IN SILICO MOLECULAR MODELING AND DOCKING STUDIES OF NANO COMPOSITES COMPOUND TO REGULATION, INHIBITION AND TREATMENT LEAF AND STEM WHEAT RUST

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ABSTRACT

Puccinia graminis f. sp. tritici (Pgt) and P. triticina (Pt), the causal agents of stem and leaf rust, respectively form new physiological races that significantly reduce growth and yield of wheat cultivars. Therefore, seeking for exploring if there an inhibition effect of the Nano Composites compound on leaf and stem rust to regulation, inhibition and treatment leaf and stem wheat rust objectives to continuously produce new wheat pesticides resistant to stem and leaf rust. The aim of the study was to finding natural and Nano compounds to control, treatment and regulation of wheat rust. In this study we used molecular modeling and docking for the two vital proteins in stem and leaf wheat rust MAP kinase 1 [Puccinia triticina] and PGTG Puccinia graminis f. sp. Tritici*. In the silico analysis, the two vital proteins activity is suppressed and inhibited In this work the chitosan and chitosan –Cu which selected for the study are considered as safe compounds the compounds showed interaction with the MAPK1 and PGAT proteins Thus the bioactive compounds that are interacting with the target can be used as a potent inhibitor to block the action of our proteins.*

Keywords: Leaf rust, nanoparticle, molecular docking, stem rust

PENDAHULUAN

Wheat (*Triticum aestivum*) is an important domestic and international economic food grain source. The demand for wheat specially for developing countries is projected to increase to 60% by 2050 (FAO, 2016). This increase demand for wheat is very serious, especially in the context of climate changes that would lead to decrease the final productivity by 29% (Rosergrant et al., 1995). Moreover, wheat is attack by three rust diseases, i.e. stripe, leaf and stem rust. The later caused by *Puccinia graminis* Pers. f. sp. tritici (Eriks. & E. Henn.) is a destructive disease of wheat crops all over the world under appropriate environmental conditions when a disease becomes epidemic (Naseri and Sabeti, 2020). Wheat stem rust fungus attacks wheat plants, especially those planted lately leading to block the vascular system, plant stunting and finally causes up to 100% yield losses , due to damage grain and tillers (Boukhatem, 2002).

In Egypt, stem rust causes 1.96 to 8.21% losses in the yield of local wheat varieties (Ashmmawy et al., 2013). In the last two decades, the stem rust disease has a reason again due to arise of the new race Ug99. About 90% of wheat crop worldwide are susceptible to this destructive race (Singh et al. 2011) leading to up to 70% yield losses. In Kenya, yield losses due to stem rust exhibited up to 100% reduction (Njau et al, 2010). In Egypt, up to 95% of the local commercial varieties are susceptible or highly susceptible to race Ug99, while only a few older varieties showing some level of "adult plant resistance" (APR) to this race and it's variants that have been detected in Kenya (Njau et al, 2010; Wanyera et al, 2006). More recently, more than 15 confirmed Ug99 races have been reported in Africa, (Singh et al. 2015) and rapidly spread to another countries in Africa (Kenya, Ethiopia, Sudan, Tanzania, South Africa, Zimbabwe, Mozambique, Eritrea and Egypt) and Middle East (Yemen, Iran and Pakistan) (Wanyera *et al* 2006, Nazari *et al* 2009, Pretorios *et al* 2010, Wolday *et al* 2011 and Abou-Zeid *et al* 2014). It is expected that the Ug99 could be attack cereal gown in another geographical regions around the world in the future.

