton JM. PROCHECK: validation of protein structure coordinates, in International Tables of Crystallography, Volume F. Crystallography of Biological Macromolecules, eds. M.G. Rossmann & E. Arnold, Dordrecht, Kluwer Academic Publishers, The Netherlands. 2001: 722-725 (2001).
17. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis.1997, 18: 2714-23.
18. Dominguez-Villa FX, Duran-Iturbide NA, Avila-Zaeeaga JG. Synthesis, molecular docking and insilico ADME/Tox profiling studies of new 1-aryl-5-(3 azidopropyl)-indol-4-ones:potential inhibitors of SARS CoC-2 main protease. Bioorg Chem. 2021, 106: 104497.