Rust fungi, belonging to the genus Puccinia, are among the most economically

destructive biotrophic phytopathogenic fungi infecting cereal crops. Members of this genus pose a serious threat to global wheat production which is a major staple food for mankind in many parts of the world. Wheat is a host to three important rust pathogen species: *Puccinia graminis* f. sp. *tritici* (Pgt), *Puccinia striiformis* f. sp. *tritici* (Pst) and *Puccinia triticina* (Pt), which are the causal agents of stem rust, stripe rust and leaf rust, respectively (Bolton *et al*., 2008; Chen *et al*., 2014; Singh *et al*., 2011). Of the three rust diseases, wheat leaf rust (WLR, caused by Pt) is comparatively more prevalent occurring regularly wherever wheat is grown (Kolmer, 2005). Because of its adaptation to diverse climatic conditions and widespread occurrence, leaf rust results in greater total annual losses worldwide than stem and stripe rusts (Huerta-Espino *et al*., 2011). Under severe epidemic conditions, *Pt* can inflict yield losses ranging from 10% to 70% (Herrera-Foessel *et al*., 2006). Fungicides are usually applied for management of rust diseases, but financial costs and adverse environmental impacts are associated with chemical inputs. In addition, a major concern with excessive application of antimicrobial compounds is a high risk of reduced sensitivity or development of resistant pathogen populations. So The identification of pathogenic proteins MAP kinase 1 [*Puccinia triticina*] (leaf rust) and PGTG *Puccinia graminis* f. sp. *Tritici* (stem rust) that are cased the pathogenicity on the leaf and stem rust has been a central aim of research now day .The evolution in the field of Cheminformatics has ushered in various approaches to identify the therapeutic properties of chemical compounds stored in the chemical databases or new Nano Composites compound.

MATERIALS AND METHODS

Hardware

Notebook with spesification Intel(R) Core(TM) i3-4005U CPU @1,70 GHz, 4 GB memory, 64-bit Operating System, Operating System Windows 7.

Software

SAMSON software 2020 (https://www.samson-connect.net/), SWISSMODLE server (https://swissmodel.expasy.org/), Discovery studio (https://discover.3ds.com/discovery-studio-visualizer-download).

Material

The ligands/compounds were used in this study are Chitosan and cu-chitosan. Protein used in this study was obtain from GenBank ID ie MAP kinase 1 [Puccinia triticina] (AAY89655.1) and PGTG Puccinia graminis f. sp. tritici (10532062).

Methods

Database Search, Structural Modeling, and Model Validation

The proteins sequences are available in NCBI (https://www.ncbi.nlm.nih.gov/) and uniprot (https://www.uniprot.org/). in fasta format with with GenBank accession no. Table 2. the fungus proteins was selected for searching all the sequential homolog and orthodox using NCBI Blast server1 (Altschul et al., 1997) keeping the default values, and against the non-redundant protein sequences, with searching the organism. The sequences were retrieved in the FASTA format as an amino-acid length sequence and used to build. the 3D structure were built using the SWISSMODLE server (https://swissmodel.expasy.org/). The homology model of the fungs protein was achieved by using the SWISSMODLE server. A sequence of Blast-P similarities for recognition of close-related structural homologs in fungus was queried against a PDB database14 (Berman et al., 2000). The first hit on the annotation Blast-p was obtained to identify the templates in which (PDB temple ID, identity, QMEAN). The Protein Data Bank collected the PDB file of the templates (PDB). Found by BLAST was the alignment data. The SWISSMODLE server began with the target sequence file, the alignment file, and the PDB file for the prototype, all the temples proteins. The fungus proteins with a QMEAN score have been developed for the development of models. In addition, for final confirmation, the protein model with an below -4 score has been chosen. The optimized models MAP kinase 1 [Puccinia triticina] and PGTG Puccinia graminis f. sp. Tritici was found to be suitable based on several qualitative backgrounds including the ERRAT, PROCHECK (PDB Sum), and Verify-3D. The Ramachandran plot which evaluated that the predicted models were closer to the template (91% , 93%) residues lying in the favored regions). The

ERRAT score values (91.56 % , 78.43%) and Verify-3D results were good enough signifying the consistency of the model prediction and explained that the predicted model was reliable and satisfactory, as it was reported that, for a model having good resolution ,the ideal score values for Verify-3D should be 80%, and that for the ERRAT around 95% (Colovos and Yeates, 1993).

Preparation of Protein and Ligands

The fungus protein was selected as a target receptor protein and was imported to the 3Drefine server to make energy minimization for 2 proteins (http://sysbio.rnet.missouri.edu/3Drefine/) for pre-docking; all water molecules and ligands were removed while hydrogen atoms were added to the target protein. The docking system was built using SAMSON software 2020 (https://www.samson-connect.net/) .and then imported to SAMSON The structures were prepared using protein preparation wizard of the Autodock Vina extension (http://vina.scripps.edu/). Using SAMSON 2020 software. A receptor grid X,Yand Z value of proteins are in table 2 was generated besd on the blind docking to explore the effect of the ligands on the proteins . The ligand (chitosan) were retrieved from the PubChem database id in Table 2 . and the chitosan with CU are prepared and designed in chemdraw https://www.perkinelmer.com/category/chemdraw) in pdb format, then converted into MOL2 format by using openbable software (http://openbabel.org/wiki/Main_Page). Then The energy minimization was done at neutral pH 7.0 ± 2.0.in SAMSON software.

Binding site prediction, Protein-ligand docking

SAMSON software (https://www.samson-connect.net/) is used for binding site prediction. It uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites. This software uses Autodocking Vina as element to maximizing the accuracy of these predictions while minimizing the computer time (Trott e al., 2010). The program works based on the quantum mechanics. It predicts the potential affinity, molecular structure, geometry optimization of structure, vibration frequencies of coordinates of atoms, bond length and bond angle. Following by the exhaustive search, 100 poses were analyzed and the best scoring poses were used to calculate the binding affinity of the ligands. The ligands that tightly bind to target protein with high score were selected Table 2 The proteins were docked against the compounds chitosan and cu using SAMSON software (https://www.samson-connect.net/). The interaction was carried out to find the favorable binding geometries of the ligand with the proteins by using Discovery studio software (https://discover.3ds.com/discovery-studio-visualizer-download) to get the interaction of the Docked protein ligand complexes with high score to the predicted active site.

Protein ligand interaction using SAMSON and Discovery studio software

The ligands were docked with the target protein, and the best docking poses were identifying. Figures 3-4 shows 3D structure of the binding poses of the compounds with the interacting residues of the active site of the proteins.

RESULTS AND DISCUSSION

Database Search, Structural Modelling, and Model Validation

The homology of the query protein sequences with the target proteins from pdb . The query coverage of proteins sequences showed MAP kinase 1 [Puccinia triticina] and PGTG Puccinia graminis f. sp. tritici (84% , 12) query coverage's with (56.14 , 56.15) identity with the template proteins (2vkn.1.A , 2fa2.1.A) they used as a template proteins for homology modeling of our target proteins.

Table 1. show the proteins with their GenBanck id with the template proteins with there sequence identity with quarry coverage of the our protein with the template proteins with the validation method which is QMEAN.

Protein	GenBank/ID:	Template	Seq Identity	Coverage	QMEAN
MAP kinase 1 [Puccinia triticina]	AAY89655.1	2fa2.1.A	56.14	0.84	-1.53
PGTG *Puccinia graminis f. sp. tritici*	10532062	2vkn.1.A	56.14	0.12	-0.69

Structural Modeling, in silico Characterization, and Model Validation

The SWISSMODLE server generated (25 ,35) predictive models for MAP kinase 1 [Puccinia triticina] **and PGTG** *Puccinia graminis f. sp. tritici* proteins with different (QMEAN) score values. The model with low values for QMEAN score(-1.53 and -0.69) were selected as a final model for *in silico* characterization and docking studies. The selected model was verified for their stereochemical quality assessment. Furthermore, in each case of qualitative assessment, a comparative study was done with experimentally solved crystal structures, to check the quality, reliability, accuracy, stability and compatibility of the computationally predicted protein. The Ramachandran plot obtained through The PROCHECK module of the PDBSum server revealed that the predicted model.

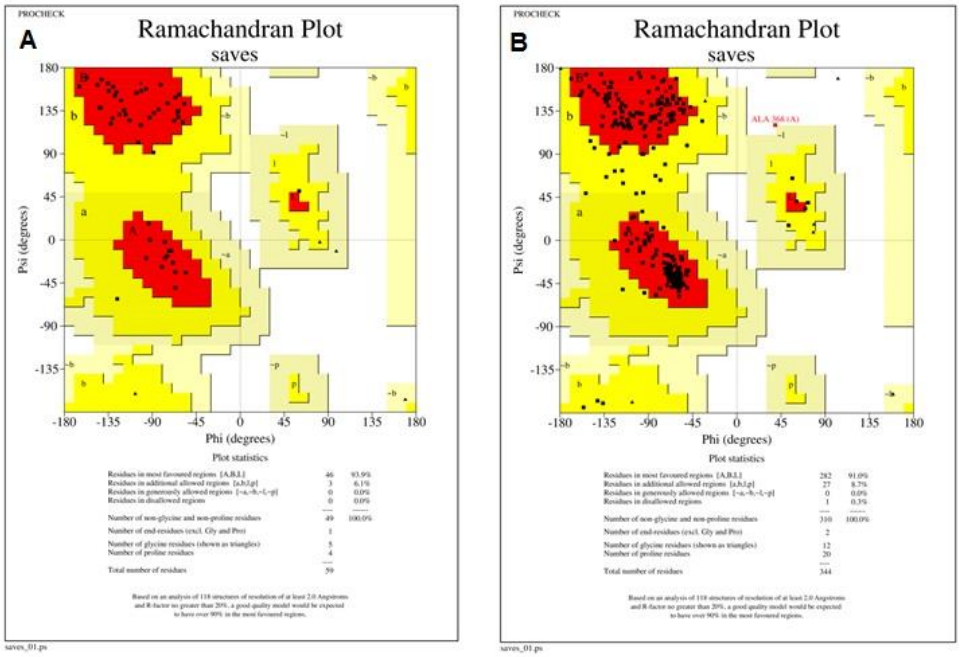

Figure 1. Ramachandran plot based on The PROCHECK module of the PDBSum server for proteins models of MAP kinase 1 [Puccinia triticina] (A) and PGTG *Puccinia graminis f. sp. Tritici* (B)

The PROCHECK module of the PDBSum server, further justified the stereochemical goodness of the predicted models MAP kinase 1 [*Puccinia triticina*] and PGTG *Puccinia graminis f. sp. Tritici* , with 91% , 93% residues accommodating in the most favored regions (A, B, and L) and only 8.7%, 6.1 % residues occupied in the additionally allowed regions (a, b, l, and p) representatively .This confirms the predicted models quality MAP kinase 1 [Puccinia triticina] and PGTG *Puccinia graminis f. sp. tritici* had good stereochemical quality and was close to the template structure. the ProQ is a neural network-based predictor based on a number of structural features predicts the quality of protein model. The ERRAT score for the modeled structure was found to be 96.22%, 78.4314 representetevely

The Verify 3D evaluated that the predicted proteins MAP kinase 1 [Puccinia triticina] and PGTG *Puccinia graminis f. sp. tritici* have 96.22%, 81.36% of the residues have averaged 3D-1D score >= 0.2 Pass At least 80% of the amino acids have scored >= 0.2 in the 3D/1D profil.

Binding site prediction, Protein-ligand docking

The predicted model was visualized through the visualization module of the Discovery Studio 3.0 (Figure 2). The ligand binding sites identified in the target proteins structure.

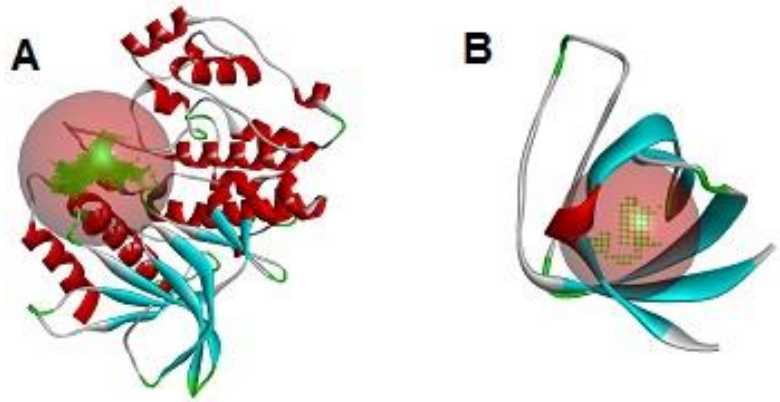

Figure 2. represent the binding site of the MAKP1 of the fungus and B its the PGTG protein The binding sites inside the proteins, the protein with red balls showing active sites

Active Site Prediction and Protein–Fungicide Interaction

The putative ligand binding sites (both major and minor) for the predicted protein were identified through Discovery studio software. The modeled proteins MAPK1 and PGAT was docked with all the chitosan and chitosan with cu to generate their binding mode which done to refine the best pose with allowed conformational change in the MAPK1 and PGAT. We have evaluated the protein–fungicide interaction through Discovery studio software. It was found that the tool have discrepancies in results for accurate pose prediction among the various putative docking poses, revealed through scoring functions, which might leads into conclusion that, docking scores are not sufficiently precise to represent the protein ligand binding affinity (Suenaga et al, 2012).

The MPAK1 its bind to chitosan with ΔG_{bind} and docking score are -6.9 kcal/mol with the interactive binding site residues (ARG106 , ASP192 , GLU210 , GLY212 , LEU213 , GLU223 , THR224 , GLY225 , MET227 , VAL231 , ALA232 and ARG234) , for the MPAK1 with chitosan -CU the ΔG_{bind} are -6.6 kcal/mol with the interactive binding site residues (ARG106, LYS194, GLU223, MET227, GLU229, VAL231, ALA232, GLN247 and GLY273) . then the PGAT its bind to chitosan with ΔG_{bind} -4.6 kcal/mol with the interactive binding site residues (TYR441 , PRO448, ASN449, SER483, SER478, GLN470, LYS467) , and the PGAT its bind to chitosan- CU the ΔG_{bind} are -5.6 kcal/mol with the interactive binding site residues (ALA443, PRO448, ASN449, GLY452, PHE453, GLN470, ARG472, GLY476, GLU477, SER478).

Table 2. Show Binding Energy

Target	Ligands	Binding energy	X,Y , Z value
MAP kinase 1 [Puccinia triticina]	Chitosan	-6.6	center_x = 8.6875 center_y = 7.9130 center_z = 26.0847
	cu-chitosan	-6.9	
PGTG Puccinia graminis f. sp. tritici	Chitosan	-4.6	center_x = 11.3055 center_y =22.877 center_z = 1.3282
	cu-chitosan	-5.6	

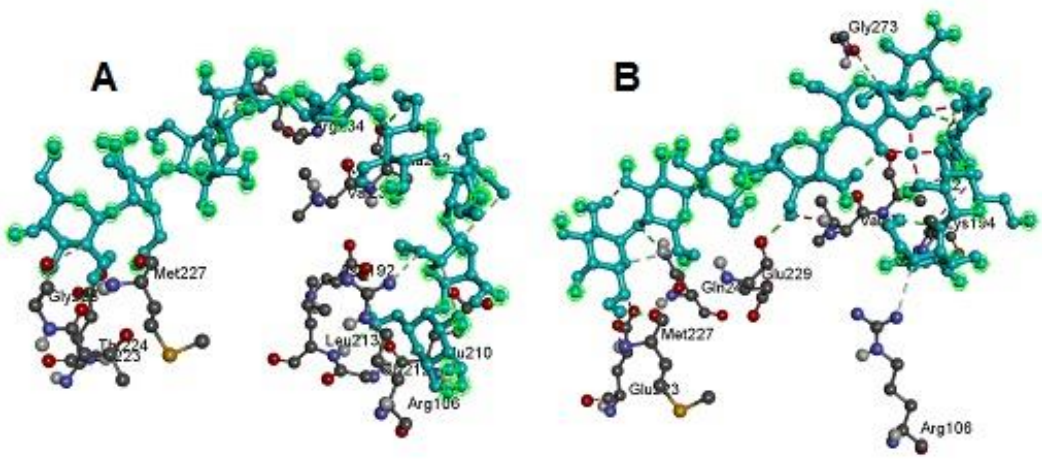

Figure 3. (A) The 3D surface view of MAPK1 modeled protein with chitosan reveals only the interactive residues on the binding site of a protein. (B) chitosan MAPK1 –Cu was the interactive residue of binding sites of the protein binding sites (active sites).

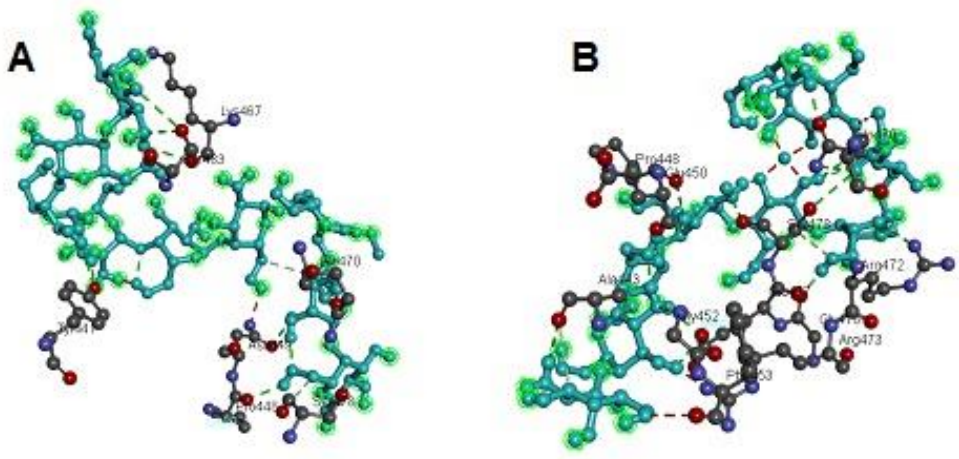

Figure 4. (A) The 3D surface view of PGAT modeled protein with chitosan reveals only the interactive residues on the binding site of a protein. (B) PGAT chitosan –Cu was the interactive residue of binding sites of the protein binding sites (active sites).

This best docking poses shows how the ligand molecule fits into the binding region of the target protein. Intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the MAPK1 and PGAT proteins–inhibitor complexes. all of the ligands (chitosan and chitosan –Cu) had a greater binding affinity with the target proteins MAPK1 and PGAT proteins. Inhibition was measured by the binding energy of the best ligand pose measured in kcal/mol. The binding pose and their energy are listed in Table 2.

CONCLUSION

Gain of wheat lines tolerance to stem rust caused by *P. graminis f. sp. tritici* and leaf rust caused by *P. triticina* are urgent because of the rareness of resistance genes against these global wheat destructive diseases. Thus, the world needs to identify new inhibitors resources. The Nano chitosan and chitosan with cu in Egypt can use to reduce Egyptian wheat rust, thus it may be exploited as a new source of rust treatment for gaining and developing novel inhibitor for wheat rust Chitosan NPs have previously been reported as immune modulator through induction of antioxidant/defense enzymes activity in tea

and finger millet plants (11, 19). In the silico analysis, the MAP kinase 1 [Puccinia triticina] and PGTG Puccinia graminis f. sp. tritici activity is suppressed and inhibited In this work the chitosan and chitosan –Cu which selected for the study are considered as safe compounds the compounds showed interaction with the MAPK1 and PGAT proteins Thus the bioactive compounds that are interacting with the target can be used as a potent inhibitor to block the action of our proteins.

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